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## Field Trial Evaluation of Levofloxacin and Erythropoietin in Treatment of Hemorrhagic Enteritis in Dogs

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**Abstract:** Enteric fever was a marked problem all over the world in domestic pets and it plays a major role in zoonosis to contact human. The target of our investigation to confirm diagnosis of enteric fever caused by *S. paratyphi A* using rapid Widal test and isolation and identification of the causative organism. Also the present study was aimed to evaluate clinically and clinicopathologically by a field work-up, the therapeutic value of levofloxacin and human erythropoietin. The present study was carried out on fourteen dogs (9 diseased dogs and 5 apparently healthy dogs). Clinical manifestations were severe watery bloody diarrhea (n.=3), acute diarrhea with occult blood in fecal examination (n.=4), high fever (n.=7), prolonged capillary times (n.=7), enophthalmos (n.=7), loss of skin turgor (n.=7), increased respiratory and pulse rates (n.=7). Clinical examination was revealed abdominal distension (n.=3), hepatomegally (n.=2), abdominal tenderness (n.=7), tympanic sound (n.=7) and increased peristaltic movements (n.=7). Clinical remission of signs occurred after treatment with levofloxacin within 14 days. Widal agglutination test was displayed the titer of 7 positive cases ranged from 1:80-1:320 and 2 negative cases (1:40). Isolation and identification was revealed *S. paratyphi A* (100%) and *S. paratyphi B* (22.2%). Significant decreases of PCV %, hemoglobin concentration, erythrocytes and leucocytes count was recorded which was became significant increases after treatment within 8 weeks. Significant increase of ALT activities was denoted hepatic involvement during the infection. Erythropoietin exerted an excellent therapeutic effect within 4- 8 weeks on basis of erythrogram investigations. It was concluded that combination of levofloxacin and human erythropoietin was the first record for treatment of hemorrhagic enteritis caused by *S. paratyphi A* in companion dogs in Egypt. (n.= number)

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**Keywords:** Dogs, *S. paratyphi A*, Widal, hemorrhagic, enteritis, levofloxacin, Erythropoietin

### 1. Introduction:

The risk of *Salmonella* infections are the zoonotic importance as it can be transferred between human and animals. Many infections are due to ingestion of contaminated food. A distinction is made between enteritis *Salmonella* and typhoid/paratyphoid *Salmonella*, where the latter because of a special virulence factor and a capsule protein (virulence antigen) can cause serious illness, such as *Salmonella enterica subsp. enterica* serovar typhi (*Salmonella typhi*). It is adapted to humans and does not occur in animals (Crump et al., 2004 and Porwollik, 2011). Enteric fever, that is typhoid and paratyphoid fevers, is the common name for infections caused by *Salmonella enterica* serotypes typhi and paratyphi (Crump et al., 2004).

*Salmonella* infection, especially a carrier state in dogs is very important to public health, because dogs are usually reared in contact with humans (Bagcigil et al., 2007). In foreign countries, there have been some reports on transmission of *Salmonella* from dogs to humans. In Japan, there have been many reports on isolation of *Salmonella* from apparently healthy dogs. *Salmonellosis* is a zoonotic disease affects both animals and humans; it

is a potentially fatal systemic infection and its clinical symptom mainly related to septicemia and enteritis

*Salmonella* infection in humans usually occurs from pets, other humans and from ingesting contaminated water or animal food products, most often eggs, poultry, and meat (Bhan et al., 2005, Centers for Disease control and prevention, 2005 and Swanson et al., 2007). Any food can become contaminated by feces. After the ingestion of organisms, the likelihood of infection developing, as well as the severity of infection, is related to the dose and virulence of the *Salmonella* strain and the status of host defense mechanisms.

*Salmonellae* are motile, gram-negative, non-spore-forming bacilli and can be differentiated into more than 2000 serotypes (serovars) by their somatic (O) antigens. There are six serogroups: A, B, C1, C2, D, and E. There are three species of *Salmonellae* that cause paratyphoid (*Salmonella enterica* serovar paratyphi): *Salmonella paratyphi A*, *S. paratyphi B* (or *S. schotmulleri*) and *S. paratyphi C* (*S. hirschfeldii*) after Maskalyk (2003), Bhan et al. (2005) and Effa & Bukirwa (2008). As a result of modern sewage and water treatment facilities, these diseases have become rare in developed countries but

remain a problem in countries without adequate sanitation and a safe water supply (Steffen et al., 2003).

The extent of infection of *Salmonella* species depends on the bacterial type and its strain. *Salmonella* possesses two antigens or weapons to protect it and cause destruction, these include Heat stable cell wall lipopolysaccharide known as Somatic or 'O' antigens and flagellar or H antigens formed from structural proteins, which make up the hair like filaments from the movement of the bacteria. (Lesser and Miller, 2001)

After ingestion of *Salmonella* species along with contaminated food, the bacterium overcomes the body's defense mechanism of gastric acidity (Connor and Schwartz, 2005). However, their main place of settlement is in the intestine where they cause inflammation and ulcers (Rene and Pines, 2002). Thus there is often a lesion in the intestinal tract with the consequent danger of haemorrhage and intestinal perforation (Turk and Porter, 1982). Perforation of the intestine which is followed by the leakage of the intestinal contents into the abdominal cavity (peritonitis) is a frequent cause of death from typhoid fever (Rene and Pines, 2002). The disease presents a clinical dilemma because of its varied manifestations and serious complications to various body organs (Qasmi et al., 2010)

Diagnosis of Salmonellosis is carried out by isolation of *Salmonella* spp. from blood, feces, urine or other body fluids (Porwollik, 2011). Moreover, salmonella infections can be diagnosed using serological tests such as the traditional Widal test ; "a test involving agglutination of typhoid bacilli when they are mixed with serum containing typhoid antibodies from an individual having typhoid fever; which may be used to detect the presence of *Salmonella* typhi and *S. paratyphi*." Newer diagnostic serological tests (e.g.IDL Tubex test, Typhidot test, IgM dipstick test) have been reported to give improved sensitivity and specificity in initial evaluations, but are not yet widely utilized. However, these tests have some limitations as a positive test result is dependent on an antibody response by the animal which may take up to 7-10 days to develop, in addition to the antigenic cross- reactions (Chin, 2000).

From clinicopathological concern, severe leucopenia and thrombocytopenia are common in typhoid and paratyphoid fever with moderately raised liver enzymes (Okafor, 2005). The present paper deals with cases of *Salmonella* Paratyphi A infection of a household dogs manifesting severe bloody diarrhea. Bacteriological examination, Widal agglutination test as well as hematological and biochemical analyses were studied in these cases.

The present study was aimed to diagnose the infection caused by *Salmonella* (paratyphoid fever) and to choose a satisfactory therapeutic approach. Differential diagnosis of hemorrhagic diarrhea caused by paratyphoid fever is difficult as large number of etiologies and need careful history taking, professional clinical examination and laboratory investigations. Causes of acute diarrhea were tremendous as internal worms, *Coccidia*, *Giardia*, *Entamoeba histolytica*, *Salmonellae*, *Campylobacter*, *Clostridium perfringens*, Corona virus, Parvo virus, Canine distemper, acute hepatic failure (canine hepatitis virus), acute renal failure (leptospirosis) and acute pancreatitis. But life threatening hemorrhagic enteritis was hookworms, whipworms, Canine Parvovirus, acute pancreatitis, acute hepatic failure (canine hepatitis virus), acute renal failure (leptospirosis), *Campylobacter*, *Clostridium perfringens* and *Salmonellae* (Burrows et al., 1995 and Leib & Monroe, 1997).

## 2. Materials and Methods:

### Animals

The present study was carried out on fourteen dogs which were admitted to veterinary clinics in Giza governorate (9 diseased dogs and 5 apparently healthy dogs). All dogs were ranged from 3.8- 13.2 years. The breeds of diseased dogs were 5 Labrador retriever dogs (3 females and 2 males), 2 German shepherd dogs (one female and one male) and one Greatdane dog (one male), while the breeds of apparently healthy dogs were 3 Labrador retriever dogs (2 females and one male) and 2 German shepherd dogs (2 males). Dogs' weight ranged from 37.5- 47 kg. An accurate medical history of previous treatments and routine health care, such as deworming and vaccination programs was recorded. All investigated dogs were vaccinated annually against Canine distemper virus, Corona virus, Parvo virus, Adeno virus 2, Parainfluenza and *L. icterohemorhagica* and *L. canicola* (by Pfizer) and received Drontal® plus (50 mg praziquantel, 150 mg Febantel, 144 mg pyrantel- Embonat, made in Germany by Bayer) as internal worm prophylaxis. The diseased dogs were fed on a commercial dog food (German formula). Thorough diagnostic plan must be followed to reach a diagnosis efficiently and evaluated clinically every one week after treatment. Clinical examination was performed by inspection, palpation, percussion and auscultation of abdomen (intestines). Respiratory rate (rate/ min), pulse rate (rate/ min), rectal temperature (°C), lymph nodes and mucous membranes were thoroughly examined and evaluated (Leib and Monroe, 1997).

**Experimental design and sampling:**

The blood samples were obtained from anterior median vein. Two blood samples were collected from each dog. The first blood sample was collected on EDTA which used for hematological examination and the second blood sample was collected into plain centrifuge tube for serum separation for biochemical analysis and Widal test. The same blood samples were taken at 4 and 8 weeks post treatment (p.t.) from all dogs except one case died at first day pre-treatment.

**Fecal examination:**

Fecal samples were examined macroscopically for presence of blood, undigested food or mucous. Thin fecal smears were examined under the microscope for any parasitic ova or coccidia oocysts (Thiopont et al., 1986). The dogs were re-examined at 4 and 8 weeks post treatment (p.t.).

**Widal agglutination test:**

Serological identification was done by using polyvalent and monovalent O and H antisera (Difco) according to Membrebe, and Chua (1999).

**Salmonella isolation and identification:**

Fecal swabs were collected from infected and non infected dogs at day 0 and 4 weeks (p.t.) for Salmonella isolation and identification. The inoculated Tetrathionate broth tubes with fecal swabs were incubated for 16 hours at 37°C. A loopfull of the broth was streaked onto XLD, MacConkey and SS agar plates and incubated at 37°C for 36-48 hours and the suspected colonies were identified morphologically, Gram's staining and biochemically using the API-20E kit system (Biomeraux, France) after Morifnigo et al. (1986) and Chirino-Trejo (1999).

**Hematological examination:**

Estimation of erythrocytic count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV) and; total and differential leukocytic counts were performed according to Fieldman et al. (2000). Biochemical analysis:

Serum samples were used for determination of the activities of Alanine amino transferase (ALT) according to Reitman & Frankel (1957). Activities of both of alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were studied according to Babson et al. (1966) and Dumas & Biggs (1972), respectively. Bilirubin was measured according to Doumas et al. (1973). Total proteins and serum albumin according to Weichselbaun (1964) and Dumas & Biggs (1972) respectively. Serum globulin

was calculated by subtracting the obtained values of albumin from values of total proteins. Blood urea nitrogen and serum creatinine were assayed according to Patton & Grauch (1977) and Fabiny & Eringhausen (1971) respectively. Serum biochemical parameters were assayed using commercial diagnostic kits supplied by Stanbio-Laboratory, USA.

Intensive therapeutic plan must be managed specially in life threatening diarrhea. Fluid therapy was the first emergency treatment by intravenous injection of dextrose® 5% (dextrose 5% solution, by Otsuka) which was calculated on basis of body weight and skin fold test, in addition to the use of rehydran N® sachets (KCl, CaCl<sub>2</sub> and NaCl, by CID) after Leib & Monroee (1997). Tavanic® tablets (Levofloxacin 500 mg/ tablet, by Aventis) used as broad spectrum antibacterial, one tablet /24 hours for 5 days (Nichterlein et al., 1998). Erypoietin® vial (Epoetin beta, recombinant humane erythropoietin, by Amoun) in a dose of 1 vial weekly/ 8 treatments beside adjunct therapy by Sytron® syrup (Iron 27.5 mg/ 5ml and sodium iron edelate 190 mg/ 5ml, by PD) in a dose of 5 ml bid for treatment of anemic cases for 8 weeks and Halorange plus® syrup (Vitamins A, E, D, K and C, by Tetra.) in a dose of 5 ml bid for 3 weeks (Oishi et al., 1995).

Statistical analysis was performed by statistical Package for Social Sciences (SPSS). Mean and standard deviation are descriptive values for quantitative data. ANOVA (Analysis Of Variance) was used for testing means of more than two groups by computer program according to the method described by Irwan (1996).

**3. Results:**

Clinical presentation was severe and sudden. 7 Sick dogs arisen suddenly the acute enteritis, low performance, high fever, loss of appetite, the diarrhea, the draining water mucoid excrement and critically 3 ill dogs (senile dogs) arranged the bloody diarrhea. Signs of dehydration (7 cases) were recorded as dry mucous membranes, loss of skin turgor, prolonged capillary refill times and enophthalmos. Mucous membranes were pale, dirty finally because of dehydration, one case then showed signs of shock then died.

Careful clinical examination of abdomen revealed abdominal distension (3 cases) on inspection, abdominal tenderness on palpation (7 cases), tympanic sound on percussion (7 cases), hypermotility (increased peristaltic movements in 7 cases) on auscultation prior treatment and clinical remission of signs after 8- 14 days. Popliteal lymph nodes were of normal size, movable, normal local temperature, symmetrical, firm in consistency, non lobulated in both groups. Mucous membranes were

pale (2 cases), congested (5 cases), rosy red (2 dogs of diseased group and 5 dogs of apparently healthy group) and improvement of color of mucous membranes after 22- 38 days. There were significant

increases in respiratory rate, pulse rate, rectal temperature and these parameters returned to normal within 18- 28 days (Table 1).

**Table (1): Respiratory rate, pulse rate and rectal temperature evaluation prior treatment in non infected and infected groups**

Parameters	Non infected group	Infected group
Respiratory rate (rate/ min.)	28 ± 2.8	57 ± 2.3**
Pulse rate (rate/ min.)	76 ± 4.4	109 ± 3.4**
Rectal temperature (°C)	38.1 ± 0.3	40 ± 0.4**

\*\*Significant at P value 0.01

Contact human (owners and attendants) were apparently healthy and showed no signs of paratyphoid or other diseases during 3 months of field evaluation.

#### Fecal examination:

The fecal samples were examined macroscopically and Samples showed blood and mucous with offensive odour in the infected group at day zero (pretreatment). Microscopically neither parasitic ova nor coccidia oocysts were detected in the specimens but there was only occult blood in 4 cases. Widal agglutination test

Seven cases out of nine were positive for salmonella paratyhi A by Widal agglutination test, the titer of positive cases ranged from 1:80 (4 out of 9,

44.4%)-1:160 (2 out of 9, 22.2%), only one case that died after 4<sup>th</sup> weeks post treatment showed a titer of 1:320 (one out of 9, 11.1%). Two cases were negative for salmonella paratyhi A by Widal agglutination test (1:40, 22.2%)

#### Salmonella isolation and identification:

Bacteriological examination of the 9 fecal swabs collected from the infected dogs' revealed presence of Salmonellae in all infected dogs. Isolated Salmonellae from the examined fecal swabs collected from infected dogs were identified as *S. paratyphi A* and *S. paratyphi B* (Table 2). The most prevalent Salmonella species was *S. Paratyphi A*, in all cases from the infected dogs (100%) but *S. paratyphi B* (22.2%). All fecal swabs collected from apparently healthy group and infected group at 4<sup>th</sup> weeks post treatment (p.t.) were bacteriologically negative.

**Table (2): Isolated Salmonellae paratyphi A and S. paratyphi B in different dog breeds**

Dog breed	<i>S. paratyphi A</i>	<i>S. paratyphi B</i>
German Shepherd	2 (100%)	0 (0%)
Labrador retriever	5 (100%)	2 (40%)
Greatdane	One (100%)	0 (0%)

#### Erythrogram

Mean values of the erythrogram [packed cell volume (PCV %), hemoglobin concentration (Hb), erythrocytes count (RBCs), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC)] of different experimental groups are illustrated in table (3).

In comparison to the mean values of apparently healthy dogs (non infected group) to the infected group at day zero (pretreatment), PCV %, Hb concentration, RBCs, and platelet count values showed significant decreases while, MCV and MCHC values revealed no significant changes. Microscopical examination of the stained blood film revealed hypochromacia in addition to the appearance of target cells. At 4<sup>th</sup> and 8<sup>th</sup> weeks (p.t.), PCV %, Hb concentration, RBCs, MCHC showed significant

increases. Platelet count values showed insignificant change at 4<sup>th</sup> and 8<sup>th</sup> weeks (p.t.) in comparison to non infected group.

#### Leukogram

Mean values of the leukogram [total leukocyte count (TLC), neutrophil, lymphocyte, eosinophil and monocyte counts] of different groups are illustrated in table (3).

Compared to the apparently healthy group, the infected group at day zero (pretreatment) showed significant leucopenia with significant neutropenia and lymphopenia while, at 4<sup>th</sup> and 8<sup>th</sup> weeks (p.t.) showed significant increase in leukogram when compared to the infected group at day zero (pretreatment).

**Table (3): Erythrogram and Leukogram in dogs of non-infected and infected groups (at zero day, 4<sup>th</sup> week and 8<sup>th</sup> week p.t.) with *S. paratyphi***

Parameters	Non infected dogs	Zero day	4 <sup>th</sup> week (p.t.)	8 <sup>th</sup> week (p.t.)	LSD
PCV (%)	43.33 ± 1.53 <sup>a</sup>	32.33 ± 2.52 <sup>c</sup>	38.67 ± 1.53 <sup>b</sup>	45.33 ± 2.08 <sup>a</sup>	3.69
Hb (g/dl)	15.27 ± 0.55 <sup>a</sup>	11.10 ± 0.75 <sup>c</sup>	14.15 ± 0.25 <sup>b</sup>	15.65 ± 0.30 <sup>a</sup>	0.95
RBCs (x10 <sup>6</sup> /μl)	6.98 ± 0.16 <sup>a</sup>	5.23 ± 0.35 <sup>c</sup>	5.95 ± 0.27 <sup>b</sup>	7.13 ± 0.18 <sup>a</sup>	0.47
MCV (fl)	62.06 ± 0.87 <sup>bc</sup>	61.73 ± 0.68 <sup>c</sup>	64.99 ± 0.51 <sup>a</sup>	63.55 ± 1.35 <sup>ab</sup>	1.71
MCHC (g%)	35.23 ± 0.31 <sup>ab</sup>	34.33 ± 0.65 <sup>b</sup>	36.47 ± 0.64 <sup>a</sup>	34.55 ± 0.96 <sup>b</sup>	1.28
Platelets (x10 <sup>3</sup> /μl)	229.00 ± 75.72 <sup>a</sup>	123.33 ± 10.41 <sup>b</sup>	209.33 ± 61.98 <sup>ab</sup>	264.33 ± 29.26 <sup>a</sup>	96.65
TLC (x10 <sup>3</sup> /μl)	8.80 ± 0.52 <sup>a</sup>	4.04 ± 0.86 <sup>c</sup>	6.96 ± 0.33 <sup>b</sup>	9.03 ± 1.15 <sup>a</sup>	1.47
Neut. (x10 <sup>3</sup> /μl)	5.01 ± 0.29 <sup>a</sup>	1.81 ± 0.39 <sup>c</sup>	3.96 ± 0.19 <sup>b</sup>	5.14 ± 0.65 <sup>a</sup>	0.84
Lymph. (x10 <sup>3</sup> /μl)	2.99 ± 0.18 <sup>a</sup>	1.93 ± 0.41 <sup>b</sup>	2.36 ± 0.11 <sup>b</sup>	3.06 ± 0.39 <sup>a</sup>	0.57
Mono. (x10 <sup>3</sup> /μl)	0.61 ± 0.04 <sup>a</sup>	0.20 ± 0.04 <sup>c</sup>	0.48 ± 0.02 <sup>b</sup>	0.63 ± 0.08 <sup>a</sup>	0.09
Esino. (x10 <sup>3</sup> /μl)	0.17 ± 0.01 <sup>a</sup>	0.08 ± 0.02 <sup>c</sup>	0.13 ± 0.01 <sup>b</sup>	0.19 ± 0.02 <sup>a</sup>	0.03

Means with different superscripts (a, b,c) are significantly different at P value < 0.05

#### Serum biochemical evaluation

Statistical analysis of different serum biochemical parameters is illustrated in table (4)

The results of the infected group at day zero, 4<sup>th</sup> and 8<sup>th</sup> weeks (p.t.) showed insignificant changes in BUN and creatinine. Serum total protein concentrations showed significant decrease in A/G ratio which attributed to the presence of significant hypoalbuminemia. The hepatic enzymes (ALT, ALP

and GGT) were significantly increased than the control group in association with the hyperbilirubinemia. On the other hand, the infected group at 4<sup>th</sup> and 8<sup>th</sup> weeks (p.t.) showed significant decreases in all serum biochemical parameters when compared to infected group at day zero (pretreatment) except serum total protein and albumin concentrations showed significant increases.

**Table (4): Serum biochemical parameters in dogs of non-infected and infected groups (at zero day, 4<sup>th</sup> week and 8<sup>th</sup> week p.t.) with *S. paratyphi***

Parameters	Non infected dogs	Zero day	4 <sup>th</sup> week (p.t.)	8 <sup>th</sup> week (p.t.)	LSD
Total proteins (g/dl)	8.40 ± 0.34 <sup>a</sup>	6.49 ± 0.23 <sup>c</sup>	7.74 ± 0.24 <sup>b</sup>	8.69 ± 0.15 <sup>a</sup>	0.47
Albumin (g/dl)	3.79 ± 0.14 <sup>a</sup>	2.17 ± 0.15 <sup>b</sup>	3.61 ± 0.32 <sup>a</sup>	3.91 ± 0.07 <sup>a</sup>	0.36
Globulin (g/dl)	4.61 ± 0.21 <sup>ab</sup>	4.33 ± 0.19 <sup>bc</sup>	4.12 ± 0.22 <sup>c</sup>	4.78 ± 0.22 <sup>a</sup>	0.39
A/G ratio	0.82 ± 0.01 <sup>a</sup>	0.51 ± 0.04 <sup>b</sup>	0.87 ± 0.12 <sup>a</sup>	0.82 ± 0.05 <sup>a</sup>	0.13
ALT	26.00 ± 5.29 <sup>b</sup>	40.00 ± 5.00 <sup>a</sup>	30.33 ± 3.21 <sup>b</sup>	25.67 ± 4.93 <sup>b</sup>	8.81
GGT	38.00 ± 3.61 <sup>b</sup>	57.00 ± 3.00 <sup>a</sup>	40.00 ± 2.00 <sup>b</sup>	37.33 ± 5.86 <sup>b</sup>	7.31
ALP	26.67 ± 12.66 <sup>b</sup>	58.33 ± 10.07 <sup>a</sup>	32.33 ± 5.86 <sup>b</sup>	26.67 ± 10.69 <sup>b</sup>	19.07
T.bilirubin (mg/dl)	0.23 ± 0.05 <sup>c</sup>	0.39 ± 0.02 <sup>a</sup>	0.31 ± 0.01 <sup>b</sup>	0.29 ± 0.02 <sup>b</sup>	0.05
Urea (mg/dl)	18.67 ± 1.53 <sup>a</sup>	18.33 ± 4.16 <sup>a</sup>	17.00 ± 3.61 <sup>a</sup>	19.00 ± 2.65 <sup>a</sup>	5.93
Creatinine (mg/dl)	1.10 ± 0.10 <sup>a</sup>	1.00 ± 0.10 <sup>a</sup>	1.00 ± 0.20 <sup>a</sup>	1.03 ± 0.15 <sup>a</sup>	0.27

Means with different superscripts (a, b,c) are significantly different at P value < 0.05

#### 4. Discussion

Enteric fever is a major global public health problem and growing international travel leads to an increase in the risk of contracting infectious diseases that are endemic in the country of destination (Crump et al., 2004, Bhan et al., 2005 and Swanson et al., 2007).

Many people believe that they can feed their dogs any type of meat; raw or cooked, and they will be able to devour and enjoy it, while most of the time that may be not true, sometimes eating raw meats can affect the dog health, especially if he already has an underlying problem to begin with. Dogs normally can fight off bacteria, but if they have a weak immune

system because of other health problems or senility, they may end up getting really sick by eating raw meat and get salmonella (Bagcigil et al., 2007).

Contact human (owners and attendants) in the present field study were apparently healthy and showed no signs of paratyphoid or other diseases during 3 months of field evaluation as restrict hygienic measures and quarantine of infected dogs.

In Dogs, they can get salmonella poisoning in a number of ways, including eating raw or uncooked meat or eggs, accessing rotten food in a trash can or coming into contact with a bird feeder or bird feces (song birds and pigeons often carry salmonella bacteria) after Bagcigil et al. (2007).

The rock pigeon (*Columba livia*) may serve as a reservoir for several pathogenic agents that can be transmitted to poultry, wildlife, domesticated pets, and/or humans via excreta, secretions, or dust from feathers. In addition, ingestion of infected pigeons by wild and domestic animals can also transmit these pathogenic agents (Eliane et al., 2010).

Clinical presentations recorded in the present research work were severe watery bloody diarrhea, high fever, prolonged capillary times, enophthalmos, loss of skin turgor, increased respiratory and pulse rates. Clinical examination was revealed abdominal distension, hepatomegally, abdominal tenderness, tympanic sound and increased peristaltic movements. Stimulation of crypt enterocytes can result in secretion of large volumes of fluid that exceed the absorptive ability of the intestine. This occurs most commonly with infectious diseases as salmonellosis and colibacillosis, but byproducts of bacterial overgrowth can also stimulate intestinal secretion. Byproducts of bacterial overgrowth can cause abnormal motility (Burrows et al., 1995).

In our study seven cases out of nine were positive for salmonella paratyphi A by Widal agglutination test (Widal test is positive if the titer is 1:80 or more), the titer of positive cases ranged from 1:80-1:160, while by bacteriological examination *Salmonella paratyphi A* (100%) and *S. Paratyphi B* (22.2 %) were identified and the most prevalent Salmonella species was *S. Paratyphi A*, in all cases from the infected dogs. The titer of the same 7 cases became stable (1: 80) after 4 and 8 weeks of treatment and negative culture results after 4 weeks denoted a state of immunity. Crump et al. (2004) and Su et al. (2004) recorded that of the three types of *S. paratyphi* (A, B, and C), B is the most common. In other study, 39 isolates of *Salmonella Paratyphi B* from animals/environment and one of human origin were characterized to understand epidemiological distribution of this pathogen (Agarwal et al., 2003).

Widal agglutination test alone is not a very specific test for diagnosis of salmonella

infection, since the dogs are often exposed to other bacteria (e.g. *Salmonella enteritidis*, *Salmonella typhimurium* and some types of *E. coli*) in this species that induce cross-reactivity; many animals have antibodies against these enteric pathogens, which also react with the antigens in the Widal test, causing a false-positive result. Test results need to be interpreted carefully in the light of past history of enteric fever, typhoid vaccination, and the general level of antibodies in the populations in endemic areas of the world. Widal agglutination test should be used as an adjunct to culture methods for definitive and accurate diagnosis for salmonella infection (Chew et al., 1992 and Olopoenia & King, 2000).

One case died at first day of treatment showed a titer of 1:320 in Widal agglutination test, it may be explained that the bacterium overcomes the body's defense mechanism as recorded by Connor & Schwartz (2005) which mentioned that after ingestion of *Salmonella* species along with contaminated food causing severe haemorrhage and intestinal perforation after settlement of the bacterium in the intestine. Perforation of the intestine followed by leakage of the intestinal contents into the abdominal cavity was resulted in severe peritonitis and death (Rene & Pines, 2002).

The significant leucopenia commonly observed in paratyphoid fever could be attributed to invasion of haemopoietic organs such as lymph nodes, spleen, tonsils and bone marrow by *S. paratyphi A* which radically slowed down the rate of granulopoiesis. The invasion of the above organs by *S. paratyphi A* which can also depress the rate of haematopoiesis may also explain the observed non regenerative anemia (normocytic normochromic anemia) and thrombocytopenia seen in typhoid and paratyphoid patients. The anemia and thrombocytopenia may be also resulted from prolonged bleeding lesions in the intestinal tract which manifested by the bloody diarrhea (Connor & Schwartz, 2005 and Okafor, 2005).

Analysis of serum liver enzymes revealed significant increase in serum alanine amino transferase (ALT). Increased activities of ALP and GGT in association with the hyperbilirubinemia observed in *Salmonella* infected groups, these changes denoted hepatic involvement which may be attributed to entering of salmonella to the small intestine wall, and then *Salmonella* invaded through the lymphatic system and after a period of multiplication invades the blood stream (Khosla 1990, Schwartz, 1994 and Rajagopal et al., 2002). From blood stream the bacteria invaded the liver, gall bladder, spleen, kidney and bone marrow where it multiplied and caused infection of these organs. Decreased serum albumin and total protein due to loss of protein from the



intestinal mucosa, to impaired dietary intake or may be attributed to its reduced production as a result of hepatic involvement (Chin, 2000)

Soon after the diagnosis by rapid Widal test, we prescribed orally 500 mg of Levofloxacin to the dog once daily for 5 days, Commercially available recombinant human erythropoietin (r-HuEPO) at a dosage of 1000 U/dog, once per week until PCV achieved the target range with iron supplement within 8 weeks. Significant increases of respiratory and pulse rates were attributed to anemia so they were improved after treatment by erythropoietin and iron. Control measures such as cleaning and disinfection of the environment were done on daily basis. Also, fluid therapy was very important for rehydration. Vit. A, E and C as adjunct therapy improved healing process of intestinal mucosa. A disinfectant solution containing sodium hypochlorite was sprayed on the concrete floor and the kennel, where diarrheal feces were excreted during the incidence (Oishi, 1995 and Nichtelein et al., 1998). Clinical remission of clinical signs within 14 days of treatment denoted suitable antibacterial with a satisfactory dose and an appropriate duration.

After 4<sup>th</sup> weeks (p.t), fecal samples were collected for bacteriological examination from all dogs at the same time, and they were negative for Salmonella. Treatment with Levofloxacin (fluoroquinolone) appeared active against bacteria only in 5 days. It functions by inhibiting DNA gyrase, which is an enzyme necessary to separate replicated DNA, thereby inhibiting cell division. Moreover, fluoroquinolones have emerged as the mainstay of therapy for invasive infection associated with typhoidal and nontyphoidal Salmonella enteric serotypes (Booker et al., 2005). Also in tissue culture cells infected with *S. typhimurium* and paratyphimurium, levofloxacin was slightly more effective than ciprofloxacin in animal models of infection so, levofloxacin is a candidate for the treatment of infections caused by facultative intracellular Gram-positive and Gram-negative bacteria. (Nichterlein et al., 1998, Huang & Dupont, 2005 and Kedhiravan et al., 2005).

At 4<sup>th</sup> and 8<sup>th</sup> weeks (p.t), Mean PCV, RBCs count and Hb concentration increased significantly and gradually during the first 4<sup>th</sup> weeks of treatment. Erythrocyte indices of dogs in our work-up showed a normocytic and normochromic anemia at the pretreatment period and during 8<sup>th</sup> weeks of treatment with r-HuEPO they became normal. All dogs received daily oral iron supplementation during treatment with r-HuEPO.

In the present study, treatment with r-HuEPO in anemic dogs appeared to stimulate erythrocyte

production. The response to r-HuEPO was found since the first week of treatment at the initial dosage of approximately 1000 U/ dog per week. All dogs showed decrease in liver enzymes as, r-HuEPO has shown a benefit in reduction of the liver injury experimentally induced by ischemia–reperfusion in the rat (Sepodes et al., 2006). Many previous literatures have shown the effective and beneficial use of r-HuEPO for treatment of anemia in animals, including in nephrectomized dogs and anemic dogs. Treatment with r-HuEPO also reduces the clinical signs of depression and weakness, increases appetite and physical activity (Oishi et al., 1995 and Assarasakorn et al., 2008).

In conclusion, the asymptomatic intestinal carrier state (2 cases) may result from inapparent infection or may follow clinical disease. It is usually self-limited to several weeks to months, with the incidence of positive stool cultures rapidly decreasing over time. But in infected cases, after an incubation period of 5 to 21 days (generally 7 to 14 days), fever and malaise developed. A small proportion of dogs may have diarrhea during the incubation period. The fever tends to rise in stepwise fashion over the first few days to a week and then became sustained, usually at 39.4 to 40°C or higher. After 2 weeks of illness, the severe complications of intestinal hemorrhage or perforation may be observed as in dead case. The illness usually resolves by the end of the fourth week in an untreated patient. Relapse may occur in untreated as well as treated patients, but the illness is milder than the original episode as recorded by Huang & Dupont (2005). The most affected cases were 3 senile dogs (immunocompromized) and from them one case died. Also, Nicolas et al. (2009) mentioned that mortality from Salmonella is not uncommon and is most likely to occur in the very young, the very old, and the immunocompromized.

## 5. Conclusion:

The present investigation emphasized the potent spread of Salmonellae bacilli in developed countries. Paratyphoid infection in the present study was incriminated to be the cause of hemorrhagic diarrhea in dogs. Since the dog can be a very important source of human Salmonella infection, strict control measures should be taken when the animal is confirmed to be infected with the pathogen. In the present investigation, control measures such as cleaning and disinfection of the surrounded environment have been done successfully because the dog was kept mainly on the concrete floor. In conclusion, Levofloxacin (fluoroquinolone) in our investigation were the first record as prescription against paratyphoid infection in companion dogs in Egypt. On the other hand, treatment with r-HuEPO

stimulated erythrocyte production in dogs with normocytic normochromic anemia seen in paratyphoid cases. PCV reached the target range within 8 weeks of treatment.

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#### 5. References:

- Agarwal, Meenu, Singh, B.R., Santoshi, M.C., Singh, V.P., Chandra, Mudit (2003). A study on biotyping, bacteriocin typing and drug resistogram of *Salmonella paratyphi B* isolates from animals, their products and environment. *Indian journal of Comparative Microbiology, Immunology and Infectious Diseases* 24, 2.
- Assarasakorn S. & Kaewthamasorn M. & Niwetpathomwat A. (2008). A retrospective study of clinical use of recombinant human erythropoietin for treatment of anemia in dogs with renal failure in Bangkok, Thailand. *Comp Clin Pathol* 17,165– 170.
- Babson, L.A., Greeley, S.J., Coleman, C.M. & Philips, G.D. (1966). Colorimetric method for estimation of serum alkaline phosphatase. *Clinical Chemistry*, 12: 482-490.
- Bagcigil A., Ikiz S., Dokuzeylul B., Basaran B., Or, E. & Ozgur, N. (2007). Fecal shedding of salmonella spp in dogs. *J. Vet. Med. Sci.* 69 (7): 775- 777
- Bhan, M.K., Bahl, R. & Bhatnagar, S. (2005). Typhoid and paratyphoid fever. *Lancet*. 366, 749-762.
- Booker, B. M., Smith, P. F., Forrest, A., Bullock, J., Kelchlin, P., Bhavnani, S. M., Ronald, N., Jones & Ambrose P. G. (2005). Application of an in vitro infection model and simulation for reevaluation of fluoroquinolone break points for *Salmonella enterica* serotype typhi. *Antimicrob. Agents Chemother.* 49 (5): 1775– 1781.
- Burrows, C.F., Batt, R.M. & Sherding, R.G. (1995). Diseases of the small intestine. In: Ettinger, S.J., Feldman, E.C., eds. *Textbook of Veterinary Internal Medicine. Philadelphia, W.B. Saunders.* 1169- 1232.
- Centers for Disease Control and Prevention (2005). Outbreak of multidrug-resistant *Salmonella typhimurium* associated with rodents purchased at retail pet stores– United States, December 2003 – October 2004. *MMWR Morb. Mortal Wkly Rep.* 54, 429- 433.
- Chew, S.K., Cnizi, M.S.P., Irim, Y.S. & Monteiro, E.H. (1992). Diagnostic value of the widal test for typhoid fever in Singapore. *Jour. Trop. Med. Hygie.* 95, 288– 291
- Chin, J. (2000). Control of Communicable Diseases Manual, 17th edition. *American Public Health Association.*
- Chirino-Trejo, J.M. (1999). A more sensitive procedure for the detection of *Salmonella* carriers in swine. *Proc. Western Can. Assoc. Swine Pract.* 50- 53.
- Connor B. A. & Schwartz E. (2005). Typhoid and paratyphoid fever in travelers. *Lancet Infect. Dis.* 5, 623–28
- Crump, J.A., Luby, S.P. & Mintz, E.D. (2004). The global burden of typhoid fever. *Bull World Health Organ.* 82, 346- 353.
- Doumas, B.T., Perry, B.W., Sasse, E.A. & Straumfjord, J.V. (1973). Colorimetric method for estimation of bilirubin. *Clinical Chemistry*, 19, 984- 993.
- Dumas, B.T. & Biggs, H.G. (1972). "Standard Methods of Clinical Chemistry". Academic Press, N.Y., U.S.A.
- Effa, E.E. & Bukirwa, H. (2008). Azithromycin for treating uncomplicated typhoid and paratyphoid fever (enteric fever). *Cohrane Database of Systemic Reviews* Issue 4. Art No. CD006083, pages 2-3.
- Eliane de Sousa, Angelo Berchieri Júnior, Aramis Augusto Pinto, Rosangela Zacarias Machado, Adriano de Oliveira Torres Carrasco, José Antonio Marciano, Karin Werther (2010). Prevalence of *Salmonella* spp. Antibodies to *Toxoplasma gondii*, and Newcastle Disease Virus in Feral Pigeons (*Columba livia*) in the City of Jaboticabal, Brazil. *Journal of Zoo and Wildlife Medicine* 41 (4): 603- 607.
- Fabiny, D.L. & Ertingshausen, G. (1971). Colorimetric method for estimation of creatinine. *Clin. Chem.* 17: 696.
- Fieldman, B.F.; Zinkl, J.G. & Jain, N.C. (2000). "Schalm's Veterinary Hematology" 5<sup>th</sup> ed. Lea and Fibiger, Philadelphia, USA.
- Huang, D.B. & DuPont, H.L. (2005). Problem pathogens: Extra-intestinal complications of *Salmonella enterica* serotype typhi infection. *Lancet. Infect. Dis.* 5, 341- 348.
- Irwan, T.M. (1996). In Applied Linear Statistical models, Neter, Kutner, Neachtsheim Wasserman. Fourth edition.

22. Kadiravan, T., Wig, N., Kapil, A., Kabra, S.K., Renuka, K. & Misra A (2005). Clinical outcomes in typhoid fever: adverse impact of infection with nalidixic acid-resistant *Salmonella typhi*. *B.M.C. Infect. Dis.* 5 (1): 37.
23. Khosla, S.N. (1990). Typhoid hepatitis. *Postgrad. Med. J.* 66: 923– 25.
24. Leib, M.E. & Monroe W.E. (1997). Diseases of the intestines. *Practical Small Animal Internal Medicine* 685- 760.
25. Lesser, C.F. & Miller S.I. (2001). Salmonellosis. In: Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL editor (s). *Harrison's Principles of Internal Medicine. 15th Edition.* Vol. 1, New York:
26. Maskalyk, J. (2003). Typhoid fever. *Canadian Medical Association Journal* 169 (2):132.
27. Membrebe, F.A. & Chua, J.A. (1999). The clinical utility of typhoid in the diagnosis of typhoid fever. *Philipp J Microbiol Infect Dis* 28,1- 4
28. Morifnigo, M.A., Borrego, J.J. & Romero, P. (1986). Comparative study of different methods for detection and enumeration of *Salmonella* spp. in natural waters. *J. Appl. Bacteriol.* 61:169-176.
29. Nichterlein, T., Bornitz, F., Kretschmar, M. & Hof, H. (1998). Successful treatment of murine listeriosis and salmonellosis with levofloxacin. *J. Chemother.* 10 (4): 313- 9.
30. Nicolas Lapaque, Thierry Walzer, Stéphane Méresse, Eric Vivier & John Trowsdale (2009). *Interactions between human NK cells and macrophages in response to Salmonella infection.* The Journal of Immunology, Volume: 182, Issue: 7.
31. Oishi, A., Hamada, S., Sakamoto, H. & Shimizu, R. (1995). Preventive effects of recombinant human erythropoietin administration on anemia associated with repeated hemodialysis in nephrectomized dogs. *J. Vet. Med. Sci.* 57, 989– 990.
32. Okafor, A.I. (2005). Haematological alterations due to typhoid fever in Enugu Urban- Nigeria. *Malaysian Journal of Microbiology*, 3 (2): 19- 22
33. Olopoenia, L.A. & King A.L. (2000). Widal agglutination test – 100 years later: still plagued by controversy. *Postgrad. Med. J.* 76, 80– 84.
34. Patton, C.G. & Crouch, S.R. (1977). Enzymatic colorimetric method of urea determination. *Anal. Chem.* 49, 464- 469.
35. Porwollik, S. (2011). *Salmonella: from genome to function.* Caister Academic Press ISBN 978- 1- 904455- 73- 8.
36. Qasmi, S., Ahmed, A., Zaidi, S. & Ahmed, O. (2010). Incidence and diagnosis of typhoid/ paratyphoid fever in Karachi. *Infectious Diseases Journal of Pakistan* 19 (3):196- 198
37. Rajagopal, A., Ramasamy, R., Mahendran, G. & Thomas, M. (2002). Hepatic abscess complicating paratyphoid infection. *Tropical Gastroenterology*; 23 (4):181– 2.
38. Reitman, S. & Frankel, S. (1957). A colorimetric method for the determination of oxaloacetic transaminase and serum glutamic pyruvic transaminase. *Am. J. Clin. Path.* 28: 56– 60.
39. Rene, D. & Pines, M. (2002). Health and Diseases In: *Time Life International* pp 22-23; N. V. Publishers, Netherland 54- 55.
40. Schwartz, E., Jenks, N.P., Shlim, D.R. (1994). 'Typhoid hepatitis' or typhoid fever and acute viral hepatitis. *Trans. R. Soc. Trop. Med. Hyg.* 88, 437– 38.
41. Sepodes, B., Maio, R., Pinto, R., Sharples, E., Oliveira, P., McDonald, M., Yaqoob, M., Thiemermann C. & Mota-Filipe, H. (2006). Recombinant human erythropoietin protects the liver from hepatic ischemia– reperfusion injury in the rat. *Transpl. Int.* 19: 919– 926.
42. Steffen, R., de Bernardis, C. & Banos, A. (2003). Travel epidemiology– a global perspective. *Int. J. Antimicrob. Agents* 21, 89- 95.
43. Su, L.H., Chiu, C.H., Chu, C. & Ou, J.T. (2004). Antimicrobial resistance in nontyphoid *Salmonella* serotypes: A global challenge. *Clin Infect Dis* , 39:546-551.
44. Swanson, S.J., Snider, C., Braden, C.R., Boxrud, D., Wunschmann, A., Rudroff, J.A., Lockett, J. & Smith, K.E. (2007). Multidrug-resistant *Salmonella enterica* serotype typhimurium associated with pet rodents. *N. Engl. J. Med.* 356, 21- 28.
45. Thiopont, D., Rochette, F., Vanparijs, O.F.J. (1986). Fecal floatation concentration, direct method. *Diagnosing Helminthiasis by coprological examination second edition textbook* 32- 34.
46. Turk, D.C. & Porter, I.A. (1982). Medical Microbiology 3rd ed. *English Universities Press Ltd* pp. 30-35.
47. Weichselbraun, D.E. (1964): Colorimetric method for determination of total proteins. *Am. J. Clin. Path.* 40, 16-40.

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## Agricultural Labor among School Children in Rural Assiut, Egypt

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**Abstract: Background:** Child labor is alarmingly prevalent in Egypt, a country with a population of nearly 75 millions; there are some 1.5 million (12%) child workers between 6 and 15 years. Agricultural work poses several characteristics that are risky for health: exposure to extremes of weather, close contact with animals and plants, extensive use of chemical and biological products, difficult working postures and lengthy hours and use of hazardous agricultural tools and machinery.

**Aim of the study:** Determine the causes of agricultural child labor in Koom Abousheel village, Assiut, Egypt and highlight some of its related health problems. **Methods:** Descriptive cross sectional study included 630 randomly selected students enrolled in primary and preparatory schools aged from 6 to 17 years old in Koom Abousheel village 2008-2009. Data collection from the target population using structured personal interview. Anthropometric measures: weight, height and body mass index were measured. Laboratory investigations including urine and stool examination were done to detect parasitic infections in these children. Urine samples were collected, centrifuged and examined microscopically. Stool examination was done macroscopically and stored in two different fixative (10% formalin and sodium acetate-acetic acid-formalin), concentrated and examined microscopically by: direct wet mount using saline, iodine and lacto-phenol cotton blue and stained by modified Kinyoun acid-fast stain. Data analyzed using SPSS version 16. **Results:** 52.7% of the studied students worked in agricultural duties and 73.2% of them began work at early age (less than 10 years). Boys were more often involved in labor activities than girls. About half of the worked students helped their fathers in their work. Worked students reported negative impact on their education and social life. Health hazards at the farm such as exposure to high temperature, animal bite, and injuries were prevalent among them. Parasitic infections were more prevalent in worked students (69.3 %). The detected parasites were *Ascaris lumbricoides*, *Ancylostoma douduenale*, *Hymenolepis nana*, *Enterobius vermicularis*, *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, *Blastocystis hominis* and *Entamoeba coli*. **Conclusion:** The problem of child labor is serious. In rural areas especially, children work as cheap labor because their parents are poor and do not earn enough to support the family, thus the problem of child labor will persist and it exposes the children to many health hazards and risks. Improving legislation and enforcement measures to establish a legal minimum age for work, community education and mobilization are essential and have been the traditional response to the problem of child labor.

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**Keywords:** Child labor – agricultural work– occupational health problems - school students

### 1. Introduction

Child labor is a persistent problem throughout the world, especially in developing countries. It is prevalent in rural areas of those countries where income poverty is widespread (ILO, 1997). Child labor, according to ILO conventions, is work that harms children's well-being and hinders their education, development and future livelihoods. One out of six children in the world is involved in child labor, doing work that is damaging to his or her mental, physical and emotional development (ILO, 2004). Because children's bodies and minds are still growing and developing, exposure to workplace hazards and risks can be more devastating and long-lasting for them (Hurst, 2007).

According to the International Labor Organization's new report on child labor (ILO, 2006); of nearly 250 million children engaged in child labor around the world, the vast majority 70 percent are working in agriculture. Child agricultural workers, who frequently work for long hours in scorching heat, haul heavy loads of produce, are exposed to toxic pesticides, and suffer high rates of injury from sharp knives and other

dangerous tools (Fassa *et al.*, 1999). Their work is grueling and harsh, and violates their rights to health, education, and protection from work that is hazardous or exploitative (FAO, 2006; Cole, 2006). The number of children working in agriculture is nearly ten times that of children involved in factory works such as garment manufacturing, carpet-weaving, or soccer-ball stitching. Yet despite their numbers and the difficult nature of their work, children working in agriculture have received little attention compared to child labor in manufacturing for export (ILO, 2004). The International Labor Organization goal of eliminating all of the worst forms of child labor by 2016 will only be possible if more work is done in agriculture (Hurst, 2007).

Child labor is an important global issue associated with poverty, unbalanced economic growth, inadequate educational opportunities, gender inequality, inadequate regulations to restrict child labor, lack of public awareness and a range of health risks (Parker, 1997; Forastieri, 2002). Most child laborers begin working at a very young age, are malnourished, and work long hours in hazardous occupations; frequently they do

not attend school. They receive very low wages or are unpaid, and their income or help is usually essential for family survival. It is estimated that, in developing countries, at least 90% of economically active children in rural areas are employed in agriculture (Human Rights Watch Reports, 2006; Forastieri, 1997; Wilk, 1993).

An investigation in Egypt, Ecuador, India, and the United States, Human Rights Watch has found that the children working in agriculture are endangered and exploited on a daily basis. Despite the vast differences among these four countries, many of the risks and abuses faced by child agricultural workers were strikingly similar (Human Rights Watch Reports, 2002).

The most obvious economic impact of child labor at the family level is an increase in household income as a short term. While the long term, is under accumulation of human capital caused by low school attendance and poor health which is a serious negative consequence of child labor, representing a missed opportunity to enhance the productivity and future earnings capacity of the next generation (Heady, 2003).

The Egyptian Child Law (Law No. 12, 1996) prohibits the employment of children below the age of fourteen. However, children between the ages of twelve and fourteen are allowed to receive vocational training from employers and to take part in seasonal agricultural work, provided that the work is not hazardous to their health and growth, and does not interfere with their studies. The child Law limits the workday for these children to six hours, only four of which may be consecutive, and requires the provision of one or more breaks (Bureau of International Labor Affairs, 2004).

Child labor is also addressed in the Egyptian Labor Code Law No. 12, 2003, which states that "employing children under the age of fourteen or children who have not completed elementary education yet, is prohibited". However, the provisions of this law do not apply to children working in agricultural labor, which is one of the most problematic forms of child labor in Egypt (Bureau of International Labor Affairs, 2004).

Poverty is the main cause of child labor in Egypt. About 17% of the population lives in poverty with almost 12 million people having difficulty meeting their basic needs. Rural Upper Egypt is the poorest region (Mattar, 2007). Also, there is a low standard of health and medical services in rural areas. This particularly is true in developing countries compared with developed countries. Health status in rural areas is below that found in urban centers in both developed and developing countries (Forastieri, 1999).

Seeing vulnerable children risking their lives and harming their health is disturbing and painful to everyone. Although child labor is globally recognized as a health problem, research into the health impacts of child labor in Egypt has been limited to some urban areas (El-Gilany *et al.*, 2007; Kishk *et al.*, 2004).

The present study aimed to:

- Determine the causes of agricultural school child labor in Koom Abousheel village, Assiut, Upper Egypt.

- Study the socioeconomic and demographic backgrounds of the target children.
- Determine the predictors of agricultural child labor.
- Highlight the work related health hazards of the agricultural child labor; including parasitic infection in these children.

## 2. Materials and Methods

### Methods

A cross sectional study was carried out during the academic year 2008-2009 in Koom Abousheel village, Abnoub District which is located in north Assiut. **The total population of the village according to the census 2006 was 17,394.** The study was conducted on students enrolled in primary and preparatory schools, aged from 6 to 17 years. Sample size has been calculated using the following equation:  $n = (Z^2 * p * "p) / D^2$  (Lwanga and Lemeshow, 1991). The suggested prevalence of the child labor between 6 to 15 years was 12% (Kishk *et al.*, 2004). A value of 2.5 is chosen as the acceptable limit of precision (D) at 95% level of confidence. Accordingly, sample size is estimated to be 500+ 10% students to guard against non-respondent's rate.

In the randomly selected village, there is an educational campus that includes two primary and two preparatory schools. The four schools were included in the study, total number of the students in the selected schools was 3,529 (2,275 in primary schools and 1,254 in preparatory schools), 15 classes were selected by simple random sampling (8 classes in primary schools and 7 classes in preparatory schools). All students in the class, who were present on the day of the survey, were included. The mean class density in the selected schools was 45.6 students. The actual number of enlisted students in the randomly selected classes was 684 of which 630 (92%) successfully completed the questionnaires making a dropout rate of 8% (54). Absenteeism was the reason for dropout.

A semi-structured questionnaire gathered information concerning: personal data, family background, questions about work and work environment, health hazards of the work. Field pre-testing of the questionnaire was carried out on a sample of 30 students who were not in the sample; the necessary modification in the sheets was done.

Formal administrative approvals were taken before the start of the fieldwork. Students were briefed about the study, encouraged to participate. The researchers stressed on the issue of confidentiality and all students were requested to fill out the questionnaires anonymously. To control for variations in reading ability, the questionnaire was read aloud to students; the instrument required between 45 and 60 minutes. Student participation was voluntary, however, no student refused to cooperate in the research. Verbal and written instructions reminded students of the importance of giving honest answers. As regard the young students aged 6- 8 years, home visits were done to complete the questionnaires by personal interviews.

Weight and height were measured. Body mass index was used to determine the degree of obesity. Obesity was defined as BMI more than or equal 30 Kg/m<sup>2</sup> (WHO, 2004).

### Parasitological methods

630 urine and stool samples were collected from the children after procuring informed permission from the school and parental authorities. Students were instructed about what they will do. Urine and stool samples were collected at schools in dry, clean, leak proof plastic disposable cups with lids labeled with name, age, date, class and sex of the student. The collection rate was 2-3 classes per visit. All samples were transported within half an hour to parasitology laboratory, Department of parasitology, Faculty of Medicine, Assiut University, Assiut, Egypt. Urine samples were examined in the same day, it were poured in a conical centrifuge tube and immediately centrifuged at 400 rpm and the sediment examined by wet mount.

Stool specimens were examined as the following: Macroscopic examination of faeces was performed to detect adult worms, segments of tapeworms, larvae, blood and mucus (if present). Stool consistency (i.e. formed, soft, loose or watery) was reported. Each stool sample after macroscopic examination was divided into 2 parts, one was well mixed and stored in 10% formalin and the other was well mixed and stored in Sodium Acetate- acetic acid- formalin (SAF) at 4°C, (one portion stool with three portion preservative) using suitable containers labeled with the same information. After the same time preservation of both preservatives; each stool specimen was concentrated using formalin-ethyl acetate sedimentation and zinc sulfate floatation concentration methods. Direct microscopic examination of saline, iodine and lacto-phenol cotton blue (LPCB) wet mounts preparation of stool specimens were prepared to microscopically screen for helminthes eggs or larvae, protozoal trophozoites, cysts and oocysts. The saline wet mount preparations were examined first and then the iodine wet mounts and lastly the LPCB wet mounts. These wet mounts were microscopically screened initially by using a low-power objective and then using high-power objectives of a compound light microscope at the same time for the two preservatives of each sample. Stool samples were stained using modified Kinyoun acid-fast staining technique for coccidian parasites (Parija *et al.*, 2003).

Reports were sent to the school administrators and school health teams including the names of the positive students and the detected parasites for proper treatment.

Data were analyzed using SPSS (version 16). The frequencies, percentages, the mean and standard deviation were computed. Chi-squared test was used as the test of significance;  $P < 0.05$  was considered significant. Body Mass Index was calculated by using BMI percentile by nutritional program EPInfo 2000.

### 3. Results:

A total number of 630 students were included in this study; 298 (47.3%) boys and 332 (52.7%) girls. The

age of the respondents ranged from 6 to 17 years with mean of 11.5. As regards smoking behavior, 1.4% of students were smokers. It was found that 332 (52.7%) of the students were working in agricultural duties (Table 1).

Table (2) shows the family characteristics of the studied students. 93.5% of the students lived with both parents. The family of the vast majority of the students was well constructed. Nearly, 6% of the students reported that their fathers are drug abusers.

A greater percent of mothers fall in the illiterate category more than the fathers (43.3% and 17.8%, respectively) (Table 3). About 58% of the student's parents were employers and 13% were farmers. Most of the student's mothers were housewives. Father's work was the source of the family income in 72%. Only 1.4% of the families depended on siblings work as a source of the income (Table 4, Figure 1).

The study revealed that male students are more involved in agricultural work than female students (60.2% versus 39.8%) about three quarters of the working students began the work at age below 10 years. The most important cause of the students' work was helping their fathers in the work and sharing in the family expenses. It was noticed that about two thirds of the working children were unpaid. Gather grass and dig up represent the common types of agriculture activities reported by working children. More than half of the working students go to the farm on their feet because the farm is near the home. The majority of the students (80%) worked in the farm not more than three hours (Table 5, Figure 2).

By univariate analysis, the strong predictors of students work, were age, sex (be a male), education of the parents (Table 6).

Regarding the social effect of agricultural labor on students, Table (7) shows that the majority (89.2%) of the studied working students had friends. More than two thirds of the working children had a spare time for playing. Nearly, one fifth of them reported that they didn't have time to talk with their families and to know their problems. Also, 4.5% of them were facing problems in dealing with their labor peer and land owner in the farm as verbal assault and physical harm. It was found that 37% of the working students absent several times from school.

Table (8) presents the psychological effects as 80.1% of the working students felt happy in farm working. About two thirds of them reported that the work affected their personalities. Reported positive effects such as: they grew up and were responsible (61.2%) and they were self dependent (45%). While the most reported negative effects of agricultural labor were being nervous (16.5%) and speak abusive words (2.4%). Concerning the behavior of the land owner toward any mistakes in the farm work, 15.7% of the working children reported that he said to them abusive words, while 9.3% of them reported hitting. Less than half of the working students felt that they are better than their schoolmates who did not work.

A statistically significance difference was found between educational achievement and the working status

of students (Table 9). As 63% of working students failed in the previous year in comparison with 37% of not working students and nearly 69% of working students succeeded with failure in some subjects versus 31% of not working students.

As shown in Table (10), the most reported work hazards among working children were exposure to high temperature, sharp instrument, wild animals, dust, heavy machines and equipments, harmful insects and reptiles, noise and pesticides. More than one third of the working students had a history of injuries. The most common reported physical problems were back pain, general weakness and fatigue and headache (34.6%, 21.4%,18.7%, respectively).

Table (11) illustrates that the most common last type of injury was "cut wound" (62%). Regarding site of injury, hand injury represented 45.8% of the injuries.

Table (12) shows a statistically significant difference between working students and not working students in their Body Mass Index.

Parasitological (urine and stool) examination revealed that school children were exposed to many parasitic infections (Table 13). The infection was 69.3% in working children while it was 41.9 % in not working children. The infection rate was 33.6% in males and 19.4% in females in working children. While it was equal in both sex in not working children (13.4%). The infection rate was 49.6 % in the age group less than 15 years old and it was 23.4% in the age group 15 years old and more. The parasitic infection rate was 12.4% when the number of individuals in the household 10 or more,

8.2% when they were from 6-9 and 4.7% when they were 2-5. The detected gastrointestinal parasitic stages were *Ascaris lumbricoides* fertilized eggs, *Giardia lamblia* cysts and trophozoites, *Entamoeba coli* cysts and trophozoites, *Ancylostoma doudenale* eggs, *Entamoeba histolytica* cysts, *Cryptosporidium parvum* oocysts, *Blastocystis hominis* cysts and *Hymenolepis nana* eggs, in order of frequencies. *Enterobius vermicularis* eggs were detected in 2.1 % urine samples of female working students and 1.3% of not working ones (Table13).

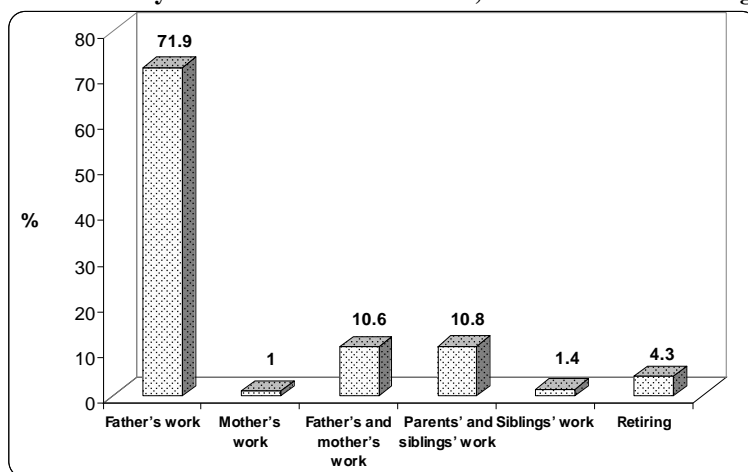
The trophozoite stage of both *Giardia lamblia* and *Entamoeba coli*, *Entamoeba histolytica* cysts and *Cryptosporidium parvum* oocysts were detected in loose or watery stool of the examined stool samples. Single infection was detected in 65% of positive cases while 23% showed double infection and 12% showed multiple infections. Double infection was detected between *Giardia lamblia* and *Cryptosporidium parvum* and multiple infections between *Ascaris lumbricoides*, *Giardia lamblia* and *Entamoeba histolytica*. 75% of the collected stool samples were well formed, 20% were loose and 5% were watery in consistency.

The more detectable parasitic stages in the examined stool specimens were in SAF preserved samples rather than 10% formalin preserved samples. In LPCB wet mounts cysts, trophozoites, and eggs of the identified parasitic species stained blue which facilitate their identification. Modified Kinyoun acid-fast staining technique for coccidian parasites facilitated the identification and confirmed the detection of *Cryptosporidium parvum* oocyst in stool specimens.

**Table (1): Personal characteristics of respondent students, Koom Abousheel village, 2008-2009**

Variable	Number (n= 630)	Percent
<b>Sex</b>		
• Male	298	47.3
• Female	332	52.7
<b>Age in years</b>		
• 6-	203	32.2
• 10-	258	41.0
• 14- 17	169	26.8
<b>Mean ± SD</b>	<b>11.4 ± 2.5</b>	
<b>Religion</b>		
• Moslem	625	99.2
• Christian	5	0.8
<b>Smoking</b>		
• Yes	9	1.4
• No	621	98.6
<b>Type of smoking</b>		
• Cigarettes	7	77.8
• Gouza	2	22.2
<b>Working in agricultural duties</b>		
• Yes	332	52.7
• No	298	47.3
<b>Academic performance of the students in the last academic year *</b>		
• Succeed	461	79.6
• Succeed with failure in some subjects	91	15.7
• Failed	27	4.7

\* 51 students were in the first year of primary education

**Fig. (1): Source of family income of studied students, Koom Abousheel village, 2008-2009****Table (2): Family characteristics of studied students, Koom Abousheel village, 2008-2009**

Variable	Number (n= 630)	Percent
<b>Family construction</b>		
• Intact family	602	95.6
• Divorced parents	10	1.6
• One or both parents died	18	2.9
<b>Living with whom</b>		
• Both parents	589	93.5
• Mother	28	4.4
• Father	7	1.1
• Others	6	1.0
<b>Father is drug abuser/ drunker °</b>		
• Yes	38	6.1
• No	580	93.9
<b>Ordering among living siblings:</b>		
• 1 <sup>st</sup>	113	17.9
• 2 <sup>nd</sup> or 3 <sup>rd</sup>	248	39.4
• 4 <sup>th</sup> or 5 <sup>th</sup>	166	26.3
• 6 <sup>th</sup> +	103	16.3
<b>Number of brothers</b>		
• None	24	3.8
• 1-3	499	79.2
• 4+	107	17.0
<b>Number of sisters</b>		
• None	38	6.0
• 1-3	449	71.3
• 4+	143	22.7
<b>Number of individuals in the household</b>		
• 2-5	82	13.0
• 6-9	425	67.5
• 10+	123	19.5
<b>Number of the house' rooms</b>		
• 1-3	319	50.6
• 4-6	241	38.3
• 7+	70	11.1
<b>Crowding index (Mean ± SD)</b>	2.4 ± 1.3	

° 12 fathers were died



**Table (3): Educational level of the parents of studied students, Koom Abousheel village, 2008-2009**

Educational level	Father's educational level °		Mother's educational level °°	
	Number	Percent	Number	Percent
Illiterate	110	17.8	270	43.3
Read and write	91	14.7	44	7.1
Completed basic education (Primary + Preparatory)	99	16.0	68	10.9
Completed Secondary school (General/Technical)	167	27.0	136	21.8
Higher education	65	10.5	38	6.1
Do not know	86	13.9	68	10.9
<b>Total</b>	<b>618</b>	<b>100.0</b>	<b>624</b>	<b>100.0</b>

° 12 fathers were died

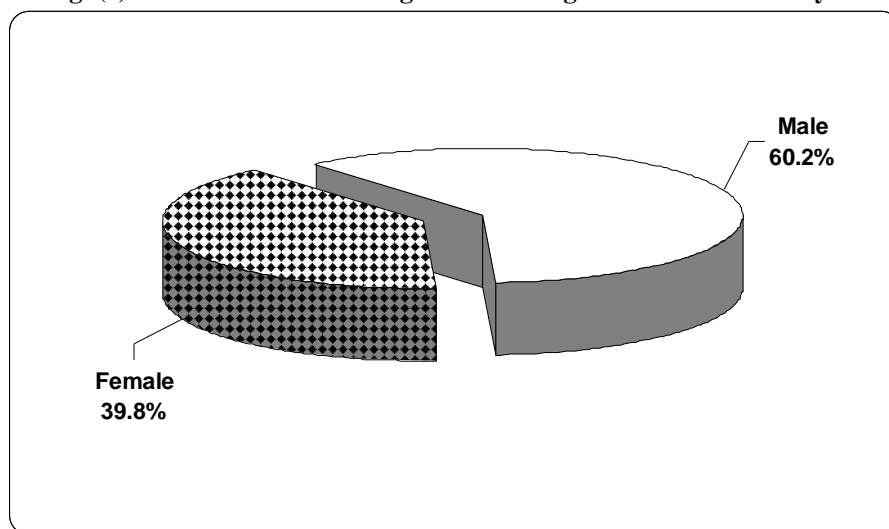
°° 6 mothers were died

**Table (4): Occupation of the parents of the studied students, Koom Abousheel village, 2008-2009**

	Number	Percent
<b>Father's occupation: *</b>		
Farmer	80	12.9
Employer	358	57.9
Skilled workers	25	4.0
Unskilled workers	45	7.3
Free business	56	9.1
Retired	24	3.9
Does not work	13	2.1
Does not know	17	2.8
<b>Total</b>	<b>618</b>	<b>100.0</b>
<b>Mother's occupation: **</b>		
Housewives	531	85.1
Employee	93	14.9
<b>Total</b>	<b>624</b>	<b>100.0</b>

\* 12 fathers were died

\*\* 6 mothers were died

**Fig. (2): Distribution of working students in agricultural activities by sex**

**Table (5): Characteristics of working students in agricultural activities in farm, Koom Abousheel village, 2008-2009**

Variable	Number (n= 332)	Percent
<b>Age at first work:</b>		
• < 10 years	243	73.2
• 10 years	89	26.8
<b>Cause of working: °</b>		
• Help my father and share in the family in expenses	256	77.1
• Occupying spare time	97	29.2
• Buying my special needs	36	10.8
• Response to the parents	26	7.8
<b>Types of agricultural activities:°</b>		
• Gather grass	216	65.1
• Dig up	108	32.5
• Irrigate water	83	25.0
• Collect crop	39	11.7
• Move the crop	33	9.9
• Help my father generally	59	17.8
<b>Worked in past 7 days:</b>		
• Paid	18	5.4
• Unpaid	200	60.2
• Did not work	114	34.3
<b>Distance between farm and house:</b>		
• Near the house	169	50.9
• Distant from the house	163	49.1
<b>How do you go to the farm? °</b>		
• On feet	190	57.2
• By donkey/other animals	115	34.6
• By bicycle	50	15.1
• By transportation	27	8.1
<b>Time spend in working in the farm:</b>		
• 1-3 hours/ day	266	80.1
• 4-6 hours/ day	53	16.0
• More than 6 hours/ day	13	3.9
<b>Work break:</b>		
• Yes	249	75.0
• No	8.3	25.0
<b>Time of work break: (minutes)</b>		
• Mean ± SD	19.3 ± 8.2	
• Range	5 – 30	

° More than one answer was allowed

**Table (6): Univariate analysis of predictors of students work, Koom Abousheel village, 2008-2009**

Predictor	Working (n=332)		Not working (n=298)		P-value
	No.	%	No.	%	
<b>Age</b>					
• < 15 years	293	51.0	281	49.0	0.008*
• 15 years and more	39	69.6	17	30.4	
<b>Sex</b>					
• Male	200	67.1	98	32.9	0.000*
• Female	132	39.8	200	60.2	
<b>Ordering among living siblings</b>					
• 1 <sup>st</sup>	46	40.7	67	59.3	0.046*
• 2 <sup>nd</sup> or 3 <sup>rd</sup>	136	54.8	112	45.2	
• 4 <sup>th</sup> or 5 <sup>th</sup>	92	55.4	74	44.6	
• 6 <sup>th</sup> +	58	56.3	45	43.7	
<b>Father's education:<sup>o</sup></b>					
• Illiterate	59	53.6	51	46.4	0.033*
• Read and write	57	62.6	34	37.4	
• Completed basic education (Primary + Preparatory)	57	57.6	42	42.4	
• Completed Secondary school (General/Technical)	69	41.3	98	58.7	
• Higher education	36	55.4	29	44.6	
• Do not know	47	54.7	39	45.3	
<b>Mother's education:<sup>oo</sup></b>					
• Illiterate	156	57.8	114	42.2	0.001*
• Read and write	26	59.1	18	40.9	
• Completed basic education (Primary + Preparatory)	43	63.2	25	36.8	
• Completed Secondary school (General/Technical)	49	36.0	87	64.0	
• Higher education	17	44.7	21	55.3	
• Do not know	37	54.4	31	45.6	
<b>Father's occupation:<sup>o</sup></b>					
• Farmer	54	67.5	26	32.5	0.264
• Employer	185	51.7	173	48.3	
• Skilled workers	11	44.0	14	56.0	
• Unskilled workers	20	44.4	25	55.6	
• Free business	27	48.2	29	51.8	
• Retired	13	54.2	11	45.8	
• Does not work	7	53.8	6	46.2	
• Does not know	8	47.1	9	52.9	
<b>Mother's occupation:<sup>oo</sup></b>					
• Housewife	285	53.7	246	46.3	0.328
• Employer	43	46.2	50	53.8	

<sup>o</sup> 7 fathers of working children and 5 fathers of not working were died.

<sup>oo</sup> 4 mothers of working children and 2 mothers of not working were died.

\* Statistical significant difference

**Table (7): Social effects of agricultural work on working students, Koom Abousheel village, 2008-2009**

Social effect	Number (n=332)	Percent
<b>Are you having friends?</b>		
• Yes	296	89.2
• No	36	10.8
<b>Do you have spare time to play?</b>		
• Yes	246	74.1
• No	86	25.9
<b>Where do you go in the holiday? °</b>		
• Stay at home	125	37.7
• In the street	36	10.8
• Wandering	50	15.1
• Café shop	121	36.4
• Go to the Club	5	1.5
• Visit my relatives	97	29.2
<b>Do you have time to talk with your family and to know their problems?</b>		
• Yes	266	80.1
• No	66	19.9
<b>How does the work affect your schooling?</b>		
• I absent many times from school	123	37.0
• I can not concentrate in the class	122	36.7
• I failed	87	26.2
<b>Do you facing any problem in dealing with your friends and teachers in the school?</b>		
• Yes	<b>38</b>	<b>11.4</b>
• No	294	88.6
<b>If yes, what are these problems?</b>		
• Problems with school mates	12	31.6
• Problems with teacher	12	31.6
• Physical violence	8	21.1
• Emotional problems	3	7.9
• Homework	3	7.9
<b>Do you facing any problem in dealing with your friends and landowner in the farm?</b>		
• Yes	<b>15</b>	<b>4.5</b>
• No	317	95.5
<b>If yes, what are these problems?</b>		
• Physical harm	11	73.3
• Verbal assault	4	26.7

° More than one answer was allowed

**Table (8): Psychological effects of agricultural work on working students, Koom Abousheel village, 2008-2009**

Psychological effect	Number (n=332)	Percent
<b>Are you happy with your work in the farm?</b>		
• Yes	<b>266</b>	<b>80.1</b>
• No	66	19.9
<b>Does the work affect your personality?</b>		
• Yes	<b>206</b>	<b>62.0</b>
• No	126	38.0
<b>Positive effect:°</b>		
• I grew up and be responsible	126	61.2
• I depend on my self	94	45.6
• I feel with my importance and earn money	28	13.6
• I am be calm	63	30.6
<b>Negative effect:°</b>		
• I am nervous	34	16.5
• I speak abusive words	5	2.4
• Night weakness	22	10.7
• I run away from school	4	1.9
<b>If you do a mistake in the farm, how does the landowner deal with you? °</b>		
• Hits me	31	9.3
• Talks me abusive words	52	15.7
• Negotiate	249	75.0
• Does not give me money	2	0.6
• Complains from me to my family	7	2.1
• Faired me	7	2.1
<b>Feeling toward not working schoolmates:</b>		
• I Feel that I am better than them	158	47.6
• I Feel that they are better than me	75	22.6
• I sense of injustice	99	29.8

° More than one answer were allowed

**Table (9): Educational achievement of children in the last year in Koom Abousheel village, 2008-2009**

Educational achievement	Working (n=332)		Not working (n=298)		P-value
	No.	%	No.	%	
Successfully pass	251	54.4	210	45.6	<b>0.028*</b>
Success with failure in some subjects	63	69.2	28	30.8	
Failed	17	63.0	10	37.0	
Total °	331	57.2	248	42.8	

° 51 children were in the first class of primary education (not applicable).

\* Statistical significant difference

**Table (10): Occupational and health hazards of agricultural work on working students, Koom Abousheel village, 2008-2009**

Variable	Number (n=332)	Percent
<b>Work hazards:<sup>o</sup></b>		
• High temperature	167	50.3
• Sharp instruments	161	48.5
• Exposure to wild animals	125	37.7
• Exposure to dust	80	24.1
• Machines and heavy equipment	74	22.3
• Exposure to harmful insects and reptiles	69	20.8
• Noise	61	18.4
• Chemicals/ pesticides	55	16.6
• Exposed electric wire	15	4.5
<b>Work injuries and accidents:<sup>o</sup></b>		
• Cuts	158	47.6
• Sun stroke	117	35.2
• Foreign body in the eye	94	28.3
• Falls from height	56	16.9
• Animal bite/kick	45	13.6
• Corporal punishment by employer	20	6.0
• Fracture/ contusion	15	4.5
• Drowning in the canal	12	3.6
• Electric shock	6	1.8
<b>Health problems and diseases:<sup>o</sup></b>		
• Back pain	115	34.6
• Generalized weakness and fatigue	71	21.4
• Headache	62	18.7
• Eye problems	36	10.8
• Skin problems	31	9.3
• Respiratory problems	27	8.1
• Ear and hearing problems	19	5.7

<sup>o</sup> More than one answer was allowed

**Table (11): The last injury among children in farm, Koom Abousheel village, 2008-2009**

Variable	Number (332)	Percent
<b>Type of injury<sup>o</sup>:</b>		
• Cut wounds	291	87.7
• Contusions	42	12.7
• Fractures	19	5.7
• Sprains	4	1.2
• Dislocation	16	4.8
• Others	10	3.0
<b>The injured part:</b>		
• Upper extremities	20	6.0
• Lower extremities	84	25.3
• Head & Neck	30	9.0
• Chest/ Back	21	6.3
• Hands	152	45.8
• Eyes	13	3.9
• Nose	12	3.6
<b>Admission to the hospital:</b>		
• Yes	25	7.5
• No	307	92.5
<b>Days of hospitalization:</b>		
• 1-2	9	36.0
• 3-4	9	36.0
• 5 or more	7	28.0
<b>Days of absenteeism from the school:</b>		
• 0 day	316	95.2
• < 7 days	9	2.7
• 8 days or more	7	2.1
<b>Presence of deformity or disability:</b>		
• Yes	12	3.6
• No	320	96.4

<sup>o</sup> More than one answer was allowed

**Table (12): Body Mass Index of studied students, Koom Abousheel village, 2008-2009**

BMI percentile	Working (n= 332)		Not working (n= 298)		P-value
	No.	%	No.	%	
Underweight	26	65.0	14	35.0	<b>0.000*</b>
Normal	277	56.6	212	43.4	
Overweight	23	34.8	43	65.2	
Obese	6	17.1	29	82.9	
<b>Mean ± SD</b>	18.02 ± 2.59		18.15 ± 2.82		<b>0.566</b>

\* Statistical significant difference

**Table (13): Laboratory investigations of students, Koom Abousheel village, 2008-2009**

Laboratory investigations	Working (n=332)		Not working (n=298)	
	Number	Percent	Number	Percent
<b>Urine examination:</b>				
• Negative	325	97.9	294	98.7
• <i>Enterobius vermicularis</i>	7	2.1	4	1.3
<b>Stool examination:<sup>o</sup></b>				
• <i>Ascaris lumbricoides</i>	48	14.5	29	9.7
• <i>Giardia lamblia</i>	41	12.3	25	8.4
• <i>Entamoeba coli</i>	41	12.3	20	6.7
• <i>Ancylostoma doudenale</i>	39	11.7	19	6.4
• <i>Entamoeba histolytica</i>	34	10.2	17	5.7
• <i>Cryptosporidium parvum</i>	14	4.2	7	2.3
• <i>Blastocystis hominis</i>	9	2.7	6	2.0
• <i>Hymenolepis nana</i>	4	1.2	2	0.7

<sup>o</sup> More than one type was found

#### 4. Discussion:

There are a number of health and safety issues directly related to child workers. First of all, children are not the same as adults physically and emotionally. Child workers are at a greater risk than adult workers of suffering from work-related problems. Furthermore, occupational hazards and work conditions may have permanent effects on the development of children who work. In spite of these facts, little investigations are available concerning the actual conditions under which these children work (Forastieri, 2002).

The present study found that 52.7% of students worked in agricultural duties. This percentage is lower than that found by ILO statistics from 20 developing countries, the proportion of children aged 5 to 14 was 74% (73.3% of boys and 78.8% of girls) (Admassie, 2003; Kebebew, 1998). El-Gilany and his colleagues (2007) conducted a study about labor among students aged 13–18 years in Mansoura, and found that the proportion of students who worked during the year prior to the study was 36.1%. This could be explained as in many rural areas where farm work is the main job; many parents believe that children will receive more useful training by working on farms than they would in the classroom. In Egypt, economic and social factors have been cited as being responsible for the increasing prevalence of child labor. Although the Egyptian child labor law (1996) bans the employment of children who are less than 14 years of age, The result of the current study revealed that more than two thirds of working children started work below age of 10 years and the majority of them worked from 1 to 3 hours per day, while Mathews and his colleagues found that children worked about 12 hours per day (Mathews *et al.*, 2003). In 2006 a survey about child labor in agriculture conducted by Human Rights Watch in developing countries, revealed that the vast majority worked between nine and thirteen hours per day.

The present study revealed that child labor is gender biased as 60% of them were males and 40% were females. The difference is higher in comparison with other country. For instance, Duraisamy (1997) reported that 5.2% of the male children aged 5-14 years in India were economically active on a full-time basis, versus 3.4% of the girls of the same age group. Working children usually come from low-income households (Mattar, 2007). Concerning the underlying causes of labor reported by the working children, socioeconomic standards of the families represents the majority of stated causes; similarly, in a survey conducted in Egypt by Helmy and Ismail (2005) they reported that poverty is often a chief cause of child labor. Over half of the children surveyed who acted as sole or partial breadwinners asserted that they worked in order to support their households and meet basic household requirements. These findings were consistent with other studies reported by Itani (2009), Özen (2008), Köksal (1992) and Mangir (1992).

According to findings of a survey conducted by UNICEF (2002) in collaboration with the National Centre for Sociological and Criminology Research in Egypt (NCSCR), half of the working children in Egypt claimed that they had to work in order to increase their family income and 33% said that they work to support themselves.

In the present study, child labor increases with age. The low level of family education is identified as one of the most important factors in determining child labor. Girls are less likely to go to school and to work compared to boys. This is similar with the findings found by Wahba (1999).

The relationship between family background and child labor is fairly established in empirical literature (El-Gilany *et al.*, 2007). In our study, low level of parents' educational attainment was an important factor



in increasing the likelihood of children labor. This is in agreement with other studies (El-Gilany et al., 2007; Wahba, 2001; Al-Wehedy, 2002; Lashine et al, 1996).

The nature of agriculture work exposes child laborers to many risks and dangers, including long hours in scorching heat, malnutrition, hauling heavy loads, exposure to pesticides and injury from sharp knives and other dangerous tools (Human Rights Watch, 2002). Gamlin and Hesketh (2007) proved that the rural nature of farm work exposes children to extreme climatic conditions, physical hazards, animals and insects, parasites and infection. The results of the current study supported these findings. Our results revealed that working children are exposed to different kinds of risks and hazards as high temperature, sharp instruments, and exposure to wild animals. These results were consistent with the findings of Larson-Bright and other researchers (2007), who observed increased risks of injury for agricultural working children compared to non working children. On the other hand, Graitcer and Lerer did not find any health problems in working Egyptian children (Graitcer & Lerer, 2002).

The size of the negative impact on future productivity of child laborers obviously depends on the degree in which work affected their school attendance and progress and their accumulation of working skills (Galli, 2001). Our findings indicate that student labor has a profound effect on education and achievement. About 37% of working children absent many times from school and cannot concentrate in the class and more than quarter of them failed in the school. These unfortunate results are directly related to the fact that the child works several hours at the work, is exhausted and cannot concentrate or perform in school. These results were consistent with the findings of Heady (2003), Leinberger and his colleagues (2005) and El-Gilany et al. (2007) who found that students who worked were more likely to have failed at least one grade in school. Inadequate income and work stress and injuries may affect student health; that in turn can affect academic performance (Robinson, 1996)

British studies have suggested that students working long hours were likely to obtain lower grades and more likely not to complete their studies. Some students found it hard to balance employment and course work (UNISON and YMCA England, 2003). This was corroborated in research conducted by UNICEF (2008) and its conclusion is that Labor often interferes with children's education.

The present study revealed that working children experience positive effect of their work as they were self-dependent and earn money. Similarly, Blair (1992) and Lachowski (2009) reported that positive effect of children's work are perceived primarily in the perspective of education and socialising this aspect is underlined especially by parents who indicate that by taking part in agricultural work activities children learn responsibility, reliability, work ethics, new skills and coping with problems.

Omokhodion and his colleagues (2006) asked 225 Nigerian children about the benefits of working and

found that 36% of working children felt that work provide a source of income for them, 23% indicated that it was a way of helping their parents and 17% thought it was part of their training to be responsible adults. Bad company, ill health and road traffic accidents were the perceived ill effects of child labor. 47% of children perceived themselves as less fortunate than their peers. 24% thought that child labor was a sign of deprivation

Parasitological examination revealed that working school children were exposed to parasitic infections (69.3 %) more than not working groups (41.9 %). The infection rate was higher in males (33.6 %) than in females (33.6 %) in working children because males were included in farm works more than females, while it was equal in both sex in not working children (13.4%). This finding is similar with that reported by Kishk and his colleagues (2004). High prevalence of parasitic infections among working children is probably due to their frequent contacts with infected and contaminated waterways and soil at work. Also lack of awareness of health risks and lack of adequate health care services is an important factor for spread of parasitic infections.

The infection was higher in the age group less than 15 years old than the age group 15 years old and more. This could be attributed to the poor hygiene of this young age group. The parasitic infection was commoner in the students of the overcrowded families than the less crowded ones. This is an additional factor which helped in transmission of parasitic infections among these children. The present study detected that; the common parasitic infections were *Ascaris lumbricoides*, *Giardia lamblia* and *Ancylostoma doudenale* and these parasites are commonly associated with infected waterways and soil contact. El-Gilany et al., (1999) explained that farm workers commonly to eat unwashed or improperly washed row vegetables or not wash their hands before eating at farms.

Gastrointestinal parasites were the commonest parasitic infection in all studied school children. The detected parasitic stages were *Ascaris lumbricoides* fertilized eggs, *Giardia lamblia* cysts and trophozoites, *Entamoeba coli* cysts and trophozoites, *Ancylostoma doudenale* eggs, *Entamoeba histolytica* cysts, *Cryptosporidium parvum* oocysts, *Blastocystis hominis* cysts and *Hymenolepis nana* eggs. A similar study was conducted by El-Masry et al., (2007) who reported nearly the same parasitic infections among rural school children in Sohag Governorate and they added that most these parasitic infections caused anemia to these children which could affect their school performance. *Enterobius vermicularis* eggs were detected only in urine samples of female working students and this is common fact for this parasite to be rarely detected in stool samples.

The trophozite stages of *Giardia lamblia* and *Entamoeba coli*, *Entamoeba histolytica* cysts and *Cryptosporidium parvum* oocysts were detected in loose or watery stool of the examined stool samples. This was suspected; because these parasites either cause diarrhea or pass with diarrheal stool. Single infection was the commonest finding in the present study, while double

infection and multiple infections were also reported. Double infections were detected between *Giardia lamblia* and *Cryptosporidium parvum* and multiple infections between *Ascaris lumbricoides*, *Giardia lamblia* and *Entamoeba histolytica*. These were in agreement with the study done by Kishk *et al.*, (2004). Helminth infection rats were nearly equal to protozoal infection rats, in the present study, which indicated that; the infection was a mixture of soil and water pollutions.

Schistosome parasites were not detected in the present study either due to: regular treatment of these children with antischistosomal drugs provided to them by the Ministry of Health and Population or the need to other methods e.g., Kato-Katz stool examination technique for detecting this parasite in stool. El-Masry *et al.*, (2007) recorded schistosomal and intestinal parasitic infections among rural school children using this technique.

Fixatives play an important role in the preservation and transport of human faecal specimens and in the accurate diagnosis of parasitic diseases. Studies by Johnston *et al.*, (2000) found that the SAF fixative works well in concentration procedures of stool samples.

The more detected parasitic stages in the examined stool specimens were in SAF preserved samples rather than 10% formalin preserved samples. Regular intervals in examination of the preserved stool specimens were done to fix the preservative effects on different parasitic stages. In LPCB wet mounts cysts, trophozoites, and eggs of the identified parasitic species stained blue which facilitate their identification. Finding in the present study in agreement with that of Parija and Prabhakar (1995), who stated that; LPCB-stained trophozoites, cysts and helminthic eggs could easily be detected and identified in LPCB wet mounts of stools. The stain is recommended for routine use in the wet mount preparation of stools in a parasitology laboratory.

There are some limitations to this study. Some students were quite young and their perception of the work hazards may not have been accurate. The questions were explained to students and unclear points were discussed. In addition, the researchers did not visit the work sites to check for hazards. This was very difficult due to the wide spread of the farm sites and many students work in family farms.

## 5. Conclusion and Recommendations

The present study concluded that the phenomenon of student labor is common in our rural and children work in agriculture in Koom Abousheel for many reasons as to share in the family expenses and occupying spare time. It affects students physically, educationally and socially.

Working student exposed to many health hazards injuries, animal bites, high temperature. The detected parasites were *Ascaris lumbricoides*, *Ancylostoma doudenale*, *Hymenolepis nana*, *Enterobius vermicularis*, *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, *Blastocystis hominis* and *Entamoeba coli*. The majority of them started work below age of 10 years.

## The main recommendations are summarized as the following:

- Comprehensive surveys should be undertaken to determine the scope and scale of child labor in the agriculture sector, the number and nature of injuries or illnesses suffered by children working in agriculture.
- Ensure that all workers including children receive full information and training from their employers about occupational illnesses and injuries related to agriculture work.
- Ensure that all children and their families are aware of the right of children. School education for children should be a priority even when the harsh economic realities in their families force parents to send them to work outside the home.
- Measures should be taken to ensure the effective implementation of convention 182. Such measures should include the allocation of resources to provide for a sufficient number of labor inspectors targeting child labor in agriculture.
- Ensure that primary health care is provided to working children.
- Teachers can become effective agents in providing information to potential child workers, working children, their families and the community as a whole.
- Health education needs to focus the attention on children through the inclusion of information on prevention of accidents and both endemic and work related health hazards in the school curriculum.
- Improving personal and environmental hygiene and regular screening, treatment and health education for students as regard parasitic infections in Egypt is recommended.

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## References:

1. Admassie, A. (2003): "Child labor and schooling in the context of subsistence rural economy: can they be compatible". International Journal of Education, 23 (2), 167-168.
2. Al-Wehedy, A. (2002): "Female working children in Mansoura city, Egypt: A socioepidemiologic study". Egyptian Journal of Occupational Medicine; 26 (2): 203-222.
3. Blair, L. (1992): "Children's participation in household labor: Child socializations versus the need for household labor". Journal of Youth and Adolescents; 21(2): 241-258.

4. Bureau of International Labor Affairs (2004): "Advancing the campaign against child labor: Effort at country level". At website: <http://www.dol.gov/ilab/media/reports/iclp/advancing1/html/egypt.htm>.
5. Cole, D. (2006): "Understanding the links between agriculture and health". International Food Policy Research Institute, at website: [www.ifpri.org](http://www.ifpri.org).
6. Duraisamy, M. (1997): "Changes in child labor over space and time in India, 1981-1991", in *The Indian Journal of Labor Economics*; 40 (4): 809-818.
7. Egyptian Child Law (1996): Quoted from Article (64) of the Convention on the Rights of Child, *Official Journal*; (13): 53-54.
8. El-Gilany, A.; Khalil, A. and El-Wehady, A. (2007): "Epidemiology and hazards of student labor in Mansoura, Egypt". *Eastern Mediterranean Health Journal*; 13(2): 347-363.
9. El-Masry H. M.; Ahmed Y. A.; Hassan A. A.; Zaky S.; Abd-Allah E. S. And Abdel-Rahem M. A.(2007): "Prevalence, Risk Factors and Impacts of Schistosomal and Intestinal Parasitic Infections Among Rural School Children in Sohag Governorate". *The Egyptian Journal of Hospital Medicine Vol.*, 29: 616– 630.
10. FAO (2006): "Agriculture account". FAO Newsroom. Food and Agriculture Organization of the United Nations. At website: [www.fao.org](http://www.fao.org)
11. Fassa, A.; Facchini, L.; Dall'Agnol, M. and Christiani, D. (1999): "Child Labor and Health: Problems and Perspectives". *International Journal of Occupational and Environmental Health Research Paper No. 160*
12. Forastieri, I. (1999): "The IOL program on occupational safety and health in agriculture". International Labor Office, Geneva. At website: [www.E:/ILO/Safework-safetyandhealthinAgriculture.htm](http://www.E:/ILO/Safework-safetyandhealthinAgriculture.htm).
13. Forastieri, V. (1997): "Children at Work: Health and Safety Risks". Geneva, Switzerland: International Labor Office. ix, 138.
14. Forastieri, V. (2002): "Children at work: Health and safety risks". Second edition ILO- child labor. International Labor Office Geneva. ix, 169.
15. Galli, R. (2001): "The Economic Impact of Child Labor". The International Institute for Labor Studies Geneva.
16. Gamlin, J. and Hesketh, T. (2007): "Child work in agriculture: Acute and chronic health hazards". *Children, Youth and Environments*; 17 (4): 1-23.
17. Graitcer, L. and Lerer, B. (2002): "The impact of child labor on health: report of a field investigation in Egypt". Washington DC: World Bank. At website: <http://www.worldbank.org/children/>
18. Heady, C. (2003): "The effect of child labor on learning achievement". *World Dev.* 31:385–398.
19. Helmy, M. and Ismail, M. (2005): "Learning through work: the interface between education and child labor". Commissioned for PPIC-Work., pp 4. ILO Convention 182.<http://www.ilocarib.org.tt/childlabour/c182.htm>.
20. Human Rights Watch (2002): "Backgrounder: Child Labor in Agriculture". Children's Rights. Available Online at <http://hrw.org/backgrounder/crp/back0610.htm>.
21. Human Rights Watch (2006): "350 Fifth Avenue", New York, NY 10118-3299 USA
22. Hurst, P. (2007): "Health and child labor in agriculture". *Food Nutrition Bulletin.*; 28(2 Suppl): 364-371.
23. ILO (1997): "The minimum age for employment". International Labor Organization, Convention No.138.
24. ILO (2004): "Facts on child labor". International Labor Organization. At website: [www.ilo.org/childlabor](http://www.ilo.org/childlabor).
25. ILO (2006): "Facts on child labor". International Labor Organization. At website: [www.ilo.org/childlabor](http://www.ilo.org/childlabor).
26. Itani, N. (2009): Child Labor in Egypt. Master thesis. University of Southern Denmark, Faculty of Humanities. Egypt.
27. Johnston, S. M.; Bishop, H.; Wahlquist, S.; Moura, H.; Oliveira, N. and Nguyen-Dinh, M.( 2000): "Evaluation of Commercially Available Preservatives for Laboratory Detection of Helminths and Protozoa in Human Fecal Specimens." *Journal of Clinical Microbiology.*, vol 38;( 5): 1959-1964.
28. Kebebew, A. (1998): "Statistics on working children and hazardous child labor in brief". Geneva, Switzerland: International Labor Organization. At website: <http://www.learns.org.au/clp/archive/index/htm>
29. Kishk, F.; El-Masry, A.; Abd-Allah S. and Gaber, H. (2004): "Vulnerability of working children to environmental health risks: a case study in a village in rural Egypt". *Global Forum for Health Research*, (Forum 8). Mexico city, Mexico, 16-20 November 2004.
30. Köksal, A. (1992): "A comparative study on the work conditions and problems of 15-18 year-old working children who do and do not attend apprentice training centers". Master's thesis (unpublished). Ankara University. Ankara.
31. Lachowski S., (2009): "Engagement of children in agricultural work activities –scale and consequences of the phenomenon". *Annual Agriculture and Environmental Medicine*; 16: 129-135.
32. Larson-Bright, M.; Gerberich S. Alexander, B.; Gurney, J.; Masten, S.; Church,T.; Ryan, A. and Renier, C. (2007): "Work practices and childhood agricultural injury". *Injury Prevention*; 13 (6): 409 - 415.
33. Lashine, S.; Metwally, H. and Bahry, M. (1996): "Child labor in two districts in Cairo". *Egyptian Journal of Community Medicine*; 14 (1):15–21.

34. Leinberger-Jabari, A.; Parker, D. and Oberg, C. (2005): "Child Labor, Gender, and Health". *Public Health*; 120: 642-647.
35. Lwanga, S. and Lemeshow, S. (1991): "Sample size determination in health studies". A practical manual, WHO, Geneva. p 80
36. Mangır, M. (1992): "A study on the working reasons of students attending apprentice training centers in Ankara and factors affecting these reasons". Ankara University Faculty of Agriculture Publications. Ankara.
37. Mathews, R.; Reism C. and Iacopino, V. (2003): "Child labor: A matter of health and human rights". *Journal of Ambulance Care Manage*, 26(2):181-182
38. Mattar, M. (2007): "Child labor in Egypt: Scope and appropriate legal responses". At website: [www.protectionproject.org/](http://www.protectionproject.org/)
39. Omokhodion, F. ; Omokhodion, S.; .Odusote ,T. (2006): "Perceptions of child labor among working children in Ibadan, Nigeria". *Child: Care, Health and Development*; 32 (3): 281–286.
40. Özen, U. (2008): "Reasons why the attendees of Ankara Ostim apprentice training center are left outside of formal education and their views about work life". Master's thesis (unpublished). Ankara University, Ankara.
41. Parija, S. C. and P. K. Prabhakar, P. K.(1995): "Evaluation of Lacto-Phenol Cotton Blue for Wet Mount Preparation of Feces". *Journal of Clinical Microbiology*. Vol 33;( 4): 1019-1021.
42. Parija, S.; Bhattacharya, S. and Padhan, P. (2003): "Thick stool smear wet mount examination: a new approach in stool microscopy". *Tropical Doctors*. 33(3):173.
43. Parker D (1997): "Health effect on child labor". *Lancet*; 350:1395–1396.
44. Robinson, L. (1996): "School students and part-time work. Melbourne". Australian Council for Educational Research, (LSAY Research Report No. 2).
45. UNICEF (2002): "Survey in collaboration with the National Centre for Sociological and Criminology Research (NCSCR). At website: [www.unicef.org](http://www.unicef.org)
46. UNICEF (2008). "Statistics by Area/Child Protection". Available Online at <http://www.childinfo.org/labour.html> Accessed on 8-26-08.
47. UNISON and YMCA England (2003): "Not just child's pay. Why younger workers need a minimum wage". Submission to the low pay commission, London. UK, UNISON and YMCA England, [Ahttp://www.unison.org.uk/acrobat/B1085](http://www.unison.org.uk/acrobat/B1085)
48. Wahba J. (2001): "Child labor and poverty transmission: no room for dreams". A report on Egypt. Cairo, Economic Research Forum for the Arab Countries, Iran and Turkey.
49. WHO (2004): "Appropriate body mass index for Asian populations and its implications for policy and intervention strategies". *The Lancet*; 157-163.
50. Wilk, V. (1993): "Child Labor: Health Hazards and Remedies ".*American Journal of Industrial Medicine*; 24 (3): 283–290.

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## The role of rural women in agricultural activities

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**Abstract:** Women form great part of total workforce that needed for agriculture part at universe, as one of the intangible factors at agriculture economy. So, statistics that was represented in relation to extent of women's activity is very lower than real extent. Because in this statistics, mostly, seasonal jobs, part time job, no wage job and their housekeeping activities, aren't considered. rural women , have different roles and duties such as husband , mother, crops producer , participate at ranching activities, planting ,maintaining , harvesting , processing , marketing and preparing food . Rural women maybe venturing to culture cash products, while cultivating subsistence products and if they have no farm land, they have to work for others instead receiving wage. We can consider such women as agriculture propagator, production expert and even in some case as policy maker. Other than activity at agriculture field, women's participation at rural development is critical and is considered in order to supply adequate and needed food.

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**Keywords:** Rural women, Agriculture

### Introduction:

A positive relationship between women's participation in agricultural and non-agricultural employment of men can be seen, so that in some countries men migration to cities or bringing them on a day wage jobs has led them responsibilities in the absence of their husbands take charge of 30 to 40 percent of work related to home and agriculture. In some areas this figure reaches to 70 percent. Number of factors also led to a kind of common gender division of labor, especially in rural societies and one the most veteran of these factors is a particular power and ability of women to provide sustenance (Ghaffari, 2005).

Means participation of women in all stages of development, evaluate needs, identify problems, planning, management, implementation and evaluation is. Equity participation in a patriarchal society was not easily achieved, such matter requires the participation of women, especially rural women in projects is the way that they are concerned. Rural population of Iran always different roles in the production and distribution have been responsible. Agricultural sector, supplier of about one third of employment, food needs of more than Chharpnjm country, half of exports, do not need the agricultural products industry and one-fifth of GDP countries.

Considering the role of women in the family play, as the main axis can development be considered according to the tangible realities of the world unfortunately Azmvqyty women have adverse effects

on international level. For example, differences in policy, investment and receive equal rights for the work phenomenon is universal. Participation rate of women in economic activities sufficient to confirm the lack of women's affairs and their added value, because rural women in all stages of agricultural production and livestock production, and general affairs of all men to work alongside, and often pay a small Fraqtshan the mere production of handicrafts such as carpets and kilims.

Macroeconomic view of employment of different aspects such as creating income, production and entrepreneurship, science and technology development, etc. is important, and gives the dignity, status and social position and a sense of confidence from the social viewpoint of man. Working and use of inherent forces, skills and knowledge and personal management to begin to work and to accomplish the activity, are not specific to particular groups. Men and women can work in a community and yet affect it with job situations that are provided to them or they themselves create.

Employment rate, like any social and economic variables directly or indirectly are affected by many factors among which can be reported the production rate, investment, wage levels, price level, government policies and foreign trade. Each of these factors may be positive or negative effects to be followed. Such factors and how they impact on employment rates, have a great influence in planning and policy and making coherent and efficient policy formulation

According to women's role at family, they can be considered as base of development and progress and unfortunately according to universal tangible realities, they possess unfavorable position at international level (Changizi Ashtiani, 2003).

For example, difference at levels of policy making, investing and receiving salary for equal activity, are universal phenomena. extent of women's participation at economic activities, extent of women's activity at economic activities, is confirmation on lack of adequate attention to women's affair and their added value, because rural women work alongside men, at all levels of producing agriculture crops and livestock products and generally all affairs, and also spend their little leisure time for handicrafts such as rugs and carpets and etc. so it is necessary to establish self acknowledgement fields, directing women's economic and social ability and programming to attract their participation at different activities. At rural area, women have more significant role on family economy and inside activities and cause economic prosperity of society. yet, women couldn't gain their real position as active citizens who have talent for participation at economic, politic, social and cultural arena at most countries, especially developing country, and still their activities in economic calculations aren't considered, and they be considered as intangible workforce. Disappointing estimation about number of active rural women and underestimate about extent of their participation at economic activities is confirmation on lack of adequate attention to women's affairs and their added value. they are major force to create revolution and potential sources to progress rural economy and increasing extent of growth rate of producing food productions, although traditionally, farming and ranching, has been male profession, but women's role was never restricted to house and family, so they are active outside (farming, ranching, forestry) other than inside activity (Balali, 2005).

while assessing historical flow of development, we find that trends to human dimensions after failure to fast industrialization, forced programmers and policy makers to revise their thoughts and consider occupation, population and adequate employing of workforce especially women who form half of population, as major goals of development (Fami, 2001).

Government and national organizations attention to this issue began from mid 1970's. After world conference in Mexico, year 1975 was named as "woman year" and after that year from 1975 until 1985 was named as "women decade", and their certain needs were considered.

In this regard, vast studies were performed and it became clear that most of development programs including Green Revolution and high yielding varieties, as caused increasing productions, had negative affects on women's occupation and has increased their duties. At 1997, world conference on rural development (WCARRD) was held by participation of 145 representatives from different counties in Roma and identified problems which women faced with. Main goal of this conference was: to support rural women as producers and their certain preferences at access to productive sources especially technologies that be able to decrease extent and hardship of their duties and lead to increase their efficiency (FAO, 1998).

Growing women's participation at labor market is one of development indexes of each society and represents increase of women's favorites to different aspect of participation at economic-social activities. Women's activities at its different dimensions at developed countries which rapidly changing by modern technology and difference at role and their functions is obvious even among poor countries. Attending to this point that women are at basic center of development, is very important. Because they control most of non-monetary economy by rearing generation, providing workforce and managing and performing family affairs and also subsistence agriculture. While always, women's productive role at agriculture has been introduces as concealed form and rarely manifested economically and socially, and maybe be most intangible participants at economic process (Emadi, 2001).

Although at all societies, rural women were introduces as one important factor for achieve to rural development goals but base on different economic, social and cultural reasons, they were considered less by programmers and practitioners, practically. Women as one intangible factor at agricultural economy, form great share of all human workforce needed for agriculture part, across the world (Ghaffari, 2000).

Economical-social studies have proven that various obstacles are at them way of women's role that majors are as follow (Samadi Afshar, 2004):

#### **1- individual obstacles :**

- 1-1 - Low literacy that further numerous problems, lead to lack of self confidence, inferiority feeling and resignation.
- 1-2 - high extent activities and increased work inside and outside due to various reasons including men seasonal migration and seasonal variation of rural women's activity (nursery, home affairs, farming, handicrafts and ranching)

1-3 - Having low information , knowledge and their applied scientific knowledge

1-4 - Malnutrition and low coefficient health and low individual health (Rivera, 2001).

### 2- family obstacles :

2-1- lack of knowledge about how to behave with girls and women

2-2- family biases and husband and father disagreement with women's attendance at social and economic activities due to various cultural reasons and even unwillingness to loss house workforce

2-3- negative attitude and belief about their abilities

2-4- gender discriminations

2-5- economic poverty of family (Varzgar, 2001).

### 3- social obstacles :

3-1- bad customs and traditions such as superstitious and fatalism and ...

3-2- patriarchy and disagreeing with women's attendance at group activities

3-3- low women's access to credits and facilities, inputs and production factors

3-3-being far of accessibility of extensional services and or orienting social activities and participatory programs toward men

5-3- limited number of female experts to educate rural women

6-3- problems of access to services and social and health facilities

7-3- low extent of rural women's wage compared to men

8-3- low attendance of women NGOs to track their issues and problems

9-3- low women's attendance at rural management (Banihashem, 1999)

### Conclusion and discussion:

One of the issues that government should pay attention to is rural development issue especially at undeveloped countries. in this countries due to lack of proper policy making to improve quality of people life level of these areas , villagers migration to cities has increased considerably and led to urbanization growth and emergence of problems and also psychological , social , cultural and economical abnormalities especially at agriculture and ranching part . Also method for growth and rural development growth, require research at this field which can help government in order to economic, social and cultural programming and policy making. Creating local organizations and regional institutions with affective women's attendance and villager participation to solve problems are among important and affective substances that should be considered in regional programming. at developing and changing process of developed economy system of agriculture, value of women's activity changed as form of money which

previously was as no wage workforce at family , and was given to her. Other than agriculture part (i.e. industry and public services) which are main field of women's work, rural women's participation is very important. The most important issues about women's social and political participation are participating at programming, decision making, performing decisions and valuing results.

Mr. Aghaee in on research as "rural women's role at economy of agriculture and their success at agriculture development programs " further assessing their status at different countries and also emphasis on their participation at production activity of family , has expressed factors that led to ignorance of their role at economy of agriculture .

Lahsae Zade at research as "assessing Iranian rural women's role at economy arena: first assessed their position at occupation structure then has compared it with rural men's occupation base. He expressed that rural women have equal importance compared to men.

Safiri, in his doctoral dissertation as "assessing quantitative and qualitative women's occupation ant its relation to economic development" has considered some of their problems of occupation due to obstacles which refers to structure of countries. And some contain social-economic and cultural obstacles.

Rasool Purarabi , in his thesis as "assessing women at economic activities in rural area of Ramsar " has shown that more than 96% of rural women , at least had participated at economic activity that was supplement for family income . But they don't participate at basic decision making of family, in spite of their affective role and vast attendance at economy of family, and also they enjoy owning production factors, less.

Development Realization is impossible without women's participation at different social-economic aspect. Therefore, to understand unknown, researchers should strive and take basic step in this regard. Some programs should be provided at national level as long term projects at the field of education and Cultural Revolution in order to create needed knowledge in society and in women to identify their rights, education and extending modern techniques, creating infrastructure facilities and also rural development.

Since, village is suitable place for agriculture and additional related activities, so it can be said that women's role at village and possibility to institutionalize proper infrastructure, we able to have suitable perspective toward development process.

Agriculture part as one of the most important productive parts of country have critical responsibility in preparing needed food security that can help this part to access its major goal according

to efficiency of workforce up to proper level . In this regard, women play critical role. Nevertheless, they couldn't represent their abilities at this field, due to various limitations which women face. Among this especial attention to this group of society and preparing them supporting, educational and extensional services for them can help to remove their vast future problems , according to major role of this forgot group at agriculture activities and finally lead to increase and improve their efficiency about agriculture and consequently lead to increase welfare and comfort of rural society .

In order to be able to remove obstacles and problems of women's activity at villages , we should reinforce stamina if women's work by one exact and codified programming in order to be able to progress at one correct direction.

villager access to education at different level , possibility to enjoy suitable occupation opportunities and also industrial , technical and healthcare equipment has caused that cities go out from concentration and attraction of inside and outside capitals , and so possibility of fair distribution of resources and facilities between city and village be provided , and government instead of bear heavy cost of urban population , spend these costs for rural development and support rural women whom get more damage while face lack of facility and compared to men enjoy less migration rate and also have to adopt existing conditions and use available facilities . In today world, it is impossible to achieve development goals without applying abilities of half of people of society (i.e. women).

Women at most countries, have low access to economic resources at the field of economic activity. They should reinforce them at this field by supplying economic facilities. Another part that changed women's attendance at economic affairs is agriculture activities. Opportunities which they gain at this part can have important impact on economic function and related social relations

Same discussions were presented about identifying women's role on environment changes (especially in preserving natural sources) that related to women's life and job. Women's access to agriculture credits, because increasing and improving their efficiency at agriculture. Women's membership at cooperatives, also help them to receive facilities in order to supply needed inputs of agriculture, sale productions and make some production with aim of increasing efficiency. Most of researches found that women's education is related to their agriculture efficiency. Indeed, years which women used educational programs, related to their productions meaningfully. So, by identifying their needs, demands and interests and also by determining their

issues, resources and preferences, we should prepare proper extensional and educational programs for them.

Also literacy programs and generally their basic education should be considered specifically with aim of better women's enjoyment of extensional and educational programs. And also access opportunities to different resources and needed inputs at agriculture activity should be provide for them. Development programs for rural women mostly have certain importance that should be considered at extension activity.

Empowering women is one of principal discussions of development process for many countries of today world. existing factors contain women's education , their ownership sample , their occupation opportunities and function of labor market but if we go beyond this rather classic variables , these factors also contain occupational relations nature , how to behave family and generally society with economic women's activity and economic and social conditions which encourage or prevent change at these moods .

Last conclusion is that men and women, play role at agriculture programs and rural development but each has different needs and knowledge base on kind of their activities, since total people activities were done to supply their needs and so governments should consider regional programming in their policy making and programming. This issue dose not achieved unless by identify climate, population, cultural, economic and politic constituents of each region and also kind of relation of these constituents with constituents outside village and region.

These kinds of study and recognition have provided causes of better programming and adopted with needs of region, and prevent loss of investment. If education, health, occupation, cure and ... facilities be provided in village and improving rural life level be considered, so migration would be regulated. At the other hand, protecting agriculture and livestock products and local industry, and attracting well condition markets for it, by governments, can be affective for villager's interest about rural life. Finally, positive attitude of development programmers, would help significantly to improve condition of one benefited rural family, and would act as a factor to diminish gap between urban family and rural family.

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**References:**

1. Amiri, Soodabeh. Female centered sustainable human development. *Journal of Agricultural and Development Economics*, 2000, No. 9.
2. Arefnia, kh (2004). Informal economy, in reality unknown issues of rural women. *Journal of Agricultural Economics and Development*, the third year, special role of women in agriculture. Tehran: Ney publishing.
3. Azkia, M and amiri A. (2006). Article "The role of micro-credit in empowerment and job creation released prisoners." Conference on rural development and poverty reduction, agricultural banks, Tehran.
4. Banihashem, F. Rural women, education, association and participation. *Jihad Journal village*, 14 years, No. 310, 1999, p. 21.
5. Balali, L. Mission Trip Reports samples producing rural women (rural women's efforts Affairs Ministry of Agriculture) to India and meeting with the board of directors and senior managers National Bank of Agriculture and Rural Development (NABARD) self-employment Women's Association (SEWA), and the Empowerment Institute rural women (CARE), 2005.
6. Changizi Ashtiani, M .Including the share of women in producing countries. *Journal of Agricultural Economics and Development*, the third year, special role of women in agriculture. Tehran: Ministry of Agriculture publications, 2003, Pp 83-81.
7. Emadi, M. H. Women and political participation. Center for Women's Participation President, Publishing Olive Leaf, 2001.
8. Fami. Sh. Analytical process to determine the educational needs - extension of rural women (Part I). *Jihad Magazine*, 2001, No. 243-242.
9. FAO. Women in agricultural development. (Translated by: Saleh GH ancestry). Publisher: Management studies and studies and promoting people's participation Deputy Agriculture (the former). Pp 46-42, 1998.
10. Farghdan, M. Cultural Arts Festival the first report of rural women. *Monthly Jihad*, 2001, No. 243-242.
11. Ghaffari, GH. The role of women and social development. *Women's Magazine*, 2000, No. 10, p. 15.
12. Kamali, A. (2004). Participation of women in development approach prerequisites and obstacles. *Women's Research Quarterly*, No. 1, Tehran: Press Center Tehran University women's studies. P. 14.
13. Khani, F (2000). Position and role of women in rural development process (case study of Gilan province). PhD thesis, Terabit Modarres University.
14. Lahsaeizadeh, a (2004). Class base of rural women in Iran. *Journal of Agricultural Economics and Development*, conference papers, women participation and Agriculture 1400, Journal No. 3, Publishing Ministry of Agriculture. P. 14.
15. Lahsaeizadeh, A. Sociology of rural development. Tehran: Publication Publication Days, 2000, p. 58.
16. Nawab Akbar, F. The role of rural women in the past decade. *Journal of Agricultural Economics and Development*, conference papers, women participation and Agriculture 1400, Journal No. 3, Publishing Ministry of Agriculture, 1997, P. 186.
17. Rivera, W.M. and corning, S.L. Empowering women through agricultural extension: a global perspective, *journal of extension*, 28(4), 2001.
18. Samadi Afshar, S. Factors affecting rural women's participation in training programs and extension services in agriculture in West Azarbaijan Province 82-81. MSc thesis, Islamic Azad University, Science and Research, 2004.
19. Varzgar, sh. and azizi. M. Evaluation of labor force participation of rural women in cotton production and its related factors in the region and dome of Gorgan, 2001, P. 318.

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## Importance of indigenous knowledge in rural areas

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**Abstract:** Indigenous knowledge of each nation has enabled them to supply their needs from natural sources without reducing these sources. So, indigenous knowledge collection of world is valuable source of practices and time-tested tool that would be useful for sustainable development of all societies. At third world countries, unconsidered triumph of world development policies has led to various social, economic, cultural and environmental issues (Agrawal, 2002). Imbalance population growth, non-sustainable efficiency of natural sources and unequal distribution of resources, goods and services made involved societies in confusing issues and impasses. In these countries, inappropriate sampling of abroad countries and inordinate imports (e.g. heterogeneous and non-indigenous technology) devastated independent collection of micro local systems, and instead has established heterogeneous and dependent system to global economy system, that obviously couldn't supply people's needs. Since, this development process is formed without considering social, cultural and environmental consequents so isn't continuing and human have to find strategies which can make development sustainable and humane (Popzan, 2002).

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**Keywords:** indigenous knowledge, rural

### Introduction:

During 1950 and 1960 decades, native (indigenous) knowledge was an inefficiency and absolute barrier for development. Nevertheless, now indigenous knowledge is recognized as a basic source. Indeed this knowledge was an answer to failure of great developmental theories by great countries and it was as a technical oriented solution for changing most peasants and farmers view in the world. (Agrawal, 2002).

The lack of indigenous knowledge about indigenous practices in many technologies in the developing countries will lead to failure. So attention to indigenous knowledge as a knowledge that is result of many thousand years experience is important in development of villages. Rural benefactors, the people who had communion in development of villages, can take efficient steps in rural development if they pay attention in the process of rural schematization for its development. Indigenous knowledge has different aspects, such as hygiene and treatment, medical plants, linguistics, livestock and agriculture, art and mystery and unprofessional things (Farrokhi and Yaghoubi, 2002).

Indigenous knowledge is local knowledge that is restricted to one specific culture and/or certain society. Indigenous knowledge is different with scientific knowledge that was established by universities and scientific communities. This

knowledge is basis for decision making at field of agriculture, health, education, food and natural sources (Warren, 1993).

Indigenous knowledge is set of all knowledge and skills that people enjoy in one geographical area (in one environmental conditions) that most of their skills and knowledge be transmitted to next generation, and new generation would be adapted with them and add to it (Merrewij, 1999).

Since, each knowledge is consequent of individual interaction with environment, so indigenous knowledge is consequent of indigenous people interaction with their environment. Chambers with emphasis on people's role at development process believes that "rural people's knowledge" term is more eloquent than other terms for indigenous knowledge. Our purpose of rural people are producer farmers, input buyers, agriculture production sellers and etc. "people" in above phrase emphasis that this knowledge is more verbal and less has been written. This word also referred to whole knowledge system which contains concepts, beliefs, and attitudes and also contains gain, store and transmitting knowledge process (Rajasekaran, and Babu, 1996).

### Features of indigenous knowledge

Some of these features are as follow:

Indigenous knowledge is holistic: indigenous knowledge is gained by sense and inspiration force

and leads information unity. In spite of formal knowledge that is aural, visual and analytic.

Indigenous knowledge is verbal: writing and documenting indigenous knowledge would make it out of reach of villagers who can add to it, if it would not follow applied activities.

Indigenous knowledge is practical: it is possible to write about indigenous knowledge but it is impossible to educate and learn it through books and articles. Only way to learn it is close view and follow professor.

Indigenous knowledge isn't explanatory: it isn't possible to expect one master (e.g. mason, apothecary, farmer) to explain his method efficiency in a way that is apprehensible to us (literate people)

Indigenous knowledge is local: villager's knowledge has formed in itself environmental and climate framework. Effective indigenous knowledge at one geographical area isn't necessarily effective at other area (Nowroozi, A and Alagha, 2000).

Indigenous knowledge is general : while, formal knowledge emphasis is on saving time and removing ideas and also monopoly of knowledge at universities and research institutes , but indigenous knowledge is , receptive , incentive and needs to more people's participation at learning , developing and add to it. Furthermore, in verbal cultures, it is impossible to separate science from world and even include it to computer and book. Every human are important in indigenous knowledge.

Indigenous knowledge is deteriorating quickly: by every death of old indigenous people, great knowledge resources would be lost also, so every action toward gathering indigenous knowledge is necessary.

Learning by doing: repeating action in order to sustain and enforce indigenous knowledge through "learning by doing" is one of features of indigenous knowledge in real operation environment (Emadi and Abbasi, 2001)

Villager's knowledge and especially indigenous knowledge systems have various dimensions that is include linguistic knowledge, zoology, ecology, climate, agriculture, ranching and professional skills. Range and value of this knowledge hasn't been considered. Four aspects of various dimensions of rural knowledge were selected and were analyzed, In order to change attitudes and reformer's behavior of rural development. These dimensions are: agriculture operations, rural knowledge about nature, rural people's aptitudes and abilities and their experiences (Razavi, 2002).

In Chambers' opinion , indigenous knowledge or rural knowledge has various dimensions that he classified them to four parts in order to explain more and better about diversity of indigenous knowledge

that are as follow : A: farming activity ; B: knowledge in relation to nature ; C : indigenous people's aptitude and ability ; D: indigenous people's test . indigenous people's knowledge originated from exact viewing of environment; since indigenous villagers have direct contact with phenomenon and also see all different processes at nature so have especial aptitude and ability compared to outside people . Maybe least known aspect of indigenous villager's knowledge is essence of tests that they do which maybe these tests are available to choose "bests" and some other for "minimizing risks" (Dewes, 1998).

### Characters of indigenous knowledge:

The characters of indigenous knowledge like the definition of this knowledge are presented by experts in different ways which we will explain about them as follow:

#### 1- it is based on experience:

Indigenous knowledge is the result of people's experience during many centuries.

#### 2- it was tested during centuries by working on it.

#### 3- it is compatible with indigenous environment and culture:

Indigenous knowledge was created through native societies and it was formed according to their needs and during time the things which were not compatible with indigenous environment were omitted, so what was remained was compatible with the environment and culture of that society (Amiri Ardekani and 2003).

#### 4- it is dynamic and is changing:

Simultaneously with changing indigenous culture, the indigenous knowledge was changing too.

#### 5- the knowledge of rural people was not technical:

This knowledge was consisted of rural people's wishes, values and preferences.

#### 6- the rural people's knowledge is not statistic:

This knowledge was formed according to people's culture, social and economic history. The history which was written by these rural people shows that their manner and activities were efficient in changing of their conditions.

#### 7- rural people's knowledge is not enough.

Maybe the rural people are knowledgeable but they like to know more and more. Because they want to be powerful in their discussions with political, economical and social forces who made these people poverty before give them technology (Zare and Yaghoubi, 2003).

#### 8- rural people's knowledge has root on their political economy and is more important in political field.

The advantages that rural people can get from indigenous knowledge are the knowledge that is created and released locally and is on their authority and also depends on main factors in regional politic economy (land distribution, marketing relations, and vertical links and ...). So improvement of their livelihoods depends on interferences which were made to pervade on these main factors.

#### **9- most of the rural people are public-oriented**

Mostly, they have a little information about many things which is in contrast with academic educations. Specialist people in universities have deep knowledge in little fields (of course some of these native people are specialist too) (Razavi, 1999).

#### **10- indigenous knowledge systems are holist:**

Local people consider the other people's problems as their problems and try to solve these problems in a whole frame with using their knowledge.

#### **11- indigenous knowledge systems combine the culture and religious believes.**

Religious believes as a part of indigenous knowledge are not separated from technical knowledge and these believes effect on people' do and don't

#### **12- indigenous knowledge systems prefer the less risk to most profit**

Escaping of risk is important for native people, for example a native person usually keeps some goats for possible cases such as disease of his children and he and he didn't expect any incomes of these cases.

#### **Comparison of native and modern knowledge**

Native knowledge is different from modern knowledge in some cases that we will explain them as follow:

- Modern knowledge is reductionism (atomistic) but native knowledge is holist
- Native knowledge is reductionism (atomistic) and modern knowledge is holist
- By using native knowledge we can reach to a sustainable agriculture and modern knowledge doesn't have this feature.
- Government organizations have known native knowledge unreliable but modern knowledge is supported by scientific organization and institutions.
- Native knowledge is available for rural people but modern knowledge is not (Rajasekaran and et al, 1996).

#### **Compilation of native and modern knowledge:**

Many experts believe that for making a sustainable development, native and modern knowledge should be combined. Nowadays, so much efforts have done to make use of native knowledge but main part of

these efforts were done for derivation and making it scientific (Burger, 1997).

Amiri Ardakani and Shah vali (2003) believe that the undesirable outcomes of development on people and rural environment is the result of using new science by scientist, so by blending and making relation between modern and native knowledge we can solve this problem.

Millar believe that by combining native and modern knowledge we can make trust between researchers and rural people, because by using this way researchers and rural people know themselves as a partner that are responsible for a common process and product. Millar believe that the trust is the reason for future development (Penny, 2001).

Experts believe that there is no way to reach sustainable development except to combine native and modern knowledge.

Native and modern knowledge will complicate when:

- 1- We solve structural barriers such as political, economical, cultural and social difficulties.
- 2- We correct the thoughts on educational systems by emphasizing on learning and thought process and also correct the thoughts on research systems by emphasizing on audience and beneficiaries needs.
- 3- We solve communication barriers that cause inactivity on relation process and steady and dynamic flow of knowledge between peasants, experts and scholars. (Emadi and Amiri Ardakani. 2004).

Nowadays, making scientific native knowledge in agriculture had devoted important part of native knowledge researches to itself. Creation forestry cultivation system which is taken from indigenous exploitation pattern in forested region is the result of making scientific native knowledge. Stimulus cultivation of fruit trees with other production was usual by farmers in developing countries from one hundred years before (Louise, 2000).

Environmental problems because of forest destruction, made scientist interested to use of forestry's methods and ways and forced them to make these ways scientific. Scientist had specified the physical and biological compatibility between different species and it is output according to laboratory studies and has identified compatible trees and productions. Then they supplied package sets by new title such as forestry cultivation, multi-cultivation and ecological agriculture systems and give them to farmers in commercial and formulated packages. Making native knowledge scientific is meaning to find its efficiency scientific reasons. In

the process of making native knowledge scientific, most of the experts and researchers are not aware of cultural aspects of ways and native methods. If derivation of native knowledge and making it scientific was without attention to cultural aspects and governing values on indigenous society, it couldn't be acceptable among native people.

Experiences show that native people would not accept methods which are not compatible with their belief and needs even if it had had scientific bank roll (Emadi and Abbasi, 2001).

### **Conclusion and discussion:**

Necessity and importance of indigenous knowledge and sustainable human development prepared field for establishing "united nation conference, about nature and development" at 1992.

this conference was established due to complaints against damaging environment in order to prepare basis for active indigenous people's participation at legislation and policy making , how to manage sources and related activities to development ; and also if people presented some suggestions about recent subjects , so find way to practice them. Failure of moved technology to rural societies also manifested necessity of considering indigenous people and their knowledge. At the other hand, considering indigenous knowledge is essential to help formal knowledge; because indigenous expert's attendance beside other experts has very critical importance. For example, indigenous peoples know condition of their regional epistemology, very well. Thus, their attendance is very affective for extending incompatible technologies with condition of region and at least, it conceives propagators to test these innovations at small scales and under natural condition and helps to extend them at larger scales, after being ensured of their appropriateness.

So, not only attendance of indigenous knowledge is necessary for applied researches but is important at compatibility researches and it enforced importance of attending to indigenous people and their knowledge. Therefore , applying affective strategy for transmitting technology has been among from affective fields at attending to indigenous people's knowledge and especially experts; because, development institutes realized positive their affects for doing this more than ever (Merrewij 1998) .

Indigenous knowledge has been manifested at sustainable process and improving extension programs at industrial countries of world, very well. Indigenous knowledge related to agriculture, medicine, food and architecture has been widely used At European countries, USA, Canada, Australia, by new names.

At one research as a name of "analyzing position of indigenous knowledge at sustainable rural development" that was done by Buzarjomhore (2005) it was signified that although there are some differences between indigenous and formal knowledge, but they should not be compared, because they are complementary of each other and it is possible to gain successes by synthesizing them that is impossible lonely. Base on new paradigms of rural development in order to solve rural problems, we should first refer to indigenous solutions and if it was working, then we should reinforce it; if not we should test and use outside solutions. Findings of one research done by Emadi and Amiri (2004), as "Synthesizing indigenous knowledge and formal knowledge as necessity for accessing to sustainable rural development", has shown that dominated belief among educated groups toward natives and their knowledge is precondition of every interaction, synthesis and relation. Creating revolution in formal education systems in order to attending empirical knowledge area is considered as one of main necessity of this synthesis that is outcome of years of researches. Researchers attention to "exploiter's accumulated experimental and historical wisdom" is one of other necessities of this revolution by using cooperative, qualitative and filed methods. Also, applying mutual extension ways and creating revolution at communication system between governmental, education-extension centers and farmers and rural people so that they be interacting, was considered as precondition and necessities. At researches as "indigenous knowledge at development process" done by Karimi (2003) , findings show that indigenous knowledge is principal factor and main source at the field of research of sustainable development , decreasing poverty , enabling local men and attracting their participation at activities and rural development programs , developing and producing appropriate technology , self-reliance of rural societies and country.

So, effort and national commitment and multi-dimensional support is very critical for recording, valuing, extending and exchanging this rich source and also preparing mechanism and practical strategy for synthesizing this knowledge with new knowledge and agricultural development programs.

Agricultural extension was identified as one powerful IT focused area, due to role variation at knowledge system and agriculture information at one hand and at the other hand due to its dependence on various exchanges among farmers, that can has great affect on rural society and developing agriculture. So that work and productions of farmers would increase by farmer's access and use of Internet and subsistence farmers at all over the world are at

developing by gaining needed knowledge and information that during time would becoming as commercial producers. Transmitting from system-cycle source of agriculture to technology-cycle system of agriculture placed more responsibility on agricultural extension because agriculture extension system is as vital technology transfer crossing to farmers at one hand and as crossing for referring feedbacks, needs and agriculture issues, researchers and policy makers of market.

What that is obvious is that extending and researching agriculture can help to sustainability through close relation to farmers, attending to their experiences, gaining their information and logical understanding of agriculture activities, attending to their vital needs for doing "demand-base" researches and extension education efforts for developing agriculture, at process of improving agriculture development.

On the research which was done by Bozarjomhari (2004) with this title "analyzing native knowledge position on rural sustainable development". It was specified that although there are many differences between native and modern knowledge but they are not in contrast with each other, because they are each other's supplement and we can't be success when we use them separately. According to new parameters in rural development, for solving rural problems, at the first we should use of native solutions and if it was not efficient, we can use and test external solutions.

Research findings which was done by Emadi and Amiri (2004) with this title " compilation of native and modern knowledge is necessary for reaching agriculture sustainable development" signify that The believe of educated people to native people and their knowledge " precondition for making them close" is called combination and compilation. Making evolution in modern system for attention to tentative knowledge is the main necessity for this compilation. Another necessity for this evolution is the researcher's attention to experimental accumulated wisdom and historical exploit by using qualitative and communion methods. Also applying compilation methods and making evolution among government, educational centers, farmers and peasant is the necessity and pre condition for combination of modern and native knowledge.

Research findings that was done by Karimi with this title " native knowledge in development process" signify that native knowledge was a essential element and important source for realization of sustainable development, poverty reduction, making local people capable and motivate them to participate in activities for agriculture and rural development, developing and product suitable

technology, rural society's self-reliance and self sufficiency. For this reason all side's try, partnership and protection for record and registration, compatibility, distribution and promotion, exchange of this resources and also suitable and scientific guidelines for compilation of this knowledge with new knowledge and rural and agricultural development plans are needed. Although native and modern knowledge are different from each other in their nature and usage, but it doesn't mean that they are in contrast with each other. Experience shows that not only native and modern knowledge are not in contrast with each other, but also they are suitable supplement in agriculture and rural development for providing developmental needs.

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**References:**

1. Agrawal. A ,(2002) "Dismantling the Divide between Indigenous and scientific knowledge "Development and change vol 26.No3.
2. Ahmed, M. 2000 .Indigenous Knowledge for Sustainable Development in the Sudan . Khartoum, Sudan. Khartoum University Press.
3. Amiri Ardakani, M. and Shahvali, M. Principles, concepts and indigenous knowledge Agriculture "series of publications and development of villages No. 34, Second Edition 2003.
4. Appleton, H., and Jeans, A. 1995" Technology from the People: Technology Transfer and Indigenous Knowledge ."Science, Technology and Development.
5. Azkia, M and Imani A, Sustainable Rural Development - Publications Information, Tehran, 2008.
6. Bouzarjmehri, Kh. indigenous farming knowledge of gender and its role in Rural Development and Research, Centre of Quarterly Tehran University Women (Women's Research), 2005.
7. Box, L. (1999), for the fun of it, Guest Column, Indigenous knowledge and Development Monitor 792; 36.
8. Brouwer, Jan. (1998). IK, IKS and ITK. Indigenous knowledge and Development Monitor. Vol.6, Issue 3, p, 13.
9. Burger, J. (1997)The Gaia Atlas of First Peoples: A Future for the Indigenous World, Penguin Books, and Ringwood.
10. Chambers, R - rural development, priority part to the poor (supporting vulnerable groups),

- translated by Mustafa Azkia, Tehran University Press, 2000.
11. Dewes, w. (1998), Introduction, p. 3 in traditional knowledge and sustainable in S. H. Davis and K. Ebbe (Eds) Proceedings of a conference held at the World Bank Washington, D.C, sept. 27-28. Environmentally Sustainable proceeding series No. 4.
  12. Emadi, M and Abbasi, E. indigenous knowledge and sustainable development of villages, the old view of a new zone, and development of village's No. 33, 2001.
  13. Emadi, M and Amiri Ardakani, M. - combining indigenous knowledge and formal knowledge, necessary to achieve sustainable development of Agriculture - Rural Development Publication No. 54, 2004.
  14. Eshraghi, G , Indigenous Knowledge and Development Planning, Journal of Forest and Rangeland, No. 40, Forest, Rangeland and Watershed country, 2004.
  15. Farrokhi, S and Yaghoubi, J. technology development through indigenous knowledge systems with agricultural research - Journal of Jihad, No. 224-225, 2002.
  16. Gigler, S, et al. (2003). ICT for Indigenous Development. Available at: [http:// topics.Developmentgateway.org/ ict/ sdm/ preview Document](http://topics.Developmentgateway.org/ict/sdm/previewDocument). Do ~ active Document Id 2003.
  17. Karami, R and Moradi, Kh. The place of research, training and promoting the preservation of indigenous knowledge, Journal of Jihad, No. 255, 2003.
  18. Kolawole, D. (2001), Local Knowledge Utilization and Sustainable rural development in the 21 St. Centuries, IK Monitor Article (9-1).
  19. Louise, G (2000), Working with indigenous knowledge (A guide for researchers), published by the International Development research Centre, po Box 8500 Ottawa. On, Canada K1G 3H9.
  20. Merrewij, A. v. (1998). Three definitions of indigenous knowledge. Indigenous knowledge and Development Monitor. Vol.6, Issue 3, p, 13.
  21. Nowroozi, A and Alagha, E. a new category of indigenous knowledge in rural development research - Journal of jihad, No. 223-222, 2000.
  22. Penny R. A (2001), Gender and Indigenous Knowledge, IK&D M, Article (9-1).
  23. Popzan, A. Design and compilation of indigenous knowledge, modern media in order to achieve a partnership approach in Kermanshah province - end of period letter PhD Tehran University Faculty of Agriculture to help Azkia and Seyed Mahmoud Hosseini. 2002.
  24. Razavi, M. Agriculture and natural resources, indigenous knowledge and combining it with modern knowledge, Jihad magazine, twenty-five years, No. 269, 2002.
  25. Rajasekaran, B.D.D. M. Warren and S.C. Babu (1996), Indigenous natural-resource management system for sustainable agricultural development- a global perspective Journal of International Development 3 (4).
  26. Smita M, (2003) Women's indigenous knowledge of forest management in Orissa, [http://www.gendermainstreamingasia.org/img/b1 .PDF](http://www.gendermainstreamingasia.org/img/b1.PDF)
  27. Warren, D. M. (1999) The role of indigenous Knowledge and biotechnology in sustainable agricultural development A Keynote Address presented at Southwestern Nigerian Regional Workshop on indigenous knowledge and Biotechnology, Obafemi Awolowo university, Ie- Ife, OsunState, Nigeria 30 July.
  28. Zare, H and Yaghoubi, J. attitude to the indigenous knowledge - Journal of jihad, No. 231-230, 2003.

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**Micro-credit for rural women in Iran**Sharareh Khodamoradi<sup>1</sup> and Mohammad Abedi<sup>2</sup><sup>1</sup> Department of Agricultural Extension Education, Science and Research Branch, Islamic Azad University, Tehran, Iran<sup>2</sup> Department of Agricultural Management, Islamic Azad University, Qaemshahr Branch, Iran\*Corresponding author: [abedi114@yahoo.com](mailto:abedi114@yahoo.com)

**Abstract:** Nowadays micro-credits and supplying micro financial resources, has changed human's life and cause to revive different societies at poorest and richest countries of world, so that we can see growth in human's power to access to common financial services. By accessing to wide range of financial tools, families according to their priorities, invest on cases such as costs of education, healthcare, healthy and good nutrition or housing. Applicants for Microfinance resources mostly involved family supervisor women, pensioners, homeless people, frugal workers, small farmers and micro entrepreneurs. These people are divided into four groups: Poor, very poor, relatively poor and vulnerable poor. Whenever repayment afford , bond terms and accessing to data , in this classification will change , in order to supply sustainable financial needs of various clients , procedures and operation structures will be develop .Generally, in most countries, micro finance sources are considered for poor women. By women's access possibility to finance services, they committed to loan and ensure its repayment and preserve their saving accounts and also enjoy insurance cover. Supplying programs for micro financial resources have strong message for families and societies. Most of qualitative and quantitative studies and researches have proven that accessing to financial services; will improve women's conditions in family and society. Women's confidence has increased and they are aware of their abilities.

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**Keywords:** Micro-credit, rural women, IRAN

**Introduction:**

Having investment (capital) independency enforce people to think about economic from different angles. He should study the ways for using capital, he must consult with authority and experienced people and he will investigate about relevant markets. Such things will help him to be authoritative & independent. But how rural women can get such independency? Are the women created inherently for housekeeping, parenting and working or is there any opportunity for rural women to show their skills in economic & social development?

It seems that experiences which are obtained from performing financial programs in some villages in the developing countries could answer clearly to such questions.

A glimpse to previous planning about rural development in the world shows that from 1950 many developing countries understood that the main reason for making their economic growth (development) slowly in their countries is the weakness of investment in the agriculture part. Although many countries by patterning from developed societies have proceeded to improve & develop their industrial agriculture part and by this action not only had irreparable damages to many traditional farmers but also the main problem (the

lack of capital sources) is also remained in the rural regions. (Rahimi, 2001).

Rural women are among those major groups at society who previously were considered less by planners, due to specific reasons in the past. And this problem is more observable at developing countries. While, by looking at women's history of economic and social life, we can find that this great group, continuously have played basic role in forming economic condition of country. This great group consistent with men have had active role at areas of social-economic activities and always have had major part on economic production of society. Nowadays, supporting family supervisor women is adopted by universal society, as politic, economic a social concern and nearly all countries applied related approaches, and however these efforts have resulted in failure, in so many cases (Banihashem, 1999). paying part of cost of life by government or charities , establishing forums to analyze family supervisor women's problems , supplying necessary facilities to grow and improve child's life quality and paying facilities to provide sustainable employment , are among most important approaches to support family supervisor women . Paying credit facilities to access sustainable employment with easy terms at limited time, is one of the most important approaches to



support family supervisor women. Because alongside supplying their continues needs, their esteem wouldn't be marred. Currently, this approach is used at many countries and positive results have emerged. (Ghaffari, 2000).

From 1970, the waves of thought about micro-credits and run of small activity in villages was one of the suitable way get increased for invest improvement in rural occupations.

The said plan because of special grants such as giving loan with low wage and no interest and with long reimbursement could give farmers this opportunity to don't rely usurers and jobber intermediaries. Indeed giving micro-credits to rural women was more effective. Because along agriculture activities which need more investments, the women with using micro-credits couldn't only show their talent in rural production, but also could improve their economic & social empowerments and they could also participate in social activities. (Chabokru et al, 2005).

Women's self-reliance and independency were the outcome of giving credits to women and in some cases were the obstacle of receiving credits by women which is necessary to explain about them shortly.

### **Cultural & social effects of rural women's financial self-reliance**

As it mentioned before the traditional culture in villages was the reason for weakening women rights and made them oppressed, it is possible that women's self-reliance & financial independency in villages make some crudities (malformation) in the family and village for a short a short time, but we can't disregard it's positive outcome in the social & cultural occasions in the long time, here we will discuss about some of these outcomes (Goetz and Sengupta, 2003)

#### **1- Preferment of women role and their social place:**

Women's financial self-reliance can increase the women's social role & place in the villages. In the new condition some of their assignment roles could change to acquisitive roles. The women should use of all their power & energy for doing their acquisitive roles. Thus they can find active view to different functions. The people & groups could increase their social place in the village with improving their social role. If their role and social place preferment be accompanied with the increasing of social intelligence & knowledge, it can have more effect culturally. (Amiri, 2000)

#### **2- Increasing self-confidence:**

Self-reliance in different life aspects can increase people's self-confidence. Rural women who are

financially independent can live peacefully. With decreasing their problems in life, their self-confidence will increase. And self-confidence is one of personality & mentally condition for being success in life.

#### **3- Family consistency:**

At the first, it seems that rural women's financial independency is not acceptable by their husband and this causes some gaps in their family's relations. But little by little these problems will be solved by increasing the rural people's knowledge. Usually poverty is one of the reasons which will destroy or decrease family's consistency. Women by working and having income can help their husband & family. (Fakhraee, 1381)

#### **4- Change in family's relation:**

The rural women with having a job and financial independency can change the viewpoint of people who live in villages and cities and they will not look at the rural women as a weak and dependent people. But also their title and place will increase among their families. So by changing people's view to the women, gently we can see some changes in their family's relation which will have respect to the women's right. By increasing women's knowledge and by introducing new rural institution which give financial & authority service to the women, their stimulus (motivation) for reaching their social rights will increase and they try more than before( Amiri, 2000).

#### **5- Making patriarchy weak in the family:**

Gently, with changing family's relation in the villages and by increasing rural people's knowledge, we can make the men and women's right equal and also we wont have patriarchy in the family, although patriarchy has historic and olden root in our villages but with improving women's position and increasing their cultural and social know ledge we can destroy patriarchy in the rural families. . (Chowdhury, 2005).

#### **6- Population and family adjustment:**

The practitioner women's view about the number of the children is different; studies show that practitioner women are interested to fewer children to the house keeper women.

By decreasing families in the village and women's financial independency we are more hopeful to adjust family's population in the future because villages have important role in the population increase in Iran. (Shaditalab 75).

#### **Micro-credit characteristics:**

##### **1- Empowerment**

Empowerment is one of the major goals of micro-credit and it's considered as a proper index to evaluate it. Creating self-reliance and self-

confidence in people, empowerment is one of the important factors to deal with poverty. It also creates social capacity.

Empowerment plans include:

1. Forming financial groups and creating social capacity
2. Education as a supplementary factor of credit-saving
3. Assigning management of credit plans to members

## 2- Stability

Stability is a fundamental characteristic for a comprehensive development program and leads to continuance of the program and makes credit-saving plans different from others.

Stability indicators:

- reduce dependence on external financial resources
- reduce trading expenses
- cut the loan subsidies (Banihashem, 1999)

## 3- creating and expanding income generating activities

A study conducted by World Bank about micro financial institutions highlights three most frequent goals:

1. Creating employment opportunities for members
2. Increasing vulnerable groups' income and productivities
3. Reduce family's dependence on agriculture in droughts' prone areas

## The role of micro-credits in poverty eradication:

The first application of micro-credit was about 20 years ago with the establishment of Grameen Bank in Bangladesh. This bank, providing credit for the poor (particularly women as 94% of its clients are them), has managed to increase income and economic welfare. Now the program is running in most parts of world especially Asia, Africa and Latin America. One interesting point is that unlike prior perceptions, the poor covered by micro-credit programs has been very successful in paying back their loans.

In the countries that credits are provided in a proper financial manner, not only it has increased production and income but also it has encouraged poor to save a part of their income. These savings can be an important support for the institutes providing micro-credits and can be a financial base for more loans and all these result in institutes' financial dependence.

With the new way of micro-credit payments, in addition to covering poor's financial needs, a combination of other services and facilities are available for them; such as saving accounts, educational services, and cooperation possibilities (Goetz and Sengupta, 2003).

If rural women could provide a job for them by getting credits, loan and other financial convenience, through their income they can get self-reliance or financial independency and we will see social, cultural & economic change in village. The question here is that if these changes have positive or negative aspects in the village? It's natural that every change in social phenomenon has both positive and negative aspect, but which is Important here is that which aspect is more than the other and it depends to different condition in various societies. In our rural society there is an especial social & cultural kind that it's outcome maybe different and in some case inconsistent. With these actions rural women could be in idealistic economic condition and they could live with out dependency to their husband's income. In most of the villages in Iran there is patriarchy in the families which is not acceptable for the most of the rural people and groups. When rural women became financially independent, it's acceptable to see its cultural & social outcomes.

Giving the right that women make decision, independency to their family, increasing the cultural knowledge among them& making relation with new institutions, having independency in making decision about marriage, occupation, migration & something like this are the right that women have got it.

## Micro-credits:

One of the raised strategy , in order to accelerate investment process and reinforcing financial foundations , and saving , at deprived and rural areas , has been empowering and eradicating poverty of rural societies through efficiency with emphasize on applying micro-credits (Shahnaj and Sajedur, 2009).

Micro-loans as useful tool to fight against poverty and starvation, has proven its capabilities and values to develop these areas. These tools have ability to change and improve human's life, especially poor peoples. Micro loans , saving accounts , and giving various bank services , cause this belief in low income and poor family that , by accessing to these services , their income will increase ,so they can protect themselves against barriers of unexpected problems and their current level of life and also invest on nutrition , housing and their children's education.( Varzgar and azizi, 2001)

Accessing to these conditions is among main goals of third millennium program (i.e. eradicating absolute poverty of human societies).

Nowadays micro-credits and supplying micro financial resources, has changed human's life and cause to revive different societies at poorest and richest countries of world, so that we can see growth in human's power to access to common financial services. By accessing to wide range of financial tools, families according to their priorities, invest on cases such as costs of education, healthcare, healthy and good nutrition or housing.

Applicants for Microfinance resources mostly involved family supervisor women, pensioners, homeless people, frugal workers, small farmers and micro entrepreneurs. These people are divided into four groups: Poor, very poor, relatively poor and vulnerable poor.

Whenever repayment afford, bond terms and accessing to data, in this classification will change, in order to supply sustainable financial needs of various clients, procedures and operation structures will be develop. (Fami, 2001)

Generally, in most countries, micro finance sources are considered for poor women. By women's access possibility to finance services, they committed to loan and ensure its repayment and preserve their saving accounts and also enjoy insurance cover. Supplying programs for micro financial resources have strong message for families and societies. Most of qualitative and quantitative studies and researches have proven that accessing to financial services; will improve women's conditions in family and society. Women's confidence has increased and they are aware of their abilities. (Banihashem, 1999)

Thus, it has proven that supplying financial services for poor peoples is powerful tool to decrease poverty so that make them able to establish finance, increase income and decrease vulnerability against economic pressures.

In micro-credits programs other than offering and distributing micro loans, there are also small savings and deposits so that they are designed as form of saving-credit programs. The existing term in phrase "micro-credits" points to two basic concepts that is due to dominant perspective on this approach. First term (i.e. credits) points to rural areas and lack of access for many villagers to formal resources that are one of their major problems. And at system of micro-credits, are tried to decrease poor families' access barriers to credit sources and also to increase effectiveness of these markets. Second term (i.e. micro) emphasize on deficiency of development, according to classic economist's method. Emphasizing on concept of "micro" means revising

recommendations of market economy at rural society's development.

Generally, goals of micro-credits programs are: (Moazami 2005)

- a- increasing access coefficient of low income rural women to credit facilities
- b- considering and focus on low income rural women groups
- c- empowering rural women to enjoy needed job skills
- d- empowering rural women to deal with group works and cooperative activity
- e- equipping non-productive villager's saving (women) to effective and productive investment
- f- planning in order to perform projects that are based on capacities and facilities of that area
- g- breaking poverty cycle and saving rural family
- h- Developing employment and stabilizing jobs which faced financial crisis

#### Discussion and results:

Overall in many countries financial plans mostly focus on women. Women, provided with financial facilities, will receive a loan, guarantee to pay it back, keep their saving account and also they'll have insurance coverage. Micro-financial plans have an important message for families and communities. Many studies have proven that women's access to mentioned facilities may improve their conditions in family and society; it also helps them feel more self-confident and makes them aware of their own abilities. Thus providing micro-credit services for the poor in society is a powerful tool to reduce poverty and so that they are able to create assets, earn more money and become less vulnerable against the economic pressure. Of about 1.3 billion poor in the world there are 900 million poor women, this obviously shows that poverty has a feminine face. According to UN's development fund, 10% of world's income and less than 10% of world's assets belongs to women. While a majority of them never possess the capital needed for their activities, women still play an important role in the economic development of country. Therefore women draw the micro-credit policy maker's attention more than others. Choosing women as the main target of micro-credit plans is an effective strategy to eradicate poverty; because their income will upgrade the family welfare; furthermore earning money improves their social status. In some countries this choice is influenced by society's attitude and culture (Araghzadeh, 2002).

For instance founder of Grumman Bank of Bangladesh, Mohammad Yunes, has stated that: "women have plans for themselves, their children, and their family life; they always have an overlook while men just look for fun" to explain why 94% of their clients are women.

Women's access to micro-credits have shown that their income benefit to improve their family and provide livelihood. In addition to all these another reason of women being the target of micro-credit plans is that women have higher loan recovery rates. Totally, expanding women's access to micro-credits may lead to many useful results which in economy is mentioned as "virtuous spiral"; because their access to micro-credits results in family welfare and in a broader point it'll improve community's welfare and shall be increased welfare this process is repeated.

In researches that conducted by Nanda (2004) became clear that women participation in credits programs had positive effects on their demand about health care. Fiona Steele and et al (2008) in researches that conducted as called "influences of credits programs on empowering women at Bangladesh", found that women who joined to credits programs, have participated in more educational programs and have married with more educated men and also they have saved more and they had more cash.

Shahnaj and Chaudhury(2009) in research as "credits and its role on empowering women" concluded that there is meaningful relation between attending in credits programs and empowering women, at economical dimensions.

Jameela (2010) presented that credit programs has shown lot of affects on empowering women so that has increased their social, politic and economic ability.

Thus it is obvious that credits programs and its educational and empowering programs can be affective on social, humane and economic development or rural society, if it be associated with proper and gradual practices and base on reciprocal communications principles and apply opinion of local society.

A study conducted by Chabokru et al (1384) shows the crucial importance of micro-credits for farmers who do not possess physical financial assets (land, building, livestock, well...) and work in agricultural sector because of environmental conditions (such as living in a village) or because it's their ancestral occupation.

So today, women's participation in sustainable economic, social, and cultural development in rural areas is not optional but an essential matter. Those communities that have not seriously considered the necessity of participation faced failures and delayed community's development, welfare and security process. In any community, village, or social group, broad participation of every women in decision-making and any other matter related to national or local development programs, is a key variable in social sciences and in the last few decades, it has interested many scholars of socio-economic and especially cultural issues, and is considered as one of the most fundamental democratic rights of women in a society. As we know in

a popular participation, all people are given the opportunity to participate in planning and decision making for their society and for their own future. When in practice women feel that they can be involved in planning, policy making and deciding or solving problems in the society certainly they'll feel more solidarity and become more interested in social, economic, and cultural development programs.

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#### References:

1. Amiri, S. Female centered sustainable human development. Journal of Agricultural and Development Economics, 2000, No. 9.
2. Arab-Mazar, A. and Jamshidi. M. T. (2005). Article "The role of agricultural banks in financing agricultural micro-credit." Conference on rural development and poverty reduction, agricultural banks, Tehran.
3. Araghzadeh, M. institutions active in the field of providing financial services to rural women. Conference Proceedings rural women micro-credit. (Volume II), 2002. 167-153.
4. Bakhshoodeh M. and Habibullah Salami. Article "The role of agricultural banks in reducing poverty with emphasis on micro-credit." Conference on rural development and poverty reduction, agricultural banks, Tehran, 2005.
5. Balali, L. Mission Trip Reports samples producing rural women (rural women's efforts Affairs Ministry of Agriculture) to India and meeting with the board of directors and senior managers National Bank of Agriculture and Rural Development (NABARD) self-employment Women's Association (SEWA), and the Empowerment Institute rural women (CARE), 2005.
6. Banihashem, F. Rural women, education, association and participation. Jihad Journal village, 14 years, No. 310, 1999, p. 21.
7. Changizi Ashtiani, M. Including the share of women in producing countries. Journal of Agricultural Economics and Development, the third year, special role of women in agriculture. Tehran: Ministry of Agriculture publications, 2003, Pp 83-81.
8. Ellen Vor der Bruegge, Maureen Plas, Christopher Dunford and Kathleen E. Stack. Credit with education: a self-financing way to empower women, 2009.

9. Fakhraee, S. Economic and social effects of their financial reliance of women in rural communities, 2002.
10. FAO. Women in agricultural development. (Translated by: Saleh GH ancestry). Publisher: Management studies and studies and promoting people's participation Deputy Agriculture (the former). Pp 46-42, 1998.
11. Fiona Steele, Sajeda Amin and Ruchira T. Naved. The Impact of an Integrated Micro-credit Program on Women's Empowerment and Fertility Behavior in Rural Bangladesh, 2008.
12. Ghaffari, GH. The role of women and social development. Women's Magazine, 2000, No. 10, p. 15.
13. Goetz, A. and Rina Sengupta, R. "Who Takes the Credit? Gender, Power, and Control over Loan Use in Rural Credit Programs in Bangladesh." *World Development* 24 (1), 2003, 45-63.
14. Jameela v. a. Micro credit, empowerment and diversion of loan use, 2010.
15. Lahsaezadeh, A. Sociology of rural development. Tehran: Publication Days, 2000, p. 58.
16. Moazami, M, Rahimi A. and Azam tayefe Heidari. "Coverage and sustainability of micro-credit programs, case study of rural women micro-credit fund" Research Center for Rural Women and Rural Affairs Ministry of Agriculture, 2005.
17. Najafi. M (2006). Participatory evaluation of rural women micro-credit fund scheme, the organization promoting education and agricultural research.
18. Nanda. P. (2004). Women's participation in rural credit programs in Bangladesh and their demand for formal health care: is there a positive impact? Center for Health and Gender Equity. USA.
19. Navab Akbar, F. The role of rural women in the past decade. Journal of Agricultural Economics and Development, conference papers, women participation and Agriculture 1400, Journal No. 3, Publishing Ministry of Agriculture, 1997, P. 186.
20. Rahmani Andalibi. S. "Need, principles, mechanisms and advantages of micro-credit programs in small business development and improvement of rural women." Conference Proceedings Volume II of rural women micro-credit and promoting people's participation Deputy Ministry of Agriculture - Bureau of Women Affairs in collaboration with Al-Zahra University, Agricultural Bank, Tehran, 2001.
21. Rahimi, A. Review of micro-credit properties. Conference Proceedings Volume II of rural women micro-credit and promoting people's participation Deputy Ministry of Agriculture - Bureau of Women Affairs in collaboration with Al-Zahra University, Agricultural Bank, Tehran, 2001.
22. Ruhai Amin, yipping li and ashrad u. Ahmad. Women's credit programs and family planning in rural Bangladesh, 2010.
23. Saadi. H, Arab Mazar A. Paper "role in accelerating the process of micro-credit in rural development: comparing two perspectives." Conference on rural development and poverty reduction, agricultural banks, Tehran, 2005.
24. Samadi Afshar, S. Factors affecting rural women's participation in training programs and extension services in agriculture in West Azerbaijan Province 82-81. MSc thesis, Islamic Azad University, Science and Research, 2004.
25. Shahnaj Praveen and Sajedur Rahman Chaudhury. Micro-credit intervention and its effects on empowerment of rural women: the brac experience, 2009.
26. Varzgar, sh. and Azizi. M. Evaluation of labor force participation of rural women in cotton production and its related factors in the region and dome of Gorgan, 2001, P. 318.

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## Effect of Reflexology on Pain and Quality of Life in a Patient with Rheumatoid Arthritis

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**Abstract:** Patients with rheumatoid arthritis (RA) face considerable physical, social and emotional disabilities. In this chronic disease, for which a cure is not yet available, improving patients' health, quality of life and reduce pain is of the utmost concern. The purpose of this work was to measure the effect reflexology has on pain and quality of life (QOL) in a patient with rheumatoid arthritis. Using an 8-week course of reflexology treatments were given to a patient who has RA. A quasi experimental research design was used with 2- month follow-up. The study was conducted in the outpatient clinic of the RA Departments at Zagazig University Hospitals. On 39 female adult patients diagnosed as having RA without deformity of bones or destruction of joints. The exclusion criterion was the presence of any other chronic illness that may affect patient's QOL as diabetes, ischemic heart disease, chronic obstructive pulmonary disease, and stroke. Perceived pain and QOL were assessed using three validated outcome measures: Bio-socio-demographic and disease (RA) characteristics, the Rheumatoid Arthritis Quality of Life (RAQOL) questionnaire, the Pain Assessment Questionnaire (Numerical Rating Scale) and Health assessment questionnaire (HAQ). The study results revealed that, improvements in patients' QOL, pain and health status at the post-intervention phase and at the follow-up phase. Satisfaction QOL scores had moderate statistically significant negative correlations with the duration of illness throughout the study phases, while the scores of the importance of QOL had weak to moderate statistically significant negative correlations with age and duration of illness. On the other hand, the poor health status scores had moderate statistically significant positive correlations with age and duration of illness throughout the study phases, while pain had no correlation with either of them. The study concludes that hands and feet reflexology applied to rheumatoid arthritis patients is effective in reducing their pain, improving their QOL and their total health status, and these positive impacts are not affected by patient's age and duration of illness. Therefore, reflexology must be considered as a complementary treatment modality in rheumatoid arthritis. It should be introduced to nursing and medical students, and in postgraduate staff development programs. Further research is recommended for the long-term effects of this treatment modality in terms of pain and disablement. Research may also extend to assess the effectiveness of as a useful modality in geriatric care and for patients with other chronic conditions.

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**Keywords:** Reflexology; Rheumatoid arthritis; Quality of life; Pain; Health assessment questionnaire

### 1. Introduction

Rheumatoid Arthritis (RA) is an ongoing, progressive auto-immune disease that affects about 1% of the general population (Palferman, 2003; Helmick et al., 2008; Rheumatoid Arthritis Fact Sheet, 2010). It affects women three times more than men (American Medical Women's Association, 2011). It is mainly a disease of the joints of the body with episodes of painful inflammation, but may also affect other organs of the body and can result in the destruction of joints, disability, and in severe cases, life threatening complications (Chorus et al., 2003). The onset of RA can occur at any age and affects women three time more than men. In general, the younger a person is when he or she develops rheumatoid arthritis, the more rapidly that disease progresses. About 10% of people with the disease become severely disabled. In addition, life

expectancy may be shortened by about 3 to 7 years, and those with severe forms of RA may die 10-15 years earlier than expected due to possible life threatening complications (National Institutes of Health, 2006; Helmick et al., 2008).

There is no cure for RA, but with early recognition and treatment, it is possible to minimize joint damage and complications of the disease. The main symptoms, disability and pain, the variability of disease-activity, alternating between improvement and exacerbation, and the chronic nature of the disease cause numerous problems which affect the quality of life. Active participation by the patient in their therapy can help to improve the disease process and its impact in terms of health results (Holman and Lorig, 2004). Because of the progressive chronic nature of the disease, treatment usually needs to be continuous, even lifelong in some cases (Jakobsson

and Hallberg, 2002). The most successful treatment plans usually use a multipronged approach, including; pain relief, buffered aspirin, salicylate medications, hydrotherapy, meditation, and mind-body exercise (British Medical Association, 2000; Gharate, 2007).

The use of complementary and alternative medicines (CAM) has increased in conventional healthcare settings (Ernst and Fugh-Berman, 2002; Pagan and Pauly, 2005). The fear of medication side-effects and desire for symptom relief are possible reasons for the increasing use of CAM by patients (Vincent and Furnham, 1999; Ross et al., 2002). With consumer interest in CAM, nurses have increasingly incorporated these modalities into their practice. For example, reflexology has been widely used in fields such as midwifery, orthopedics, neuroscience and palliative care (Stephenson et al. 2003). However, many CAM modalities lack scientific evidence to support their efficacy and safety.

The supposed theoretical support for reflexology has been developed since ancient Chinese and Egyptian times (Wang et al., 2008). It is performed using the thumb and forefinger to apply pressure to specific areas on the feet that have been claimed to correspond to the internal organs, glands and body parts (Wang et al., 2008). It is different from foot massage in that it involves more superficial contact, deeper pressure on certain parts of the foot, and resembles a caterpillar-like movement (Rose, 2006). It has been claimed that by pressing the 'reflex zones', energy blocks disturbances such that calcium, lactate or uric acid crystals are reabsorbed and later eliminated – a process referred to as 'detoxification.' It has also been suggested that reflexology may help relieve stress and tension, improve blood flow, and promote homeostasis (Wang et al., 2008).

Anecdotal evidence has shown that reflexology is beneficial in many conditions such as pre- and postnatal discomfort, pain, migraine and chronic obstructive pulmonary disease (Stephenson et al. 2003, Wilkinson et al. 2006). Other therapeutic effects, such as strengthening the immune system, improving sleep quality, and wound healing have also been claimed (Xavier, 2007). Reflexology has also been offered to cancer patients to improve the adverse physical and psychological symptoms associated with the illness or its treatments (Hodgson, 2000; Stephenson et al, 2000; Ross et al., 2002; Wright et al., 2002; Quattrin et al., 2006). In addition, the human touch accompanied by reflexology offers care and attention for patients, and this psychological comforting has been reported as one of its primary benefits (Gambles et al, 2002). However, patients' reports of benefits from reflexology may be influenced by bias in the lay literature or limited

information about the use and effectiveness of the intervention (Montbriand, 1994). As reflexology has become popular in nursing practice, its effects on pain and quality of life in a patient with rheumatoid arthritis need to be evaluated.

### **Significance of the study**

The prevalence rate of RA in Egypt is not well documented. However, by extrapolation using the worldwide reported prevalence of 1%, about 800000 Egyptians may be affected. Moreover, about 10% of people with the disease become severely disabled. In addition, their life expectancy may be shortened due to possible life threatening complications. Therefore, the magnitude and severity of the disease are high. Since there is no cure for RA, early recognition and treatment are important to minimize joint damage and complications of the disease. The use of non-pharmacological treatment modalities as reflexology may help in reduce pain and improve the quality of life of these patients.

### **Aim of the Study**

The aim of this work was to measure the effect reflexology has on pain and quality of life (QOL) in a patient with rheumatoid arthritis. The research hypotheses were that the QOL of patients with RA, their health status, and pain sensation will demonstrate statistically significant improvements after application of the reflexology intervention.

## **2. Subjects and Methods**

### **Research design:**

A quasi experimental research design was used with 2- month follow-up.

### **Research setting:**

The study was conducted in the outpatient clinic of the RA Departments at Zagazig University Hospitals.

### **Subjects:**

The subjects of this study consisted of 50 patients. The inclusion criteria were being female adult patient diagnosed as having RA without deformity of bones or destruction of joints. The exclusion criterion was the presence of any other chronic illness that may affect patient's QOL as diabetes, ischemic heart disease, chronic obstructive pulmonary disease, and stroke.

### **Tools for data collection:**

Four different tools were used to collect data about QOL, health assessment, and pain. These were included in a structured interview questionnaire form that included the following sections.

**Section I:**

Bio-socio-demographic and disease (RA) characteristics as age, marital status, level of education, occupation, residence, monthly income, as well as the duration of the disease and management.

**Section II:**

This consisted of the Arthritis Quality of Life Questionnaire sheet version IV (*Ferrans & Powers, 1998*). It is the most recent version composed of 70 questions, for assessing health-related QOL. The tool assesses four subscales of QOL: health and functioning, social and economic, psychological/spiritual, and family. Each subscale has six items on a 6-point scale which are assessed twice: once for satisfaction with the item, and once for the importance of the item. The scale for satisfaction is: very dissatisfied, moderately dissatisfied, slightly dissatisfied, slightly satisfied, moderately satisfied, and very satisfied, which are scored from 1 to 6 respectively. The scale for importance is: very unimportant, moderately unimportant, slightly unimportant, slightly important, moderately important, and very important, also scored from 1 to 6 respectively. A higher score means more QOL satisfaction and higher perception of importance.

**Section III:**

This section included the Health Assessment Questionnaire (HAQ-DI), which assesses upper extremity fine movements, lower extremity locomotor activities, and other activities of both upper and lower extremities. Scoring takes into account the use of aids and devices or assistance from another person. The tool has 20 items in eight categories of functional activities during the past week: dressing, rising, eating, walking, hygiene, reach, grip, and usual activities. Each category contains at least two specific sub-category questions. For example, under the category "walking," patients are asked about their ability to walk outdoors on flat ground and to climb up five steps. Scoring of the HAQ-DI is modeled after the American Rheumatism Association/ American College of Rheumatology functional classes (*Hoch Bergm et al., 1992; Bruce and Fries, 2005*). For each item, there is a four-level response set scored from 0 to 3, with higher scores indicating more disability (0 = without any difficulty; 1 = with some difficulty; 2 = with much difficulty; and 3 = unable to do). The highest sub-category score determines the value for each category, unless aids or devices are used. The category scores are then averaged into an overall HAQ-DI from zero to three. A higher score points to more disability.

**Section IV:**

This consisted of a Numerical Rating Scale where the user has the option to verbally rate pain severity on scale from 0 to 10; zero indicates absence of pain, while 10 represents the most intense pain possible (*Ware et al., 1988; Kagee, 2001; McGill, 2009*).

**The reflexology intervention:**

Reflexology therapy is not massage, and it is not a substitute for medical treatment. Source: The reflexology manipulations in this intervention have been adapted from the techniques taught in David Vennell's book entitled *Healing Hands: Simple and practical reflexology techniques for developing good health and inner peace (David Vennell 2007)*. A reflexology session involves pressure treatment that is most commonly administered in foot therapy sessions of approximately 20 minutes in duration. The foot therapy may be followed by a brief 15-minute hand therapy session and 5 minute for video film on reflexology treatment. No artificial devices or special equipment are associated with this therapy. If the part of the body corresponding to the reflex area is out of balance then a degree of tenderness will be felt in the foot when pressure is applied. Treatment to all of the reflex areas in both feet takes about 40 minutes and during this time the patient is sitting in a comfortable, reclining position with the feet raised. Treatment is not applied to inflamed or painful joints. After receiving a massage treatment, the patient is instructed to drink water to eliminate toxin and lactic acids developed during the massage process.

**Content validity and reliability:**

It was established by a panel of two expertises who reviewed the instruments translation from English version to Arabic and back to English the differences between expertises were calculated and proved high inter reliability ( $r=89$ ).

**Pilot Study:**

A pilot study was conducted on five RA patients from the study setting to check and ensure the clarity, applicability, relevance, and feasibility of the tools, to identify the difficulties that may be faced during the application, and to estimate the time needed for data collection. Then modifications of the tools were done to reach to the finalized form. Subjects who shared in the pilot study were not included in the main study sample.

**Administrative design and ethical considerations:**

To carry out the study, the necessary approvals were obtained from the Head of outpatient Department, and from the General Director of the



Zagazig University Hospitals. Letters were issued to them from the Faculty of Nursing, Zagazig University explaining the aim of the study in order to obtain permission and help. The study protocol was approved by the pertinent official authorities at the Faculties of Nursing and Medicine, Zagazig University. Oral informed consents were secured from each subject to participate after explaining the nature, purpose, and benefits of the study. Participants were informed that participation is voluntary, with no obligation to continue against will. Confidentiality and anonymity were ensured. The study maneuvers would improve participant's health, with no potentials of harmful effects.

#### Study maneuver:

The researchers met with participants who gave their consent and were willing to comply with the entire study protocol and interviewed them individually in the outpatient clinic. The researchers started to apply the intervention and educate the subjects on how to perform reflexology treatment. Teaching included giving a good therapeutic foot and hand massage, massaging own feet and hands for self healing, and stress management. Also, they were advised to comply with the medications prescribed by their physicians. The researchers scheduled times for attending to the clinic on Saturday, Monday, and Wednesday of each week (days for females in outpatient clinic) for physiotherapy, exercises, and reflexology treatment. They encouraged participants to attend regularly three times per week for two weeks these 40-minute sessions. They also ask the patients to perform these exercises and reflexology regularly at home. Follow-up assessment was done after 8 weeks. Data collection extended over a period of one year from June 2009 to June 2010. Available patients (39 patients) who fulfilled the inclusion and exclusion criteria were assigned to implemented nursing intervention three times per week. While patients can not received nursing intervention three times per week excluded from the study.

#### Statistical analysis:

Data entry and statistical analysis were done using SPSS 14.0 statistical software package. The non-parametric Kruskal-Wallis was used for multiple group comparisons of quantitative data as normal distribution of the data could not be assumed. Pearson correlation analysis was used for assessment of the inter-relationships among quantitative variables. Statistical significance was considered at p-value <0.05.

#### Limitations of the study:

The researchers were faced with many logistic problems and spent much effort to convince and promote the objectives of the study. Drop out cases: the total subjects number at the pre, intervention and post phases were 50 participants. While during the follow up phase 11 participants were dropout from the study for personal reasons and did not complete the study. The small number of the study sample of patients doesn't allow generalization of the result. Patients who suddenly get complications were excluded.

#### 3. Results:

The study included 39 female patients in the age range from 19 to 70 years (mean±SD 45.9±11.8 years). As Table 1 shows, the majority were illiterate (79.5%), married (82.1%), and from rural areas (74.4%). The duration of their illness ranged between less than one to 16 years with mean±SD 4.4±4.0 years. About two thirds of them had regular follow-up (66.7%).

**Table 1. Socio-demographic and diseases characteristics of patients in the study sample**

	Frequency	Percent
Age (years):		
<50	24	61.5
50+	15	38.5
Range	19-70	
Mean±SD	45.9±11.8	
Marital status:		
Single	7	17.9
Married	32	82.1
Education:		
Illiterate	31	79.5
Educated	8	20.5
Job:		
Unemployed	35	89.7
Working	4	10.3
Living:		
Alone	2	5.1
With family	31	79.5
With children	6	15.4
Residence:		
Rural	29	74.4
Urban	10	25.6
Income:		
Sufficient	20	51.3
Insufficient	19	48.7
Duration of illness (years):		
<5	25	64.1
5+	14	35.9
Range	<1-16	
Mean±SD	4.4±4.0	
Regular follow-up:		
No	13	33.3
Yes	26	66.7

Table 2 show statistically significant improvements in patients' QOL at the post-intervention phase. The improvements were also sustained at the follow-up phase. The only domain that did not improve was that related to family, both for satisfaction and importance. As for the poor health status, the areas of statistically significant improvements were those related to walking ( $p<0.001$ ), self-care ( $p=0.01$ ), reaching ( $p<0.001$ ), and pinching ( $p=0.003$ ). The improvements also persisted during the follow-up phase in these areas. Also, the pain scale scores demonstrated a statistically significant decline at the post-intervention phase, with a slight increase at the follow-up phase ( $p<0.001$ ).

Figure 1 summarizes the total scores of various parameters throughout the intervention phase. It

demonstrates significant increase in the scores of QOL (satisfaction and importance) at the post-intervention phase, which was sustained at the follow-up phase. Also, the pain and poor health status scores showed decline at the post-intervention phase, which was maintained at the follow-up phase.

Concerning the correlations with patients' age and duration of illness, Table 3 indicates that scores of satisfaction with QOL had moderate statistically significant negative correlations with the duration of illness throughout the study phases, while the scores of the importance of QOL had weak to moderate statistically significant negative correlations with age and duration of illness. On the other hand, the poor health status scores had moderate statistically significant positive correlations with age and duration of illness throughout the study phases, while pain had no correlation with either of them.

**Table 2. Changes in patients' QOL, health status, and pain scores throughout intervention phases**

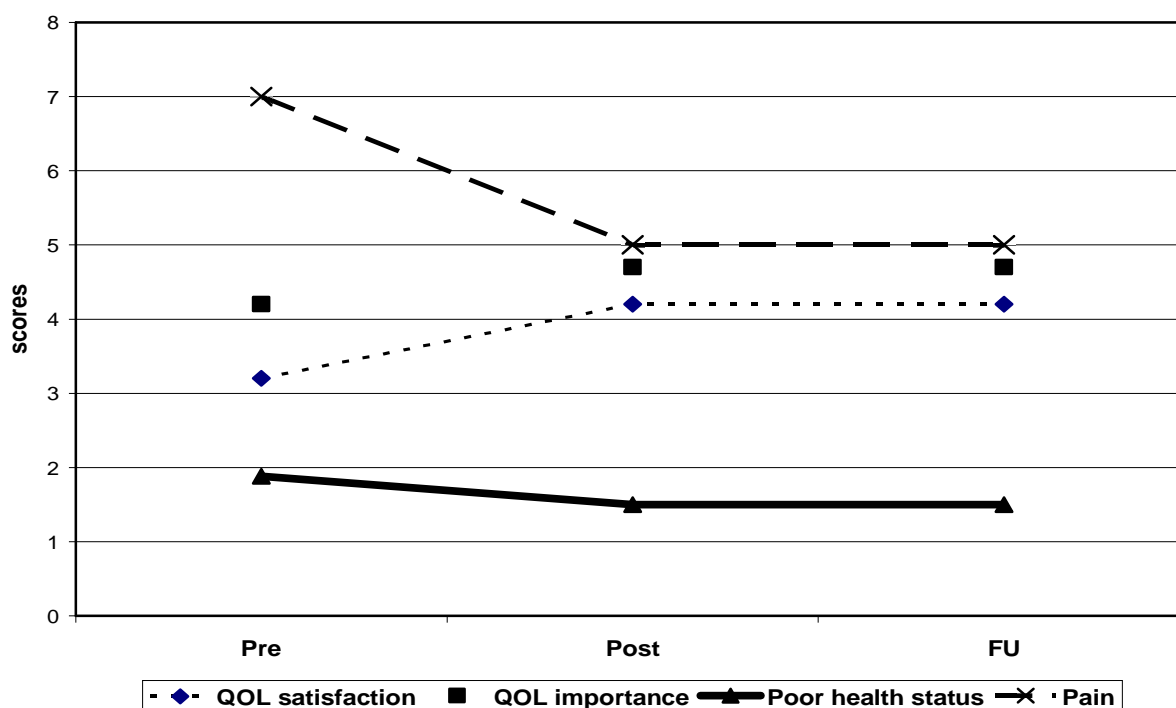
	Time						Kruskal Wallis Test	p-value
	Pre (n=39)		Post (n=39)		FU (n=39)			
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median		
QOL satisfaction:								
Health/functioning	2.9±0.9	2.50	4.2±0.4	4.10	4.2±0.4	4.10	55.69	<0.001*
Social/economic	2.7±0.8	2.60	3.4±0.6	3.30	3.4±0.6	3.30	36.16	<0.001*
Psychic./spiritual	3.4±0.7	3.30	4.7±0.3	4.60	4.7±0.3	4.60	56.82	<0.001*
Family	4.9±0.5	5.00	5.0±0.5	5.00	5.0±0.5	5.00	0.10	0.95
QOL importance:								
Health/functioning	4.2±0.9	4.40	4.9±0.5	4.90	4.9±0.5	4.85	14.50	0.001*
Social/economic	3.0±1.0	2.90	3.9±0.6	3.70	3.9±0.6	3.70	26.67	<0.001*
Psychic./spiritual	4.2±0.9	4.00	5.0±0.4	4.90	5.0±0.4	4.90	23.21	<0.001*
Family	5.0±0.9	5.20	5.3±0.5	5.40	5.3±0.5	5.40	3.41	0.18
Poor health status:								
Clothing/hygiene	1.7±0.8	2.00	1.6±0.8	2.00	1.7±0.7	2.00	0.29	0.87
Lifting	1.2±0.9	1.00	1.1±0.9	1.00	1.3±0.8	1.00	0.65	0.72
Eating	2.2±0.7	2.00	2.1±0.6	2.00	2.1±0.6	2.00	1.05	0.59
Walking	2.1±0.9	2.00	1.3±0.7	1.00	1.4±0.6	1.00	20.43	<0.001*
Self-care	1.9±1.0	2.00	1.4±0.6	1.00	1.4±0.6	1.00	8.70	0.01*
Reaching	2.2±0.7	2.00	1.6±0.5	2.00	1.6±0.5	2.00	22.57	<0.001*
Pinching	1.9±0.9	2.00	1.3±0.6	1.00	1.3±0.6	1.00	11.44	0.003*
Outdoor activities	2.7±0.5	3.00	2.6±0.5	3.00	2.6±0.5	3.00	1.77	0.41
Pain scale	6.7±1.0	7.00	5.3±0.8	5.00	5.5±0.6	5.00	44.75	<0.001*

(\*) Statistically significant at  $p<0.05$

**Table 3. Correlations of patients' scores of QOL, pain, and health status and their age and duration of illness throughout intervention phases**

	Pearson correlation coefficient					
	Pre		Post		FU	
	Age	Duration of Illness	Age	Duration of Illness	Age	Duration of Illness
QOL satisfaction	-.253	-.442**	-.166	-.418**	-.192	-.419**
QOL importance	-.638**	-.380*	-.561**	-.331*	-.592**	-.455**
Pain	-.094	.202	.017	.192	.055	.140
Poor health status	.581**	.510**	.631**	.486**	.609**	.480**

(\*) Statistically significant at  $p < 0.05$ ; (\*\*) Statistically significant at  $p < 0.01$

**Figure 1. Changes in patients' total QOL, health status, and pain scores throughout intervention phases**

#### 4. Discussion:

The present study was carried out to test the research hypothesis that the QOL of patients with RA, their health status, and pain sensation will demonstrate statistically significant improvements after application of the reflexology intervention. The study findings lead to accepting this research hypothesis since they point to statistically significant increases in QOL scores and decreases in poor health status and pain scores after implementation of the reflexology intervention. The improvements were also sustained during the follow-up phase.

In the present study, the researchers used the Short-Form 36 (SF-36), Rheumatoid Arthritis Quality of Life (RAQOL) questionnaire, Health Assessment Questionnaire (HAQ), and visual analog scales for

pain, all of them having high degrees of validity and reliability. Additionally, their validity was further confirmed in the study of rheumatoid arthritis in particular Bansback et al, 2008; (Linde et al, 2008). The authors compared the measurement properties of these tools and showed that all of them were able to discriminate between low, moderate, and severe rheumatoid arthritis activity as measured by the Disease Activity Score. These findings add to the validity of these instruments, and consequently support the results of the present study.

According to the present study findings, the application of reflexology had a positive impact on participants' QOL, health status, and pain scores throughout intervention phases. These improvements may be attributed to the process of reflexology as it

has been claimed that local finger pressure on reflex points on the hands or feet can influence the function of corresponding target organs to promote homeostasis, relaxation, and sense of human touch. Moreover, the reduction of pain intensity by reflexology may improve patients' independent involvement in personal and self-care, as well as social functioning, with further positive impact on self-esteem and QOL. Therefore, it has been recommended as a promising complementary therapy which can improve quality of life in persons with rheumatoid arthritis, and other conditions (Lynn, 2010).

The present study revealed improvements in all QOL domains except that related to family. This might be attributed to that the scores of this domain were the highest at the pre-intervention phase and in all subsequent phases. This implies that the family domain of QOL is the least affected by the disease. The finding is expected given the importance of the family solidarity and values in our community, where chronic disease patients are provided with more care in their families. In agreement with this, El-Mansoury et al. (2008), comparing loneliness among Egyptian and Dutch women with rheumatoid arthritis found that the role of the family in perceived loneliness is greater in Egypt than the Netherlands. The importance of family relations and functioning in rheumatoid arthritis has been addressed in previous studies (Cunha-Miranda et al, 2010; Strand and Khanna, 2010). Furthermore, Coty and Wallston (2010) in a study that examined the relationship of problematic social support and family functioning in women with rheumatoid arthritis. The study concluded that subjective well-being in women with rheumatoid arthritis is related to perceptions of family functioning and the amount and type of support received.

As regards health status, the reflexology intervention had significant positive impacts on certain areas but did not affect others. The areas that showed improvements were those of walking, self-care, reaching, and pinching, whereas the areas of clothing, eating, lifting, and outdoor activities were not affected. This might have two different explanations. The first is related to the severity of rheumatoid arthritis and the types of joints affected and their relation to these different activities. The other explanation is related to the proper application of reflexology and compliance with the instructions so that all functions are improved. In line with this explanation, Somchock (2006) mentioned that most of the studies addressing reflexology claimed that reflexology induced general relaxation evidenced by physiological changes has been reported, but whether its therapeutic effects were associated with pressure

on specific foot zones remains unanswered because none of the researchers used an objective indicator to verify changes in blood perfusion in the foot zones pressed. Meanwhile, the differential effects of reflexology on functional aspects of rheumatoid arthritis patients of the present study are in congruence with Wang et al. (2008) who reported variations among reviewed studies regarding the efficacy of reflexology.

Patient's age and duration of illness may also be confounding factors affecting the impact of reflexology on QOL, and health status, and pain. The study findings revealed no effect of age or duration of illness on pain throughout the intervention phases. As regards QOL, it had statistically negative significant correlations with the duration of illness in both its aspects, and with patient's age in its importance aspect. This means that patient's QOL declines with increased age and duration of illness. Similarly, the scores of poor health status increase with age and duration of illness. The findings are quite plausible given the added effects of aging, and the progress of the disease severity with increased duration as previously reported (Schneider et al., 2008; Collins et al., 2009). However, all these correlations did not change throughout the study phases, which means that patient's age and duration of illness were not confounding factors, and consequently the observed positive impact of the intervention was true. In other words, the intervention was successful in alleviating pain and improving QOL and health status regardless patient age and duration of illness.

## 5. Conclusion and recommendations

The study concludes that hands and feet reflexology applied to rheumatoid arthritis patients is effective in reducing their pain, improving their QOL and their total health status, and these positive impacts are not affected by patient's age and duration of illness. Therefore, reflexology must be considered as a complementary treatment modality in rheumatoid arthritis. It should be introduced to nursing and medical students, and in postgraduate staff development programs. Further research is recommended for the long-term effects of this treatment modality in terms of pain and disablement. Research may also extend to assess the effectiveness of as a useful modality in geriatric care and for patients with other chronic conditions.

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**6. References:**

1. American Medical Women's Association (2011): Statistics about Rheumatoid arthritis. Retrieved on 26 Jan 2011 at: [http://www.wrongdiagnosis.com/r/ rheumatoid\\_ arthritis/ Prevalence](http://www.wrongdiagnosis.com/r/ rheumatoid_ arthritis/ Prevalence)
2. Bansback N.J., Anis A.H., and Marra C.A. (2008): Patient Reported Outcomes for Rheumatoid Arthritis: Where Are We and Where Are We Going? *The Journal of Rheumatology*, 35:8 1482, 1483
3. British Medical Association (2000): Royal Pharmaceutical Society of Great Britain. British National Formulary. No 40. NSAIDs Interactions. Appendix I: Interactions. London: BMA, RPS, pp. 588-624.
4. Bruce B., and Fries J.F. (2005): The Health Assessment Questionnaire (HAQ) Clinical and Experimental Rheumatology; 23 (Suppl. 39): S14-S18.
5. Chorus A.M.J., Miedema H.S., Boonen A., and van der Linden S. (2003): Quality of life and work in patients with rheumatoid arthritis and ankylosing spondylitis of working age. *Ann Rheum Dis*; 62:1178-1184
6. Collins P., Qc AM., and Allbon P. (2009): Australian Institute of Health and Welfare 2009 A picture of rheumatoid arthritis in Australia. Arthritis series no. 9. Cat. No. PHE 110. Canberra: AIHW.1-28.
7. Coty M.B., and Wallston K.A. (2010): Problematic social support, family functioning, and subjective well-being in women with rheumatoid arthritis. *Women Health.*; 50(1):53-70.
8. Cunha-Miranda L., Costa L., and Ribeiro J.S. (2010): Near study: Needs and Expectations in Rheumatoid ARthritis - do we know our patients needs? *Acta Reumatol Port.*; 35(3):314-23.
9. David Vennell (2007): Simple Hands, Foot Reflexology Techniques. Retrieved on 14 Oct 2007 <http://healing>.
10. About.com/od/books/ig/Book-of-the-week/Healing- Hands-Reflexology.htm
11. El-Mansoury T.M., Taal E., Abdel-Nasser A.M., Riemsma R.P., Mahfouz R., Mahmoud J.A., El-Badawy S.A., Rasker J.J. (2008): Loneliness among women with rheumatoid arthritis: a cross-cultural study in the Netherlands and Egypt. *Clin Rheumatol.*; 27(9):1109-18.
12. Ernst E., and Fugh-Berman A. (2002): Complementary and alternative medicine: what is it all about? *Occupational & Environmental Medicine*; 59(2): 140-144.
13. Ferrans C. E. & Powers M. J. (1998): Ferrans and Powers Quality of Life Index (QLI). Available at <http://www.uic.edu/orgs/qli/questionnaires/pdf/QLI%20THAI%20from%20Suparat%202-07.pdf>
14. Gambles M., Crooke M., and Wilkinson S. (2002): Evaluation of a hospice based reflexology service: a qualitative audit of patient perceptions. *European Journal of Oncology Nursing*; 6(1): 37-44.
15. Gharate M.K. (2007): Rheumatoid arthritis & Complementary and Alternative Medicine. *Pharmainfo.net*; 5(Issues 5).
16. Helmick C., Felson D., Lawrence R., and Gabriel S. (2008): Estimates of the Prevalence of Arthritis and Other Rheumatic conditions in the United States. *Arthritis & Rheumatism*; 58(1):15-25.
17. Hoch Bergm C., Chang R.W., and Dwosh I. (1992): The American College of Rheumatology 1991 revised criteria for the classification of global functional status in rheumatoid arthritis. *Arthritis Rheum*; 35: 498-502.
18. Hodgson H. (2000): Does reflexology impact on cancer patients' quality of life? *Nursing Standard*; 14(31): 33-38.
19. Holman H., and Lorig K. (2004): Patient self-management: a key to effectiveness and efficiency in care of chronic disease. *Public Health Rep*; 119:239-243
20. Jakobsson U., and Hallberg I.R. (2002): Pain and quality of life among older people with rheumatoid arthritis and/or osteoarthritis: A literature review. *Journal of Clinical Nursing*; 11(4): 430-443.
21. Kagee A. (2001): Review of the SF-36 Health Survey. In Plake BS & Impara, JC. (Eds). The fourteenth mental measurements yearbook. 2001; Lincoln NE: Buros Institute of Mental Measurements.
22. Linde L., Sørensen J., Østergaard M., Hørslev-Pedersen K., Lund Hetland M. (2008): Health-related quality of life: validity, reliability, and responsiveness of SF-36, 15D, EQ-5D, RAQoL, and HAQ in patients with rheumatoid arthritis. *J Rheumatol*; 35: 1528-37.
23. Lynn B. (2010): Reflexology for Chronic Conditions: A Promising Modality, Gerontological Nurses Association of British Columbia Conference and Annual Meeting "Aging: Honoring the Journey" September 16 - 18, 2010 Coast Inn of the North 770 Brunswick Street Prince George BC V2L 2C2 Newsletter (2010) Spring edition March 2010.
24. McGill (2009): Pain Scale for Pain Assessment McGill Pain Questionnaire, From Erica Jacques, former About.com Guide Updated September 03, 2009 About.com Health's Disease and Condition

- content is reviewed by the Medical Review Board.
25. Montbriand M.J. (1994): An overview of alternate therapies chosen by patients with cancer. *Oncology Nursing Forum*; 21(9): 1547–1554.
  26. National Institutes of Health (2006): National Institute of Arthritis and Musculoskeletal and Skin Diseases
  27. Pagan J.A., and Pauly M.V. (2005): Access to conventional medical care and the use of complementary and alternative medicine. *Health Affairs*; 24(1): 255–262.
  28. Palferman T.G. (2003): Principles of rheumatoid arthritis control. *J heumatol*; 67(Suppl):10–13.
  29. Quattrin R., Zanini A., Buchini S., Turello D., Annunziata M.A., Vidotti C., Colombatti A., and Brusaferrero S. (2006): Use of reflexology foot massage to reduce anxiety in hospitalized cancer patients in chemotherapy treatment: methodology and outcomes. *Journal of Nursing Management*; 14: 96–105.
  30. Rheumatoid Arthritis Fact Sheet (2010): Arthritis Foundation news from the arthritis foundation, 1330 west Peachtree Street. Atlanta Georgia, 30309
  31. Rose G. (2006): Why do patients with rheumatoid arthritis use complementary therapies? *Musculoskeletal Care*; 4(2): 101–115.
  32. Ross C.S., Hamilton J., Macrae G., Docherty C., Gould A., and Cornbleet M.A. (2002): A pilot study to evaluate the effect of reflexology on mood and symptom rating of advanced cancer patients. *Jan: Eview Paper Efficacy of reflexology. Palliative Medicine*; 16: 544–545.
  33. Schneider M., Manabile E., and Tikly M. (2008): Social aspects of living with rheumatoid arthritis: a qualitative descriptive study in Soweto, South Africa – a low resource context, *Health and Quality of Life Outcomes*; 6:54.
  34. Somchock J. (2006): Effects of foot reflexology on reducing blood pressure in patients with hypertension, Master of Science (in Nursing) School of Nursing and Midwifery Flinders University Adelaide, Australia January 2006.
  35. Stephenson N., Dalton J.A., and Carlson J. (2003): The effect of foot reflexology on pain in patients with metastatic cancer. *Applied Nursing Research*; 16(4): 284–286.
  36. Stephenson N.L., Weinrich S.P., and Tavakoli A.S. (2000): The effects of foot reflexology on anxiety and pain in patients with breast and lung cancer. *Oncology Nursing Forum*; 27(1): 67–72.
  37. Strand V., and Khanna D. (2010): The impact of rheumatoid arthritis and treatment on patients' lives. *Clin Exp Rheumatol.*; 28(3 Suppl 59):S32-40.
  38. Vincent C., and Furnham A. (1999): Complementary medicine: state of the evidence. *Journal of the Royal Society of Medicine*; 92(4): 170–177.
  39. Wang M.Y., Tsai P.S., Lee P.H., Chang W.Y., and Yang C.M. (2008): The efficacy of reflexology: systematic review. *Journal of Advanced Nursing*; 62(5): 512–520
  40. Ware JE, Jr., and Hays RD. (1988): Methods for measuring patient satisfaction with specific medical encounters. *Medical Care*; 26:393-402.
  41. Wilkinson I.S., Prigmore S., and Rayner C.F. (2006): A randomised controlled trial examining the effects of reflexology of patients with chronic obstructive pulmonary disease (COPD). *Complementary Therapies in Clinical Practice*; 12: 141–147.
  42. Wright S., Courtney U., Donnelly C., Kenny T., and Lavin C. (2002): Clients' perceptions of the benefits of reflexology on their quality of life. *Complementary Therapies in Nursing & Midwifery*; 8: 69–76.
  43. Xavier R. (2007): Facts on reflexology (foot massage). *Nursing Journal of India*; 98(1): 11–12.

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## Effect of Regular Aerobic Exercises on Behavioral, Cognitive and Psychological Response in Patients with Attention Deficit-Hyperactivity Disorder

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**Abstract:** Background and purpose: Attention Deficit-Hyperactivity Disorder (ADHD) is a common behavioral disorder started in childhood and is characterized by one or a combination of three behaviors, named hyperactivity, inattentiveness and impulsiveness. The aim of this study was to find out the value of aerobic exercises on improvement symptoms of ADHD. Patients, Materials and Methods: This study included 84 students diagnosed as having ADHD. Their age ranged from eleven to sixteen years. The students were randomly divided into two equal groups. The exercise group received ten weeks aerobic exercises program included upper limb, lower limb, trunk and neck exercises as well as running three sessions per week( In the first four weeks the session lasted for about 40 minutes and in the last six weeks the session extended to be 50 minutes). Behavior Rating scale was used to assess the students behavior before starting and after the end of ten weeks of the exercise program. Results: Results of the exercise group revealed a significant improvement in three of the five items involved in the scale(attention, motor skills and academic and classroom behavior) with  $p < 0.05$  while there was no improvement in the control group ( $p > 0.05$ ). Conclusion: It could be concluded that regular aerobic exercises program has a positive effect in improving symptoms of ADHD.

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**Key words:** Aerobic exercise-Cognition-Behavior-Attention Deficit Hyperactivity Disorder.

### 1. Introduction

Attention deficiency hyperactivity disorder (ADHD) is one of the most common neurobehavioral disorders. Patients with ADHD have trouble paying attention, controlling impulsive behaviors (may act without thinking about what the result will be), and in some cases are overly active<sup>1</sup>.

ADHD is characterized by a persistent pattern of impulsiveness and inattention, with or without a component of hyperactivity<sup>2</sup>. This disease is diagnosed twice as frequently in boys as in girls. As they mature, adolescent and adult with ADHD are likely to develop coping mechanisms to compensate for their impairment<sup>3</sup>. Baumgardner et al.<sup>4</sup>, studied patients with ADHD and discovered morphological asymmetry in the caudate nucleus of the corpus striatum. The caudate nucleus is mainly dopaminergic and the asymmetry weakens the reception of the dopamine signal by this structure. Thus, ADHD is believed to be caused by a slight abnormality in the brain caused either genetically or by prenatal complications<sup>5</sup>.

Differences in distributed cortical and sub-cortical networks that support basic cognitive functions (such as attention, motor control and self-regulation) have been proposed as a neural basis for ADHD<sup>6</sup>. Reduced volumes within-hemisphere (corticocortical) and callosal white matter have been noted in ADHD.

These suggested the possibility that the symptoms of ADHD may be related to impaired interactions within brain networks, rather than impaired function of specialized cortical regions<sup>7</sup>.

ADHD is characterized by reductions in regions of the corpus callosum, frontal lobes, basal ganglia, and cerebellum. These networks involve input-output processing of attention, including alerting and executive functions. Deficits in attention, information processing, alerting, orienting and working memory may be mediated primarily in the prefrontal cortex<sup>8</sup>. Deficient connectivity between hemispheres induced state of over-connectivity within and between frontal hemispheres. Patients always seem to be in motion and may move around touching or playing with whatever is around, or talk continually. Some patients wiggle their feet or tap their fingers<sup>9</sup>.

Thus, self-perceptions of patients with ADHD are low with regards to their feelings on behavior, ability to get along with others and to succeed in school. Self-perceptions are poor and over time they become increasingly more doubtful about their ability to cope with academic and social issues during adolescence<sup>10,11</sup>. A growing number of practitioners and researchers suggest that a regular program of physical exercise may lead to lower stimulant doses for those who still need medication<sup>12</sup>.

Previous studies<sup>13,14</sup> suggested that the exercise regimens can help brains remain vital and healthy well. Exercise almost immediately elevates dopamine and nor-epinephrine and keeps them up for a period of time. It also helps to still the impulsivity and still the cravings for immediate gratification as it works to wake up the executive function of the frontal cortex, which in turn allows for delay, better choices, a bit more time to evaluate consequences<sup>11</sup>. The purpose of this study was to investigate the effect of regular aerobic exercises on behavioral, cognitive and psychological problems related to ADHD.

## 2. Patients and Methods:

Eighty four students from both gender (54 boys 30 girls) with ADHD participated in this study. Their age ranged from 11:16 years. They were recruited from Special Needs Schools in Riyadh (Riyadh El Salehen, El Maaref and El Rawda Schools). All patients were functionally independent, could understand well, follow orders and cooperative. The students were excluded if they had;(1) Medical or systemic problems such as hypertension, hypotension, diabetes mellitus,(2)Musculoskeletal deformities (scoliosis, kyphosis , pes cavas),(3) Neurological problems (sensory or motor deficit),(4)Orthopedic problems (including past history of trauma before application of the study at least two months),(5) Rheumatic fever,(6)Obesity.

### Instrumentation:

The Behavior Rating Scale is a modified version of Conner's Rating Scale<sup>15</sup>. The scale has been validated for screening and assessing behavioral, cognitive and psychological problems related to ADHD<sup>16</sup>. The scale provides a reliable, accurate and relatively brief measure of perception of disruptive behavior<sup>17</sup>. It consists of 25-behavior related questions, subdivided into categories for attention, motor skills, task orientation, emotional and oppositional behavior and academic and classroom behavior. The higher the score, the better behaved the student.

### Procedures:

Prior to commencement of the exercise program, the students, teachers and the parents attended an information session. The aim and the procedures of the study were explained in details to the school manager, parents and teachers. Detailed explanation about exercise program was taught to the students before starting the study. Ethical approval was received from the schools administration to participate the students in the study. The students were assigned randomly into two equal groups (control and exercise). The exercise(study)group

received exercise program for ten weeks as three sessions per week. The control group did not receive any designed exercise program.

### Evaluation protocol:

The students were evaluated using the Behavior Rating Scale Pre and post exercise program. The Scale was filled from the teachers by the interviewers. During the study, the teachers were asked to observe the students for any changes, either positive or negative, in the school behavior, psychological statement and class concentration .The teachers helped in taking the suggestions of the students, and give any ideas about the application of the session.

### Exercise protocol:

The moderate-intensity exercise program<sup>18</sup> was applied three sessions per week. Exercise program included upper limb, lower limb, trunk and neck aerobic exercises in addition to free running. Ten repetitions for each exercise increase with time, rest period two minutes between every 15 minutes.

- **In the first four weeks** the session lasted for about 40 minutes, ten minutes preparation and warm up, 20 minutes aerobic exercise and five minutes with walking between exercises, at the end five minutes walking around the school with each other for cooling down.

- **The following six weeks**, the session lasted for about 50 minutes. Ten minutes warm up, 30 minutes aerobic exercises, five minutes walking around the school building, and five minutes slow walking and stretching for cooling down. The cool down phase include relaxation exercises. The aim of the relaxation exercises was to reduce the student's heart rates back to or close to resting levels.

- **Home program**, Parents were instructed to start home program for the study group from the 6<sup>th</sup> week and continuous to the 10<sup>th</sup> week. The home program included walking half an hour out-door in the week-end.

### Statistical analysis:

Intra-rater reliability between interviewers was conducted for the first 20 interviews. To determine any significant changes in variables, all variables were subjected to analysis of variance (ANOVA) with repeated measures. Tukey's post-hoc tests were used to determine significant differences. The level of significance was set at  $p < 0.05$ .

## 3. Results:

The student's physical characteristics were: age; study group(13.9±1.6), control group (13.8±1.7 years), weight; study group (42.10±4.2 kg), control



group ( $41.11 \pm 4.1$  kg) and height; study group ( $149.46 \pm 3.8$  cm), control group ( $152.33 \pm 4.1$  cm.). There were no significant differences between both groups related to general characteristics.

Statistical analysis showed that there was an improvement in the Behavior Rating Scale scores for students in the exercise group while there was no significant changes ( $P > 0.05$ ) in behavior, cognitive or psychological problems in the control group who did not receive any exercise program (Table 1 & 2).

There were no significant differences between both groups at the beginning of the study ( $P > 0.05$ ). After cessation of the exercise program for the study group, the following behavior categories were significantly improved in the study group compared to the control group: attention ( $P = 0.005$ ), motor skills ( $P = 0.04$ ) and academic and classroom behavior ( $P = 0.001$ ). Task orientation and emotional and oppositional behavior were not significantly altered after the ten weeks of exercise program with  $P > 0.05$  (Table 3 & Fig.1).

**Table (1): Comparison between the mean values of attention, motor skills , task orientation, emotional and oppositional behavior, academic and classroom behavior before and after exercise program in the study group.**

Variable	Before Mean $\pm$ SD	After Mean $\pm$ SD	P-value
Attention	4.89 $\pm$ 1.25	8.46 $\pm$ 3.61	0.001*
Motor skills	4.11 $\pm$ 5.94	7.97 $\pm$ 3.96	0.01*
Task orientation	0.41 $\pm$ 0.87	0.35 $\pm$ 0.61	0.14
Emotional & Oppositional behavior	3.94 $\pm$ 2.61	2.71 $\pm$ 3.14	0.87
Academic & classroom behavior	22.24 $\pm$ 4.26	30.24 $\pm$ 7.27	0.008*

Significance\*  $< 0.05$ . SD: Standard deviation.

**Table (2): Comparison between the mean values of attention, motor skills , task orientation, emotional and oppositional behavior, academic and classroom behavior before and after exercise program in the control group.**

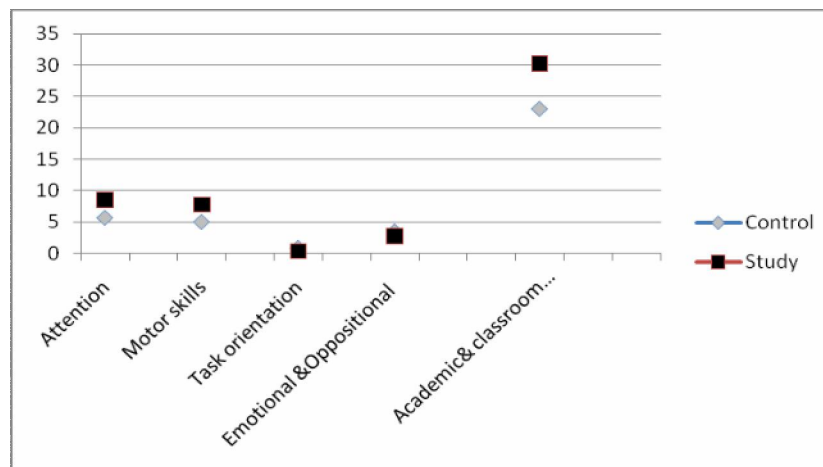
variable	Before Mean $\pm$ SD	After Mean $\pm$ SD	P-value
Attention	4.91 $\pm$ 7.68	5.62 $\pm$ 7.15	0.56
Motor skills	5.36 $\pm$ 5.95	4.95 $\pm$ 6.07	0.08
Task orientation	0.32 $\pm$ 2.86	0.82 $\pm$ 2.82	0.87
Emotional & Oppositional behavior	3.36 $\pm$ 2.54	3.45 $\pm$ 2.68	0.23
Academic & classroom behavior	22.27 $\pm$ 6.91	23.00 $\pm$ 5.83	0.21

Significance\*  $< 0.05$ . SD: Standard deviation.

**Table (3) .Comparisons between mean values of Behavior Rating Scale after exercise at the end of the study of both groups (study and control).**

variable	Study group Mean $\pm$ SD	Control group Mean $\pm$ SD	P-value
Attention	8.46 $\pm$ 3.61	5.62 $\pm$ 7.15	0.005*
Motor skills	7.97 $\pm$ 3.96	4.95 $\pm$ 6.07	0.04*
Task orientation	0.35 $\pm$ 0.61	0.82 $\pm$ 2.82	0.78
Emotional & Oppositional behavior	2.71 $\pm$ 3.14	3.45 $\pm$ 2.68	0.421
Academic & classroom behavior	30.24 $\pm$ 7.27	23.00 $\pm$ 5.83	0.001*

Significance\*  $< 0.05$ . SD: Standard deviation.



**Fig.1. Mean values of Behavior Rating Scale after exercise program in the study and control groups.**

#### 4. Discussion:

Researchers have noted the positive role of exercises in the treatment of anxiety<sup>19,20</sup>, depression, stress<sup>21</sup>, and enhancing general mood state<sup>22</sup>. Based on these previous studies, it was hypothesized that an exercise program would significantly improved the behavior in patients with ADHD compared with that of non- exercised controls. The present study examined the alterations in the behavior, cognition and psychological problems of students with ADHD after ten weeks moderate intensity exercise program. There were significant improvements of the study group in three items of five from Behavior Rating Scale (attention, motor skills and academic and classroom behavior).

The results of the present study agree with the findings of Maddigan et al.<sup>23</sup>, who concluded that exercise therapy would be effective in reducing symptoms or medication dose in attention deficit hyperactivity disorder in school age subjects who were already stabilized on medication. The improvement in inattentiveness was also supported by Went<sup>24</sup> who stated that there was positive shift observed in concentration among subjects with ADHD after they participated in therapeutic movement therapy. The results showed positive improvements on working speed, social and behavioral problems.

Possible explanation for improved behavior may therefore be that the exercise sessions encouraged co-operation in group situations and fostered tolerance and acceptance<sup>5</sup>. Hoza et al.<sup>26</sup> reported that friendship forming in ADHD students is associated with improved behavior. The social interactions in the exercise group during the exercise sessions, as well as during school may therefore resulted in improved behavior. Most students tend to be more active and attentive at the end of the program. The students in

the exercise group stated that they enjoyed this activity and the parents found this to be of benefit for them.

Taylor and Kuo<sup>11</sup> reported that exercise helps to heighten the response to stressors, that is, the students became less stressed to the same stressors when in a fit condition. The researchers also mentioned that, mood is made better by raising the levels of neurotransmitters that works as antidepressants (dopamine, nor-epinephrine, and serotonin). All of these neurotransmitters get jacked up by exercise. Exercise reenergizes depressed brains to do its job of adapting to environment.

The results of the present study agree also with the results of Tantillo et al<sup>27</sup>., who suggested that exercise is beneficial to subjects with ADHD. However, the results disagree with a few studies which have been published in this area and have either been case reports of only one or few subjects<sup>28-30</sup>, or failed to test implement valid behavioral measures<sup>31</sup>.

Coe et al.,<sup>32</sup> suggested that exercises could play a significant role in the classroom by improving attention and learning problems. Miller et al.,<sup>33</sup> found that schools that offered a more intense physical activity program tended to have pupils who demonstrated higher concentration levels. In addition, these pupils demonstrated improved reading, writing and mathematics scores. This overall positive effect on academic performance was also found when the physical activity time reduced time for academic teaching.

Dwyer et al.,<sup>34</sup> studied Physical fitness and academic achievement in sixth and seventh grade students and their study confirmed that physical fitness is generally associated with improvement of academic performance in elementary school. Aerobic fitness and behavior test scores were associated with

achievement of reading and mathematics. The authors regarded these improvements in academic achievements to the effect of exercises on behavior including attention and concentration. The study concluded also that student's physical competency improves self-esteem perceptions and emotions.

### 5. Conclusion:

It was found that ten weeks exercise program as three times per week improved behavioral-cognitive and psychological response of students with ADHD. So, exercises may be considered as an additional treatment required for improving the symptoms in patients with ADHD.

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### 6. References:

- 1- American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders: DSM-IV-TR. Washington: fourth ed., Am Psych Assoc, 2000.
- 2- Burt Sa, Krueger RF, McGue M, Iacono W. Parent-child Conflict and the comorbidity among childhood externalization disorders. *Arch. Gen. Psychiatr* 2003; 60:505-13.
- 3- Bauermeister, J., Shrout, P., Chávez, L., Rubio-Stipec, M., Ramírez, R., Padilla, L., et al. ADHD and gender: are risks and sequela of ADHD the same for boys and girls. *J. Child Psychology & Psychiatry* 2007; 48(8): 831-39.
- 4- Baumgardner T, Singer H, Denckla M, Rubin M, Abrams M, Colli M, Reiss A. Corpus callosum morphology in children with Tourette syndrome and attention deficit hyperactivity disorder. *Neurology* 1996;47: 477-82.
- 5- Braun JM, Kahn RS, Froehlich T, Auinger P, Lanphear BP. Exposures to environmental toxicants and attention deficit hyperactivity disorder in U.S. children. *Environ. Health Perspect* 2006; 114 (12): 1904-9.
- 6- Semrud-Clikeman M, Filipek PA, Biederman J, Steingard R, Kennedy D, Renshaw P, Bekken K. Attention-deficit hyperactivity disorder: Magnetic resonance imaging morphometric analysis of the corpus callosum. Solanto MV. Dopamine dysfunction in AD/HD: integrating clinical and basic neuroscience research. *Behavioural Brain Research* 2002; 130: 65-71.
- 7- Paus T, Zijdenbos A, Worsley K, Collins DL, Blumenthal J, Giedd JN, Rapoport JL, Evans AC. Structural Maturation of Neural Pathways in Children and Adolescents: In Vivo Study. *Science* 1999; 283: 1908-11.
- 8- Voller KS, Heilman KM. Motor impersistence in children with attention deficit hyperactivity disorder: evidence for right hemisphere dysfunction. *Ann. Neurol.* 1998; 24:323-31.
- 9- Cantwell DP. Attention deficit disorder: A review of the past 10 years. *J. Am. Acad child Adolesc psychiatry* 1995; 45:978-87.
- 10- Yadid G, Overstreet DH, Zangen A. Limbic dopaminergic adaptation to stressful stimulus in a rat model of depression. *Brain Res* 2001; 896:43-7.
- 11- Taylor AF, Kuo FE. Children With Attention Deficits Concentrate Better After Walk in the Park. *J. Attention Disorders* 2008; 23:36-56.
- 12- Durston S, Hulshoff PHE, Schnack HG, Buitelaar JK, Steenhuis MP, Minderaa RB, Kahn RS, van Engeland H: Magnetic resonance imaging of boys with attention-deficit/hyperactivity disorder and their unaffected siblings. *J Am Acad Child Adolesc Psychiatry* 2004; 55: 332-340.
- 13- Ward J. The Student's Guide to Cognitive Neuroscience, Psychology Press 2006; 23: 65-73.
- 14- Wood D. Habituation in Stentor produced by mechanoreceptor channel modification. *J Neuroscience* 1999; 8: 2254-64.
- 15- Conners CK, Sitarenios G, Parker JD, Epstein JN. The revised Conners rating Scale: factor structure, reliability and criterion validity. *J. Abnorm. Child psycho.* 1998; 26:257-68.
- 16- Fisher M, Newby RF. Assessment of stimulant response in ADHD children using a refined multimethod clinical protocol (special issue on child psychopharmacology). *J. Clin. Child Psychol.* 1991; 20: 232-44.
- 17- Horm WF, Lalongo N, Popovich S, Peradotto D. behavioural parent training and cognitive-behavioral self control therapy with ADDH children: Comparative and combined effects. *J. Clin. Child Psychol.* 1987; 16:57-68.
- 18- Mckune AJ, Pautz J, Lombard J. Behavioral response to exercise in children with attention-deficit hyperactivity disorder. *Sports Medicine* 2003; 2:17-21.
- 19- Norris R, Carroll D, Cochrane R. The effect of physical activity and exercise training on psychological stress and well-being in an adolescent population. *J Psychosom Res* 1992; 36:55-65.
- 20- Paluska SA, Schwenk TL. Physical activity and mental health: Current concepts. *Sports Med* 2000; 29:167-80.

- 21- Raglin JS, Morgan WP. Influence of exercise and quiet rest on state of anxiety and blood pressure. *Med Sci Sports Exerc* 1987; 19:456-83.
- 22- Potgieter JR. *Sport Psychology: Theory and practice*, Stellenbosch: Institute for Sport and Movement Studies, University of Stellenbosch, 1997.
- 23- Maddigan B, Hodgson P, Dick B. The Effects of Massage Therapy & Exercise Therapy on Children/Adolescents with Attention Deficit Hyperactivity Disorder. *Can Child and Adolescent Psychiatry Review* 2003; 2( 12 ):40-3.
- 24- Went MS. The effect of an activity program designed with intensive physical exercise on the behavior of ADHD children. Buffalo: State University of new York, 2000
- 25- Biederman, J, Faraone SV. Attention-deficit hyperactivity disorder. *Lancet* 2005; 366:237-48.
- 26- Hoza B, Mrug S, Pelham WE, Greiner AR, Gnagy EM. A friendship intervention for children with Attention- Deficit Hyperactivity Disorder: Preliminary findings. *J Attention Disorders* 2003;6:87-98.
- 27- Tantillo M, Kesich CM, Hynd GV, Dishman RK. The effects of exercise on children with attention-deficit hyperactivity disorder. *Med Sci Sports Exerc* 2002; 34:203-12.
- 28- Etscheidt MA, Ayllon T. Contingent exercise to decrease hyperactivity. *J Psychotherapy* 1987; 4:192-8.
- 29- Gaub M, Carison CL, Gender differences in ADHD: a meta analysis and critical review. *J. Am. Acad Adolesc Psychiatry* 1997;36:1036-45
- 30- Lane AM, Crone GD, Lane H. Mood changes following exercise. *Percept Mot Skills* 2002;94:732-4.
- 31- Steptoe A, Cox S. Acute effects of aerobic exercise on mood. *Health Psychol* 1988; 7:329-40.
- 32- Coe D, Pivarnik J, Womck C, Reeves M, Malina R. Effect of Physical Education and Activity Levels on Academic Achievement in Children. *Med. Sci. Sports Exerc.* 2006; 38 (8): 1515-19.
- 33- Miller P, Naglieri J, Gregoski M. Effects of aerobic exercise on overweight children's cognitive functioning: a randomized controlled trial. *J Res. Exerc. Sport* 2007; 78(5): 510-9.
- 34- Dwyer T, Sallis J, Blizzard L, Lazarus R, Dean K. Relation of Academic Performance to Physical Activity and Fitness in Children. *Pediatric Exercise Science* 2001; 13: 225-38.

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## Effect of Low Level Laser Therapy on Bone Histomorphometry in Rats

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**Abstract:** The aim of this study was to assess the histological effect of Low Level Laser Therapy (LLLT) (904 nm) on the repair of standardized bone defects on the femur of rats. Sixty male wistar rats were assigned into two equal groups. Group (A: laser group) and group (B: control group). A surgical fracture was done in middle third of femur of all rats. In group (A) a continuous wave 904 nm infrared laser was applied at dose 4 j/cm<sup>2</sup> at fracture site immediately post operative for 7 sessions, each session was 5 minutes. The animals were killed by over dose of general anesthesia on the 15th, 30th and 45th days after surgery, The specimens were processed and stained with Hematoxylin-eosin (H/E), special stain Mason trichrome and analyzed by light microscopy. The descriptive analysis of histological imaging showed greater degree of new bone formation, osteoblastic surface and collagen fiber in the irradiated group when compared with the control group. Based on the obtained results, this study concluded that LLLT was efficient in promoting bone healing, and increasing new bone formation in the process of surgically fractured femur in animal study.

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<http://www.lifesciencesite.com>.

**Key words:** Wistar rats, Low Level Laser Therapy, Bone histomorphometry, Bone repair.

### 1. Introduction

Bone histomorphometry is the only procedure, which allows for assessment of bone turnover, static and dynamic tissue and cell activities of modeling and remodeling, and mechanisms underlying bone mass changes at the tissue and cellular levels <sup>(1)</sup>. Bone repair is a proliferative physiological process, in which the body facilitates healing of bone fractures. The healing potential of bone, whether in a fracture or fusion model, is influenced by a variety of biochemical, biomechanical, cellular, hormonal, and pathological mechanisms. A continuously occurring state of bone deposition, resorption, and remodeling facilitates the healing process. Long bone fractures heal by a slow process which may be associated with significant social, domestic and financial consequences. Delayed healing may exaggerate serious problems <sup>(2)</sup>. In turn, enhanced bone healing should contribute to a reduction in health care costs in terms of earlier patient mobility and discharge from hospital and, not least, it should lessen the discomfort experienced by the patient following surgical treatment or trauma <sup>(3)</sup>. Currently, bone increment stimulus has been achieved with the application of chemical stimuli, biomaterials, and bone morphogenetic proteins as well as the use of physical stimuli, such as ultrasound, electromagnetic fields and more recently low level laser therapy (LLLT) <sup>(4)</sup>.

Laser therapy is a new approach applicable in different medical fields when bone loss occurs, including orthopedics and dentistry. It has also been used to induce soft-tissue healing, for pain relief, bone, and nerve regeneration. With regard to bone synthesis, laser exposure has been shown to increase osteoblast activity and decrease osteoclast number <sup>(5)</sup>. Stimulation with LLLT enhances the healing environment, resulting in accelerated healing of bone defects in vivo and in vitro. The healing process has three phases: a substrate phase, a proliferative phase and a remodeling phase. Most accounts of laser biostimulation suggest that its greatest effects occur during the proliferative phase <sup>(6)</sup>.

The use of LLLT in the biostimulation of bone repair has been growing steadily, and several studies have demonstrated positive results of LLLT on the healing of bone tissue <sup>(2-4)</sup>. Also, data in the literature reveal that LLLT may stimulate osseointegration and can enhance bone ingrowths and functional recovery <sup>(5, 6)</sup>. It is still difficult for one to compare studies about the action of LLLT on bone, because the experimental models and duration of treatments are very distinct <sup>(7)</sup>.

Bone healing differs substantially from soft tissue healing because of its morphology and composition. Generally bone healing processes are slower than that of soft tissue. The natural course of

bone healing follows consecutive phases that differ from each other according to the type and intensity of trauma and the extent of bone damage. Several *in vivo* and *in vitro* studies have investigated the use of laser therapy in the biomodulation of bone repair through its photochemical and photobiologic properties<sup>(6-8)</sup>. The aim of this study was to investigate the histological effect of Low Level Laser Therapy (LLLT) (904 nm) on the repair of standardized bone defects on the femur to provide patients with a more comfortable postoperative recovery and faster healing.

## 2. Materials and Methods:

This study was designed to investigate the effect of LLLT on healing of bone fracture. Sixty young adult healthy male wistar rats were used in this study. Their weight ranged from 250g to 500g. Their ages were not less than 10 weeks and not more than 15 weeks. They were kept in animal experimental house of Kasr El-Aini hospital in separate plastic cages. This plastic cage measuring 50 x 24 x 16 cm and bedded on sterilized wood chips. They were maintained under controlled temperature ( $24 \pm 2$  C), light-dark periods of 12 h, and with unrestricted access to water and commercial diet.

The rats were divided into two equal groups, each group consisted of thirty rats the first group (A) (laser group) received LLLT while the rats in second group (B) (control group) were put under general medical observation. Each group (A) and (B) was subdivided into three subgroups (1, 2 and 3) according to their killing day (every fifteen days) at day 15, 30 and 45.

### Laser unit

Laser Therapy (Phyaction 769) - Pasweg 6a - 3740 Bilzen - Belgium. Pulsed, infrared (IR) gallium arsenide, wave length 904nm. Mains voltage 110-240V (+/-10%), 50-60 HZ, Dimensions (W×d×h): 41×28×13 cm. Weight: 6kg. Clearly visible LCD display with digital display of all parameters. Convenient touch controls. Many laser probes can be supplied. Their Peak power was 16W, Pulse repetition frequency 2-30.000 HZ, and Max. average power 81,6 mW.

### Computerized optical microscope

Two image processing and analysis system were used in this study: An image processing and analysis system; Leica imaging systems Ltd; Clifton road, Clifton Road, Cambridge, CB1 3QH England. This system was used as measuring tool for all histological parameters. The microscope allowed following magnifications: x40, x100, x200, x400, and x1000. It was linked to a Panasonic color CCTV

Camera; model WV-CP 210/G; Matsushita communication industrial Co., Ltd.;1-9-5,Otemachi, Chiyoda-ka, Tokyo, Japan. The images taken by camera were linked to computer with Qwin Windows's software for analysis of images.

### Procedures:

Surgical procedure were done under general anesthesia by using an intra-peritoneal injection of a mixture of xylazine 10 mg/kg and ketamine HCL 90 mg/kg body weight<sup>(9)</sup>. The right leg of the animal was shaved and the femur exposed, then veterinarian made transverse fracture in mid shaft of femur and put two fragments close to each other, after that the skin was closed with nylon. The fractured leg was kept in elastic bandage for immobilization.

Group (A) were treated by LLLT (904nm). Laser irradiation was started immediately after the surgery procedure and it was performed on the 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 10<sup>th</sup> and 12<sup>th</sup> day's post-operative. The irradiation was performed transcutaneously at dose of 4J/cm<sup>2</sup> was applied to 4 points around fracture site anterior, posterior, laterally and medially for 5 minutes every session. All rats were treated in the same way. The animals were positioned on a table in ventral decubitus, and manually immobilized. The laser was used on their hind limb, directly on the injury, at a 90° angle. The laser pen was covered by plastic film before each application to avoid contamination.

A dose of 4 J/cm<sup>2</sup> was applied to four points around the defect giving a total of 16 J/cm<sup>2</sup> per session and a total treatment dose of 112 J/cm<sup>2</sup>. The protocol used on this study was based upon previous study carried out by Pinheiro et al, 2003<sup>(11)</sup> whom recommended doses ranging from 1.8 to 5.4 J/cm<sup>2</sup>.

### Morphometry

Animals were killed by over dose of general anesthesia on day15, 30 and 45. After the surgery procedures, the irradiated femur and control femur were immediately defleshed, dissected and fixed. Bone sample of mid shaft of femur was taken and prepared for histological examination. Measurements of bone histomorphometry include bone area, osteoblastic surface and collagen area.

The specimens were kept on 4% buffered paraformaldehyde solution for overnight. The samples were decalcified with 10% nitric acid and dehydration was followed by embedding in soft paraffin. Hard blocks were made with transverse section of 5µm thickness. The slices were stained with hematoxylin - eosin (H-E) stain which was widely used to evaluate bone changes. To evaluate

the data and compare variables in the 15th, 30th and 45th post operative day between laser group and control group morphometric analyses, images were digitized, and computer analysis was performed with a specific image processing and analysis program.

- Bone area: using midshaft transverse section, the bone area was measured at a magnification of x 40. of standard area  $7286.78\mu\text{m}^2$ ; many fields were taken to cover the whole area. At each field bone tissue were detected, previously colored on the screen using special software (Qwin windows) through the camera. The bone area was expressed in  $\text{mm}^2$ .
- Osteoblast surface: was measured with magnification of x100, and x400 in many fields of standard area  $7286.78\mu\text{m}^2$ , osteoblast surface was considered as the perimeter of trabeculae covered with osteoblastic cells. Measurement made by tracing the features of interest on screen viewed by the microscope through the camera. Osteoblast surface was expressed in  $\mu\text{m}$ .
- Bone collagen: The collagen fiber was detected by using maison trichrome. The collagen was measured with magnification of x100 in many fields of standard area  $118476, 6\mu\text{m}^2$

#### Statistical design and data analysis

All analyses were conducted using the SPSS statistical package, version 10.0. Descriptive statistics were used for the means and standard deviations. The mean of the three trials is an estimate of the true value. The standard deviation is an indication of the progression of the single measurement. Paired t-test (for parametric data in one group), independent t-test (for parametric data between two groups). Repeated measurement analysis of variance ANOVA to record between and within groups differences. The level of significance was accepted as  $p < 0.05$ .

### 3. Results:

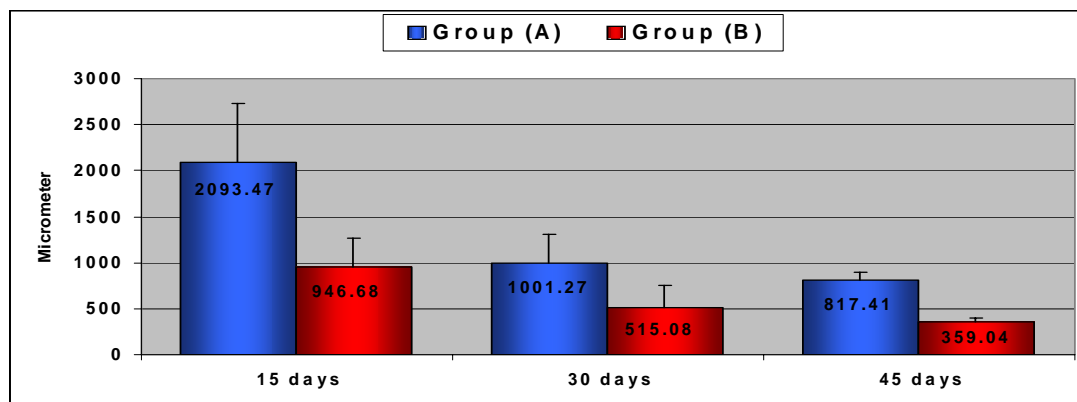
#### 1- New bone formation surface area:

Table (1) represented the independent sample t-test results for new bone formation surface area at day 15, 30, and 45 between groups A and B. There was a significant difference between both groups in new bone formation surface area at day 15 where the t-value was (5.1) and p-value was (0.0007), there was a significant difference between both groups in new bone formation surface area at day 30 where the t-value was (3.97) and p-value was (0.001), and there was a significant difference between both groups in new bone formation surface area at day 45 where the t-value was (16.28) and p-value was (0.0003) (Figure 1).

**Table (1): Independent sample t-test between groups A and B for new bone formation surface area at day 15, 30, and 45.**

New bone formation surface area ( $\mu\text{m}^2$ )	day 15	day 30	days 45
Mean difference ( $\mu\text{m}^2$ )	1146.78	486.19	458.36
t-value	5.1	3.97	16.28
P-value	0.0007	0.001	0.0003
S	S	S	S

\*SD: standard deviation, P: probability, S: significance.



**Figure (1): Mean and  $\pm$ SD of new bone formation surface area at 15, 30, and 45 days for group (A, B).**

## 2. Osteoblast surface area:

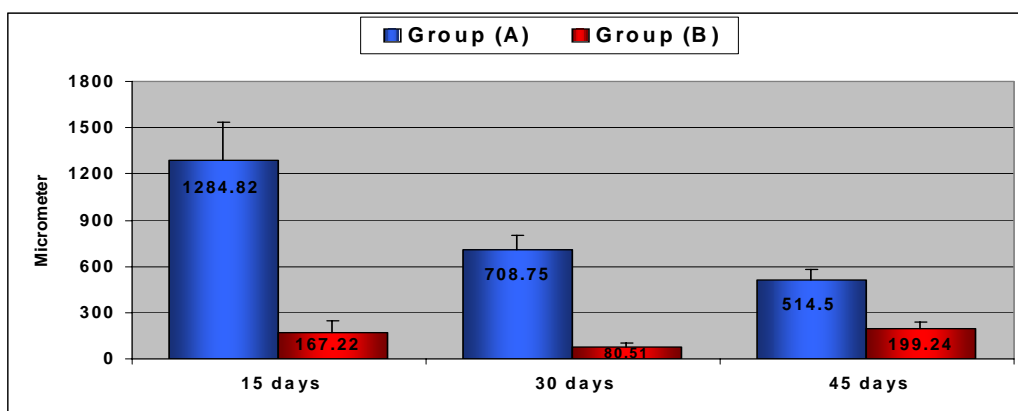
Table (2) represented the independent sample t-test results for osteoblast surface area at day 15, 30, and 45 between groups A and B. There was a significant difference between both groups in osteoblast surface area at day 15 where the t-value was (13.32) and p-value was (0.0004), (Figure 2)

there was a significant difference between both groups in osteoblast surface area at day 30 where the t-value was (20.6) and p-value was (0.0001), and there was a significant difference between both groups in osteoblast surface area at days 45 where the t-value was (12.73) and p-value was (0.0005).

**Table (2): Independent samples t-test between groups A and B for Osteoblast surface area at day 15, 30, and 45.**

Osteoblast surface area ( $\mu\text{m}^2$ )	day15	day30	Day45
Mean difference ( $\mu\text{m}^2$ )	1117.59	628.23	315.26
t-value	13.32	20.6	12.73
P-value	0.0004	0.0001	0.0005
S	S	S	S

\*SD: standard deviation, P: probability, S: significance.



**Figure (2): Mean and  $\pm$ SD of Osteoblast surface area at day 15, 30, and 45 days for group (A, B).**

## 3. Collagen surface area:

Table (3) represented the independent sample t-test results for collagen surface area at day 15, 30, and 45 between groups A and B. There was a significant difference between both groups in collagen surface area at day 15 where the t-value was (6.61) and p-value was (0.0003), In (Figure 3) there

was a significant difference between both groups in collagen surface area at day 30 where the t-value was (6.72) and p-value was (0.0002), and there was a significant difference between both groups in collagen surface area at day 45 where the t-value was (2.63) and p-value was (0.01).

**Table (3): Independent sample t-test between groups A and B for collagen surface area at day 15, 30, and 45.**

collagen surface area ( $\mu\text{m}^2$ )	day15	Day30	Day45
Mean difference ( $\mu\text{m}^2$ )	14667.5	7679.1	1283.24
t-value	6.61	6.72	2.63
P-value	0.0003	0.0002	0.01
S	S	S	S

\*SD: standard deviation, P: probability, S: significance.



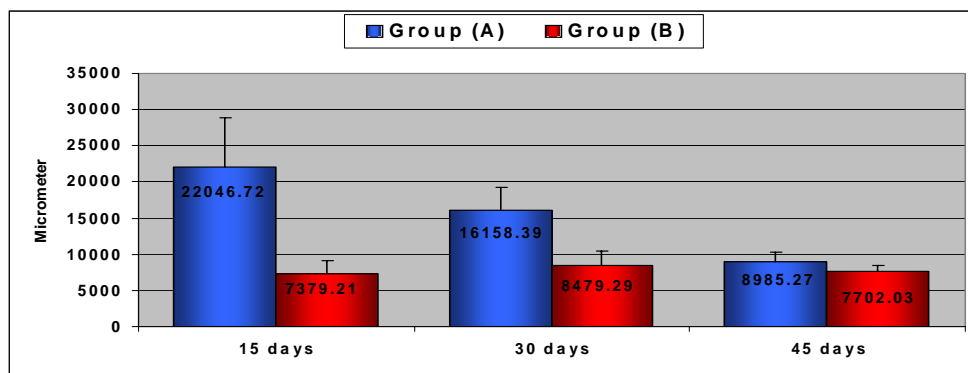


Figure (3): Mean and  $\pm$ SD of collagen surface area at day 15, 30, and 45 for group (A, B).

#### 4. Discussion:

It has been postulated that LLLT has therapeutic efficacy on various clinical conditions. The aim of this study was to explore influence of LLLT on bone repair. Biostimulation provided by LLLT is an area of controversy. Many investigations have indicated a positive effect by the use of LLLT on bone reconstruction either in vivo<sup>(8, 10)</sup> or in vitro<sup>(11, 12)</sup>. Contrarily; other researchers did not find any effect of LLLT on the healing of soft and hard tissues<sup>(13, 14)</sup>.

In current study infra red laser (IR) was chosen supported by previous several studies<sup>(14,15)</sup> which have demonstrated that IR laser therapy is the most suitable method for bone repair due to its higher penetration depth in the bone tissue when compared to visible laser light<sup>(16)</sup>. The results of previous studies also concluded that the 4J/cm<sup>2</sup> energy density provided more significant results than the 8J/cm<sup>2</sup><sup>(17)</sup>. Other experiments, have reported better tissue healing at laser exposure levels between 1 and 4 J/cm<sup>2</sup><sup>(18)</sup>.

**Nissan et al. 2006**<sup>(19)</sup> compared laser irradiation at 4 j/cm<sup>2</sup> and 10 j/cm<sup>2</sup> and concluded that a 4 j/cm<sup>2</sup> energy density significantly increased radio-calcium accumulation 2 weeks after surgery, whereas 10j/cm<sup>2</sup> had no effect. The results from **Garavello-Freitas et al. 2003**<sup>(20)</sup> showed that application of LLLT longer than 5 min (i.e. for 15 min) did not improve the bone healing process, and suggested that more future experiments were needed to be performed in order to find if stopping irradiation after the first 7 days would be more effective for bone healing.

In studies investigating the effects of laser on bone fracture consolidation, **Silva Junior et al. 2002**<sup>(10)</sup> suggest that a total dose of 16 J/cm<sup>2</sup> per session is effective in increasing osteoblast proliferation in tibial fractures of rats. In addition, **Pinheiro et al 2003 and Renno et al 2006.**<sup>(11, 21)</sup> using lower doses did not find any effect of laser on

bone consolidation. **Queiroga et al. 2008**<sup>(16)</sup> concluded in their study a significant amount of newly formed bone within 15 days showed the biomodulated effect of laser therapy in the early stages of the repair process in which there was a large quantity of cells, mainly osteoblasts and undifferentiated cells. Some in vitro studies confirm the effects of laser therapy both in the visible and invisible spectra on cells from the osteoblast alignment<sup>(8)</sup>.

All of these results agree with study findings when the animals were examined on the fifteenth day after the creation of surgical fracture, it was observed that group (A) (treated with laser) was at a more advanced stage of repair than was the other group, presenting an area of new vessel formation, and newly formed bone tissue with a large concentration of osteoblasts and the absence of inflammatory reaction. On the other hand, group (B) was at an earlier stage of repair than group (A), with a cavity lesion, lower osteoblast concentration and more evident inflammatory infiltrate.

The same positive results about increased bone formation were suggested by **Lopes et al. 2007**<sup>(22)</sup> studies, who treated bone defect placing implants, and irradiating with LLLT. They found that infrared LLLT stimulated bone healing 15, 30 and 45 days after operation. They also found the presence of organizing connective tissue, numerous blood capillaries and fibroblasts at 15 days. However, the difference in collagen fiber maturation between the two groups at 30 days was not significant and finally, there were no difference at 45 days of the experiment. Nevertheless, bone formation was higher in the irradiated group at 30 and 45 days.

Additionally, the animal experimental model seems to be a useful technique for investigating the tissue reactions to LLLT, but the results reported from such experimental models cannot safely be put into practice to humans<sup>(23)</sup>. Unfortunately, only a few studies based on humans

exist to support the positive effect of LLLT on alveolar bone regeneration <sup>(24)</sup>. This indicates the need for more human studies to help researchers securely support the positive or the negative action of LLLT. It can be supported that LLLT has the potential of beneficial effects on hard and soft regeneration under stable and no hurtful surgical conditions, irradiation with LLLT could reduce healing time and accelerate osseointegration <sup>(25)</sup>. Nevertheless, further investigations will be necessary to define stable protocols about LLLT use, such as type of laser, treatment duration, energy dose, optimum wavelength and distance from the irradiated tissues, in order to accomplish the best stimulatory action of LLLT.

The results of current study confirmed a positive effect of the soft laser in accelerating bone formation, resulting in a significant improvement in the quality of recovery and a decrease in recovery time. This is particularly important in presence of local and systemic conditions, which could retard the healing process, for example in patients with uncontrolled diabetes. In turn, such enhanced bone healing should contribute to a reduction in healthcare costs in terms of advancing patient mobility, timely discharge from hospital and reduction of the discomfort experienced by the patient following surgical treatment or trauma.

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#### 5. References:

- 1- Kalfas IH. 2001: Principles of bone healing. *Neurosurg Focus*;10:1- 4.
- 2- Otremski I, Irga A, Edelstein S, Ornoy A and Newman R 2004: Does Laser Irradiation Effect Fracture Healing. *Med. Laser Appl*; 19: 146–149.
- 3- Maawan K, Kasem N, Haanæs RH, Ellingsen EJ and Lyngstadaas P S 2004: Enhancement of bone formation in rat calvarial bone defects using low-level laser therapy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*; 97: 693-700.
- 4- Pretel H, Rosane FZ and Lizeti TO 2007: Effect of Low-Level Laser Therapy on Bone Repair: Histological Study in Rats”*Lasers in Surgery and Medicine*; 39:788–796.
- 5- Saracino S, Mozzati M, Martinasso G, Pol R, Canuto AR and Muzio G 2009: Superpulsed Laser Irradiation Increases Osteoblast Activity Via Modulation of Bone Morphogenetic Factors” *Lasers in Surgery and Medicine*; 41:298–304.
- 6- AboElsaad NS, Soory M, Gadalla LAM, Ragab LI, Dunne S and Zalata K R, 2008: Effect of soft laser and bioactive glass on bone regeneration in the treatment of infra-bony defects (a clinical study). *Lasers Med Sci*; 24(3): 227-234.
- 7- Obradović RR, Kesić L G, and Peševska S 2008: Influence of low-level laser therapy on biomaterial osseointegration: a mini-review. *Lasers Surg Med*; 14: 129–132.
- 8- Blaya DS, Pozza DH, Weber JBB and de Oliveira MG 2008: Histological study of the effect of laser therapy on bone repair. *Journal of contemporary dental practice*; 9(6): 3-7.
- 9- Gabriel YF, Dicky TC, Mason CP, and Xia G 2004: Comparison of Single and Multiple Applications of GaAlAs Laser on Rat Medial Collateral Ligament Repair. *Lasers in Surgery and Medicine*; 34, 285–289.
- 10- Silva junior AN, Pinheiro ALB, and Oliveira MG 2002: Computerized morphogenic assessment of the Low-level laser therapy on bone repair: an experimental animal study, *J Clin Laser Med Surg*; 20:83-87.
- 11- Pinheiro ABL, Limeira AF, Marzola C 2003: Effect of low level laser therapy on repair of bone defects grafted with inorganic bovine bone”. *Braz Dent J*; 14(3): 177-181.
- 12- Stein A, Benayahu D, Maltz L and Oran L 2005: Low level laser irradiation promotes proliferation and differentiation of human osteoblasts in vitro. *Photomed.laser Surg*; 23:161-166.
- 13- Luger JE, Rochkind S, Wollman Y, Kogan G, and Dekel S 1998: Effect of Low Power Laser Irradiation on the Mechanical Properties of Bone Fracture Healing in Rats *Lasers in Surgery and Medicine*; 22: 97–102.
- 14- Gur A, Sarac AJ, Cevik R and Sarac S 2004: Efficacy of 904 nm Gallium Arsenide Low Level Laser Therapy in the Management of Chronic Myofascial Pain in the Neck: A Double-Blind and Randomize-Controlled Trial. *Lasers in Surgery and Medicine*; 35: 229–235.
- 15- Bjordall, Couppé C, ChowTR, Tunér J and Ljunggren EA 2003: A systematic review of low level laser therapy with location-specific doses for pain from joint disorders. *Australian Journal of Physiotherapy*; 49:107-116.

- 16- Queiroga AS, Sousa FB, Araújo JMS, Santos SD, Sousa CF, Quintans TC, Almeida TP, Nonaka CFW, Batista LV and Limeira Junior FA 2008: Evaluation of Bone Repair in the Femur of Rats Submitted to Laser Therapy in Different Wavelengths: An Image Segmentation Method of Analysis *Laser Physics*, Vol. 18, No. 9:1087–1091.
- 17- Pinheiro ABL, and Gerbi ME 2006: Photo engineering of bone repair processes”*Photomed. Laser Surg*; 24: 169-178.
- 18- Pugliese LS, Medrado AP, and Andrade ZA 2003: The influence of low level laser therapy on biostimulation of collagen and elastic fibers”. *Pesqui. Odontol. Bras*;17:307-313.
- 19- Nissan J, Assif D, Gross MD and Yaffe A 2006: Effect of low intensity laser irradiation on surgically created bony defects in rats” *J of Oral Rehabilitation*; 33: 619-624.
- 20- Garavello-Freitas I, Baranauskas V, Joazeiro PP, Padovani CR, Dal Pai-Silva M and De Hofling MAC 2003: Low-power laser irradiation improves histomorphometrical parameters and bone matrix organization during tibia wound healing in rats” *Journal of Photochemistry and Photobiology*; 70: 81–89.
- 21- Renno CAN, de Moura FD, dos Santos NSA, Tirico RP and Parizotto NA 2006. The effects of infrared-830 nm laser on exercised osteopenic rats” *Lasers Med Sci*; 21: 202–207.
- 22- Lopes CB, Pacheco MTT, Silveira JL, Duarte J, Cangussu MCT, Pinheiro ALB 2007: The effect of the association of NIR laser therapy BMPs, and guided bone regeneration on tibial fractures treated with wire osteosynthesis: Raman spectroscopy study. *Journal of Photochemistry and Photobiology B; Biology* 89: 125–130.
- 23- Maawan K, Kasem N, Lungstadaas SP and Haanase HR 2005: laser therapy accelerates initial attachment and subsequent behavior of human oral fibroblasts cultured on titanium implant material. *Clin Oral Implants Res*; 16: 168-165.
- 24- Nagasawa A 1991: Clinical applications of LLLT in dental and oral surgery in the Urawa Clinic. *Laser therapy. An Int J of Low Level Laser Therapy*; 3(3):119-121.
- 25- Dortbudak O, Haas R and Mailath-Pokory G 2002: Effect of low power laser irradiation on bony implant sites. *Clin. Oral Implants Res*; 13: 288–292.

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## Study of the Derivatives of Benzimidazole and Applications For Organic Thin Film Transistor

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**Abstract:** This article reviews the development of the derivatives of benzimidazole and applications for organic thin film transistor. With special molecular structure that causes the gathering of holes by electric field can increase the carriers' mobility up to practical application stage.

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**Keywords:** organic thin film transistor; benzimidazole

### I. Introduction

In recent years, the materials combined with the activity of organic semiconductor field effect that can be used for organic thin film transistors (OTFTs) are being extensively studied. Originally, it was considered in some of the initial stage of slow response or more low-end products such as gas detectors, flexible detector, electronic paper, smart card and electronic label tags. However, due to the efforts of researchers, the technology of developing new materials has been improved in synthesis of the field effect that causes carrier mobility increasing up to  $0.5 \sim 2.4 \text{ cm}^2/\text{Vs}$  [1]. This carrier mobility is higher than amorphous silicon rate ( $0.5 \text{ cm}^2/\text{Vs}$ ) and reaches the practical stage. It can be applied as control elements for the pixel-access devices in active matrix displays, flat panel display applications target [2], such as liquid crystal display light valve control device (TFT-LCD) and light-emitting display pixel circuit control circuit (TFT-OLED). The amazing activity based on recent reports that the organic material (Pentacene) of the carrier mobility was substantially increased to  $10^5 \text{ cm}^2/\text{Vs}$  [3]. It is not only beyond the low-temperature poly silicon ( $300 \sim 500 \text{ cm}^2/\text{Vs}$ ) but also can compete with single crystal silicon. A new revolution for the material engineering and the development by the impending changes, the applications can be directly for the logic memory arrays, logical devices such as registers, processor and the integrated circuit etc.. Most of the silicon semiconductor industries can not develop the silicon products to the flexible substrate IC (Integrated circuits on flexible substrate) system. Even through pentacene molecule can only be obtained the phonon scattering [4, 5] at low temperature (10K), it is still excellent to reflect the capability of organic active materials being combined with a broad delocalization  $\pi$ -electron charges and which is very useful for the applications to the electronic transistor.

### II. The Development of New Materials

Based on the molecular structure, materials used for organic thin film transistor can be classified into five categories including (1) aromatic rings class (2) Thiophenes (3) compounds containing electron-donating groups (4) compounds containing electron withdrawing groups and (5) metal complexes of these compounds. The first kind of organic materials is currently the best performing compounds. The typical one is Pentacene. At room temperature, the mobile rate could reach to  $3.2 \text{ cm}^2 / \text{Vs}$ . The majority of this kind of materials belongs to organic semiconductors that are p-type, however, it also can be used as n-type organic semiconductor in conditions [6, 7].

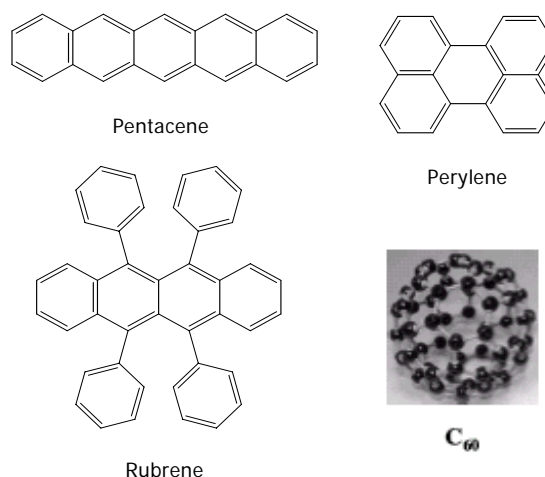


Fig. 1 Aromatic rings for organic thin film transistor materials

Study of the polycyclic aromatics, J.H. Schön, Ch. Kloc and B. Batlogg published a report in Organic Electronics in 2000 as "On the limits of intrinsic pentacene transistors" sets an example [3]. In this paper, organic semiconductor thin film devices was reported

to be constructed by using of Pantacene thin film (Fig. 2 and Fig. 3) with its essential characteristics to explore the limits of carriers' mobility. They pointed out in the report that the Pantacene with the

limited monocrystal mobility at low temperatures can reach to  $10^5 \text{ cm}^2/\text{Vs}$ . In room temperature, thermal phonon scattering leads the mobility up to  $3.2 \text{ cm}^2/\text{Vs}$  (Fig. 4) as an intrinsic property of the Pantacene.

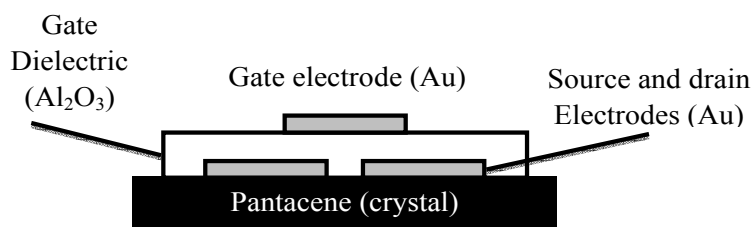


Fig. 2. Pantacene monocrystal transistor device (by J.H. Schön et al.)

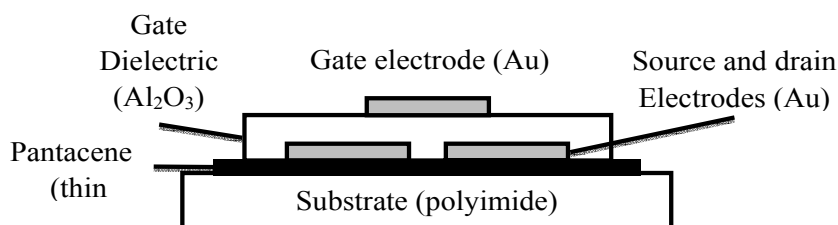


Fig. 3. Pantacene thin film transistor device (by J.H. Schön et al.)

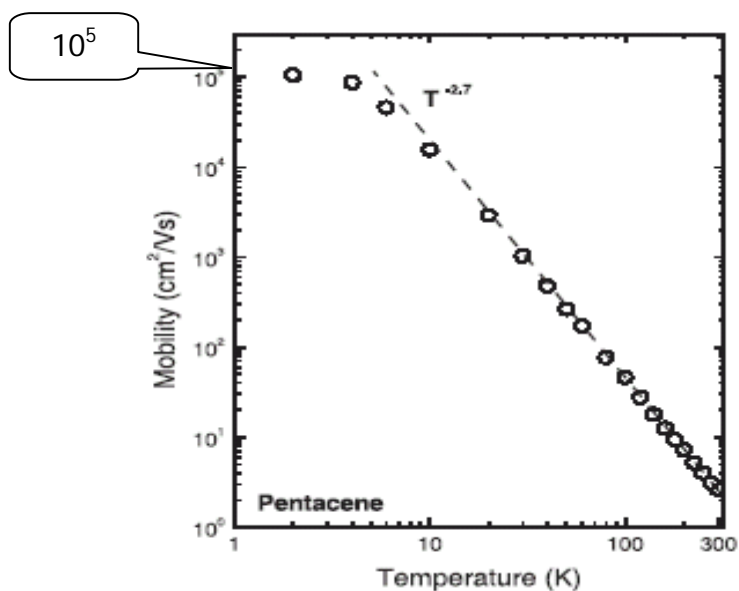


Fig. 4. Mobility vs. Temperature for Pantacene thin film transistor

Different film forming conditions for Pantacene transistor may affect its electrical characteristics (Fig. 5. and Fig. 6) majorly because the temperature influences the grain boundary energy barrier. Meanwhile, the gap of the lattice directly affect the energy barrier (Fig. 7)

so that the grain boundary mobility  $\mu_{GB}$  can be negatively affected by the barrier energy,  $\mu_{GB} = \mu_0 \exp(-E_B/kT)$ . In short, the characteristic of the crystal has the long term effect to the carriers' mobility and highly correlates the symmetry of the crystal lattice.

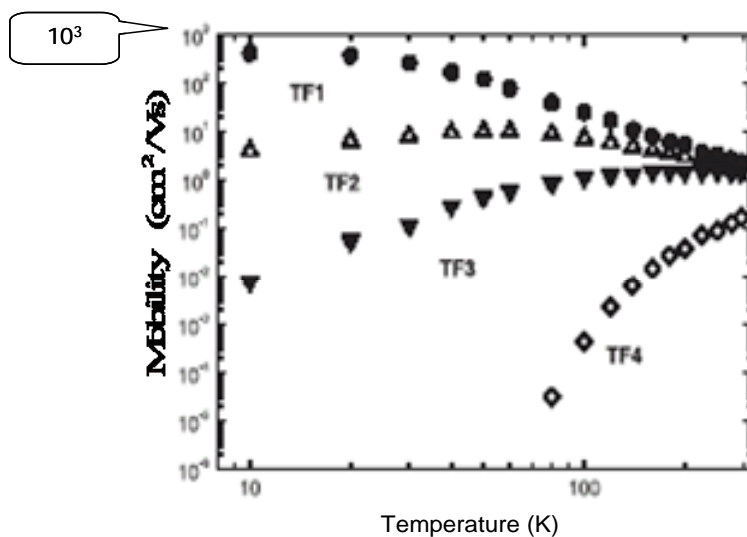


Fig. 5. The carrier-mobility in the Pantacene film transistors correlate with the temperature where TF4 (higher) to TF1 (lower) has been presented the film formation temperature on the substrate. (by J.H. Schön et al.)

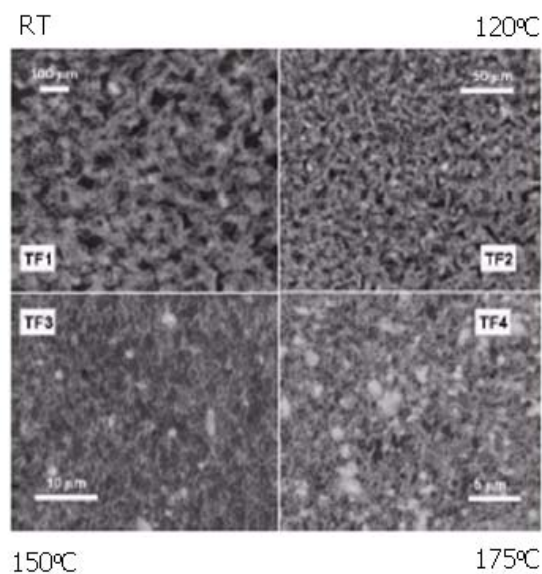


Fig. 6. The effect of the thin film formation temperature at different cons under crystallization of Pantacene (by J.H. Schön et al.)

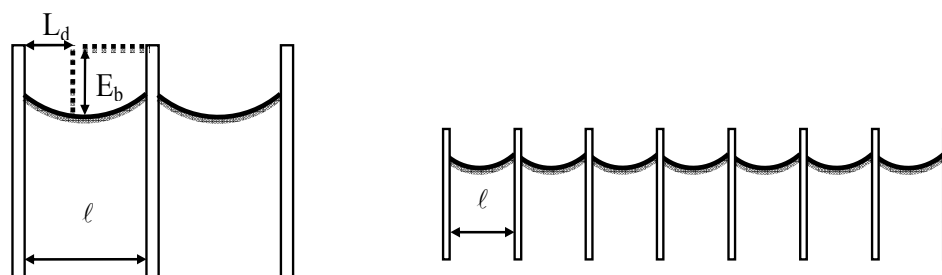


Fig. 7. The gap size of the lattice directly affects the grain barrier energy ( by G. Horowitz et al.)

In this report, if the thermal phonon scattering can be overcome, increasing the operation temperature can be applied to high carriers' mobility rate. This can be done by material molecular structure design or by lowering intra-molecular charge transport node or expand the  $\pi$  electron set of unstable domain. Additionally, planar molecular structural and symmetrical molecules will benefit the lattice stack to reduce the barrier energy so that be able to increase the carriers' mobility including phonon scattering effects dominated bulk mobility ( or called intragrain mobility)  $\mu_B$  and crystallized characteristic dominated mobility ( $\mu$ ) where  $\mu^{-1} = \mu_{GB}^{-1} + \mu_B^{-1}$ . The second type of organic thin-film transistors materials for the thiophene compounds is depicted in Fig. 8. This is a kind of molecular having structural extensibility, formed by Tatrathiphene, Polythiophene and containing various

derivative substituents. The standard type is Sexithiophene, and Octathiophene. However, the rate of mobility can only be from  $10^{-3} \text{ cm}^2/\text{Vs}$  to  $0.3 \text{ cm}^2/\text{Vs}$ . This serial compounds belongs to p-type organic semiconductor materials in majority [8-10]. The directional transmission of thiophenes is one of the hot topics for researches. Kinder et al. reported an article entitled "ordering and mobility in organic polymer carrier to thin film transistors" in 2004 [11] was an example to construct organic thin-film transistors by using of thiophene polymer. The result pointed out that the junction channel and the phenol molecular axial are related. If they oriented being in parallel, the carriers' mobility would be  $4.4 \times 10^{-3} \text{ cm}^2/\text{V-s}$ . But, if they oriented in vertical, the carriers' mobility would be  $1.1 \times 10^{-3} \text{ cm}^2/\text{V-s}$  of four times higher (Fig. 10.).

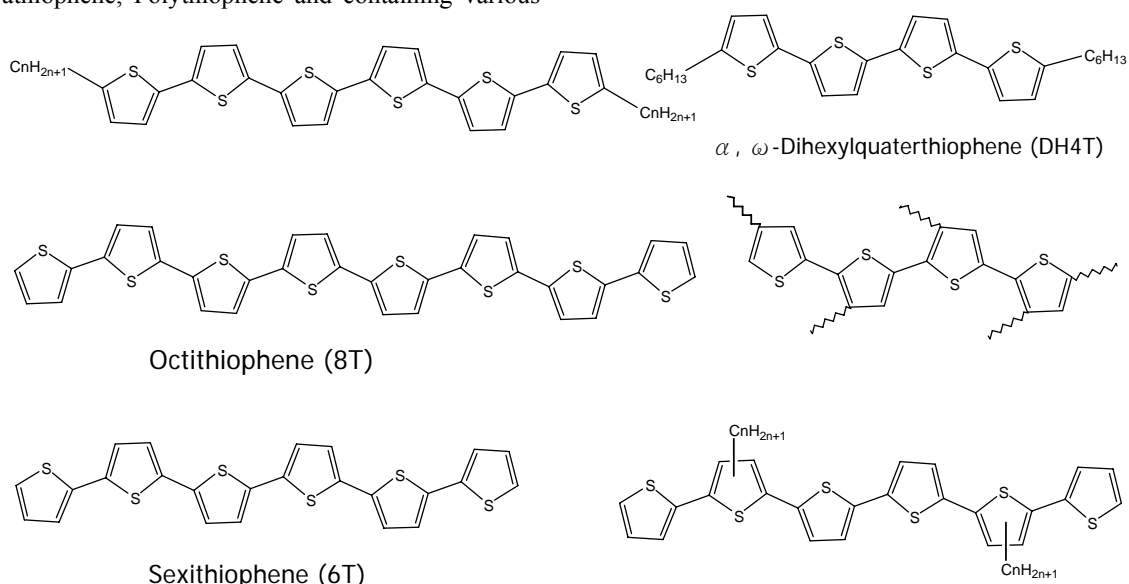


Fig. 8. The material structure of thiophene for organic thin-film transistor device

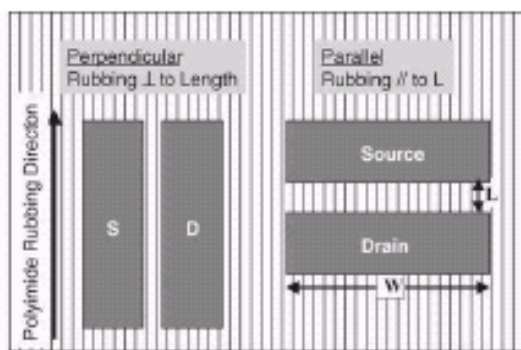


Fig. 9 The thiophene organic thin-film transistors (left: molecule axis perpendicular to the junction channel ; right: molecule axis parallel to the junction channel)

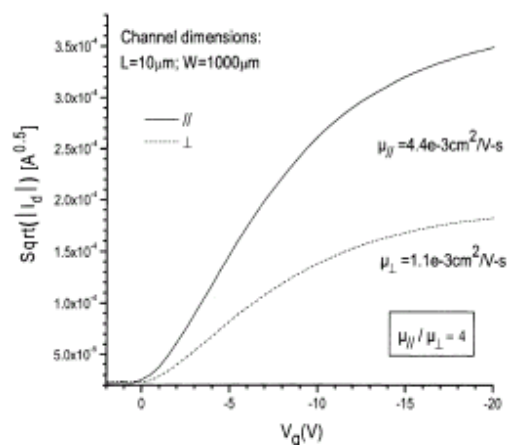


Fig. 10 The carriers' mobility of thiophene organic thin-film transistors

This article reported that the carriers moving parallel along the organic molecules  $\pi$  electronic un-localized space axis would be easier than the movement vertically along the axis. Therefore, the characteristic of carriers' mobility should be able to be justified by changing the distribution of the  $\pi$  electrons. The third type materials used for organic thin film transistor is containing electron donating group shown in Fig. 11. Those materials function as N-type. The carriers mobility is around  $1 \times 10^{-3} \text{ cm}^2/\text{Vs}$  but they have

the potential increasing the mobility if charge transfer can be strengthened. M. Iizuka et al. (200) used the compound of Tetramethyltetraselenafulvalene (TMTSF) as electron donor and Tetracyanoquinodimethane (TCNQ) as electron acceptor to design different molecular structure with strong charge transfer successfully achieved the multiple stacked organic thin film transistor where the current can be higher up to two orders (Fig. 12 and 13).

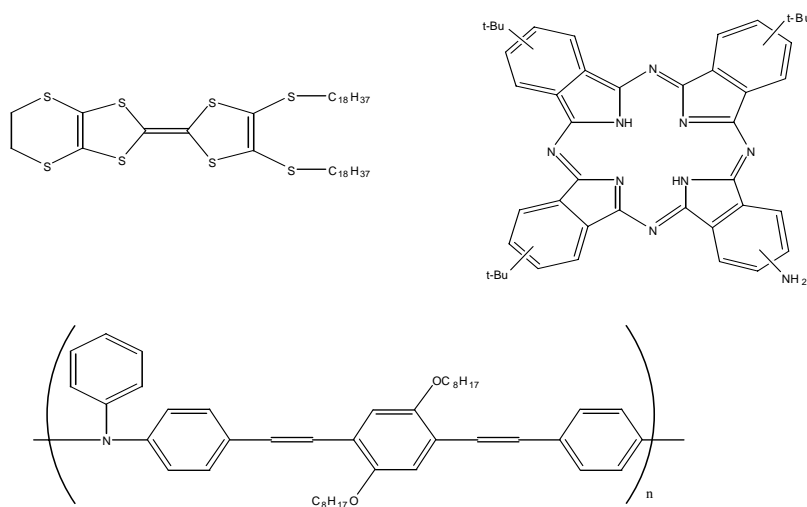


Fig. 11 Containing electron donating group compound for organic thin film transistor



The fourth type of thin film organic material is containing acceptor electron group structural compound shown in Fig. 14 [13, 14]. It functions as negative P-type material for the existing of withdrawing capability of the electron group in the

structure. So far, the carrier mobility is still very low approximately from  $1 \times 10^{-7}$  to  $1 \times 10^{-3}$   $\text{cm}^2/\text{Vs}$  but can be strengthened by charge transfer between the molecules up to two orders.

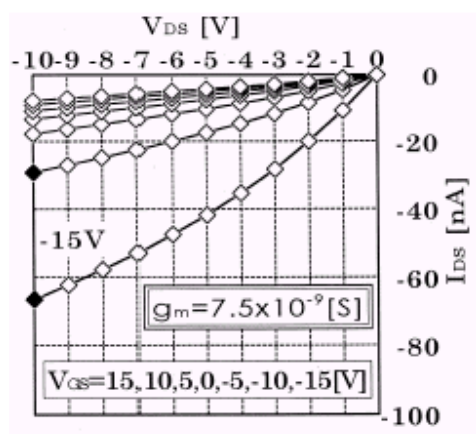


Fig. 12 I-V curve of single layer TMTSF thin film transistor (by M. Iizuka)

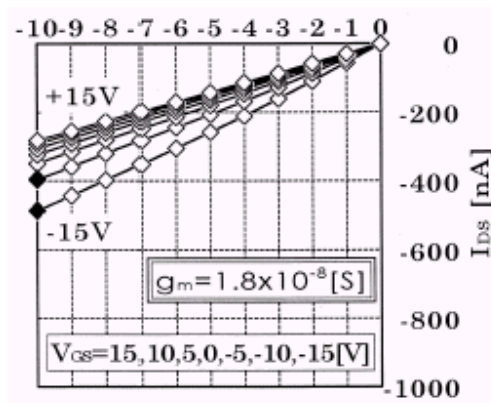


Fig. 13 I-V curve of TMTSF up-stacked TCNQ multilayer thin film transistor.

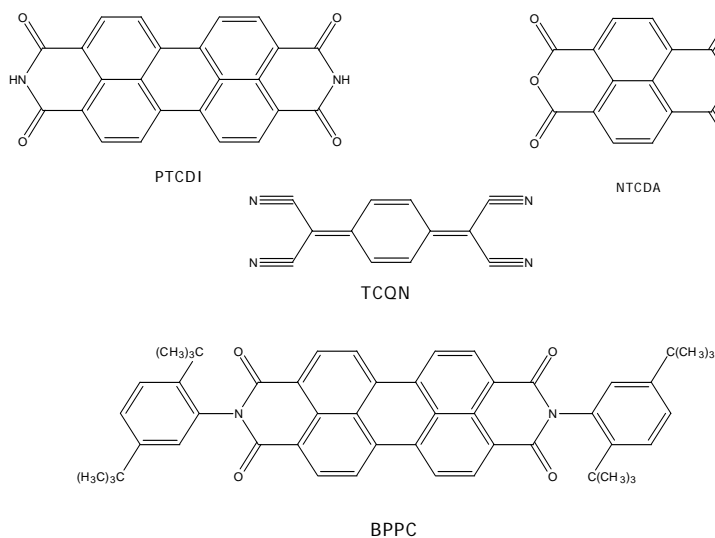


Fig. 14 Containing acceptor electron group structural compound for organic thin film transistor

The fifth class materials for organic thin-film transistors is metal complex compounds [15, 16]. This is a type of planar organic molecules.

Phthalocyanine is a standard type including CuPc,

NiPc, benzene with 16-flubendazolium copper and so on. They can function as both p-type and n-type with the carrier mobility rate from  $1 \times 10^{-5}$  to  $1 \times 10^{-7}$   $\text{cm}^2/\text{Vs}$ .

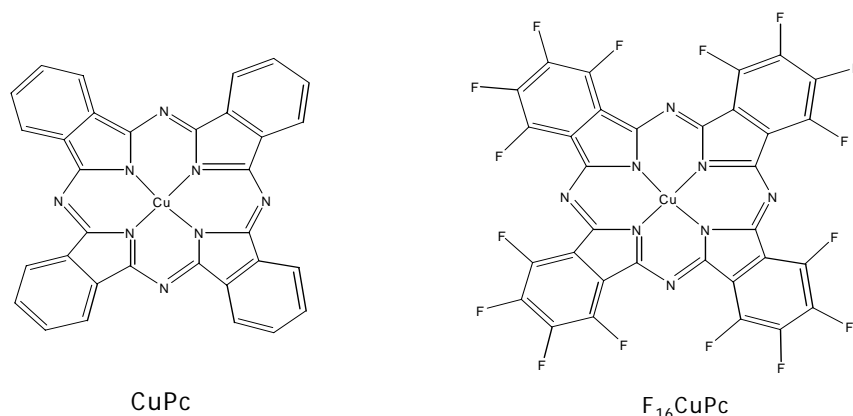


Fig. 15 Metal complex compounds used for organic thin film transistor

### III New Applications to OTFT

We have developed four derivatives of benzimidazole of new materials for thin film organic transistors such as 2-(naphtho[3,4]

imidazol-2-yl)quinoline (NIQ), 2-(benzimidazol-2-yl) quinoline (BIQ), 2-(naphtho [3,4] imidazol-2-yl) pyridine (NIP), 2-(benzimidazol-2-yl) pyridine (BIP). It is shown in Fig. 16.

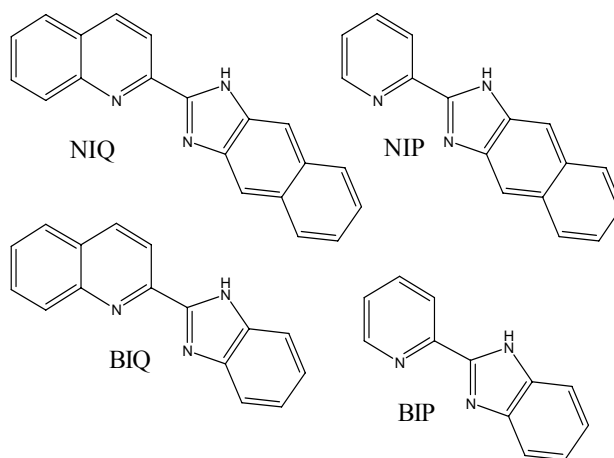


Fig. 16 New materials that we developed in our Lab for organic thin film transistor

Generally, the organic thin film design process with Bottom-Source-Drain Contact OTFT (BC-OTFT) has been used in our lab. The construction of the transistor device is to use silicon wafer being washed by soak water, acetone, distill water, isopropanol solvent and then put into sonic oscillators for 10 minutes. Taking distill water, hydrogen peroxide solution (30%), ammonia (29%) with the ratio 5:1:1 for lye and distill water, hydrogen peroxide solution (30%), hydrochloric acid (37%) ratio 5:1:1 for acid. Putting wafer in lye, acid respectively, and then put the

surpluses soaking ultrasound oscillators for ten minutes dried with nitrogen (N<sub>2</sub>) placing into vacuum drying oven 30 minutes (120°C). Complete the cleaning steps, it should be followed by six methyl two silicon nitrogen 1,1,1,3,3,3 - Hexamethyldisilazane, polyurethanes (MHDS spin) spin coating for the film. The electrodes can be deposited by thermal evaporation method. The width of the channel is 10mm and the length is 50μm. We should be very careful to avoid and check the shadow effect shown in Fig. 17

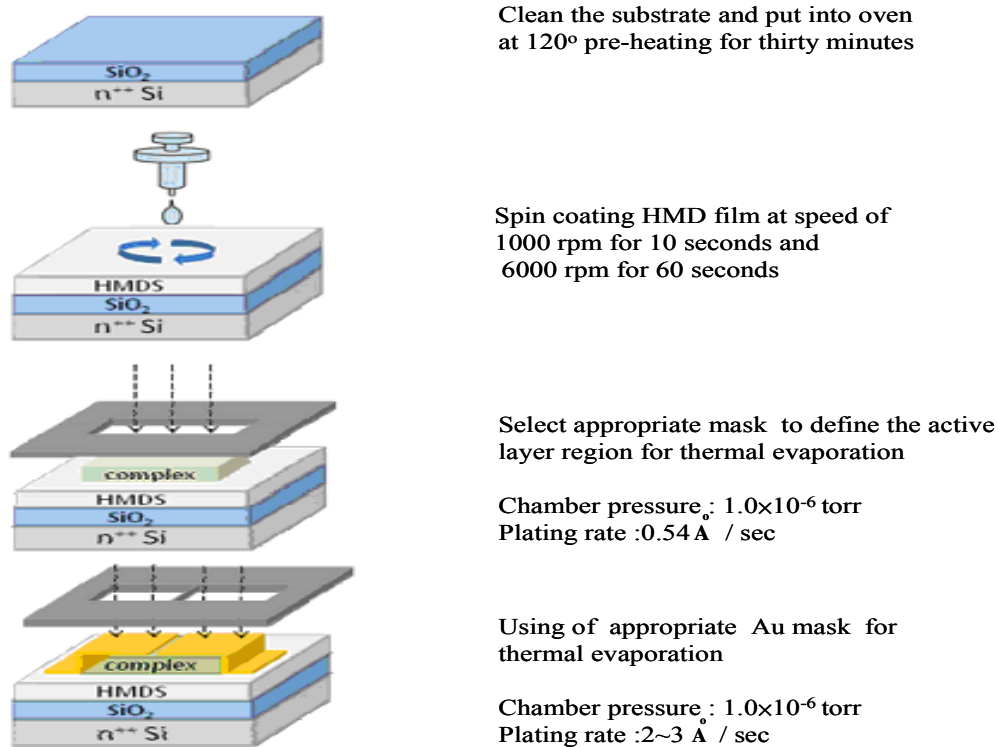


Fig. 17 Schematic drawing of the design process by using of Bottom-Source-Drain Contact OTFT

By using of the derivatives of benzimidazole as new materials for thin film organic transistors, both NIQ and BIQ molecules have internal hydrogen bonds to be as planar structural bonding and stacked as 3-D structure (Fig.18, 19). The derivatives of

benzimidazole of new materials observation of V-VIS (Fig. 20) has shown that the solidified derivatives of benzimidazole of molecules can have the effect of intermolecular charge transfer (ICT) for the effective stacking of  $\pi$  electron and  $\pi$  orbital.

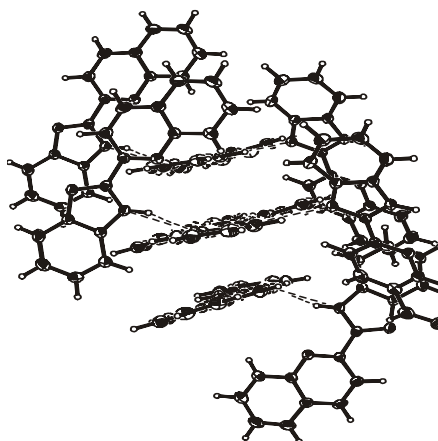


Fig. 18. 3-D stacking structure of BIQ

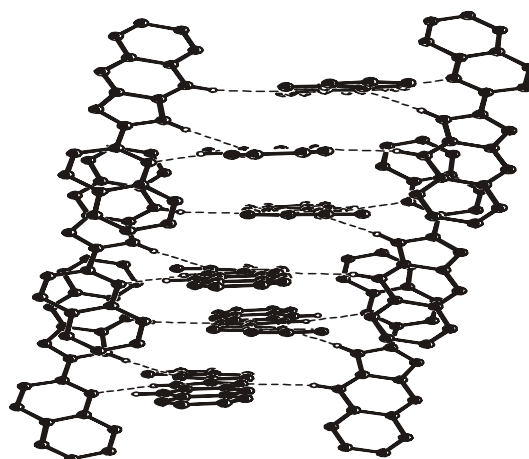


Fig. 19. 3-D stacking structure of NIQ

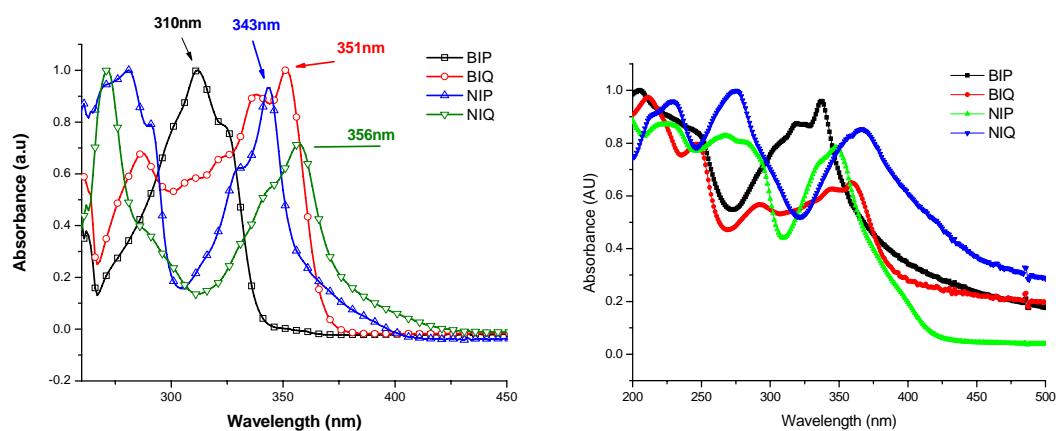


Fig. 20. The V-VIS spectrum for the derivatives of benzimidazole, left one is for solution sample and the right one is for thin film sample

Based upon the carrier mobility of organic materials is related to solidified molecules via the effect of intermolecular charge transfer (ICT) from stacking of  $\pi$  electron and  $\pi$  orbital, we further set up a reliable evaluation by checking the purity and size of grain for the new material of thin films. The optimized condition in our lab is to use the evaporation rate at 0.5

$\text{\AA}/\text{sec}$  for the thin film deposition thickness around 100 nm. We have observed those criteria are the best base for constructing the minimum roughness and most complete grain productions that can be key factor to affect the molecular characteristics and the carrier mobility. The SEM observations of all BIP, BIQ, NIP, NIQ thin films are depicted in Fig.21

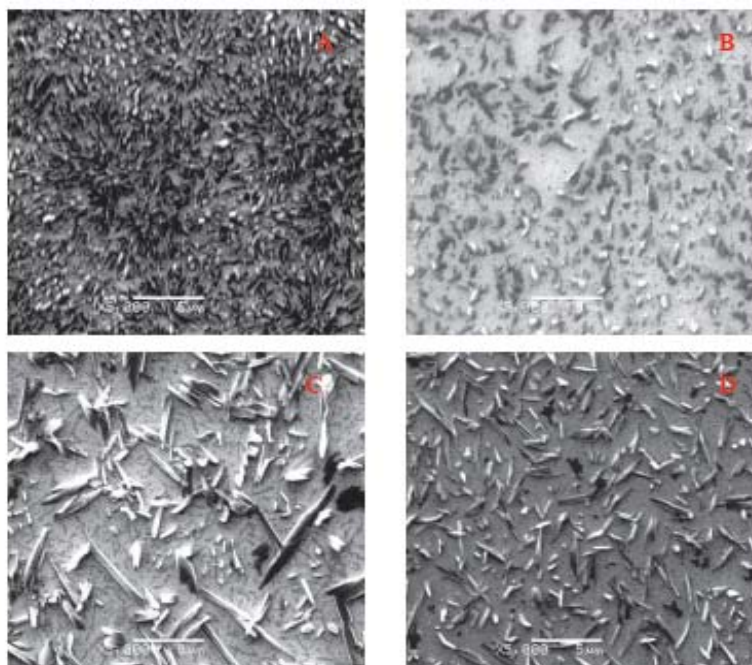


Fig. 21. SEM observation of (A) BIP device (B) BIQ device (C) NIP device (D) NIQ device with thickness of 100nm

Structure of metal-semiconductor-metal (MSM) has been used to anticipate the carrier mobility. The process is simple but effective by using of ITO thin film as electrode to predict the carrier mobility. The detail structure is like a sandwich structure as Glass/ITO/organic semiconductor/u (shown in Fig. 22). The sample thickness is 100 nm. This structure is thus

also similar to the Schottky diode. When the reverse bias is added in the structure the depletion area will be increased. Meanwhile, if bias is added unto another side, due to the ohm contact the current should be increased very quick and cause the diode being breakdown.

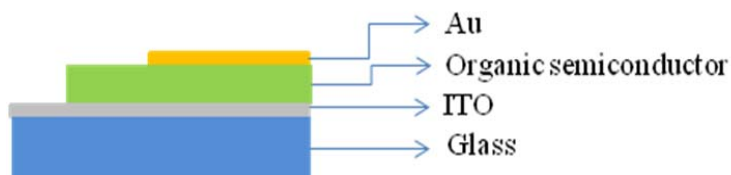


Fig. 22. Device structure of metal-Semiconductor-metal

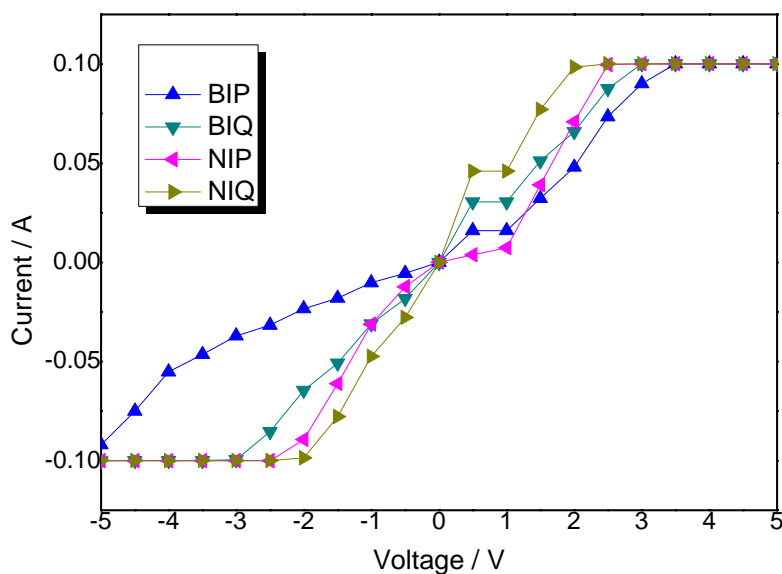


Fig. 23. I-V curve of four derivatives of benzimidazole using MSM device

From the I-V curve for MSM in Fig 23, it is revealed the resistance should be as  $BIP > BIQ > NIP > NIQ$ . Therefore, we can predict the carrier mobility could be as  $NIQ > NIP > BIQ > BIP$  which is fit our experimental results. The charge

transfer in derivatives of benzimidazole (BIP, BIQ, NIP and NIQ) can strengthen the carrier mobility. The I-V curves of derivatives of benzimidazole (BIP, BIQ, NIP and NIQ) the thin film transistor and the characteristics of transconductance are depicted in Fig. 24 ~27.

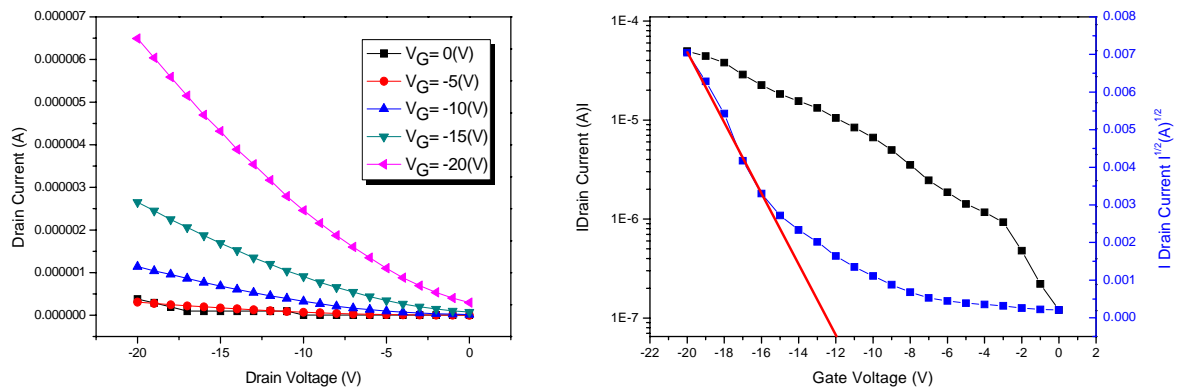


Fig. 24. I-V (left) and transconductance (right) curves of BIP thin film transistor

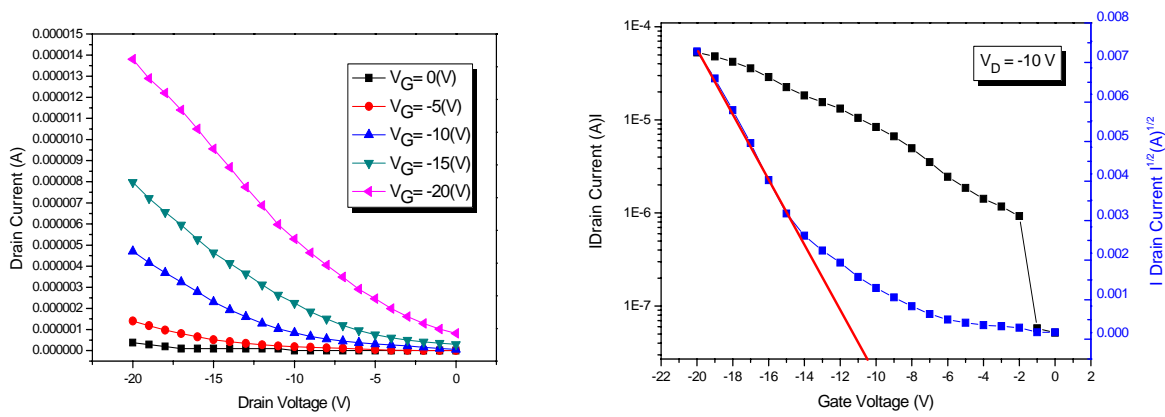


Fig. 25. I-V (left) and transconductance (right) curve of BIQ thin film transistor

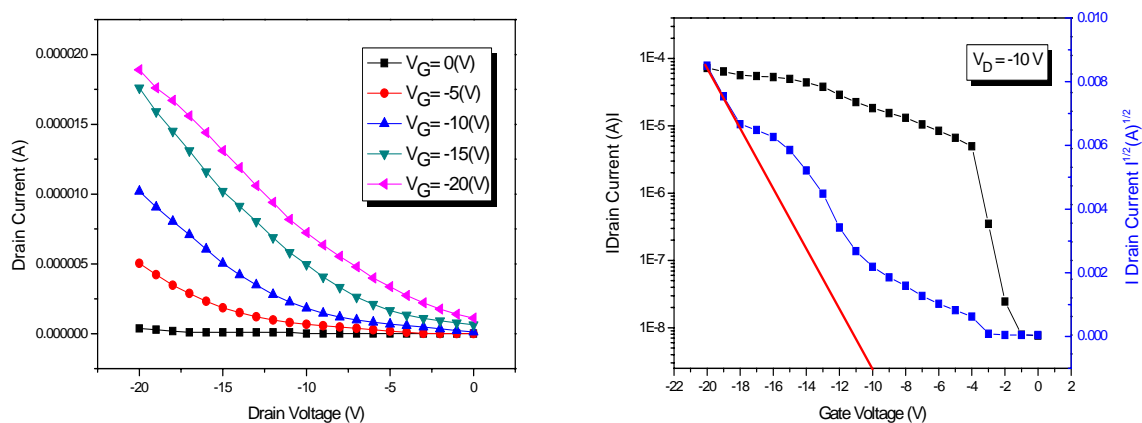


Fig. 26. I-V (left) and transconductance (right) curve of NIP thin film transistor

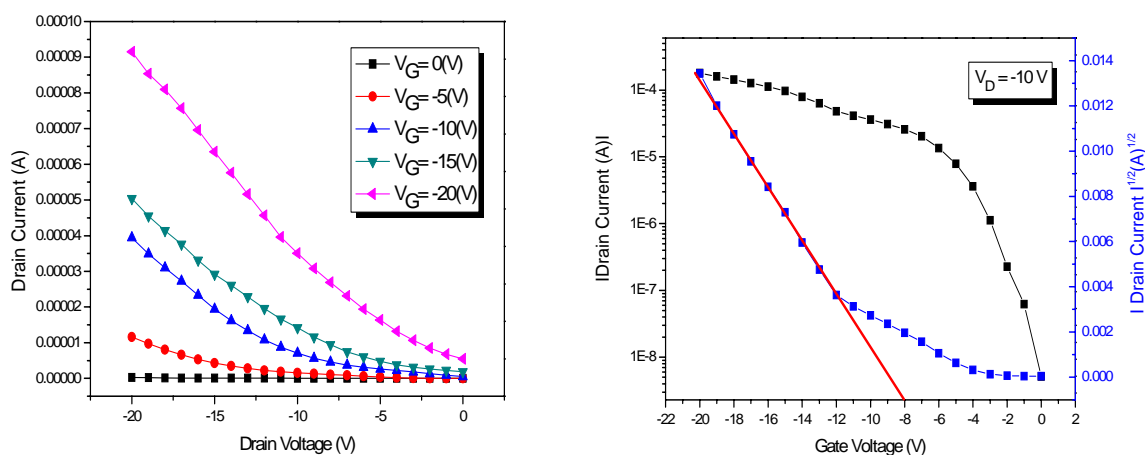


Fig. 27. I-V (left) and transconductance (right) curve of NIP thin film transistor

The observation has shown that the derivatives of benzimidazole (BIP, BIQ, NIP and NIQ) functions all as p-type material to sense the accumulation of holes

and transport enhancement mode with carrier mobility rate ( $\mu_{FET}$ ) between 0.117 to 0.336  $\text{cm}^2/\text{V}\cdot\text{s}$ .

Table I.  
Electrical characteristics under the atmosphere of the OTFT made by BIP, BIQ, NIP and NIQ

Materials	$\mu_{FET}$ ( $\text{cm}^2/\text{V}\cdot\text{s}$ )	$V_{th}$ (V)	$I_{ON}/I_{OFF}$ ratio	S (V/decade)
BIP	0.117	-12	$4.11 \times 10^2$	7.653
BIQ	0.129	-10.5	$1.02 \times 10^3$	6.645
NIP	0.230	-10	$9.45 \times 10^3$	5.031
NIQ	0.336	-8	$3.50 \times 10^4$	4.401

From the experimental results, it is observed that when we use the idea of device of MSM to predict the sequence of carrier mobility, we can get consistent outcomes. In our new derivatives of benzimidazole compounds including pyridine ring and imidazole ring, for those two aromatic groups we observe an interesting phenomena, when more aromatic rings, pyridine ring (p) and imidazole ring (I) involved, the carrier mobility increased as NIQ (2P+3I) > NIP (1P+3I) > BIQ (2P+2I) > BIP (1P+2I). In NIP (1P+3I) and BIQ (2P+2I), they have four aromatic rings.

The carrier mobility of NIP (1P+3I),  $\mu_{FET} = 0.230 \text{ cm}^2/\text{V}\cdot\text{s}$ , which is about two times more than BIQ (2P+2I),  $\mu_{FET} = 0.129 \text{ cm}^2/\text{V}\cdot\text{s}$  because the connection of pyridine ring and imidazole ring are single bond which may decrease the effective capability from the  $\pi$ - $\pi$  conjugation of aromatic. Therefore, since the NIP (1P+3I), located at imidazole ring side, there has three aromatic ring in which effective capability of  $\pi$ - $\pi$  conjugation of aromatic is larger than BIQ (2+2) two aromatic ring. Furthermore, if the numbers of pyridine ring or imidazole ring is less, the carrier

mobility can't increase lot like BIQ (2P+2I)  $\mu_{\text{FET}} = 0.129 \text{ cm}^2/\text{V}_s$ , comparatively, just be a little bit higher than BIP (1P+2I)  $\mu_{\text{FET}} = 0.117$  in about  $0.012 \text{ cm}^2/\text{V}_s$ . When the number of pyridine ring or imidazole ring is larger, the carrier mobility can increase a lot. However, if the number of pyridine of NIQ (2P+3I)  $\mu_{\text{FET}} = 0.336 \text{ cm}^2/\text{V}_s$  in compare with NIP (1P+3I)  $\mu_{\text{FET}} = 0.23$  appears higher, for instance  $0.106 \text{ cm}^2/\text{V}_s$  which is about 1.5 times more than the carrier mobility of NIP. As the carrier mobility of NIQ is  $0.336 \text{ cm}^2/\text{V}_s$ , the materials are good for OTFT. Conclusively, we can adjust the number of pyridine ring or imidazole ring to increase the carrier mobility.

### References

- [1]. Y.-Y. Lin, D.J. Gundlach, S.F. Nelson, and T.N. Jackson, IEEE Transactions on Electron Devices, Vol 44, pp.1325-1331, AUG. 1997.
- [2]. A. Tsumura, H. Koezuka, T. Ando, Appl. Phys. Lett. 49(1986) 1210.
- [3]. J.H. Schon, Ch. Kloc, B.Batlogg, Organic Electronics, 1(2000) 57.
- [4]. S.M. Sze, Physics of Semiconductor Devices, Wiley, New York, 1981, P.442.
- [5]. J.H. Schon, S. Berg, Ch. Kloc, B.Batlogg, Proceedings of the Materials Research Society Fall Meeting, Boston, 1999, Vol.599.
- [6]. Y.-Y. Lin, D.J. Gundlach, S.F. Nelson, and T.N. Jackson, IEEE Electron Devices Letters, Vol 18, pp.606-608, DEC. 1997.
- [7]. M.H. Choo, W.S. Hong, Seongil Im, Thin solid Film 420-421 (2002) 492.
- [8]. Francis Garnier, Gilles Horowitz, Denis Fichou, Abderrahim Yassar, Synthetic Metals, 81, pp.163-171, 1996.
- [9]. Luisa Torsi, Microelectronics Reliability 40 (2000) 779.
- [10]. D. Fichou, F. Demanze, G. Horowitz, R.Hajlaoui, M Constant, F. Garnier, Synthetic Metals 85 (1997) 1309.
- [11]. L. Kinder, J. Kanicki, P. Petroff, Synthetic Metals 146 (2004) 181.
- [12]. M. Iizuka, Y. Shiratori, S. Kuniyoshi, K. Kudo, K. Tanaka, Appl. Surface Science, 130, pp. 914-918. 2000
- [13]. T. Suga, M. Iizuka, S. Kuniyoshi, K. Kudo, K. Tanaka, Synthetic Metals 102 (1999) 1050.
- [14]. L. Torsi, A. Dodabalapur, N. Cioffi, L. Sabbatini, P.G. Zamboni, Sensors and Actuators B 77 (2001) 7.
- [15]. J. Yuan, J. Zhang, J. Wang, D. Yan, W. Xu, Thin Solid Film 450 (2004) 316.
- [16]. K. Kudo, M. Iizuka, S. Kuniyoshi, K. Tanaka, Thin Solid Films 393 (2001) 362.
- [17]. C. D. Dimitrakopoulos and P. R. L. Malenfant, "Organic thin film transistors for large area electronics" Adv. Mater., 2002, 14, 99-117.

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## Effect of Regular Aerobic Exercises on Behavioral, Cognitive and Psychological Response in Patients with Attention Deficit-Hyperactivity Disorder

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**Abstract:** background and purpose: Attention Deficit-Hyperactivity Disorder (ADHD) is a common behavioral disorder started in childhood and is characterized by one or a combination of three behaviors, named hyperactivity, inattentiveness and impulsiveness. The aim of this study was to find out the value of aerobic exercises on symptoms of ADHD. Patients, Materials and Methods: This study included 84 students diagnosed as having ADHD. Their age ranged from eleven to sixteen years. The students were randomly divided into two equal groups. The exercise group received ten weeks aerobic exercises program included upper limb, lower limb, trunk and neck exercises as well as running three sessions per week (In the first four weeks the session lasted for about 40 minutes and in the last six weeks the session extended to be 50 minutes). Behavior Rating scale was used to assess the students behavior before starting and after the end of ten weeks of the exercise program. Results: Results of the exercise group revealed a significant improvement in three of the five items involved in the scale (attention, motor skills and academic and classroom behavior) with  $p < 0.05$  while there was no improvement in the the control group ( $p > 0.05$ ). Conclusion: It could be concluded that regular aerobic exercises program has a positive effect in improving symptoms of ADHD.

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**Key words:** Aerobic exercise-Cognition-Behavior-Attention Deficit Hyperactivity Disorder.

### 1. Introduction

Attention deficiency hyperactivity disorder (ADHD) is one of the most common neurobehavioral disorders. Patients with ADHD have trouble paying attention, controlling impulsive behaviors (may act without thinking about what the result will be), and in some cases are overly active<sup>1</sup>.

ADHD is characterized by a persistent pattern of impulsiveness and inattention, with or without a component of hyperactivity<sup>2</sup>. This disease is diagnosed twice as frequently in boys as in girls. As they mature, adolescent and adult with ADHD are likely to develop coping mechanisms to compensate for their impairment<sup>3</sup>. Baumgardner et al.<sup>4</sup>, studied patients with ADHD and discovered morphological asymmetry in the caudate nucleus of the corpus striatum. The caudate nucleus is mainly dopaminergic and the asymmetry weakens the reception of the dopamine signal by this structure. Thus, ADHD is believed to be caused by a slight abnormality in the brain caused either genetically or by prenatal complications<sup>5</sup>.

Differences in distributed cortical and sub-cortical networks that support basic cognitive functions (such as attention, motor control and self-regulation) have been proposed as a neural basis for ADHD<sup>6</sup>. Reduced volumes within-hemisphere (corticocortical) and callosal white matter have been noted in ADHD.

These suggested the possibility that the symptoms of ADHD may be related to impaired interactions within brain networks, rather than impaired function of specialized cortical regions<sup>7</sup>.

ADHD is characterized by reductions in regions of the corpus callosum, frontal lobes, basal ganglia, and cerebellum. These networks involve input-output processing of attention, including alerting and executive functions. Deficits in attention, information processing, alerting, orienting and working memory may be mediated primarily in the prefrontal cortex<sup>8</sup>. Deficient connectivity between hemispheres induced state of over-connectivity within and between frontal hemispheres. Patients always seem to be in motion and may move around touching or playing with whatever is around, or talk continually. Some patients wiggle their feet or tap their fingers<sup>9</sup>.

Thus, self-perceptions of patients with ADHD are low with regards to their feelings on behavior, ability to get along with others and to succeed in school. Self-perceptions are poor and over time they become increasingly more doubtful about their ability to cope with academic and social issues during adolescence<sup>10,11</sup>. A growing number of practitioners and researchers suggest that a regular program of physical exercise may lead to lower stimulant doses for those who still need medication<sup>12</sup>.

Previous studies<sup>13,14</sup> suggested that the exercise regimens can help brains remain vital and healthy well. Exercise almost immediately elevates dopamine and nor-epinephrine and keeps them up for a period of time. It also helps to still the impulsivity and still the cravings for immediate gratification as it works to wake up the executive function of the frontal cortex, which in turn allows for delay, better choices, a bit more time to evaluate consequences<sup>11</sup>. The purpose of this study was to investigate the effect of regular aerobic exercises on behavioral, cognitive and psychological problems related to ADHD.

## 2. Patients and Methods:

Eighty four students from both gender (54 boys 30 girls) with ADHD participated in this study. Their age ranged from eleven to sixteen years. They were recruited from Special Needs Schools in Riyadh (Riyadh El Salehen, El Maaref and El Rawda Schools). All patients were functionally independent, could understand well, follow orders and cooperative. The students were excluded if they had;(1) Medical or systemic problems such as hypertension, hypotension, diabetes mellitus,(2)Musculoskeletal deformities (scoliosis, kyphosis , pes cavas),(3) Neurological problems (sensory or motor deficit),(4)Orthopedic problems (including past history of trauma before application of the study at least two months),(5) Rheumatic fever,(6)Obesity.

### Instrumentation:

The Behavior Rating Scale is a modified version of Conner's Rating Scale<sup>15</sup>. The scale has been validated for screening and assessing behavioral, cognitive and psychological problems related to ADHD<sup>16</sup>. The scale provides a reliable, accurate and relatively brief measure of perception of disruptive behavior<sup>17</sup>. It consists of 25-behavior related questions, subdivided into categories for attention, motor skills, task orientation, emotional and oppositional behavior and academic and classroom behavior. The higher the score, the better behaved the student.

### Procedures:

Prior to commencement of the exercise program, the students, teachers and the parents attended an information session. The aim and the procedures of the study were explained in details to the school manager, parents and teachers. Detailed explanation about exercise program was taught to the students before starting the study. Ethical approval was received from the schools administration to participate the students in the study. The students were assigned randomly into two equal groups (control and exercise). The exercise group received

exercise program for ten weeks as three sessions per week.

The control group did not receive any designed exercise program.

### Evaluation protocol:

The students were evaluated using the Behavior Rating Scale Pre and post exercise program. The Scale was filled from the teachers by the interviewers. During the study, the teachers were asked to observe the students for any changes, either positive or negative, in the school behavior, psychological statement and class concentration. The teachers helped in taking the suggestions of the students, and give any ideas about the application of the session.

### Exercise protocol:

The moderate-intensity exercise program<sup>18</sup> was applied three sessions per week. Exercise program included upper limb, lower limb, trunk and neck aerobic exercises in addition to free running. Ten repetition for each exercise increase with time, rest period two minutes between every 15 minutes.

- **In the first four weeks** the session lasted for about 40 minutes, ten minutes preparation and warm up, 20 minutes aerobic exercise and five minutes with walking between exercises, at the end five minutes walking around the school with each other for cooling down.

- **The following six weeks**, the session lasted for about 50 minutes. Ten minutes warm up, 30 minutes aerobic exercises, five minutes walking around the school building, and five minutes slow walking and stretching for cooling down. The cool down phase include relaxation exercises. The aim of the relaxation exercises was to reduce the student's heart rates back to or close to resting levels.

- **Home program**, Parents were instructed to start home program for the study group from the 6<sup>th</sup> week and continuous to the 10<sup>th</sup> week. The home program included walking half an hour out-door in the week-end.

### Statistical analysis:

Intra-rater reliability between interviewers was conducted for the first 20 interviews. To determine any significant changes in behavior, all variables were subjected to analysis of variance (ANOVA) with repeated measures. Tukey's post-hoc tests were used to determine significant differences. The level of significance was set at  $p < 0.05$ .

### 3. Results:

The students's physical characteristics were: age; study group (13.9±1.6), control group (13.8±1.7

years), weight; study group (42.10±4.2 kg.), control group (41.11±4.1 kg.) and height; study group (149.46±3.8 cm.), control group (152.33±4.1 cm.). There were no significant differences between both groups related to general characteristics.

Statistical analysis showed that there was an improvement in the Behavior Rating Scale scores for students in the exercise group while there was no significant changes ( $P > 0.05$ ) in behavior, cognitive or psychological problems in the control group who did not receive any exercise program (Table 1&2).

**Table (1): Comparison between the mean values of attention, motor skills , task orientation, emotional and oppositional behavior, academic and classroom behavior before and after exercise program in the study group.**

Variable	Before Mean± SD	After Mean± SD	P-value
Attention	4.89 ± 1.25	8.46 ± 3.61	0.001*
Motor skills	4.11 ± 5.94	7.97 ± 3.96	0.01*
Task orientation	0.41 ± 0.87	0.35 ± 0.61	0.14
Emotional &Oppositional behavior	3.94 ± 2.61	2.71 ± 3.14	0.87
Academic& classroom behavior	22.24 ± 4.26	30.24 ± 7.27	0.008*

Significance\* <0.05. SD: Standard deviation.

**Table (1): Comparison between the mean values of attention, motor skills , task orientation, emotional and oppositional behavior, academic and classroom behavior before and after exercise program in the control group.**

variable	Before Mean± SD	After Mean± SD	P-value
Attention	4.91 ± 7.68	5.62 ± 7.15	0.56
Motor skills	5.36 ± 5.95	4.95 ± 6.07	0.08
Task orientation	0.32 ± 2.86	0.82 ± 2.82	0.87
Emotional &Oppositional behavior	3.36 ± 2.54	3.45 ± 2.68	0.23
Academic& classroom behavior	22.27 ± 6.91	23.00 ± 5.83	0.21

Significance\* <0.05. SD: Standard deviation.

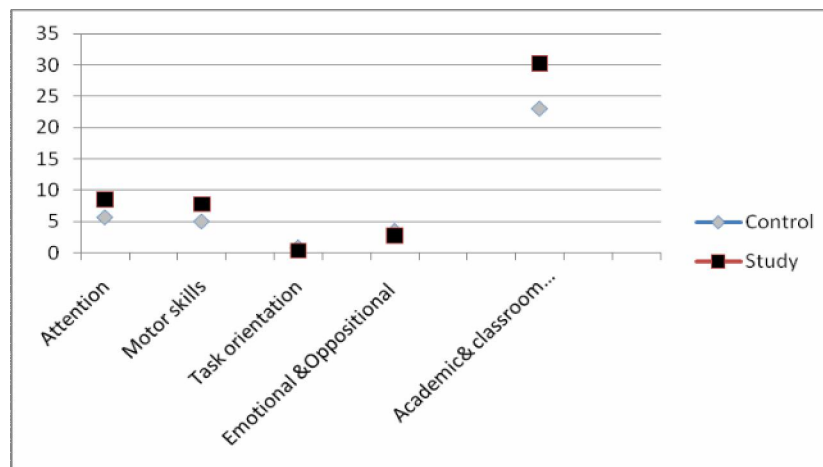
There were no significant differences between both groups at the beginning of the study ( $P > 0.05$ ). After cessation of the exercise program for the study group, the following behavior categories were significantly improved in the study group compared to the control group: attention ( $P = 0.005$ ), motor skills

( $P = 0.04$ ) and academic and classroom behavior ( $P = 0.001$ ). Task orientation and emotional and oppositional behavior were not significantly altered after the ten weeks of exercise program with  $P > 0.05$  (Table 3 & Fig.1).

**Table (3) .Comparisons between mean values of Behavior Rating Scale after exercise at the end of the study of both groups (study and control).**

variable	Study group Mean± SD	Control group Mean± SD	P-value
Attention	8.46 ± 3.61	5.62 ± 7.15	0.005*
Motor skills	7.97 ± 3.96	4.95 ± 6.07	0.04*
Task orientation	0.35 ± 0.61	0.82 ± 2.82	0.78
Emotional &Oppositional behavior	2.71 ± 3.14	3.45 ± 2.68	0.421
Academic& classroom behavior	30.24 ± 7.27	23.00 ± 5.83	0.001*

Significance\* <0.05. SD: Standard deviation.



**Fig.1. Mean values of Behavior Rating Scale after exercise program in the study and control groups.**

#### 4. Discussion:

Researchers have noted the positive role of exercises in the treatment of anxiety<sup>19,20</sup>, depression, stress<sup>21</sup>, and enhancing general mood state<sup>22</sup>. Based on these previous researches, it was hypothesized that an exercise program would significantly improved the behavior in patients with ADHD compared with that of non- exercised controls. The present study examined the alterations in the behavior, cognition and psychological problems of students with ADHD after ten weeks moderate intensity exercise program. There were significant improvements of the study group in three items of five from Behavior Rating Scale (attention, motor skills and academic and classroom behavior).

The results of the present study agree with the findings of Maddigan et al.<sup>23</sup>, who concluded that exercise therapy would be effective in reducing symptoms or medication dose in attention deficit hyperactivity disorder in school age children who were already stabilized on medication. The improvement in inattentiveness was also supported by Went<sup>24</sup> who stated that there was positive shift observed in concentration among subjects with ADHD after they participated in therapeutic movement therapy. The results showed positive improvements on working speed, social and behavioral problems.

Possible explanation for improved behavior may therefore be that the exercise sessions encouraged co-operation in group situations and fostered tolerance and acceptance<sup>5</sup>. Hoza et al.<sup>26</sup> reported that friendship forming in ADHD students is associated with improved behavior. The social interactions in the exercise group during the exercise sessions, as well as during school may therefore resulted in improved behavior. Most student tend to be more active and attentive at the end of the program. The student in the

exercise group stated that they enjoyed this activity and the parents found this to be of benefit for them.

Taylor and Kuo<sup>11</sup> reported that exercise helps to heighten the response to stressors, that is, the students became less stressed to the same stressors when in a fit condition. The researcher also mentioned that, mood is made better by raising the levels of neurotransmitters that works as antidepressants (dopamine, norepinephrine, and serotonin). All of these neurotransmitters get jacked up by exercise. Exercise reenergizes depressed brains to do its job of adapting to environment.

The results of the present study agree also with the results of Tantillo et al<sup>27</sup>, who suggested that exercise is beneficial to subjects with ADHD. However, the results disagree with a few studies which have been published in this area and have either been case reports of only one or few subjects<sup>28-30</sup>, or failed to test implement valid behavioral measures<sup>31</sup>.

Coe et al.,<sup>32</sup> suggested that exercises could play a significant role in the classroom by improving attention and learning problems. Miller et al.,<sup>33</sup> found that schools that offered a more intense physical activity program tended to have pupils who demonstrated higher concentration levels. In addition, these pupils demonstrated improved reading, writing and mathematics scores. This overall positive effect on academic performance was also found when the physical activity time reduced time for academic teaching.

Dwyer et al.,<sup>34</sup> studied Physical fitness and academic achievement in sixth and seventh grade students and their study confirmed that physical fitness is generally associated with improvement of academic performance in elementary school. Aerobic fitness and behavior test scores were associated with achievement of reading and mathematics. The authors regarded these improvements in academic

achievements to the effect of exercises on behavior including attention and concentration. The study concluded also that student's physical competency improves self-esteem perceptions and emotions.

### 5. Conclusion:

It was found that ten weeks exercise program as three times per week improved behavior of students with ADHD. So, exercises may be considered as an additional treatment required for improving the behavior in patients with ADHD.

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### 6. References:

- 1- American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders: DSM-IV-TR. Washington: fourth ed., Am Psych Assoc, 2000.
- 2- Burt Sa, Krueger RF, McGue M, Iacono W. Parent-child Conflict and the comorbidity among childhood externalization disorders. *Arch. Gen. Psychiatr* 2003; 60:505-13.
- 3- Bauermeister, J., Shrout, P., Chávez, L., Rubio-Stipec, M., Ramírez, R., Padilla, L., et al. ADHD and gender: are risks and sequela of ADHD the same for boys and girls. *J Child Psychology & Psychiatry* 2007; 48(8): 831-39.
- 4- Baumgardner T, Singer H, Denckla M, Rubin M, Abrams M, Colli M, Reiss A. Corpus callosum morphology in children with Tourette syndrome and attention deficit hyperactivity disorder. *Neurology* 1996;47: 477-82.
- 5- Braun JM, Kahn RS, Froehlich T, Auinger P, Lanphear BP. Exposures to environmental toxicants and attention deficit hyperactivity disorder in U.S. children. *Environ. Health Perspect* 2006; 114 (12): 1904-9.
- 6- Semrud-Clikeman M, Filipek PA, Biederman J, Steingard R, Kennedy D, Renshaw P, Bekken K. Attention-deficit hyperactivity disorder: Magnetic resonance imaging morphometric analysis of the corpus callosum. Solanto MV. Dopamine dysfunction in AD/HD: integrating clinical and basic neuroscience research. *Behavioural Brain Research* 2002; 130: 65-71.
- 7- Paus T, Zijdenbos A, Worsley K, Collins DL, Blumenthal J, Giedd JN, Rapoport JL, Evans AC. Structural Maturation of Neural Pathways in Children and Adolescents: In Vivo Study. *Science* 1999; 283: 1908-11.
- 8- Voller KS, Heilman KM. Motor impersistence in children with attention deficit hyperactivity disorder: evidence for right hemisphere dysfunction. *Ann. Neurol.* 1998; 24:323-31.
- 9- Cantwell DP. Attention deficit disorder: A review of the past 10 years. *J. Am. Acad child Adolesc psychiatry* 1995; 45:978-87.
- 10- Yadid G, Overstreet DH, Zangen A. Limbic dopaminergic adaptation to stressful stimulus in a rat model of depression. *Brain Res* 2001; 896:43-7.
- 11- Taylor AF, Kuo FE. Children With Attention Deficits Concentrate Better After Walk in the Park. *J. Attention Disorders* 2008; 23:36-56.
- 12- Durston S, Hulshoff PHE, Schnack HG, Buitelaar JK, Steenhuis MP, Minderaa RB, Kahn RS, van Engeland H: Magnetic resonance imaging of boys with attention-deficit/hyperactivity disorder and their unaffected siblings. *J Am Acad Child Adolesc Psychiatry* 2004; 55: 332-340.
- 13- Ward J. The Student's Guide to Cognitive Neuroscience, Psychology Press 2006; 63: 65-73.
- 14- Wood D. Habituation in Stentor produced by mechanoreceptor channel modification. *J Neuroscience* 1999; 8: 2254-64.
- 15- Conners CK, Sitarenios G, Parker JD, Epstein JN. The revised Conners rating Scale: factor structure, reliability and criterion validity. *J. Abnorm. Child psycho.* 1998; 26:257-68.
- 16- Fisher M, Newby RF. Assessment of stimulant response in ADHD children using a refined multimethod clinical protocol (special issue on child psychopharmacology). *J. Clin. Child Psychol.* 1991; 20: 232-44.
- 17- Horm WF, Lalongo N, Popovich S, Peradotto D. behavioural parent training and cognitive-behavioral self control therapy with ADHD children: Comparative and combined effects. *J. Clin. Child Psychol.* 1987; 16:57-68.
- 18- Mckune AJ, Pautz J, Lombard J. Behavioral response to exercise in children with attention-deficit hyperactivity disorder. *Sports Medicine* 2003; 2:17-21.
- 19- Norris R, Carroll D, Cochrane R. The effect of physical activity and exercise training on psychological stress and well-being in an adolescent population. *J Psychosom Res* 1992; 36:55-65.
- 20- Paluska SA, Schwenk TL. Physical activity and mental health: Current concepts. *Sports Med* 2000; 29:167-80.
- 21- Raglin JS, Morgan WP. Influence of exercise and quiet rest on state of anxiety and blood pressure. *Med Sci Sports Exerc* 1987; 19:456-83.

- 22- Potgieter JR. Sport Psychology: Theory and practice, Stellenbosch: Institute for Sport and Movement Studies, University of Stellenbosch, 1997.
- 23- Maddigan B, Hodgson P, Dick B. The Effects of Massage Therapy & Exercise Therapy on Children/Adolescents with Attention Deficit Hyperactivity Disorder. *Cand Child and Adolescent Psychiatry Review* 2003; 2( 12 ):40-3.
- 24- Went MS. The effect of an activity program designed with intensive physical exercise on the behavior of ADHD children. Buffalo: State University of new York, 2000
- 25- Biederman, J, Faraone SV. Attention-deficit hyperactivity disorder. *Lancet*2005; 366:237-48.
- 26- Hoza B, Mrug S, Pelham WE, Greiner AR, Gnagy EM. A friendship intervention for children with Attention- Deficit Hyperactivity Disorder: Preliminary findings. *J Attention Disorders* 2003;6:87-98.
- 27- Tantillo M, Kesich CM, Hynd GV, Dishman RK. The effects of exercise on children with attention-deficit hyperactivity disorder. *Med Sci Sports Exerc* 2002; 34:203-12.
- 28- Etscheidt MA, Ayllon T. Contingent exercise to decrease hyperactivity. *J Psychotherapy* 1987; 4:192-8.
- 29- Gaub M, Carison CL, Gender differences in ADHD: a meta analysis and critical review. *J. Am. Acad Adolesc Psychiatry* 1997;36:1036-45
- 30- Lane AM, Crone GD, Lane H. Mood changes following exercise. *Percept Mot Skills* 2002;94:732-4.
- 31- Steptoe A, Cox S. Acute effects of aerobic exercise on mood. *Health Psychol* 1988; 7:329-40.
- 32- Coe D, Pivarnik J, Womck C, Reeves M, Malina R. Effect of Physical Education and Activity Levels on Academic Achievement in Children. *Med. Sci. Sports Exerc.*2006; 38 (8): 1515–19.
- 33- Miller P, Naglieri J, Gregoski M. Effects of aerobic exercise on overweight children's cognitive functioning: a randomized controlled trial, *J Res. Exerc. Sport* 2007; 78(5): 510-9.
- 34- Dwyer T, Sallis J, Blizzard L, Lazarus R, Dean K. Relation of Academic Performance to Physical Activity and Fitness in Children. *Pediatric Exercise Science* 2001; 13: 225-38.

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## Molecular Diagnosis of *Schistosoma mansoni* infection in human serum and feces by using Polymerase Chain Reaction

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**Abstract:** A Fast, sensitive and specific detection of cercariae of human schistosomes is required for better definition of risk of infection. So, a novel method for the detection of *Schistosoma mansoni* in human samples that is based on the amplification of a highly repeated DNA sequence has been developed. By use of simple DNA extraction techniques and a rapid 2-step polymerase chain reaction (PCR), it was possible to amplify *S. mansoni* DNA in human fecal and serum samples. The high sensitivity of the approach enabled the detection of the parasite DNA in fecal samples containing as few as 3 eggs per gram of feces, which makes it 10 times more sensitive than the Kato-Katz examination. A detection limit of 1 fg of *Schistosoma* sp. DNA was determined when pure DNA was used as PCR template. The amplification reaction showed to be specific giving no cross-reaction with DNA from other helminths. The PCR assay developed in this study may constitute a valuable alternative for the diagnosis of the *Schistosoma* sp. infection.

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**Key words:** *Schistosoma mansoni*, molecular Diagnosis, DNA, PCR, serum, feces.

### Introduction

Human schistosomiasis, or blood flukes, currently infects several hundred million people globally. The World Health Organization estimates that hundreds of thousands of people die of schistosomiasis each year and about a billion people are at risk of exposure. Schistosomes also infect livestock and cause serious economic hardship in many third world nations, including the eastern Mediterranean and Egypt<sup>[1]</sup>. The disease is characterized by the presence of adult worms, or blood flukes, within the host's vascular system. These worms, living as male/female pairs, can survive for many years during which time the female produces hundreds of eggs per day. The study and development of new diagnostic techniques for schistosomiasis are still necessary in view of the difficulties to evaluate infection patterns accurately and to control the disease.

Definitive diagnosis of *S. mansoni* infection requires the demonstration of eggs in feces. The Kato-Katz technique<sup>[2]</sup> is currently the method of choice for parasitological diagnosis of *Schistosomiasis mansoni*, as it is relatively inexpensive and simple.<sup>[1]</sup> However, it is observed that the sensitivity of parasitological methods diminishes when prevalence and intensity of infection are low, making these methods less appropriate for low-endemic areas and in post treatment situations. In addition, parasitological methods are not sufficient

for diagnosing recent infections in which worms have not yet started to produce eggs (the prepatent period).

To address these shortcomings, antibody detection methods have been evaluated as adjuncts to fecal examinations. Comparative studies of parasitological and serological methods confirmed higher sensitivity of the latter<sup>[3]</sup>. Several authors have approached diagnosis through the specific detection of antigens or antibodies<sup>[4]</sup>. The existence of cross-reactivity with other helminthic infections, however, and its low specificity after treatment, due to the slow reduction of specific antibody levels, constitute great disadvantages of the immunodiagnostic methods.

One possible solution to this problem could be the search for circulating antigens rather than antibodies. Several circulating antigens assays have been described in different laboratories<sup>[5-7]</sup>. The high specificity is the main advantage that the detection of circulating antigens offers as compared with the antibodies' determination and the disadvantages are low sensitivity in light infections and dependence on the production of monoclonal antibodies<sup>[8,9]</sup>.

Polymerase chain reaction (PCR) has shown its usefulness in the clinical approach of a wide variety of pathogenic infections, such as human immunodeficiency viruses,<sup>[10,11]</sup> *Legionella pneumophila*<sup>[12]</sup>, *Plasmodium falciparum*<sup>[13,14]</sup>, and *Trypanosoma cruzi*<sup>[15,16]</sup>. In the study of *Schistosoma* sp., it has also been successfully used for sex

determination of the cercariae<sup>[17]</sup>, for the cloning and sequencing of specific genes,<sup>[18,19]</sup> in the determination of genetic variability and population structure of *Schistosoma* sp. strains and species<sup>[11,20]</sup>, and in the development and application of new techniques to generate expressed sequence tags<sup>[11]</sup>. Hamburguer and others<sup>[21]</sup> developed a PCR protocol that was based on the amplification of a highly repetitive DNA sequence of the parasite for monitoring *S. mansoni* infestation in water<sup>[22]</sup>. In the present article, we describe, for the first time in Egypt, the usefulness of the PCR for detecting *S. mansoni* DNA in human fecal and serum samples.

## Material and Methods

**Sample preparation:** *Schistosoma mansoni* eggs were obtained from livers of mice (Swiss albinos) 45 days after infection with 100 cercariae and stored at -20°C in 0.9% saline until use. Ten microliters of egg-saline solution containing ~2,000 eggs were diluted in 90 µL of water and vortexed for 5 min in order to break the eggs and release the miracidia, the larval form that infects the snail host. The entire volume was then used for DNA extraction.

**The DNA from other parasite species:** The DNA from 4 related helminthic parasites (*Ascaris lumbricoides*, *Ancylostoma duodenale*, *Taenia solium*, and *Trichiuris trichiuria*).

**Artificial mixtures of eggs and feces:** Four positive fecal samples (five replicates) were artificially prepared by the following procedure: 0.1 g of *S. mansoni* positive feces containing 216 eggs per gram of feces (determined by Kato-Katz stool examination) were mixed to 0.9 g of negative feces, resulting in a preparation of ~21.6 eggs per gram of feces. Tenfold dilution were subsequently performed, producing 2 more samples with estimated concentrations of ~ 2.16 and 0.216 eggs/g, respectively. The samples were then analyzed by the Kato-Katz method and stored at -70°C until use. Approximately, 100 mg of feces were diluted in 400 µL of water and vortexed for 5 min to break the *S. mansoni* eggs and release the miracidia. Insoluble material and large debris were separated by decantation, leaving the samples for 2 min at room temperature. One hundred microliters of the supernatant were used for DNA extraction.

**Patient samples:** Patient samples were obtained from the endemic area of Kafer El-Shakh, Egypt, or from the laboratory staff (Informed consent was obtained from all adult participants and from parents or legal guardians of minors).

**Stool examination:** All fecal samples used in this work were evaluated for the presence of *S. mansoni* eggs by the quantitative parasitological method of Kato-Katz.

**Patient feces:** Two hundred patient fecal samples provided feces that were previously analyzed by Kato-Katz method and contained 98–910 eggs/g. All fecal samples were stored at -70°C until use. The samples were treated in the same manner described for the artificial mixture of eggs and feces and then used for DNA extraction.

**Serum:** Two hundred positive serum samples were taken from infected patients containing 96 and 216 eggs/g. The laboratory staff provided 5 negative serum samples. All serum samples were stored at -20°C until use. Serum samples needed no previous preparation and were used directly in DNA isolation.

Free-circulating DNA was isolated, purified from 200 µL of serum with the QIAamp DNA Blood Mini Kit (QIAGEN) according to the manufacturer's instructions.

**Extraction of DNA, feces, *S. mansoni* eggs, and related helminthes:** The fecal and egg DNA were extracted through a modification of the ROSE method<sup>[24]</sup>.

Briefly, 100 µL of the fecal supernatant, or of the solution containing disrupted eggs, were diluted in 200 µL of slightly modified ROSE buffer (10 mM Tris, pH 8.0; 270 mM ethylenediamine tetraacetic acid [EDTA], pH 8.0; 1% lauryl sulfate; 1% polyvinylpyrrolidone). This mixture was incubated at 95°C for 20 min, with one rapid manual agitation after 10 min of incubation. The sample was then cooled on ice for 1 min and centrifuged at 8,000 × g for 10 min at room temperature. After that, 200 µL of the supernatant was collected in a separate tube and mixed with 500 µL of frosty ethanol (-20°C) and 10 µL of cold 3 M sodium acetate (4°C), pH 5.3, and incubated overnight at -20°C or -70°C for 2 hr. The samples were then centrifuged at 8,000 × g for 15 min, the supernatant was discarded, and the pellet washed once with 500 µL 70% ethanol (25°C) for a final 10 min at 8,000 × g centrifugation. Finally, the supernatant ethanol was discarded, and the pellet was left to dry at 37°C for 15 min, then resuspended with 100 µL of TE (10 mM Tris, pH 8.0; 1 mM EDTA, pH 8.0).

The DNA extracted from *S. mansoni* eggs and from pure total extracts of other related helminthes was quantified by spectrophotometric readings at 260 and 280 nm. All DNA samples were stored at -20°C until use.

Free-circulating DNA was purified from 200 µL of serum with the QIAamp DNA Blood Mini Kit



(QIAGEN) according to the manufacturer's instructions.

### Polymerase Chain Reaction:

Primers were designed to amplify the 121-bp tandem repeat DNA sequence of *S. mansoni* described by Hamburguer and others [22] were modified with our team of research (Figure 1). For amplification of egg-derived DNA, 1 µL of the extracted material was used as initial template. For

stool amplification, DNA samples were diluted 100 times, and 1 µL was used as template. For serum amplification, 2 µL of undiluted extracted DNA was used as template. All reactions were carried out in a Gen Amp 9700 Thermal cycler PCR in a 10-µL mixture containing: 20 mM Tris-HCl (pH 8.4), 50 mM KCl buffer; 1.5 mM MgCl<sub>2</sub>; 0.5 µM of each primer; 200 µM dNTPs and 0.75 U of *Taq* DNA polymerase.

GATCTGAATC CGACCAACCG TTCTATGAAA ATCGTTGTAT  
CTAGACTTAG GCTGGTTGGC AAGATACTTT TAGCAACATA

CTCCGAAACC ACTGGACGGA TTTTATGAT GTTTGTTTTA  
GAGGCTTTGG TGACCTGCCT AAAAATACTA CAAACAAAAT

GATTATTTGC GAGAGCGTGG GCGTTAATAT AAAACAAAGAA  
CTAATAAACG CTCTCGACC CGCAATTATA TTTTGTTCCT

Primer -1: 5'-GATCTGAATC CGACCAACCG-3' (Forward -20mer)

Primer -2: 5'-CCACGCTCTC GCAAATAATA-3' (Backward -20mer)

**Figure 1.** The DNA sequence of the *Schistosoma mansoni* tandem repeat unit used as target and the primers used for amplification. The PCR primers are internally located and are marked by underline letters. The main DNA band is expected to be 100 bp long.

The 2-step amplification cycle consisted of denaturation at 95°C for 45 sec and an annealing step at 63°C for 30 sec. A total of 35 cycles was performed. The denaturation step was prolonged for 5 min in the first cycle, and the last cycle included an extension step of 2 min. After amplification, 10 µL of the sample was electrophoresed on a 6% polyacrylamide gel, and the amplified sequences were visualized via silver staining.<sup>[25]</sup> The amplification products had their identity confirmed by DNA sequencing performed by standard techniques.

### Results:

**Sensitivity of *S. mansoni* eggs DNA detection:** To assess the sensitivity of the PCR, *S. mansoni* egg DNA was quantified and tested for amplification after serial dilutions. Where the minimum detectable DNA quantity was found to be 1 fg.

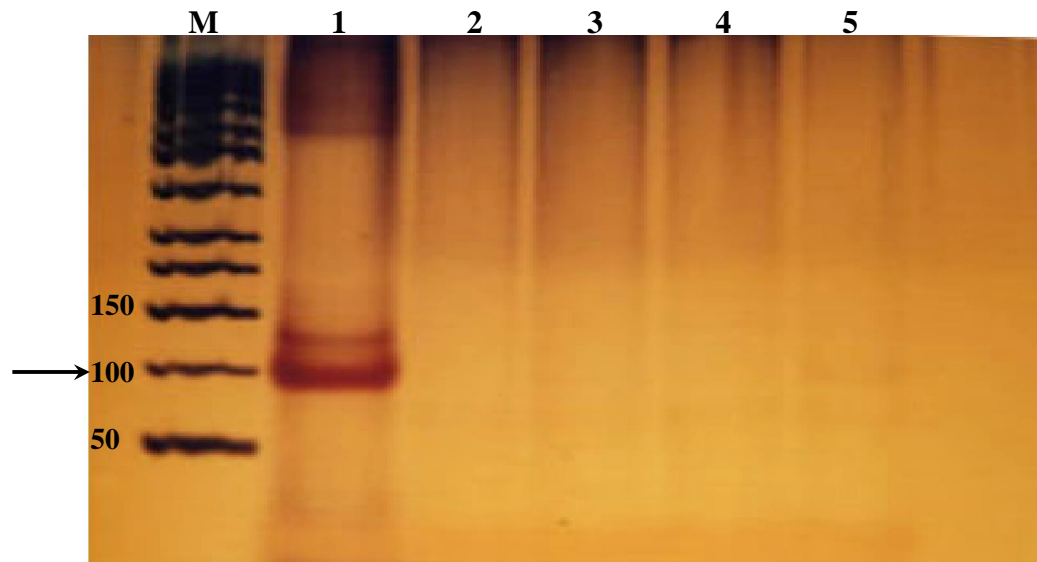
**Specificity of PCR.** The specificity of the PCR in amplifying *Schistosoma* DNA was evaluated by means of equal quantities of DNA from other related parasites under the same reaction conditions; *S. mansoni* DNA was used as positive control for the amplification reaction. As shown in **Fig. 2**, DNA amplification was not achieved for any of the other helminthic parasites evaluated.

### Comparison of PCR and Kato-Katz methods on the detection of *S. mansoni* eggs added to noninfected feces:

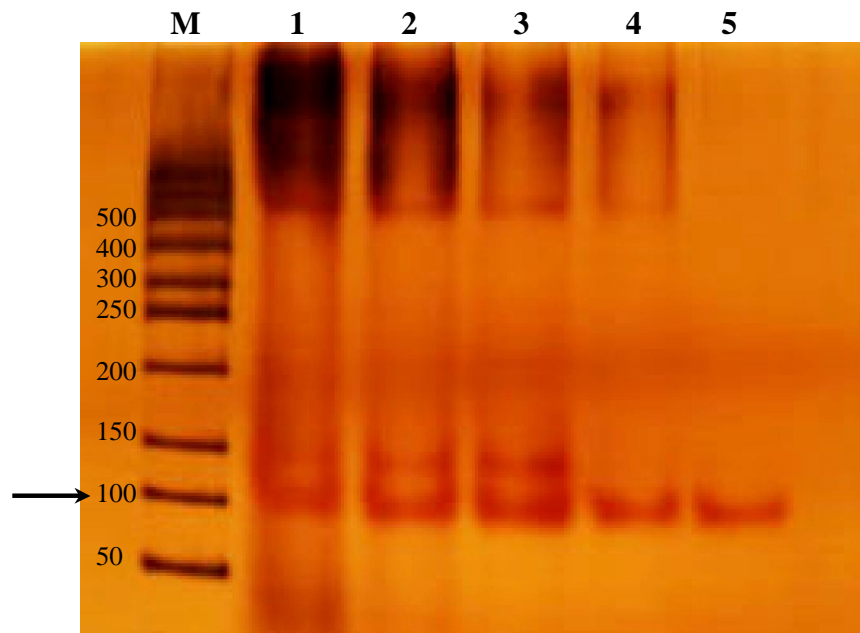
The sensitivity of the PCR and Kato-Katz methods in detecting *S. mansoni* in artificially prepared positive fecal samples (see sample preparation in Materials and Methods) was compared. **Fig. 3** shows the results obtained. The first 2 samples (216 and 21.6 eggs/g) were counted by Kato-Katz method as 216 eggs/g and 48 eggs/g, respectively, and were also positive by PCR. The last 2 samples were negative by the Kato-Katz examination, but the PCR was able to detect the *S. mansoni* DNA, also in the third sample, estimated to contain 2.16 eggs/g.

**Detection via PCR of *S. mansoni* in human clinical samples: Fecal samples.** Fresh fecal samples from *S. mansoni*- positive patients were examined by the Kato-Katz and PCR methods. As shown in **Fig. 4**, PCR was able to detect the *S. mansoni* DNA in fecal samples containing different numbers of eggs per gram, giving equally strong amplification signals.

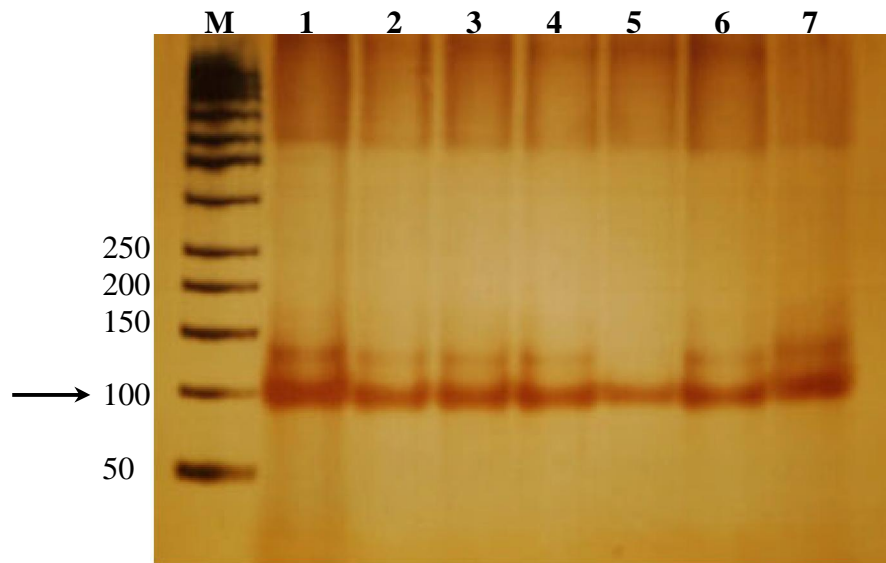
**Serum sample:** Serum samples taken from patients with positive stool examination were tested by PCR, and these also gave positive results, as shown in **Fig. 5**. No bands were seen with the negative control serums.



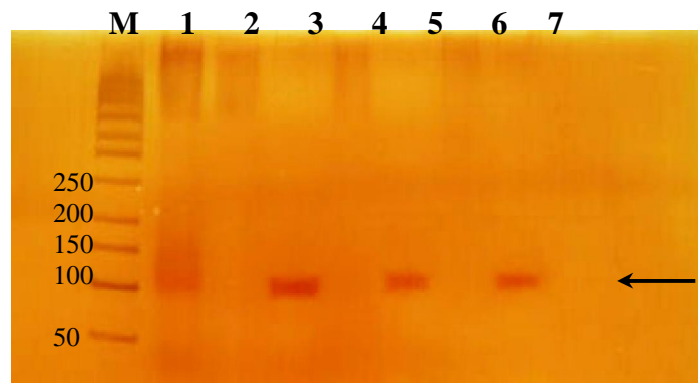
**Figure 2.** Specificity of the *Schistosoma mansoni* polymerase chain reaction (PCR); 0.1 ng of DNA from several related parasite species were tested for amplification in the same described PCR conditions. **Lane M**, molecular weight marker (Gene Ruler™ 50 bp DNA Ladder [Fermentas]). **Lane 1**, *S. mansoni*. **Lane 2**, *Ascaris lumbricoides*. **Lane 3**, *Ancylostoma duodenale*. **Lane 4**, *Taenia solium*. **Lane 5**, *Trichiuris trichiuria*.



**Figure 3.** Compared sensitivity between polymerase chain reaction (PCR) and parasitological examination in the diagnosis of *Schistosoma mansoni* on human feces. The ability of PCR and Kato-Katz technique to detect the parasite was evaluated by means of artificially positive feces (see Materials and Methods) with low numbers of egg per gram of feces. **Lane M**, molecular weight marker (Gene Ruler™ 50 bp DNA Ladder [Fermentas]). **Lane 1**, positive control (*S. mansoni* egg DNA). **Lanes 2–5**, artificially infected feces containing approximated values of 216, 21.6, 2.16, and 0.216 eggs per gram of feces. Although the parasitological examination was positive only for the first 2 samples, the PCR was positive for the first 3 samples because it was 10 times more sensitive.



**FIGURE 4.** *Schistosoma mansoni* detection in feces via polymerase chain reaction (PCR). Six human clinical feces, previously examined by Kato-Katz, were tested by PCR. **Lane M**, molecular weight marker (Gene Ruler™ 50 bp DNA Ladder [Fermentas]). **Lane 1**, positive control (*S. mansoni* egg DNA). **Lanes 2–7**, fecal samples containing 270, 445, 323, 98, 822, and 910 eggs per gram of feces.



**FIGURE 5.** *Schistosoma mansoni* polymerase chain reaction (PCR) detection in clinical serum samples. The DNA from 4 serum samples was analyzed by PCR. **Lane M**, molecular weight marker (Gene Ruler™ 50 bp DNA Ladder [Fermentas]). **Lane 1**, positive control (*S. mansoni* egg DNA). **Lanes 2, 4, 6** serum from patients with 0 (negative sample) No bands were visualized in the 3 negative samples, **lane 3** (445), **lane 5** (270), and **lane 7** (98) eggs per gram of feces, respectively.

#### Discussions:

A consistent diagnosis of schistosomiasis still depends on the parasitological demonstration of the *Schistosoma* sp. eggs in fecal samples, which is well accomplished by the Kato-Katz technique. Despite of the remarkable qualities of this diagnostic method, a more sensitive approach would be of great value in situations such as the presence of light infections. Polymerase chain reaction has been acclaimed as an

outstanding sensitive and specific diagnostic tool for many diseases, but to our knowledge, this technique has not been used for demonstrating the presence of *S. mansoni* DNA in clinical samples.

We tested the possibility of the use of PCR for the detection of *S. mansoni* DNA in human samples of serum and feces. The high sensitivity of the assay was demonstrated by its ability to achieve amplification with minimum amounts of *S. mansoni*

egg template DNA (1 fg). Hamburguer and others<sup>[20]</sup> used a different set of primers that targets the internal part of the same DNA sequence and obtained very similar results (1.2 fg) when they used adult worm DNA as template. Amplification of the *S. mansoni* tandem-repeated unit generates a ladder of PCR products, which DNA sequence determination proved to be multiples of the 100-bp long sequence (data not shown). The high sensitivity of the assay is certainly due to the unusually high copy number (600,000/cell) of the target sequence, which comprises ~ 10% of the *S. mansoni* genome.<sup>[22]</sup> The specificity of the test was demonstrated by the absence of amplification when DNA from other 4 helminthes commonly found in the same endemic areas as *S. mansoni* were used as templates (Figure 2). Figures 3 and 4 show the ability of the PCR assay in detecting the *S. mansoni* DNA in human fecal samples.

Particularly interesting was the comparison between the sensitivity of the Kato-Katz method and this new PCR approach (Figure 3). By use of an artificially prepared positive fecal sample, the PCR was able to detect the *S. mansoni* DNA in fecal samples where eggs could no longer be observed by the Kato-Katz examination (2.16 eggs/g). Because of the high number of copies of this repeated region, this technique should enable the detection of fractions of a single *S. mansoni* individual cell, instead of the entire eggs needed for microscopic detection. Polymerase chain reaction in clinical fecal samples gave strong amplification signals, regardless of the difference among the number of eggs per gram of feces (Figure 4).

The possibility of detecting *S. mansoni* DNA in the serum of infected patients is illustrated in Figure 5. These results show that serum samples can be used as a template source for schistosomiasis clinical diagnosis. The serum DNA bands were sharp enough to allow a steady interpretation, although not as strong as those seen in fecal samples, suggesting limited quantities of free-circulating DNA.

The DNA amplification assay developed in this study may constitute an unprecedented alternative to the available diagnostic techniques for the detection of *S. mansoni* infection. The value of this approach in the field needs further study.

It certainly demands a more sophisticated laboratory apparatus and a greater operational effort when compared with the Kato-Katz parasitological examination, which is better than molecular and serological techniques in terms of low costs and ease of operation. Nevertheless, our results indicate that PCR is probably more sensitive than the Kato-Katz technique and the circulating antigen detection that presents the similar sensitivity to stool examination in situation of low worm burden, medication and in low

endemic area. The high degree of specificity may make it better diagnostic alternative than serological techniques.

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#### REFERENCES

1. World Health Organization, 1994. *The Control of Schistosomiasis. Report of a WHO Expert Committee*. Technical Report Series 830. Geneva: World Health Organization.
2. Katz N, Chaves A, Pellegrino J, 1972. A simple device for quantitative stool thick smear technique in *Schistosomiasis mansoni*. *Rev Inst Med Trop Sao Paulo* 14: 337–340.
3. Doenhoff MJ, Butterworth AE, Hayes RJ, Sturrock RF, Ouma JH, Koech D, Prentice M, Bain J, 1993. Seroepidemiology and serodiagnosis of schistosomiasis in Kenya using crude and purified egg antigens of *Schistosoma mansoni* in ELISA. *Trans R Soc Trop Med Hyg* 87: 42–48.
4. Pardo, J., Carranza, C., Turrientes, M.C., Perez Arellano, J.L., Lopez Velez, R., Ramajo, V., Muro, A., 2004. Utility of *Schistosoma bovis* adult worm antigens for diagnosis of human schistosomiasis by enzymelinked immunosorbent assay and electroimmunotransfer blot techniques. *Clinical and Diagnostic Laboratory Immunology* 11, 1165– 1170.
5. De Jonge N, Rabello ALT, Krijger FW, Kremsner PG, Rocha RS, Katz N, Deelder AM, 1991. Levels of the schistosome circulating anodic and cathodic antigens in serum of schistosomiasis patients from Brazil. *Trans R Soc Trop Med Hyg* 85: 756–759.
6. Barsoum IS, Kamal KA, Bassily S, Deelder AM, Colley DG, 1991. Diagnosis of human schistosomiasis by detection of circulating cathodic antigen with a monoclonal antibody. *J Infect Dis* 164: 1010–1013.
7. Hassan MM, Badawi MA, Strand M, 1992. Circulating schistosomal antigen in diagnosis and assessment of cure in individuals infected with

- Schistosoma mansoni*. *Am J Trop Med Hyg* 46: 737–744.
8. Van Lieshout L, De Jonge N, El-Masry N, Mansour MM, Bassily S, Krijger FW, Deelder AM, 1994. Monitoring the efficacy of different doses of praziquantel by quantification of circulating antigens in serum and urine of schistosomiasis patients. *Parasitology* 108(Pt 5): 519–526.
  9. Al-Sherbiny MM, Osman AM, Hancock K, Deelder AM, Tsang VC, 1999. Application of immunodiagnostic assays: detection of antibodies and circulating antigens in human schistosomiasis and correlation with clinical findings. *Am J Trop Med Hyg* 60: 960–966
  10. Imagawa DT, Lee MH, Wolinski SM, Sano K, Morales F, Kwok S, Sninski JJ, Nishanian PG, Giorgi J, Fahey JL, Dudley J, Bisscher BR, Detels R, 1989. Human immunodeficiency virus type 1 infection of homosexual men who remain seronegative for prolonged periods. *Nat Engl J Med* 320: 1458–1462.
  11. Dias Neto E, Harrop R, Correia-Oliveira R, Pena SDJ, Wilson RA, Simpson AJG, 1996. The schistosome genome project: RNA arbitrarily primed PCR allows the accelerated generation of expressed sequence tags. *Mem Inst Oswaldo Cruz* 91: 655–657.
  12. Starnbach MN, Falkow S, Tompkins LS, 1990. Species specific detection of *Legionella pneumophila* in water by DNA amplification and hybridization. *J Clin Microbiol* 28: 1968–1973.
  13. Barker RH, Banchongaksorn T, Courval JM, Suwonkerd W, Rimwungtragoon K, Wirth D, 1992. A simple method to detect *Plasmodium falciparum* directly from blood samples using the polymerase chain reaction. *Am J Trop Med Hyg* 46: 416–426.
  14. Wooden J, Kyes S, Sibley CH, 1993. PCR and strain identification in *Plasmodium falciparum*. *Parasitol Today* 9: 303–305.
  15. Russomando G, Figueiredo A, Almiron M, Sakamoto M, Morita K, 1992. Polymerase chain reaction-based detection of *Trypanosoma cruzi* DNA in serum. *J Clin Microbiol* 30: 2864–2868.
  16. Diaz C, Nussenzweig V, Gonzales A, 1992. An improved polymerase chain reaction to detect *Trypanosoma cruzi* in blood. *Am J Trop Med Hyg* 46: 616–623.
  17. Gasser RB, Morahan G, Mitchell GF, 1991. Sexing single larval stages of *Schistosoma mansoni* by polymerase chain reaction. *Mol Biochem Parasitol* 47: 255–258.
  18. Francis P, Bickel Q, 1992. Cloning of a 21.7 kDa vaccine-dominant antigen gene of *Schistosoma mansoni* reveals an elf hand-like motif. *Mol Biochem Parasitol* 50: 215–224.
  19. Kiang D, Karim AM, LoVerde PT, 1996. Cloning the gene encoding *Schistosoma mansoni* p50, an immunophilin. *Gene* 170: 137–140.
  20. Simpson AJ, Dias Neto E, Vidigal TH, Pena HB, Carvalho OS, Pena SD, 1995. DNA polymorphism of schistosomes and their snail hosts. *Mem Inst Oswaldo Cruz* 90: 11–213.
  21. Hamburguer J, Yu-Xin X, Ramzy RM, Jourdan J, Ruppel A, 1998. Development and evaluation of a polymerase chain reaction for monitoring *Schistosoma mansoni* infestation of water. *Am J Trop Med Hyg* 59: 468–473.
  22. Hamburguer J, Turetski T, Kapeller I, Deresiewicz R, 1991. Highly repeated short DNA sequences in the genome of *Schistosoma mansoni* recognized by a species specific probe. *Mol Biochem Parasitol* 44: 73–80.
  23. Pellegrino J, Siqueira AF, 1956. Técnica de perfusão para colheita de *Schistosoma mansoni* em cobaias experimentalmente infectadas. *Rev Bras Malariol* 8: 589–597.
  24. Steiner JJ, Poklemba CJ, Fjellstrom RG, Elliott LF, 1995. A rapid one-tube genomic DNA extraction process for PCR and RAPD analyses. *Nucleic Acids Res* 23: 2569–2570.
  25. Sanguinetti CJ, Dias Neto E, Simpson AGJ, 1994. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* 17: 914–921.

3/11/2011

## Effect of Erythropoietin on Experimental Unilateral Testicular Torsion Detorsion in Rat Model

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**Abstract:** Testicular torsion is a common syndrome that could lead to infertility. In this study, we investigated the protective effect of erythropoietin on experimental unilateral testicular torsion detorsion in a rat model. Fourty male albino rats were divided into three groups: Group I (10): Sham operated rats, Group II (15) untreated torsion detorsion (T/D); torsion of the right testis by rotating the testis 720° (2 times around the longitudinal axis of the spermatic cord) in a clockwise direction for 2 hours, and after 2 hours, the right testis was detorted by rotating the testis 2 times (720°) in an anticlockwise fashion and then fixed in the right position for 30 days, Group III (15): treated torsion detorsion (T/D): the same surgical procedure was done as in group II, in addition EPO at a dose of 1000 IU/Kg was injected i.p, 3 times per week for 12 doses. After the end of the study, ECG was performed, abdominal aorta was exposed and blood samples were collected in plain tube and then centrifuged to obtain serum, to determine serum Testosterone, both testes were removed, weighed, then divided into 2 halves, one half for histopathological examination and the other half for determination of testicular malonyldialdehyde, testicular glutathione peroxidase (GPx), and testicular catalase levels. The results of the present study showed significant increase in Q wave voltage and ST segment slope in untreated T/D compared to sham operated rats. Biochemical parameters showed significant decrease in serum testosterone in untreated T/D rats compared to treated rats and to sham operated rats. In addition significant increase in right and left testicular MDA level and significant decreases in right and left testicular GPx and catalase levels compared to sham operated rats, these parameters were greatly ameliorated in treated T/D rats. These biochemical changes were further confirmed by histopathological examinations showing severe destruction of seminiferous tubules of the right testes, left testes affection were also observed but to a lesser degree in untreated T/D. EPO treatment greatly ameliorated the damage of ipsilateral and contralateral testes of T/D model. In conclusion, the protective effect of erythropoietin on testicular torsion could serve as a promising intervention to oxidative stress associated infertility problems, such as testicular torsion. [Gehane M. Hamed, Ramadan M. Ahmed, Maher M. Emara, and Manar H. Mahmoud. **Effect of Erythropoietin on Experimental Unilateral Testicular Torsion Detorsion in Rat Model.** Life Science Journal. 2011;8(2):405-412] (ISSN:1097-8135). <http://www.lifesciencesite.com>.

**Keywords:** Erythropoietin; Unilateral Testicular; Torsion; Detorsion; Rat

### 1. Introduction

Testicular torsion is an urological emergency that usually results from rotation of testis around the axis of the spermatic cord, and it is frequently observed in newborns, children and adolescents (Ergur *et al.*, 2008). Rapid diagnosis and immediate surgical treatment are essential to avoid permanent testicular damage. Delay or misdiagnosis and inappropriate treatment usually lead to male infertility (Perotti *et al.*, 2006). The main pathophysiology of testicular torsion detorsion of spermatic cord is ischemia/ reperfusion (I/R) injury of the testis (Turner *et al.*, 1993).

Under normal conditions, free radicals are produced and their effects are counterbalanced by the endogenous antioxidant system. When ROS generation exceeds the defense mechanisms capacity, oxidative stress is generated and contributes to

reversible and irreversible cell injury (Bulger & Maier, 2001).

Erythropoietin (EPO) is a glycoprotein responsible for the formation of erythrocytes in the bone marrow and it is the drug of choice in treatment of anemia. There are several studies reported the beneficial effects of exogenous EPO administration on I/R of lung, eye, kidney, spinal cord in animals (Akeora *et al.*, 2007). Moreover, EPO mRNA has been detected in brain, bone marrow, placenta, ovary and testis. In addition, functional EPO receptors (EPO-R) has been demonstrated in rodent and human placenta, brain, and kidney cells. In testis, there is basal expression of EPO mRNA which increased upon hypoxic exposure (Akeora *et al.*, 2007). It has been reported that EPO influences rat Leydig cells steroidogenesis via stimulating production of testosterone and in humans, EPO administration intravenous increases testosterone in renal failure

(Yamamoto *et al.*, 1997). In light of these data, we conducted this study in order to evaluate the protective effects of EPO on ischemic reperfusion injury in rat model subjected to torsion detorsion of the spermatic cord.

## 2. Materials and Methods:

This study was approved by the Ethics Committee FMASU 952/2011

### Experimental animals:

This study was carried out on 40 young male albino rats weighing 120- 140 gm at start of the study, rats were purchased from Experimental Animal farm of Helwan and housed 3/cage in suspended wire-mesh cages and maintained in Physiology Department animal house, Faculty of Medicine, Ain Shams University under standard conditions of boarding, at room temperature and in controlled environment of 12h light-dark cycle. All rats were fed standard rat chow before starting the experiment. The standard rat chow diet (AIN-93 M diet formulated for adult rodents) prepared according to Reeves *et al.* (1993).

### Experimental protocol:

The rats were allocated into 3 groups:

a- Group I included (n= 10) sham rats. Under ether anaesthesia, median scrotal incision was done, delivery of the right testis without twisting the testicle and fixed to the scrotum with chromic catgut sutures. Then they are submitted to a second sham under ether anaesthesia, with incision of scrotal skin sutures and fixation sutures within the scrotum.

b-Group II included (n= 15) untreated torsion detorsion (T/D): Under ether anaesthesia, median scrotal incision was done, delivery of the right testis and after division of gubernaculum, then torsion was created by rotating the testis 720° (2 times around the longitudinal axis of the spermatic cord) in a clockwise direction for 2 hours, and fixed to the scrotum with chromic catgut sutures. After 2 hours, the right testis was detorted by rotating the testis 2 times (720°) in an anticlockwise fashion after cutting the fixation sutures and then replaced into the scrotum and fixed in the right position for 30 days (modified from Ergur *et al.*, 2008). 0.5 cc 0.9% NaCl solution was injected i.p. 1/2 an hour before the detorsion and 3 times in a week (for 12 doses).

c-Group III (n= 15): treated torsion detorsion (T/D): the same surgical procedure was done as in group II, in addition EPO at a dose of 1000 IU/Kg was injected i.p. 1/2 an hour before the detorsion and 3 times in a week (for 12 doses), as described by Koseoglu *et al.* (2009).

EPO was purchased from EPREX®: is a sterile phosphate buffered Epoetinum alfa solution for parental administration, graduated prefilled syringes with needle guard (PROTECS™) containing 4000 IU/ 0.4 ml.

### Experimental procedure:

At the end of the experimental period, all rats were fastened overnight, weighed and anaesthetized with intraperitoneal thiopental sodium (40 mg/Kg bw).

### 1-ECG recording:

Needle electrodes were placed under the skin of the 4 limbs of the animal near the paws, and connected through an ECG coupler to a 2 channel oscillograph (Cardimax FX 121, Fukuda Denshi Co, LTD). The electrocardiographic tracing was recorded using standard limbs. From lead II-ECG tracing with paper speed of 25 mm/sec, heart rate (HR), P-R interval, QRS duration, QT interval, Q wave voltage, R wave voltage and ST segment deviation were measured. The heart rate was calculated using the following formula:

$$HR = \frac{7500}{\text{Distance in mm between 6 successive peaks of R waves}}$$

### 2-Orchidectomy:

The scrotal skin was incised, sutures fixing the right testis were cut, the spermatic cord was clamped with forceps, transected and ligated with 2/0 chromic catgut sutures. The same procedure was done for the contralateral left testis. Each testis was weighed then cut transversely, one half is preserved in 10% formaline for histopathological examination, and the second half was preserved in parafilm and kept at - 80°C for biochemical studies.

**3- A midline incision was made**, then the abdominal aorta was exposed and blood samples were collected in plain centrifuge tubes; to obtain serum after centrifugation at 4000 r.p.m. for 15 minutes for separation of serum and then stored at - 80° till used for determination of biochemical measurements.

### Biochemical measurements:

**a- Serum Testosterone**, Calbiotech Inc, ELISA; as described by Bricaire *et al.* (1991).

**B-Testicular malonyldialdehyde (MDA)**, according to the method described by Draper and Hadley (1990).

**c-Testicular glutathione peroxidase (GPx)**, Bio.diagnostic. com, UV method; as described by Paglia and Valentine (1967).

**d-Testicular catalase**, Bio.diagnostic. com, colorimetric method; as described by Aebi (1984).

#### 4-Histopathological examinations:

Each testis was cut transversely into 2 halves by sharp incisor. The specimens were prepared for histological study, according to the method described by *Drury and Wallington (1980)*. They were fixed in 10% neutral buffered formaline, dehydrated cleared and embedded in paraplant. Paraffin sections were cut serially at 5  $\mu$ m-thicknesses. Sections were stained by haematoxyline and eosin (Hx &E).

#### Statistical Analysis:

All statistical data and significance tests were performed by using SPSS (Statistical Program for Social Science) statistical package (SPSS Inc) version 8.0.1 (*Armitage & Berry, 1987*). Statistical significance was determined by one-way ANOVA (analysis of variance) for differences between means of different groups; further analysis was made by LSD (least significance difference) multiple-range test to find intergroupal differences; a probability of  $P < 0.05$  was considered statistically significant.

#### 3. Results:

Concerning ECG parameters, significant increase ( $P < 0.05$ ) in Q wave voltage and increase in ST segment slope were observed in untreated T/D rats compared to sham operated rats (Table 1).

Concerning testicular weights, significant decreases ( $P < 0.05$ ) in right testicular weights were observed in group II compared to both group I and III, upon calculation of right testicular weight/ body weight ratio, the significance was still present between group II and III. While non significant differences were observed in left testicular weights and left testicular weight/ body weight ratio among the 3 studied groups (Table 2).

Concerning lipid peroxidation, the untreated T/D rats promoted significant increases ( $P < 0.05$ ) in right and left testicular MDA compared to treated T/D and to sham operated rats. Upon erythropoietin administration to T/D rats, significant increases ( $P < 0.05$ ) were observed in right and left testicular GPx and catalase levels compared to untreated T/D rats (Table 3).

Concerning serum testosterone level, significant decrease ( $P < 0.05$ ) in serum testosterone was observed in untreated T/D rats compared to sham operated rats. Upon treatment with EPO to T/D rats, serum testosterone showed significant increase ( $P < 0.05$ ) compared to untreated T/D rats, although the level was still significantly lower than sham operated rats (table 4).

Histopathological examination of both right and left testes of sham operated group revealed multiple rounded seminiferous tubules with regular outlines. They were lined by multiple layers of germinal

epithelium at different stages of spermatogenesis. The interstitial spaces between the tubules contained Leydig cells and some blood capillaries (Figure 2). In untreated T/D, histopathological examination of the testicular sections of right testes of this group showed seminiferous tubules with irregular outlines. Some tubules showed complete loss of the germinal epithelium with acidophilic exudates in the lumen. Other tubule showed few layers of germinal epithelium with multiple spaces in between the cells. The lumen showed complete loss of the sperm. The interstitial spaces were filled by cellular infiltration most probably fibroblast and fluid exudates (Figure 3). On the other hand, examination of sections of the left testes showed irregular outlines and destructed some seminiferous tubules. The germinal epithelium revealed diminution of spermatogenic cells with areas of cellular loss and intercellular spaces in between the cells. The lumen revealed very few spermatids and rare sperm. The interstitial space showed acidophilic exudates, congested blood vessels and some Leydig cells (Figure 4). Histopathological examination of testicular sections of right testes of treated T/D rats showed some seminiferous tubules appearing with regular lining, multiple layers of spermatogenic cells, abundant whorly appearance of sperm flagella filling their lumina and some Leydig cells in the interstitial spaces. However, some tubules showed cellular infiltration in their lumina. The most prominent observation were the presence of enlarged congested blood vessels in the interstitial spaces (Figure 5). On the other hand, examination of the sections of the left testes of this group showed multiple seminiferous tubules with regular outline and spermatogenic cells at different stages of spermatogenesis. Also, enlarged congested blood vessels and cellular exudates in the interstitial spaces were also seen (Figure 6).

#### 4. Discussion:

The results of the present study demonstrated that I/R or torsion of the right testes for 2 hours followed by 30 days detorsion caused testicular necrosis and marked destruction of seminiferous tubules and decreased testicular weight in ipsilateral testes, as well as diminution of spermatogenic cells with areas of cellular loss and intercellular spaces in between the cells in contralateral left testes compared to sham operated rats. While treatment with EPO provided a beneficial effect to the ipsilateral and contralateral testes by decreasing necrosis and atrophy and improving spermatogenesis as shown by histopathological examinations and further proved by significant increases in serum testosterone level in treated T/D rats compared to untreated T/D rats.



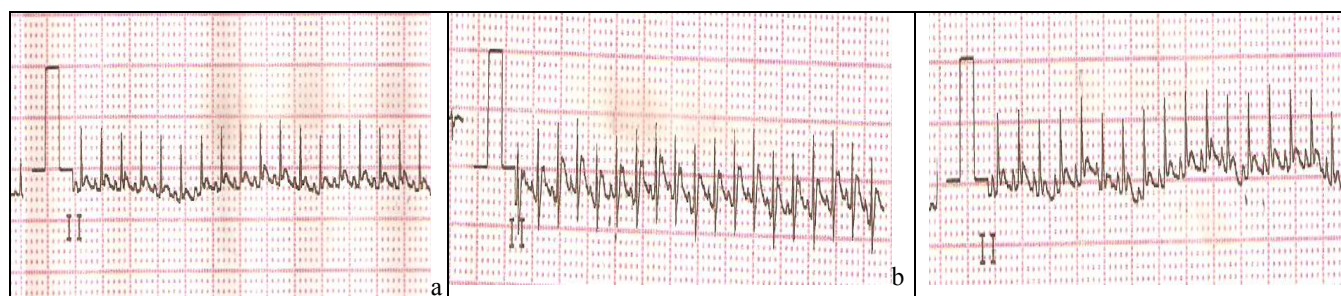
**Table (1): Changes in ECG parameters; heart rate (HR, beats/min), P-R interval (msec), QRS wave (msec), QT (msec), Q wave ( $\mu\text{v}$ ), R wave ( $\mu\text{v}$ ), and ST segment elevation ( $\mu\text{v}$ ) in the three studied groups.**

Groups	HR (beats/min)	P-R (msec)	QRS (msec)	QT (msec)	Q ( $\mu\text{v}$ )	R ( $\mu\text{v}$ )	S ( $\mu\text{v}$ )
-Sham (10)	377 $\pm$ 22	54 $\pm$ 2.7	40 $\pm$ 2.9	80 $\pm$ 3.7	43 $\pm$ 3.8	575 $\pm$ 37.5	55 $\pm$ 3.3
-Untreated T/D (15)	387 $\pm$ 10.7	58 $\pm$ 1.5	39 $\pm$ 0.7	88 $\pm$ 2.6	62 $\pm$ 5.4*	547 $\pm$ 23.6	73 $\pm$ 4.5*
-Treated T/D (15)	359 $\pm$ 13.2	53 $\pm$ 3.2	41 $\pm$ 2.3	90 $\pm$ 4.4	53 $\pm$ 3.3	560 $\pm$ 21.4	58 $\pm$ 3.2**
P	NS	NS	NS	NS	< 0.05	NS	< 0.05

P: Significance by 1-way ANOVA among the 3 studied groups.

\*: Significance by LSD at  $P < 0.05$  from sham-operated group \*\* : Significance by LSD at  $P < 0.05$  from untreated T/D group.

In parenthesis is the number of rats. Results are expressed as Mean  $\pm$  SEM. NS: not significant.

**Figure (1): ECG records (lead II) of: a) Sham-operated b) Untreated T/D c) Treated T/D.****Table (2): Changes in right testis weight (RTW, g), right testis weight/ body weight (RTW/BW, mg/g), left testis weight (LTW, g), left testis weight/ body weight (LTW/BW, mg/g) in the three studied groups.**

Groups	RTW (g)	RTW/BW (mg/g)	LTW (g)	LTW/BW (mg/g)
-Sham (10)	0.62 $\pm$ 0.05	3.1 $\pm$ 0.2	0.64 $\pm$ 0.49	3.2 $\pm$ 0.3
-Untreated T/D (15)	0.53 $\pm$ 0.02*	2.7 $\pm$ 0.1	0.65 $\pm$ 0.01	3.3 $\pm$ 0.07
-Treated T/D (15)	0.64 $\pm$ 0.01**	3.2 $\pm$ 0.08**	0.68 $\pm$ 0.01	3.4 $\pm$ 0.06
P	< 0.05	< 0.05	NS	NS

P: Significance by 1-way ANOVA among the 3 studied groups. \*: Significance by LSD at  $P < 0.05$  from sham-operated group.

\*\* : Significance by LSD at  $P < 0.05$  from untreated T/D group.

In parenthesis is the number of rats. Results are expressed as Mean  $\pm$  SEM. NS: not significant.

**Table (3): Effect of ischemia/reperfusion injury and EPO on malondialdehyde (MDA,  $\mu\text{mol/g}$  tissue), glutathione peroxidase (GPx, mU/mg protein), and catalase (CAT, U/mg protein) levels in ipsilateral (right= R) and contralateral (left= L) testicular tissues in the three studied groups.**

Groups	MDA ( $\mu\text{mol/g}$ ) R	MDA ( $\mu\text{mol/g}$ ) L	GPx (mU/mg) R	GPx (mU/mg) L	CAT (U/mg) R	CAT (U/mg) L
-Sham (10)	5.3 $\pm$ 0.2	5 $\pm$ 0.2	7.5 $\pm$ 0.3	7.5 $\pm$ 0.3	64.3 $\pm$ 4.5	62.9 $\pm$ 3.8
-Untreated T/D (15)	9.6 $\pm$ 0.5*	6.4 $\pm$ 0.4*	4.2 $\pm$ 0.3*	5.9 $\pm$ 0.3*	33.6 $\pm$ 2.3*	41.9 $\pm$ 2.3*
-Treated T/D (15)	6.6 $\pm$ 0.4**	5.3 $\pm$ 0.3**	6.4 $\pm$ 0.2**,**	7.3 $\pm$ 0.3**	45.3 $\pm$ 2.8**,**	53.9 $\pm$ 3**,**
P	< 0.001	< 0.05	< 0.001	< 0.05	< 0.001	< 0.001

P: Significance by 1-way ANOVA among the 3 studied groups. \*: Significance by LSD at  $P < 0.05$  from sham-operated group.

\*\* : Significance by LSD at  $P < 0.05$  from untreated T/D group.

In parenthesis is the number of rats. Results are expressed as Mean  $\pm$  SEM. NS: not significant.

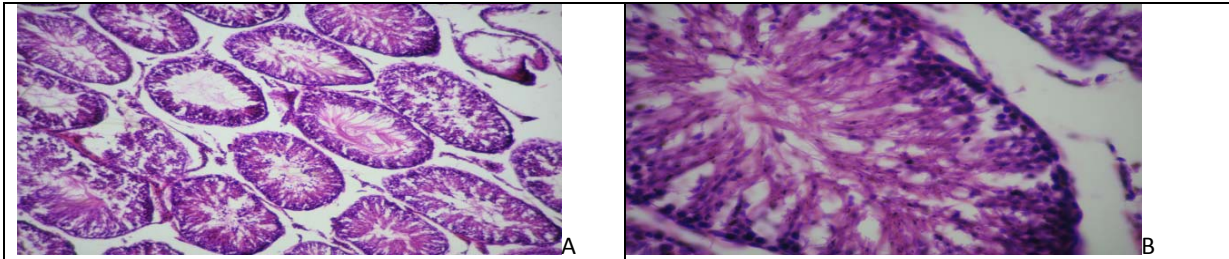
**Table (4): Changes of serum testosterone levels in the three studied groups.**

Groups	Testosterone level (pg/ml)
-Sham (10)	2.9 $\pm$ 0.2
-Untreated T/D (15)	1.1 $\pm$ 0.07*
-Treated T/D (15)	1.8 $\pm$ 0.09**,**
P	< 0.001

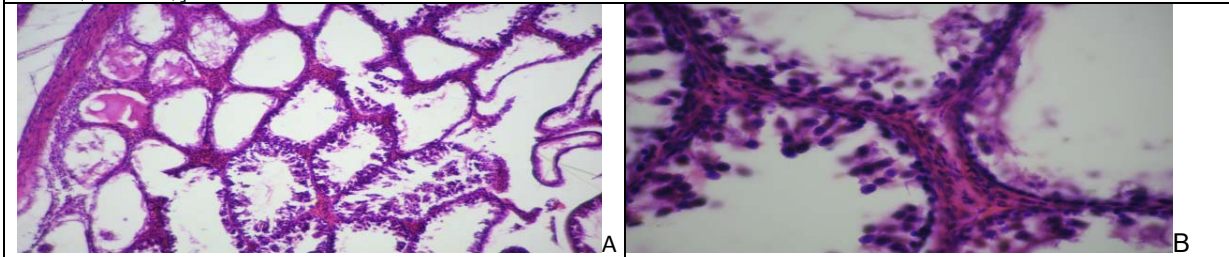
P: Significance by 1-way ANOVA among the 3 studied groups. \*: Significance by LSD at  $P < 0.05$  from sham-operated group.

\*\* : Significance by LSD at  $P < 0.05$  from untreated T/D group.

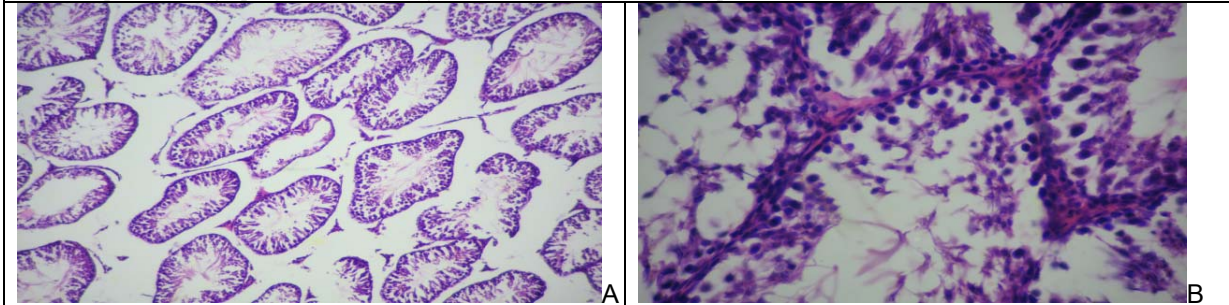
In parenthesis is the number of rats. Results are expressed as Mean  $\pm$  SEM. NS: not significant.



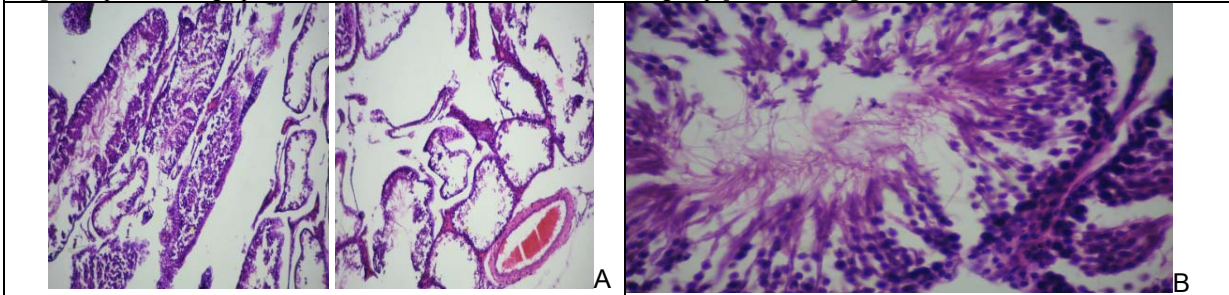
**Fig.2:** A photomicrograph of a T.S in the testis of sham operated group [Hx & E, magnification A 100X (right testis) & B 400X (left testis)].



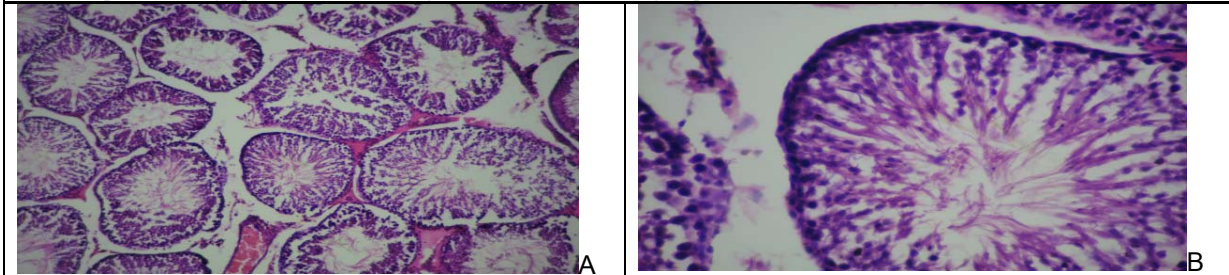
**Fig.3:** A photomicrograph of a T.S in the right testis of untreated T/D group [Hx & E, magnification A 100X & B 400X].



**Fig.4:** A photomicrograph of a T.S in the left testis of untreated T/D group [Hx & E, magnification A 100X & B 400X].



**Fig.5:** A photomicrograph of a T.S in the right testis of treated T/D group [Hx & E, magnification A 100X & B 400X].



**Fig.6:** A photomicrograph of a T.S in the left testis of treated T/D group [Hx & E, magnification A 100X & B 400X].

The ECG is one of clinical tests used for diagnosis of cardiovascular diseases (Okin *et al.*, 2004). Abnormalities in the ECG were detected in untreated T/D rats such as significant elevation in the slope of ST segment compared to both sham-operated and treated T/D rats, as well as significant increase in Q wave voltage in untreated T/D rats compared to sham operated rats; which are important in diagnosis of ventricular injury or infarction. This observation indicates that testosterone confers cardioprotection against ischaemic insult. This finding is also in agreement with a previous observation that testosterone is associated with a decreased susceptibility to myocardial ischaemia (Callies *et al.*, 2003). The study of Tsang *et al.* (2008) showed that the protective effect of testosterone is due to its direct action on the myocardium in addition to the beneficial effects of androgen, such as dilation of the coronary artery (Weidemann and Hanke, 2002). Moreover, testosterone enhances the effects of stimulation of both  $\alpha$ 1- and  $\beta$ 1-adrenoceptors, thus enhancing both cardioprotection and injury, respectively. It has been shown that endogenous testosterone induces cytoprotection via activating cardiac mitochondrial ATP-sensitive  $K^+$  channels (Er *et al.*, 2004). Two studies have shown that chronic administration of testosterone at physiological concentrations enhances  $Ca^{2+}$  influx via the L-type  $Ca^{2+}$  channel in neonatal (Michels *et al.*, 2006) and adult (Er *et al.*, 2007) rat ventricular myocytes. In the adult ventricular myocyte, testosterone also increases the  $Ca^{2+}$  spark, indicating an increase in  $Ca^{2+}$  release from the sarcoplasmic reticulum. It has also been shown that testosterone increases the  $Na^+-Ca^{2+}$  exchange mRNA level in the heart, suggesting increased activity of the exchanger, which is responsible for  $Ca^{2+}$  removal (Golden *et al.*, 2004). So testosterone may alter  $Ca^{2+}$  homeostasis, thus attenuating the  $[Ca^{2+}]_i$  overload in response to ischaemic insult and conferring cardioprotection (Tsang *et al.*, 2008).

Torsion of the testis initially results in obstruction of venous blood flow of the spermatic cord with secondary edema and haemorrhage, then edema results in ischaemia and arterial obstruction leading to necrosis (Koseoglu *et al.*, 2009). A possible mechanism of torsion detorsion is ischemic reperfusion injury. In fact, although that restoration of the blood flow to the testis is essential for tissue salvage, but it may have detrimental effects (Minutoli *et al.*, 2005). As it has been reported that reperfusion of an ischemic tissue leads to ROS generation which arise from xanthine oxidase activation or from leukocytes penetration in the interstitial tissue (Granger & Korthuis, 1995). Also, activation of NOS which can cause testicular damage and atrophy and

stimulation of proinflammatory cytokines (Filho *et al.*, 2004). Normally, they are counterbalanced by the endogenous antioxidant system. When ROS production exceeds the capacity of the defense mechanisms, cell damage occurs (Lysiak *et al.*, 2002). Moreover, upon reperfusion of the tissue, oxygen needed for the conversion of hypoxanthine to uric acid becomes available resulting in generation of enormous amount of free radicals which react with lipid in the cell and mitochondrial membranes leading to disruption of integrity of the cell (Reilly *et al.*, 1991).

As previously known, sperms are highly sensitive to oxidative stress and particularly to lipid peroxidation due to their high content of polyunsaturated fatty acids in the plasma membrane. The fatty acids are an essential requirement for the male germ cell to maintain sperm functions (Henkel, 2005). ROS can also decrease the enzymatic defenses of the spermatozoa (Michael *et al.*, 2009). Increased oxidative stress damages the sperm membranes, proteins and DNA (Sanoka and Kurpisz, 2004). Also, oxidative stress induces peroxidative damage to the plasma membrane of sperm (Visser and Heynes, 2003), which causes loss of sperm function (Kato *et al.*, 2001). The effect of oxidative stress of I/R of right testes are demonstrated by significant increases in right and left testicular MDA levels and significant decreases in right and left testicular glutathione peroxidase and catalase levels compared to both sham and treated T/D rats. These results are in agreement with the study of Ergur *et al.* (2008) who reported that darbepoetin  $\alpha$ ; a novel EPO protein caused similar effects in torsioned testes. The significant increase in MDA level (indirect method for measurement of ROS activity) in untreated T/D rats compared to both sham and treated rats proved that I/R of the testes promotes severe cell membrane peroxidation (Guimaraes *et al.*, 2007). The antioxidant effect of EPO is previously supported by the findings that EPO protects against oxidative damage via inhibition of lipid peroxidation and restoration of cytosolic catalase and GPx activities in erythrocytes. Erythropoietin also increases free radical scavengers activities in cultured mouse astrocytes. Thus, EPO may serve the role of direct antioxidant by scavenging oxyradicals and indirect antioxidant by stimulating the other antioxidant defensive mechanisms (Palmer *et al.*, 1990). It is also possible that EPO exerts EPO-R independent cytoprotective actions via antioxidation (Akeora *et al.*, 2007).

EPO is a hormone secreted in response to hypoxia mainly by the kidneys, also the liver has a significant role in erythropoietin mRNA formation (Koseglu *et al.*, 2009). Moreover, it has been reported

that testosterone production was stimulated by EPO in rat Leydig cells and this finding might be the cause of suggestion that EPO can act directly on human Leydig cells (Buemi *et al.*, 2002). Furthermore, erythropoietin is important in the development of new blood vessels, for example, in the muscle of athletes training at high altitudes, also it is found in ovary, uterus and testis (Lappin, 2003). In the present study EPO treatment caused an increase in vascularization in between seminiferous tubules and this may suggest a vasoproliferative and neoangiogenic ability of EPO as previously described by Koseglu *et al.* (2009).

Histopathological examination of sections of right testes in untreated T/D rats showed loss of germinal epithelium, loss of spermatid, absence of the sperm which denotes absence of spermatogenesis. Dark (deep stained) nuclei indicating inactive cells. Also, presence of cellular infiltration, exudates, congested blood vessels are further proofs of the dysfunction of the right testes. Contralateral left testes affection are also observed, one theory postulates decrease in blood supply of contralateral testis as a reflex to an afferent stimulus (Andiran *et al.*, 2000), other theories proposed autoimmune reaction, release of acrosomal enzymes from contralateral testis (Uguralp *et al.*, 2004), and apoptosis (Pampal *et al.*, 2010). So the preservation of twisted testes by the detorsion procedure might cause further deterioration by I/R injury, indicating the importance of the removal of the damaged testis to decrease histopathological damage of the contralateral side, or using a therapy to decrease the injurious effect of I/R. In the present study, EPO treated T/D rats showed marked improvement of the general histopathological picture of the right T/D as well as of the left contralateral testes which may be explained by the direct stimulating effect of EPO on germ cell proliferation and its antiapoptotic signaling. The exact mechanism of the protective effect of EPO against I/R injury is not fully understood. Akeora *et al.* in their study reported that EPO protection on brain tissues exposed to oxidative stress is secondary to the inhibition of caspases 3, 8 and 9 and hence an antiapoptotic effect of EPO (Akeora *et al.*, 2007). Both sperm concentration and motility were reduced on 30 days after one hour torsion in rats in the study of Payabvash *et al.* (2008). The detrimental effects of IR on the sperm concentration suggest an interference with spermatogenesis; ischemia may induce necrotic degeneration of testicular tissues, which may contribute to loss of spermatogenesis in testis.

In conclusion we can suggest that erythropoietin treatment can be used beside surgical correction as a novel therapeutic approach in T/D of the testis to ameliorate the injurious effect of ischemia

reperfusion, and to be an alternative therapy to T/D instead of removal of ipsilateral testicular torsion.

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#### 5. References:

1. Aebi H. (1984): Catalase in vitro. *Methods Enzymol.* 105: 121-126.
2. Akeora B.; Altug M.E.; Kontas T. and Atik E. (2007): The protective effect of darbepoietin alfa on experimental testicular torsion and detorsion injury. *Int. J. Urol.* 14: 846-50.
3. Andiran F.; Okur D.H.; Kilink A.; Gedikoglu G.; Kilink K. and Tanyel F.C. (2000): Do experimentally induced ipsilateral testicular torsion, vas deferens obstruction, intra-abdominal testis or venous obstruction damage the contralateral testis through a common mechanism?. *BJU Intern.* 85:330-335.
4. Armitage P. and Berry G. (1987): *Statistical Methods in Medical Reserve in left ventricular hypertrophy.* *Hypertension* 5: 192-197.
5. Bricaire C.; Raynaud A.; Benotmane A.; Clair F.; Paniel B.; Mowszowicz I.; Wright F.; Moreau J.F.; Kuttann F.; and Mauvais-Jarvis P. (1991): Selective venous catheterization in the evaluation of hyperandrogenism. *J. Endocrinol. Invest.* 14:946-956.
6. Buemi M.; Vaccaro M.; Sturiale A.; Galeano M.R.; Sansotta C.; Cavallari V.; Floccari F.; D'Amico D.; Torre V.; Calapai G.; Frisina N.; Guarneri F. and Vermiglio G. (2002): Recombinant human erythropoietin influences revascularization and healing in a rat model of random ischemic flaps. *Acta Derm. Venereol.* 82 (6): 411- 417.
7. Bulger E.M. and Maier R.V. (2001): Antioxidants in critical illness. *Arch Surg.* 136: 1201-1207.
8. Callies F.; Stromer H.; Schwinger R.H.; Bolck B.; Hu K.; Frantz S.; Leupolal A.; Beer S.; Allolio B. and Bonz A.W. (2003): Administration of testosterone is associated with a reduced susceptibility to myocardial ischemia. *Endocrinology.* 144:4478-4483.
9. Draper H.H. and Hadley M. (1990): Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol.*; 186: 421-431.
10. Drury R.A. and Wallington E.A. (1980): *Carleton's Histological Techniques.* Fifth Edition Oxford University, p 139.
11. Er F.; Michels G.; Brandt M.C.; Khan I.; Haase H.; Eicks M.; Lindner M. and Hoppe U.C. (2007): Impact of testosterone on cardiac L-type calcium channels and Ca<sup>2+</sup> sparks: acute actions antagonize chronic effects. *Cell Calcium.* 41:467-477.
12. Er F.; Michels G.; Gassanov N.; Rivero F. and Hoppe U.C. (2004): Testosterone induces cytoprotection by activating ATP-sensitive K<sup>+</sup> channels in the cardiac mitochondrial inner membrane. *Circulation.* 110:3100-3107.

13. Ergur B.U.; Kiray M.; Pekcetin C.; Bagriyanik H.A. and Erbil G. (2008): Protective effect of erythropoietin pretreatment in testicular ischemia-reperfusion injury in rats. *Journal of Pediatr. Surg.* 43: 722-728.
14. Filho D.W.; Torres M.A.; Bordin A.L.; Crezcynski-Pasa T.B. and Boveris A. (2004): Spermatic cord torsion, reactive oxygen and nitrogen species and ischemic reperfusion injury. *Mol. Aspects Med.* 25 (1-2): 199-210.
15. Gnimaraes S.B.; Aragao A.A.; Santos J.M.; Kimura Ode S.; Barbosa P.H. and Vasconcelos P.R. (2007): Oxidative stress induced by torsion of the spermatic cord in young rats. *Acta Circ. Bras.* 22: 30-33.
16. Golden K.L.; Marsh J.D. and Jiang Y. (2004): Testosterone regulates mRNA levels of calcium regulatory proteins in cardiac myocytes. *Horm. Metab. Res.* 36:197-202.
17. Granger D.N. and Korthis R.J. (1995): Physiological mechanisms of postischemic tissue injury. *Annu. Rev. Physiol.* 57: 311-32.
18. Henkel R. (2005): The impact of oxidants on sperm function. *Andrologia* 37: 205-206.
19. Kato M.; Makino S.; Kimura H.; Ota T.; Furuhashi T. and Nagamura Y. (2001): Sperm motion analysis in rats treated with adriamycin and its applicability to male reproductive toxicity studies. *J. Toxicol. Sci.* 26: 51-59.
20. Köseoğlu B.; Yılmaz E.; Ceylan K.; Uzun E.; Bayram I. and Hizh F. (2009): The protective effect of erythropoietin on testicular torsion/detorsion: an experimental study. *Int. J. Urol.* 41: 85-91.
21. Lappin T. (2003): The cellular biology of erythropoietin receptors. *The Oncologist* 8: (suppl 1)15-18.
22. Lysiak J.J.; Nguyen Q.A. and Turner T.T. (2002): Peptide and nonpeptide reactive oxygen scavengers provide partial rescue of the testis after torsion. *J. Androl.* 23 (3): 400-409.
23. Michael J.A.; Alexopoulos C.; Pontiki E.A.; Hadjipavlou-Litina D.J.; Saratsis P.; Ververidis H.N. and Boscos C.M. (2009): Effect of antioxidant supplementation in semen extenders on semen quality and reactive oxygen species of chilled canine spermatozoa. *Anim. Reprod. Sci.* 112: 119-135.
24. Michels G.; Er F.; Eicks M.; Herzig S. and Hoppe U.C. (2006): Long-term and immediate effect of testosterone on single T-type calcium channel in neonatal rat cardiomyocytes. *Endocrinology.* 147:5160-5169.
25. Minutoli L.; Antonuccio P.; Romeo C.; Nicòtina P.A.; Bitto A.; Arena S.; Polito F.; Altavilla D.; Turiano N.; Cutrupi A.; Zuccarello B. and Squadrito F. (2005): Evidence for a role of mitogen activated protein kinase 3/ mitogen activated protein kinase in the development of testicular ischaemia reperfusion injury. *Biol. Reprod.* 73 (4): 730-36.
26. Okin P.M.; Devereux R.B.; Nieminen M.S.; Jern S.; Oikarinen L.; Viitasalo M.; Toivonen L.; Kjeldsen S.E.; Julins S.; Snapinn S. and Dahlöf B. (2004): Electrocardiography strain pattern and prediction of cardiovascular morbidity and mortality in hypertensive patients. *Hypertension* 44: 48-54.
27. Paglia D.E. and Valentine W.N. (1967): Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* 10: 158-169.
28. Palmer C.; Vannucci R.C. and Towfighi J. (1990): Reduction of perinatal hypoxic ischemic brain damage with allopurinol. *Pediatr. Res.* 27: 332-36.
29. Pampal A.; Ozen I.O.; Ekingen G.; Demirogullari B.; Helvacioğlu F. and Take G. (2010): The morphological evaluation of ipsilateral and contralateral vasa deferentia in a rat model of unilateral spermatic cord torsion. *Pediatr. Surg. Int.* 26: 287-292.
30. Payabvash S.; Kiumehr S.; Tavangar S.M. and Dehpour A.R. (2008): Ethyl pyruvate reduces germ cell specific apoptosis and oxidative stress in rat model of testicular torsion/detorsion. *J. Pediatr. Surg.* 43: 705-712.
31. Perotti M.; Badger W.; Prader S. and Moran M.E. (2006): Medical malpractice in urology, 1985 to 2004: 469 consecutive cases closed with indemnity payment. *J. Urol.* 176 (5): 2154-7.
32. Reeves P.G.; Nielson F.H.; and Fahey G.C.Jr. (1993): AIN 93 Purified diets for laboratory rodents: Final report of the American Institute of Nutrition and HOC Writing Committee on the Reformation of the AIN 76 A rodent diet. *J. Nutr.* 123: 1939-1952.
33. Reilly P.M.; Schiller H.J. and Bulkley G.B. (1991): Pharmacologic approach to tissue injury mediated by free radicals and other reactive oxygen metabolites. *Am. J. Surg.* 161 (4) 488-503.
34. Sanocka D. and Kurpisz M. (2004): Reactive oxygen species and sperm cells. *Reprod Biol. Endocrinol.* 2: 12.
35. Tsang S.; Wu S.; Liu J. and Wong T.M. (2008): Testosterone protects rat hearts against ischaemic insults by enhancing the effects of  $\alpha$ 1-adrenoceptor stimulation. *Br. J. Pharmacol.* February; 153(4):693-709.
36. Turner T.T. and Brown K.J. (1993): Spermatic cord torsion: loss of spermatogenesis despite return blood flow. *Biol. Reprod.* 49: 401-407.
37. Uguralp S.; Karabulut A.B.; Mizrak B.; Kaymaz F.; Kiziltay A. and Hasirci N. (2004): The effect of sustained and local administration of epidermal growth factor on improving bilateral testicular tissue after torsion. *Urol. Res.* 32: 323-331.
38. Visser A.J. and Heynes C.E. (2003): Testicular function after torsion of the spermatic cord. *BJU Int.* 92: 200-203.
39. Weidemann W. and Hanke H. (2002): Cardiovascular effects of androgens. *Cardiovasc Drug Rev.* 20:175-198.
40. Yamamoto Y.; Sofikitis N. and Miyagawa I. (1997): Effects of erythropoietin, bromocryptine and hydralazine on testicular function in rats with chronic renal failure. *Andrologia* 29 (3): 141-144.

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## Laser versus Nerve and Tendon Gliding Exercise in Treating Carpal Tunnel Syndrome

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**Abstract:** carpal tunnel syndrome (CTS) is a highly prevalent entrapment neuropathy with a major impact on hand functions. The purpose of this study was to compare the clinical effect of low level laser (LLL) with nerve and tendon gliding exercise as a conservative treatment for carpal tunnel syndrome. Methods: Thirty female patients with mild to moderate carpal tunnel syndrome; ranged in age from 30-45 years, participated in this study. Patients were randomly divided into two groups of equal number; patients in group (A) received low level laser, while those in group (B) received nerve and tendon gliding exercises. Treatment was conducted three times / week for two successive months for both groups. Outcomes were assessed at the baseline and at the end of the two months using visual analogue Scale, grip strength measurement and nerve conduction studies. Results: Both groups showed a statistically significant reduction in pain, improvement of the grip strength and nerve conduction in favor of the group (A), that showed significant differences in all measured variables compared with group (B). Conclusion: LLLT has to be more effective treatment option than nerve and tendon gliding exercises for treatment of mild to moderate CTS. Further studies are recommended to investigate the combined effects of both interventions for treating CTS.

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**Key Words:** carpal tunnel syndrome – low level laser –nerve gliding exercises –tendon gliding exercises.

### 1. Introduction

Carpal tunnel syndrome (CTS) is one of the most common upper limb entrapment neuropathies particularly in the adult women. <sup>(1)</sup> The prevalence of CTS varies from 0.6% to 16% in the general population. <sup>(2)</sup> It has been estimated that CTS is one of the work related disabilities in the industrial countries that recently become a growing reason for workers' compensation claims due to work absence, induced social impact, and increased load to the health care system. <sup>(1)</sup>

The pathophysiology of CTS is thought to be a constellation of symptoms associated with localized compression of the median nerve at the wrist resulting in ischemia or mechanical injury which impaired nerve conduction velocity. <sup>(3)</sup> This condition may result from space-occupying lesions within the tunnel, such as cysts, tumors, osteophytes, fracture callus, or hypertrophic synovial tissue. It has also been tied to metabolic or systemic conditions such as thyroid disease, diabetes mellitus, rheumatoid arthritis, alcoholism, and pregnancy. <sup>(4)</sup>

In addition to compression and systematic disease, CTS has also attributed to a decrease in longitudinal excursion of the median nerve as it passes through the carpal tunnel. <sup>(5)</sup> Although most cases of CTS are idiopathic, its incidence is much higher in certain occupations involved daily high

velocity and high-force manual labor and repetitive work as construction, meat-packing and assembly line trades as well as those that perform long periods of precise hand movements. <sup>(6, 7)</sup> Patients with CTS typically reported symptoms of numbness, tingling, paraesthesia and nocturnal burning pain involving the fingers innervated by the median nerve. Symptoms are worst at night and often wake the patient. The clinical signs may include a decrease in both light and discriminative touch sensation, and in advanced cases, a loss of grip and pinch strength. <sup>(8)</sup>

Early in the course of CTS, no morphologic changes are observable in the median nerve, neurological findings are reversible, and symptoms are intermittent. Prolonged or frequent episodes of elevated pressure in the carpal tunnel may result in segmental demyelination and more constant and severe symptoms, occasionally with weakness. When there is prolonged ischemia, axonal injury, and nerve dysfunction may be irreversible. <sup>(9)</sup>

The management of CTS is based on relieving the pressures from the median nerve. Several treatments are used with considerable controversy surrounding the optimal management modalities. Despite the superiority of surgical release of transverse carpal ligament in the management of patients with advanced CTS, conservative management is the treatment of choice for patients

with mild to moderate CTS.<sup>(10)</sup> The currently recommended conservative treatment options are splinting, modifications in working conditions, local cortico-steroid injections, non-steroidal anti-inflammatory drugs, ultrasound therapy, and iontophoretic applications.<sup>(11,12)</sup> However, systematic reviews indicate that there is either limited evidence for the long term effects of these conservative treatment modalities<sup>(13,14)</sup> the long term effects are considered to be poor.<sup>(10)</sup>

Occasionally, alternative modalities for treatment of CTS are carried out. These include the introduction of low level laser therapy (LLLT)<sup>(15, 16)</sup>. Many investigators demonstrated the potential effects of laser therapy in treating CTS. These include effects on acetylcholine esterase enzyme, stimulation of fibroblast proliferation, increase microcirculation, stimulation of RNA, DNA, and ATP synthesis, increase cellular oxygen consumption and increase endorphin production.<sup>(16,17,18)</sup> However the issues about optimum dose regimens, schedules, cost effectiveness and clarifying which patients will likely respond to LLLT are needed to be enlightened by future controlled trials.<sup>(19)</sup>

Recently, several narrative reviews have advocated nerve and tendon gliding exercises as a biologically plausible alternative for traditionally advocated treatment modalities in the conservative management of CTS.<sup>(19,20,21)</sup> There is evidence that median nerve excursion can be influenced by neural gliding techniques, as demonstrated in a cadaveric study.<sup>(22)</sup> nerve and tendon gliding technique based mainly and attempts to take the nerve throughout the available range of motion, potentially affecting the nerve both mechanically and physiologically.<sup>(5,22)</sup>

The beneficial effects of these exercises may include improving the actual excursion of the nerve, reducing symptoms by allowing the nerve to move freely, decreasing adhesions, direct mobilization of the nerve, facilitation of venous return, edema dispersal, This technique may also help to oxygenate the nerve, decreasing ischemic pain, decrease of pressure inside the perineurium, and decrease of carpal tunnel pressure.<sup>(23,24)</sup> Though these exercises are commonly prescribed, there has been little research performed to support their use and justify their clinical value in combined with other conservative management.<sup>(25)</sup>

So the aim of this study was comparing the clinical efficacy of low level laser versus nerve and tendon gliding exercise for treating CTS.

## 2. Materials and methods:

### Subjects

30 female patients their age ranged from 35 to 45 years with mild to moderate CTS. The clinical

and electrophysiological evidence of CTS lasted over 3 months. All patients had unilateral involvement, and they were right handed. The local ethics committee approved the study protocol. The aim and methods of the study were explained to all patients before their informed consent was given. All patients with history of pain, paresthesia or numbness in the median nerve distribution, nocturnal pain, and night waking were enrolled in the study. Inclusion criteria were: 1) positive Phalen's test, 2) positive Tinnel's test, and 3) standard electrophysiological criteria including prolongation of nerve conduction velocity (i.e., motor latency > 4 ms or sensory latency > 3.5 ms and sensory conduction velocity < 39m/sec).

Patients were excluded if they had secondary entrapment neuropathies; electroneurographic and clinical signs of axonal degeneration of the median nerve, history of treatment with LLLT for CTS, or had required regular analgesic or anti-inflammatory drugs. Patients with a history of steroid injection into the carpal tunnel, thyroid disease, diabetes, pregnancy or systemic peripheral neuropathy were excluded as well. When the patients satisfied the inclusion criteria, they were randomly divided into two groups. Numbers were given to them. Group(A) consisted of the patients with odd numbers, and group ( B) consisted of the patients with even numbers.

Group A: (n = 15) received low level laser therapy and group B :( n = 15) received nerve and tendon gliding exercises.

### Interventions

#### 1) For group (A):

Low-level laser therapy was administered by applying low intensity infrared laser (Enraf, Endolaser) with a wave length 830 nm and output power was 30mw. The laser probe (1 cm diameter) was applied directly and perpendicularly on five points over the course of the median nerve on the volar side at the wrist where it localized superficially.<sup>(26)</sup> The dose per tender points was (1.8 J). The total dose per treatment was (9 J) and accumulated dose for ten treatments was 72 joule. The treatment was conducted over the course of 4 weeks for 10 minutes/ day, 2times \week. To standardize the total dosage that each subject received, a thin clear plastic template with 1cm grids was placed over the wrist and palm. The template was placed at an identical location at each session. A total of 5 points across the median nerve trace were irradiated with the laser probe.<sup>(27)</sup>

#### 2) For group (B):

The patients in group II were instructed to perform nerve and tendon gliding exercises

developed by Totten and Hunter.<sup>(28)</sup> A brochure describing exercises was given to all patients in this group. During tendon gliding exercises, the fingers were placed in five discrete positions. Those were (straight, hook, fist, table top, and straight fist). Each position kept for seven seconds. During the median nerve gliding exercise, the median nerve was mobilized by putting the hand and wrist in six different positions. These exercise carried out with the patient in a sitting position that varied according to the patient's ability to relax the proximal musculature as a following:

**Position 1:** Exercises were begun with the wrist in a neutral position (0 degrees) and the fingers and thumb in the full flexion position. The distal median nerve was placed in a relatively relaxed position.

**Position 2:** With the wrist kept in the neutral position, the fingers were brought to extension with the thumb in a neutral position. Tension in the distal segment of the nerves in the digits was increased.

**Position 3:** With maintenance of finger extension and the neutral position of the thumb, wrist extension was added to the exercises. The area of greatest excursion was accessed as the wrist was extended.

**Position 4:** While keeping the wrist and fingers extended, the thumb was extended. The median nerve branch to the thumb was included in this exercise.

**Position 5:** With the wrist, fingers, and thumb kept in extension, the forearm was brought into supination. This added tension to the more proximal portion of the median nerve in the forearm.

**Position 6:** With extension of the wrist, fingers, and thumb and supination of the forearm, slight tension was applied to the thumb with the other hand. During these exercises, the neck and the shoulder were in a neutral position, and the elbow was in supination and 90 degrees of flexion. Each position was maintained for 5 sec. The exercises were applied as 3 times a day. Each exercise was repeated 10 times the exercises program was continued for two month.<sup>(29)</sup>

### Outcome Measures

Patients were assessed at the baseline and at the end of treatment sessions to compare between the effects of the two treatment protocols. The main outcomes measures include: pain assessment, grip strength measurement and nerve conduction studies (NCS).

Pain intensity was measured by means of a visual analogue scale (VAS), which is a calibrated scale on which the patients could indicate their assessment along a 10 cm line ranging from 0 ('no

pain at all') to 10 ('the most severe pain that I can imagine).

Hand grip strength measured with a handheld dynamometer. The patient's positioning was standardized with his/her elbow was flexed 90 and the average force of three consecutive trials was calculated. The dynamometer was initially standard and its sensitivity was controlled regularly by standard weights.

The nerve conduction studies of the median nerve were performed with a portable electromyography (Medelec Synergy; Oxford Instruments Medical, Surrey, UK). The room temperature was maintained around 30–31°C. For the motor nerve conduction studies; a pair of surface recording electrodes placed on the abductor pollicis brevis muscle. The stimulating electrodes were placed at the wrist proximal to carpal tunnel for the distal segment stimulation, and at the elbow for the proximal segment stimulation. The distal motor latency was measured from the onset of the stimulating artifact to the onset of the compound muscle action potential. The nerve conduction velocity was also calculated to rule out any median nerve lesions such as poly-neuropathy.<sup>(30)</sup>

In the study of sensory nerve conduction, a pair of ring electrodes was placed on the index finger for recording, and the sensory nerve was stimulated antidromically at the same site used for distal motor stimulation. Sensory distal latency was measured from the stimulating artifact to the peak of sensory nerve action potential.<sup>(31)</sup>

The outcome measuring parameters were statically analyzed by descriptive statistics (mean and stander deviation). The paired and unpaired t-test was used to compare the pre and post- treatment values of measuring outcomes within the group and between the two groups respectively. The p value of less than 0.05 was taken as significant.

### 3. Results

Thirty female patients with unilateral CTS, aged from (35 to 45) years who fulfilled the inclusion criteria agreed to participate in our study and were randomly allocated to either the laser (group A) or exercise (group B) treatment groups. Demographic characteristics and clinical features of both groups before the treatment are shown in (Table 1). There was no significant difference between the two groups regarding age, weight and symptoms duration ( $P > 0.05$ ).



**Table 1. Demographic Data of 30 Patients in the Two Groups**

Characteristics	Group A (n=15) mean $\pm$ SD	Group B (n=15) mean $\pm$ SD	P- VALUE
Age (years)	38.07 $\pm$ 2.25	38.47 $\pm$ 2.29	0.418
Weight (kg)	82.6 $\pm$ 17.23	73.47 $\pm$ 8.45	0.075
Duration (years)	2.31 $\pm$ 1.93	1.65 $\pm$ 1.3	0.276

Pre-treatment and post-treatment intra-group analysis revealed that, there was a significant decrease in pain value according to VAS, significant

increase in the average grip strength, and significant improvement in NCS, in favor of Laser group (Table2).

**Table 2: Statistical analysis of treatment outcomes between two groups**

Variables	Group A		t-value	p-value	Group B		t-value	p-value
	Pre-treatment	Post treatment			Pre-treatment	Post treatment		
	Mean $\pm$ SD	Mean $\pm$ SD			Mean $\pm$ SD	Mean $\pm$ SD		
VAS	7.13 $\pm$ 1.3	2.86 $\pm$ 1.30	8.49	0.0001*	7.533 $\pm$ 1.5	5.2 $\pm$ 1.52	5.25	0.0001*
Grip strength	9.40 $\pm$ 2.13	16.20 $\pm$ 2.27	16.3	0.0001*	9.73 $\pm$ 2.12	11.6 $\pm$ 2.92	6.08	0.0001*
MDL	4.53 $\pm$ 0.35	3.54 $\pm$ 0.35	10.88	0.0001	4.86 $\pm$ 0.3	4.36 $\pm$ 0.61	3.93	0.0015*
SDL	4.44 $\pm$ 0.49	3.43 $\pm$ 0.25	6.55	0.0001*	4.01 $\pm$ 0.22	3.76 $\pm$ 0.32	4.67	0.0001*
SCV	34.81 $\pm$ 1.64	40.81 $\pm$ 1.67	16.63	0.0001*	35.51 $\pm$ 0.85	39.07 $\pm$ 1.52	11.43	0.0002*

VAS . Visual analogue scale

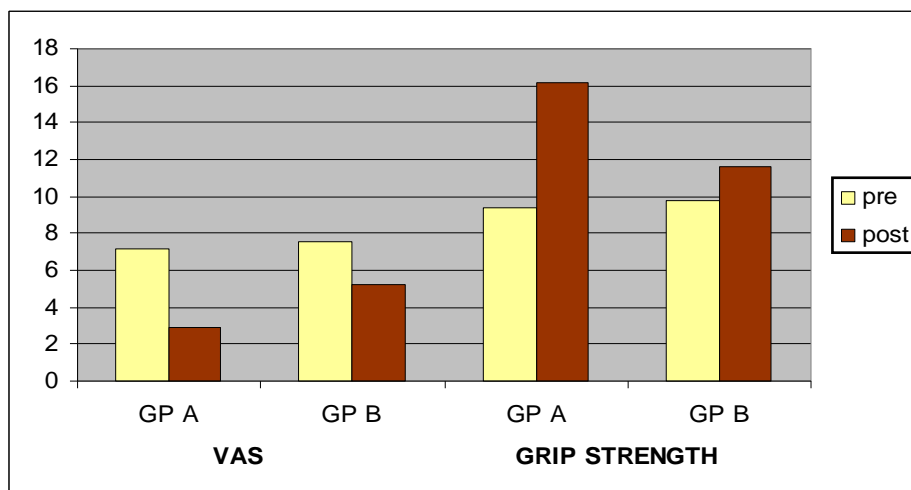
MDL. motor distal latency

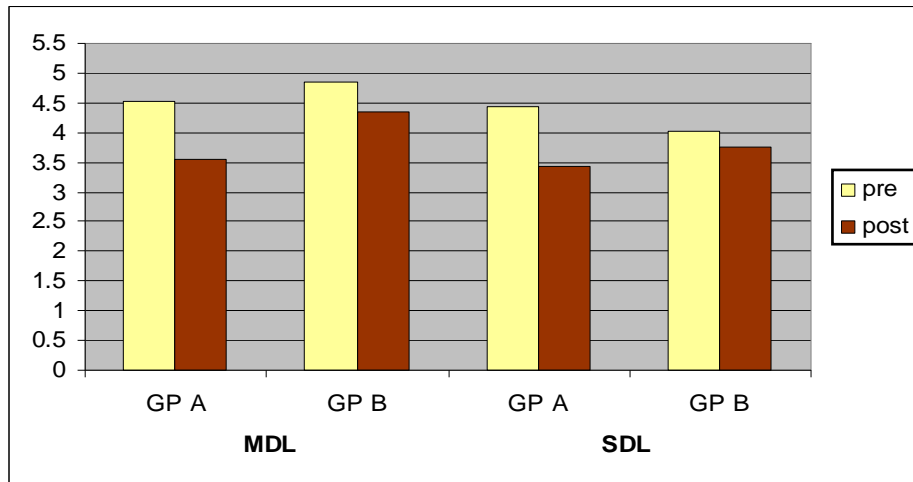
SCV. Sensory conduction velocity

SDL. sensory distal latency

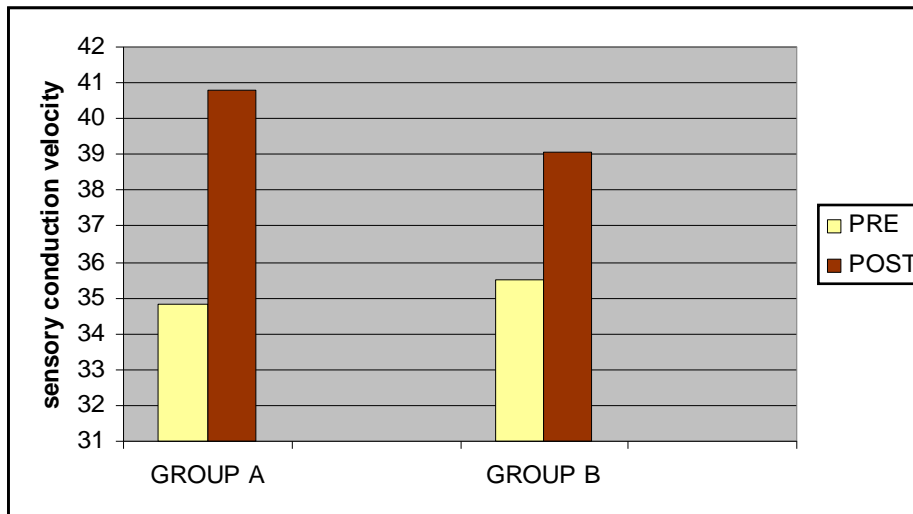
Comparing the difference between post-treatment outcome measurements related to pain , grip strength and nerve conduction studies (MDL,SDL AND SCV) between two groups revealed

that there was a statistically significant reduction in VAS score, increasing grip strength and improvement of NCS in laser group compared with exercise group ( $p < 0.05$ ). ( fig.1,2,3).

**Fig1. Mean values of VAS and Grip strength of the two groups pre and post treatment**



**Fig2. Mean values of Motor and Sensory distal latencies for the two groups pre and post treatment**



**Fig3. Mean values of Sensory Conduction Velocity for the two groups pre and post treatment**

#### 4. Discussion

The current study was conducted to compare the clinical efficacy of LLLT versus nerve and tendon gliding exercises in treating CTS. In the era of CTS researches, various studies advocate the conservative management for mild to moderate cases of CTS.<sup>(19,32)</sup> There may be possible reasons for choosing conservative methods that including: recurrent symptoms of CTS in 10 % to 19% of patients following surgical release, with up to 12% requiring re-exploration ; there is probability of the spontaneous recovery in some patients with CTS related to pregnancy, in addition to potential complication of surgery compared with safety , non invasive and cost effect of the non surgical management.<sup>(33,34)</sup> Kaplan et al. tried to define more accurately those patients likely to respond to non-surgical treatments by identifying five risk factors:

patient age greater than 50 years, the presence of symptoms for 10 months or more, constant paresthesias, presence of associated trigger fingers and/or positive results of phalen's test after 30s. These five factors help in predicting the outcome of conservative management of CTS.<sup>(35)</sup>

Regarding these risk factors, our patients mean age was under 50 years, and the duration of symptoms was less than 10 months and none of them had trigger fingers. The other possible reason for the favorable results obtained in our study could be the absence of thenar atrophy in those patients. There is no consensus with regard to the choice of initial treatment for CTS. The American Academy of Neurology advises non-invasive treatment first, i.e. wrist splints, modification of activities, NSAIDs or diuretics and using invasive steroid injections or open

carpal tunnel release only if noninvasive treatment have turned out to be ineffective.<sup>(36)</sup>

LLLT has gained a considerable attention as a trial of alternative modalities for CTS. There are many methodological differences among the studies regarding the laser type, dose regimens, and outcome measures for treating CTS. Naeser et al. Investigated the effects of real or sham LLLT plus microamperes transcutaneous electric nerve stimulation with helium neon laser to acupuncture points on the finger and hands. A significant decrease was observed in pain, sensory latency, and Phalen's and Tinel's signs after real laser treatment.<sup>(37)</sup>

Based on the finding of our study, there was a significant difference in the mean value of pain, grip strength and NCS between groups after treatment. Therefore, the dosage and treatment time with the 830-nm laser used in our study may be sufficient to provide continuous energy to accelerate repair, and to decrease the inflammatory response. These results are consistent with the findings of Chang et al who investigated the therapeutic effects of the 830-nm diode laser in a placebo-controlled study, the results revealed that pain scores of laser group had reduced significantly after 2 wk of treatment, and after 2 wk of follow-up, there were significant differences in hand and finger grip strength testing in favor of laser group.<sup>(30)</sup> Irvine et al. used different laser dosage over the carpal tunnel, whereas the control group was treated with sham laser. It was found a significant symptomatic improvement in both the control and laser groups and no significant difference was detected in any of the outcome measures between the two groups.<sup>(38)</sup>

In addition to Evcik et al. who compared placebo laser with a randomized trial, adding hand splint for both groups. The results revealed that statistically significant improvements were found in sensory nerve velocity and sensory and motor distal latencies in the laser group compared with the other group.<sup>(39)</sup> As well as Shooshtari et al. who evaluated the effects of laser therapy (9–11 J/cm<sup>2</sup>) with a sham-controlled study and were found a significant improvement in clinical symptoms, NCV findings, and hand grip in laser group while there were no significant changes in sham laser except changes in clinical symptoms.<sup>(40)</sup>

Also these findings were in agreement with Dincer et al. who compared splinting, splinting plus ultrasound, and splinting with ultrasound plus low level laser therapy. It was seen that the combinations of the ultrasound and low-level laser therapy with splinting seems more effective than only splinting in the CTS treatment.<sup>(41)</sup>

With the progressive achievement in the field of LLLT, the healing effect of damaged tissue was confirmed in this study by reliving of pain, increasing

of grip strength and NCS compared with the exercise group. Previous studies have shown that laser energy may induce athermal photochemical reaction that alters the pain threshold of nociceptors. Evidence abounds that phototherapy modulate inflammation by reducing prostaglandin concentration, inhibiting cyclo-oxygenase in vitro, reducing tumor necrosis factor, increase production of adenosine triphosphate, which increases cell metabolism, and increases the production of serotonin and endorphins (the body's endogenous pain relievers).<sup>(42, 43)</sup>

The exact mechanisms by which laser energy reducing pain and inflammation continue to be evolve. It has been shown that phototherapy increases local and systemic microcirculation thereby reducing swelling and pain. Others have suggested that LLLT could relief pain by modulating the key mediators of inflammation similar to the effect of non steroidal anti-inflammatory drugs and steroids. There are many possibilities could explain why there were increased grip strength in the laser group than the exercises group, on possible explanation may be the consistently linear relationship between the repair of the nerve and the subsidence of inflammation and swelling of the carpal ligament.<sup>(25)</sup>

Identifying the specific pathogenesis of CTS may be important in determining the true efficacy of nerve and tendon gliding exercises. Though the most current and available evidence suggested that patients with CTS due to mechanical compression of the median nerve whose are likely to benefit from this type of exercise. The following etiologies are suggested to elicit a compression of the neurovascular system as it passes through the carpal tunnel: ischemia, decrease in longitudinal excursion of the median nerve, and mechanical compression or injury to involved carpal structures.<sup>(44)</sup> Nerve and tendon gliding exercises may relieve ischemic pain by contributing to the delivery of oxygenated blood to the median nerve at its distal site within the wrist and hand. Therefore, gliding exercises are recommended as an important mainstay in the conservative management of CTS of mechanical nature.<sup>(45, 46)</sup>

In light of these research findings, nerve and tendon gliding exercises applied in the present study, resulted in significant reduction of pain increase in grip strength, and median nerve conduction; however, the significant difference between both laser an exercises group does not provide sufficient reason to recommend these exercises as the best available conservative treatment of CTS, but might play an important role in enhancing the effectiveness of conservative treatments.

## 5. Conclusion

The current study addressed the superiority of LLLT as a non invasive treatment option for mild to moderate CTS. Incorporation of nerve and tendon gliding exercises as a cost-effective intervention in treating CTS might play an important role in enhancing the effectiveness of the conservative treatments and delay the need for surgery. Further researches are required to investigate the long-term efficacy of LLLT versus exercises using different laser parameters, and whether the combination of these two treatments is superior to either treatment alone.

Future recommendations for more research include well-controlled randomized clinical trials, follow-up periods, and combination with other physical therapy modalities. While the efficacy of neural gliding techniques for the treatment of CTS is not clear, trends toward pain and symptom reduction, improved sensation, and improved function and strength, combined with the low monetary and temporal cost of the treatment, make this treatment a reasonable option for clinicians in treating individuals with CTS.

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#### 5. References:

1. Hamamoto P., Leite F., Ruiz T., de Lima L. A Systematic Review of Anti-inflammatory for Mild to Moderate CTS. *J Clinical Neuromuscular Disease*. 2009; 11(1):22-30.
2. Gerritsen A., de Krom M., Struijs M. Conservative treatment options for carpal tunnel syndrome: a systematic review of randomised controlled trials. *J Neurol* 2002; 249: 272–80.
3. Werner R., Andary M. Carpal tunnel syndrome: pathophysiology and clinical neurophysiology. *Neurophysiology Clinics*. 2002; 113:1373-1381.
4. Von Schroeder H., Botte M. Carpal tunnel syndrome. *Hand Clin*. 1996; 12:643-655.
5. Hough A., Moore A., Jones M. Reduced longitudinal excursion of the median nerve in carpal tunnel syndrome. *Arch Phys Med Rehabil*. 2007; 88: 569-576.
6. Gell N., Werner R., Franzblau A., Ulin S., Armstrong, T. A longitudinal study of industrial and clerical workers: incidence of carpal tunnel syndrome and assessment of risk factors. *Journal of Occupational Rehabilitation*. 2005; 15 (1), 47–55.
7. BonWglioli R., Mattioli S., Fiorentini G · Francesca Graziosi F., Curti S., · Francesco S. Relationship between repetitive work and the prevalence of carpal tunnel syndrome in part-time and full-time female supermarket cash- iers: a quasi- experimental study. *Int Arch Occup Environ Health* .2007; 80:248–253.
8. Moscony AMB. Common periph- eral nerve problems. In: Cooper C, ed. *Fundamentals of Hand Therapy: Clinical Reasoning and Treatment Guidelines for Common Diagnoses of the Upper Extremity*. Philadelphia: Mosby Elsevier; 2007:201-250.
9. Katz j. Simmons p. Carpal Tunnel Syndrome. *The New England Journal of Medicine*.2002; 346(23): 1807-12.
10. Wilson J., Sevier T. A review of treatment for carpal tunnel synd- rome. *Disabil Rehabil* . 2003; 25: 113–139.
11. Hayes E., Carney K., Wolf J. Carpal tunnel syndrome. In: Ma- ckin EJ, Callahan AD, Skirven TM, Schneider LH, Osterman AL, eds. *Rehabilitation of the Hand and Upper Extremity*. St Louis: Mosby; 2002:643-657.
12. Burke F., Ellis J., McKenna H., Bradley M. Primary care manag- ement of carpal tunnel syndrome. *Postgrad Med J*. 2003; 79:433-437.
13. Gerritsen A., De Krom M., Struijs M. Conservative treatment options for carpal tunnel syndrome: a systematic review of randomized controlled trials. *J Neurol*.2002; 249:272–280.
14. O'Connor D., Marshall S., Massy-Westropp N. Nonsurgical treatment (other than steroid injection) for carpal tunnel syndrome. *Cochrane Database Syst Rev*.2003: Issue 1, Art No: CD003219.
15. Irvine J., Chong S., Amirjani N., Chan K. Double-blind randomized controlled trial of low-level laser therapy in carpal tunnel syndrome. *Muscle Nerve*.2004; 30 (2):182–187.
16. Kujawa J., Zavodnik L., Zavodnik I., Bryszewska M. Low intensity near infrared laser radiation induces changes of acetylcholine esterase activity of human erythrocytes. *Lasers Surg Med*.2003; 21(6):351–355.
17. Matson E., Perira A., Eduardo P., Marques M. Effect of low power laser irradiation on cell growth and procollagen synthesis in cultured fibroblasts. *Lasers Surg Med*.2002; 31(4):263–267.
18. Walker J. Relief from chronic pain by low power laser irradiation. *Neuroscience Letters*. 1983; 43:339–344.
19. Baysal O., Altay Z., Ozcan C., Ertemk., Yologlu S., Kayhan A.Comparison of three conser- vative treatment protocols in carpal tunnel syndrome. *J Clin Pract*, 2006; 60 (7): 820–828.
20. Michlovitz S. Conservative interv- entions for carpal tunnel syndrome. *J Orthop Sports Phys Ther*.2004; 34:589–600.
21. Muller M., Tsui D., Schnurr R. Effectiveness of hand therapy interventions in primary manag- ement of carpal tunnel syndrome: a systematic review. *J Hand Ther*. 2004; 17:210–228.

22. Ettema A., Zhao C., Amadio P., O'Byrne M., An K. Gliding characteristics of flexor tendon and tenosynovium in carpal tunnel syndrome: a pilot study. *Clin Anat.* 2007; 20:292-299.
23. Burke F., Ellis J., McKenna H. Primary care management of carpal tunnel syndrome. *Postgrad Med J.* 2003; 79:433-437.
24. Seradge H, Jia Y., Owens W. In vivo measurement of carpal tunnel pressure in the functioning hand. *J Hand Surg.* 1995; 20:855-859.
25. Medina J., Yancosek K. Neural Gliding Techniques for the Treatment of Carpal Tunnel Syndrome: A Systematic Review. *J Sport Rehabilitation.* 2008; 17:324-341.
26. Bakhtiary A., Rashidy-Pour A. Ultrasound and laser therapy in the treatment of carpal tunnel syndrome. *Aust J Physiother.* 2004; 50 (3): 147-151.
27. Ekima A., Armagana O., Tascioglu F., Onera C, Colakb M. Effect of low level laser therapy in rheumatoid arthritis patients with carpal tunnel syndrome. *SWISS MED WKLY.* 2007; 137:347-352.
28. Totten P., Hunter J. Therapeutic techniques to enhance nerve gliding in thoracic outlet syndrome and carpal tunnel syndrome. *Hand Clin* 1991; 7:505-520.
29. Pinar L., Enhos A., Ada S., Güngör N. Can We Use Nerve Gliding Exercises in Women With Carpal Tunnel Syndrome? *Advances in Therapy.* 2005; 22 (5):467-473.
30. Chang W., Wu J., Jiang J., Yeh C., Tsai C. Carpal tunnel syndrome treated with a diode laser: a controlled treatment of the transverse carpal ligament. *Photomed Laser Surg.* 2008; 26:551-557.
31. Levine D., Simmons B., Koris M.: A self-administered questionnaire for the assessment of severity of symptoms and functional status in carpal tunnel syndrome. *J Bone Joint Surg (Am)* 1993; 75: 1585-92.
32. Bardak A., Alp M., Erhan B., Paker N., Kaya B., Onal A. Evaluation of the Clinical Efficacy of Conservative Treatment in Management of Carpal Tunnel Syndrome. *Adv Ther.* 2009; 26(1):107-116.
33. Botte M., von Schroeder H., Abrams R., Gellman H. Recurrent carpal tunnel syndrome. *Hand Clin.* 1996; 12(4):731-743.
34. Stolp-Smith K., Pascoe M., Ogburn P. Carpal tunnel syndrome in pregnancy: frequency, severity, and prognosis. *Arch Phys Med Rehabil.* 1998; 79(10):1285-1287.
35. Kaplan S., Glickel S., Eaton R. Predictive factors in the non-surgical treatment of carpal tunnel syndrome. *J Hand Surg (Br)* 1990; 15:106-108.
36. American Academy of Neurology. Practice parameter for carpal tunnel syndrome (summary statement). Report of the Quality Standards Subcommittee of American Academy of Neurology. *Neurology.* 1993; 43: 2406-09.
37. Naeser M., Hahn K., Lieberman B., Branco K. Carpal tunnel syndrome pain treated with low-level laser and microamperes transcutaneous electric nerve stimulation: a controlled study. *Arch Phys Med Rehabil.* 2002; 83(7):978-988.
38. Irvine J., Chong S., Amirjani N., Chan K. Double-blind randomized controlled trial of low-level laser therapy in carpal tunnel syndrome. *Muscle Nerve.* 2004; 30(2):182-187.
39. Evcik D., Kavuncu V., Cakir T., Subasi V., Yaman M. Laser therapy in the treatment of carpal tunnel syndrome: a randomized controlled trial. *Photomed Laser Surg.* 2007; 25(1):34-39.
40. Shooshtari S., Badiie V., Taghizadeh S., Nematollahi A., Amanollahi A., Grami M. The effects of low level laser in clinical outcome and neurophysiological results of carpal tunnel syndrome. *Electromyogr Clin Neurophysiol.* 2008; 48(5):229-231.
41. Dincer U., Cakar E., Kiralp M., Kilac H., Dursun H. The effectiveness of conservative treatments of carpal tunnel syndrome: splinting, ultrasound, and low-level laser therapies. *Photomed Laser Surg.* 2009; 27: 119-125.
42. Amat A., Rigau J., Waynant R. Modification of the intrinsic fluorescence and the biochemical behavior of ATP after irradiation with visible and near-infrared laser light. *J. Photochem. Photobiol.* 2005; B. 81, 26-32.
43. Master E., Master A., and Master A. The biomedical effects of laser application. *Lasers Surg. Med.* 1985; 5, 31-39.
44. Butler D. Mobilisation of the Nervous System. 1st ed. Melbourne: Churchill Livingstone; 1991.
45. Szabo R., Bay B., Sharkey N. Median nerve displacement through the carpal canal. *J Hand Surg (Am)* 1994; 19: 901-906.
46. Rozmaryn M, Dovel S, Rothman E, Gorman K, Olvey K, Bartko J. Nerve and tendon gliding exercises and the conservative management of carpal tunnel syndrome. *J Hand Ther.* 1998; 11:171-179

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**Andragogy implications and differences with children education**<sup>1</sup> Mohammad Abedi, <sup>2</sup> Ali Badragheh<sup>1,2</sup> Department of Agricultural Economic, Islamic Azad University, Marvdasht Branch, Marvdasht, Iran\*Corresponding author: [abedi114@yahoo.com](mailto:abedi114@yahoo.com)

**Abstract:** Teaching adults can be very challenging, but also very rewarding. Most teachers would agree that the benefits derived from a successful adult education program in agriculture far outweigh the costs. In addition to the direct benefits to adult participants, the teacher, the school, the community, and the secondary program also benefit from a quality adult education program in agriculture. Adults in agriculture use a number of sources to gain new information that can be used to help them solve problems. Persons employed in agriculture utilize newspapers, magazines, newsletters, radio, television, government publications, internet, and meetings to gather information which can be directly utilized in their business activities. In many communities, the agriculture teacher is the primary source of agricultural information.

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**Keywords:** Andragogy , education, adult education

**Introduction:**

The field of adult education and literacy is plagued by confusion about definitions. Over the years definitions have evolved from provisions in federal law and initiatives of groups advocating particular methodologies or the needs of specific adult populations. The result is that definitions tend to merge statements about the goals to be achieved (e.g., improving the literacy of a particular population) with a particular means (e.g., adult basic education) to achieve the goal.

Adult illiteracy feeds the state's unemployment, its welfare rolls, and the correctional institutions. Adult illiteracy severely hinders the life chances of young children, undermines school reform, and limits the opportunities for postsecondary education.

Despite landmark reforms in public schools, too many Kentuckians continue to drop out of school, thereby perpetuating the chronic problem of adult illiteracy. Too many young Kentucky parents are unable to read and lack the basic literacy necessary to provide the necessary stimulating, supportive family environments for young children. It is known that children's literacy levels are strongly linked to the educational level of their parents and that children of parents who are unemployed and have not completed high school are five times more likely to drop out.

Only the negative consequences are obvious: getting more education often means leaving one's family and community for jobs and opportunities for advancement somewhere else. The future of Kentucky depends on uplifting the quality of life and

economy of all of Kentucky. The social and economic costs of neglect of large parts of the state will drag down the rest of the state and seriously hinder its capacity to compete in the global economy.

Much like strategies to curb epidemic, strategies to reduce illiteracy and raise the educational attainment of Kentucky's population must include both short-term efforts to face the immediate crises as well as long-term strategies to get at the underlying causes. Short-term crises include the imperative to keep helping welfare clients make the transition from welfare to work within the constraints of federal and state mandates and the need to train workers for immediate employer demands. Long-term prevention must address the underlying, persistent problems of the state's economic structure as well as the low awareness--if not appreciation--among segments of the population of the vital connection among education, employment, and improved standards of living.

Therefore, it is helpful to distinguish between at least these dimensions of the issue:

1. "Literacy" refers to the knowledge, skills, and competencies of individuals. The federal Adult Education and Family Literacy Act (Title II of the Workforce Investment Act)<sup>1</sup> defines literacy as "an individual's ability to read, write, speak in English, compute and solve problems, at levels of proficiency necessary to function on the job, in the family of the individual, and in society." Literacy is often defined in terms of specific domains such as "basic academic skills," "workplace skills," "life skills," "parenting skills," or skills necessary to exercise one's rights and

responsibilities for citizenship. Different dimensions of literacy are often categorized by terms that cluster several dimensions of literacy important for different clients.

#### **Meaning adult:**

Adult who is able to recognize their needs. He is who knows what will. Refers to individual adults in their lives cross and understand their responsibilities and has accepted the role is social. Adult learners are often those that distinguish each other and have many different targets at the same time and will follow a common challenge to fulfill the goals of building self motivation vectors as educational materials to learn and use the forge.

#### **Concept of adult education:**

Several definitions of adult education has been done Community

- Adult Education is a)in the following examples are given of them. conscious effort by public institutions or voluntary organizations to promote community awareness comes action.
- adult education teaching is typically specific age group above the legal age) limits as formal and informal, voluntary and at different levels of time, place
- Adult Education is a process in which people who)and education is presented. somehow been cut course they consciously to change or advance their skills in information and do organized activities.
- Adult education includes all formal and informal training and volunteer after) school, which by experienced educators and aware of the system.

Educational materials on adult education with daily life, needs, goals, aspirations and past experiences of adults and their relationship helps to results learned in life and career are used.

#### **Comparison of adult education in various countries:**

In developed countries, adult education is a form of informal education for people above 24 years is presented. In fact, a means of expanding knowledge, skills and abilities of adults. In these countries, adult education helps adults to variable conditions of political, social, economic and cultural adjustment, and pay to fix their shortcomings.

In developing countries and backward because the problems in primary education, lack of resources and facilities, poverty, social existence, economic and

cultural concept of adult education is different. In such countries the concept of adult education, literacy education is.

Concept of adult education in revolutionary countries, is a combination of these two concepts. Changes in these countries due to social, political and cultural revolution, resulting from, literacy and continuing education necessary to find because of the revolution, there is cultural poverty on the other hand the implementation of development plans and the need for skilled personnel are expert. General adult education system based on economic conditions - social and cultural community is different and each specific goals will follow. General objectives of adult education and literacy in two categories is divided into professional education.

#### **Literacy goals include:**

- Providing primary education in childhood that adults were deprived
- raising awareness for adults;
- knowledge bases and adults about their cultural heritage;
- increase confidence in adults.

#### **Professional education goals include:**

- Equipped with the necessary skills to adults living;
- providing the necessary manpower for the country's goals;
- achieving social equality and equity and eliminate the existing differences between different classes.

#### **Adult characteristics:**

To understand the characteristics of adult learners, their mental and physical condition should be considered in the following referred to some of them.

#### **Operating speed:**

slow reaction in adults is natural that necessarily means reducing the logic and practice skills, not due to weakness and increased awareness of natural forces and their skills.

#### **Consciousness:**

No stimulus and incentives encouraging, despite inhibiting stimuli, slow transfer rate, mental, and weak inhibitors of natural forces (mostly visual and auditory) are factors that slow reaction affect individual mental and cognitive activities, but never able to understand, understanding and learning ability (which varies with the speed of learning) is not relevant.

#### **Health:**

What is most age, longer duration is necessary to be heard by listening issue. Why is that when elderly

people and old could not hear well, their confidence and vulnerable to the possibility that negative beliefs about their find, they are great. Visual abilities can be like other people, usually decreases with age.

#### **Background of knowledge - skills and beliefs of adults:**

adults, social experiences, many have already learned different values and beliefs in their pronouns have stabilized, so changes in the new act very cautiously. The idea of such a manner that skill and applying them older and longer life is, Similar resistance to accept new ideas will be more and more severe. Thus, the adult criteria for the built and paid for their ideas and beliefs that are forming. Because of these criteria and the beliefs that they are afraid of failure, Therefore, to prevent it, sometimes against the resistance of new phenomena are only the material taught and its face that make reinforced concrete and tangible interference situation is.

#### **Adult Education Features (compared to regular education) :**

1 - Flexibility in time:

the sense that a religion course in hours and days of the appeal.

2 - Flexibility in place:

people can live without taking their training to take place. (Transfers): a case for training in various locations will provide access to individuals.

3- Flexibility of age:

the curriculum will be developed for different age range.

4 - Flexibility in accepting

5 - along with being able to adult education and job responsibilities:

during that time working in the training classes to attend. In other words, they are part of their training to be considered.

#### **Issues Beyond the Department of Adult Education and Literacy**

Beyond the issues relating directly to DAEL(Department of Adult Education and Literacy), the task force heard a number of concerns about the Commonwealth's overall approach to adult literacy.

- Lack of coherent statewide leadership and coordination among multiple complementary initiatives aimed at the same problem.
- Lack of continuity in state leadership. Cited in particular was the difficulty sustaining a high level commitment to the issue long enough to make a difference because of changes in priorities of the state's political leaders. A high level of turnover in the leadership of the Department of Adult

Education and Literacy has also contributed to the instability.

- Tendency to think of adult education as a separate categorical program rather than a strategy that cuts across the mission and responsibility of multiple Commonwealth programs and initiatives (e.g., early childhood education, welfare reform, economic development, and corrections).
- Multiple uncoordinated categorical federal initiatives that tend to drive (and fragment) policy for an overall state effort that is largely funded by Kentucky.
- A tendency to commingle and confuse different functions. The most important distinction is between functions focused on the needs of clients (adult learners, employers, communities, regions, and the Commonwealth as a whole) and functions associated with the operations and performance of providers. It is important that each of these functions receive attention, yet the tendency is for one (e.g., overseeing a network of providers) to drive out attention to overall system strategy.
- Inadequate coordination of services to meet the needs of individual adults, communities, employers, and regions is hindered by:
  - Vertical financing and regulatory relationships between separate federal and state programs and local providers and administrative units. These vertical relationships can hinder the horizontal coordination of services for individual adult learners, communities, and employers.
  - Turf wars among providers, local politics, and long-standing conflicts among neighboring counties.
- Inadequate links with and leverage of other public and private initiatives and investments to reach the target population. Major sources of help include employers, postsecondary education, and workforce development.
- Lack of a state financing policy and strategy for provider performance incentives and collaboration, and tax and other employer incentives for leverage of non-state resources.
- Lack of programmatic and administrative flexibility to meet the rapidly changing needs of adult learners, employers, regional economies, and communities.

#### **Conclusion:**

Program. Offering adult education programs helps to keep farmers and agribusiness employees



better informed of current trends and provides them with opportunities to learn new skills and improve existing ones.

Teaching adults can be very challenging, but also very rewarding. Most teachers would agree that the benefits derived from a successful adult education program in agriculture far outweigh the costs. In addition to the direct benefits to adult participants, the teacher, the school, the community, and the secondary program also benefit from a quality adult education program in agriculture.

Adults in agriculture use a number of sources to gain new information that can be used to help them solve problems. Persons employed in agriculture utilize newspapers, magazines, newsletters, radio, television, government publications, internet, and meetings to gather information which can be directly utilized in their business activities. In many communities, the agriculture teacher is the primary source of agricultural information.

Successful adult education programs develop and utilize an Agricultural Education Program Advisory Committee to assess the informational needs of adults in the community. Agriculture teachers should utilize the expertise and communications link, which an effective advisory committee provides. Specifically, the advisory committee should be asked to provide advice regarding planning, conducting, and evaluating the adult education program in agriculture.

Adult education programs in agriculture should emphasize practical application of the information presented. Topics and information included in adult programs should be provided which fulfills needs of the local community. Providing information which cannot be applied to solve a local problem or address a local issue will generally be viewed as frivolous and over time will result in decreased interest (i.e. participation) in the adult education program.

The role of the agriculture teacher should be as a facilitator of the learning process. Most adults reject the traditional teacher-student relationship, which is necessary to maintain in secondary programs. Teachers should be encouraged to view themselves as partners with adult participants in the learning process. The democratic philosophy of shared responsibility for planning, conducting, and evaluating adult education programs distinguishes adult education from secondary education.

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**Reference:**

1. Cranton, P. (1996). *Professional Development as Transformative Learning*. San Francisco: Jossey-Bass.
2. Creighton S. (2000). *Participation trends and patterns in adult education: 1991-1999*. United States: National Center for Education Statistics.
3. Egan, K. (1992). *Imagination in Teaching and Learning*. Chicago: University of Chicago Press.
4. Fabry, D. L., & Higgs, J. R. (1997). Barriers to the effective use of technology in education: Current status. *Journal of Educational Computing Research*, 17(4), 385-395.
5. Fletcher, W. E., & Deeds, J. P. (1994). Computer anxiety and other factors preventing computer use among United States secondary agricultural educators. *Journal of Agricultural Education*, 35(2), 16-21.
6. Frye, N. (1993). *The Educated Imagination*. Toronto: Canadian Broadcasting Corporation.
7. Ginsburg, L. (1998). Integrating technology into adult learning. In C. Hopey (Ed.), *Technology, basic skills, and adult education: Getting ready and moving forward* (Information Series No. 372, pp. 37-45). Columbus, OH: Center on Education and Training for Employment. (ERIC Document Reproduction Service No. ED 423 420).
8. Glenn, A. D. (1997). Technology and the continuing education of classroom teachers. *Peabody Journal of Education*, 72(1), 122-128.
9. Habermas, Jurgen. (1991). *Knowledge and Human Interests*. Boston: Beacon Press.
10. Hair, J. F., Anderson, R. E., Tatham, R. L., & Black, W. C. (1998). *Multivariate data analysis* (5th ed.). Upper Saddle River, NJ: Prentice Hall.
11. Hanson, Karen. (1988). Prospects for the Good Life: Education and Perceptive Imagination. In K. Egan and D. Nadaner (Eds.), *Imagination and Education*. New York: Teachers College Press.
12. Isahak Haron & Doraisamy, J. (1992). *Lifelong education in Malaysia: A general survey*. Thesis Mas. UM. 10. 1-13. Kuala Lumpur: Universiti Malaya.
13. Kim K. (2000). *Participation in adult education in the United States, 1998-1999*.

- U.S. Dept. Of Education, Office of Educational Research and Improvement.
14. King, K. P. (1999). Unleashing technology in the classroom: What adult basic education teachers and organizations need to know. *Adult Basic Education*, 9(3), 162-175.
  15. King, K. P. (2003). Learning the new technologies: Strategies for success. In K. P. King & P. Lawler (Eds.), *New perspectives on designing and implementing professional development of teachers of adults. New directions for adult and continuing education* (Vol. 98, pp. 49-57). San Francisco: Jossey-Bass.
  16. Knowles, M. S. (1999). *The making of adult educator: An autobiographical journey*. 1st Edn. San Francisco: Jossey-Bass Inc. Pub.
  17. Kolb, David A. (1993). *Experiential learning: Experience as the source of learning and development*. 1st Edn. United States: FT Press.
  18. Kotrlik, J.W., & Smith, M. N. (1999). Computer anxiety levels of vocational agriculture and other vocational teachers. In M. F. Burnett (Ed.), *Proceedings, national agricultural education research meeting* (pp. 1-9). Columbus, OH: American Association for Agricultural Education.
  19. Krajnc, A. (1999). Andragogy. In Collin, J. T. (Ed.), *Lifelong education for adults: An international handbook*. 1st Edn. New York: Pergamon Press.
  20. Lang, J. M. (1998). *Technology in adult basic and literacy education: A rationale and framework for planning* (Research report). Cheney: Eastern Washington University, Instructional Media and Technology. Retrieved on November 14, 2003, from <http://cehd.ewu.edu/education/GraduateExamples/JML98Educ601.html>
  21. Jordan, W. R., & Follman, J. M. (1993). *Using technology to improve teaching and learning. Hot topics: Usable research*. Palatka, FL: Northeast Florida Educational Consortium, Southeastern Regional Vision for Education. (ERIC Document Reproduction Service ED 355 930).
  22. Mazanah Muhamad & Associates. (2001). *Adult and continuing education in Malaysia*. 1st Edn. Kuala Lumpur: Universiti Putra Malaysia.
  23. Merriam, S.B., Baumgarther, L.M., & Caffarella, R.S. (1999). *Learning in adulthood: A comprehensive guide*. 2nd Edn. San Francisco: Jossey-Bass Pub.
  24. Mezirow, Jack and Associates (Eds.) (1990). *Fostering Critical Reflection in Adulthood: A Guide to Transformative and Emancipatory Education*. San Francisco: Jossey-Bass.
  25. Moore, M. G., & Kearsley, G. (1996). *Distance education: A systems view*. Belmont, CA: Wadsworth.
  26. Office of Technology Assessment, U.S. Congress. (1993). *Adult literacy and new technologies: Tools for a lifetime* (Final Report No. OTA-SET-550). Washington, DC: Government Printing Office.
  27. Pratt, D.D. (1993). *Andragogy after twenty-five years: New directions for adult and continuing education*. Journal Articles. San Francisco: Jossey-Bass Inc. Pub.
  28. Olgren, C. H. (2000). Learning strategies for learning technologies. In E. J. Burge (Ed.), *The strategic use of learning technologies. New directions in adult and continuing education* (Vol. 88, pp. 7-16). San Francisco: Jossey-Bass.
  29. Russell, A. (1995). Stages in learning new technology: Naive adult email users. *Computers and Technology*, 25(4), 173-178.
  30. Timmermann, S. (1998). The role of information technology in older adult learning. In J. C. Fisher & M. A. Wolf (Eds.), *Using learning to meet the challenges of older adults. New directions for adult and continuing education* (Vol. 77, pp. 61-71). San Francisco: Jossey-Bass.
  31. UNESCO. (1999). *The Hamburg Declaration. Fifth international conference on adult education* (Confitea V). Paris: UNESCO
  32. Williams, Oscar. (Ed.) (1990). *A Little Treasury of Modern Poetry* (3rd Edition). New York: Charles Scribner's.

4/13/2011

**Application of distance learning in adult education**<sup>1</sup>Ali Badragheh, <sup>2</sup>Mohammad Abedi<sup>1,2</sup> Department of Agricultural Economic, Islamic Azad University, Marvdasht Branch, Marvdasht, Iran\*Corresponding author: [abedi114@yahoo.com](mailto:abedi114@yahoo.com)

**Abstract:** There are two types of programs offered by distance education schools: synchronous learning programs and asynchronous learning programs. With synchronous learning, distance education students must log on to the school's website at a set time. Often, they interact with their peers and professors via group chats, web seminars, video conferencing, and phone call-ins. With asynchronous learning, distance education students complete all coursework on their own time. They often learn via assignment sheets, message boards, email, pre-recorded video lectures, mp3s, and traditional mail correspondence. Many students find that distance education courses give them the freedom to complete a degree while meeting their personal and professional obligations. Motivated learners are often able to complete distance education degrees in a fraction of the time often required. Distance education courses also allow students to network with participants from all over the nation. On the downside, distance education courses do not offer the face-to-face interaction found in traditional classrooms. Some students find that they struggle to stay motivated and meet deadlines due to the independent nature of distance education courses.

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**Keywords:** distance learning, adult education

**Introduction:**

Adult who is able to recognize their needs? He is who knows what will. Refers to individual adults in their lives cross and understand their responsibilities and has accepted the role is social. Adult learners are often those that distinguish each other and have many different targets at the same time and will follow a common challenge to fulfill the goals of building self motivation vectors as educational materials to learn and use the forge. Adult illiteracy is like a disease that infects virtually every dimension of Kentucky life. Adult illiteracy saps the energy and capability of Kentucky's people and its economy. Adult illiteracy feeds the state's unemployment, its welfare rolls, and the correctional institutions. Adult illiteracy severely hinders the life chances of young children, undermines school reform, and limits the opportunities for postsecondary education. Despite landmark reforms in public schools, too many Kentuckians continue to drop out of school, thereby perpetuating the chronic problem of adult illiteracy. Too many young Kentucky parents are unable to read and lack the basic literacy necessary to provide the necessary stimulating, supportive family environments for young children. It is known that children's literacy levels are strongly linked to the educational level of their parents and that children of parents who are unemployed and have not completed high school are five times more likely to drop out. To be successful, the Commonwealth's strategies must

energize and gain the commitment of all the state's political, education, business, and civic leaders. No strategy will succeed unless it engages leaders in each community and county to identify needs and develop programs and services appropriate to the community's unique circumstances. The most serious challenge will be to motivate low-skilled, under-educated adults within the working age population to seek further education. Simply expanding the number of providers and programs will not necessarily increase demand from the populations and communities where the needs are greatest. Deepseated social, economic and cultural barriers—many dating back generations—lead people to undervalue education. In addition, in many counties it is difficult for people to see a direct relationship between better education and better-paying jobs. Either there are no jobs available or many existing employers do little to emphasize the connection between better education and the possibilities for getting a job, keeping a job, or earning a higher wage. For many, getting more education and earning a high school diploma or a college degree has little positive meaning.

Only the negative consequences are obvious: getting more education often means leaving one's family and community for jobs and opportunities for advancement somewhere else. The future of Kentucky depends on uplifting the quality of life and economy of all of Kentucky. The social and

economic costs of neglect of large parts of the state will drag down the rest of the state and seriously hinder its capacity to compete in the global economy.

The field of adult education and literacy is plagued by confusion about definitions. Over the years definitions have evolved from provisions in federal law and initiatives of groups advocating particular methodologies or the needs of specific adult populations. The result is that definitions tend to merge statements about the goals to be achieved (e.g., improving the literacy of a particular population) with a particular means (e.g., adult basic education) to achieve the goal. Therefore, it is helpful to distinguish between at least these dimensions of the issue:

1. "Literacy" refers to the knowledge, skills, and competencies of individuals. The federal Adult Education and Family Literacy Act (Title II of the Workforce Investment Act)<sup>1</sup> defines literacy as "an individual's ability to read, write, speak in English, compute and solve problems, at levels of proficiency necessary to function on the job, in the family of the individual, and in society." Literacy is often defined in terms of specific domains such as "basic academic skills," "workplace skills," "life skills," "parenting skills," or skills

necessary to exercise one's rights and responsibilities for citizenship. Different dimensions of literacy are often categorized by terms that cluster several dimensions of literacy important for different clients. Examples include workplace literacy (combining both basic academic skills and workplace skills), and family literacy (combining basic academic skills and other skills essential for successful parenting).

2. "Education attainment" usually refers to the numbers of years of schooling completed or the level of credential (e.g., high school diploma or associate degree) an individual has obtained. Despite concerns about the meaning of credentials, there is a strong correlation between educational attainment and literacy.

3. "Literacy initiatives" often are defined in terms of the needs of a particular target group. These may be parents of young children, youth who have dropped out of high school without earning a high school diploma, welfare recipients, persons with limited English-speaking ability, incarcerated adults, or adults in the workforce.

Getting a college education can be difficult for people with inflammatory bowel disease (IBD). Frequent trips to the restroom, exhaustion, doctor visits, and medication side effects are all barriers to the traditional college experience.

What if you could get the degree without ever setting foot on a campus? You can do just that through distance or virtual learning.

Distance learning has been around for a long time (we've all seen the commercials on TV). While there is still prejudice surrounding some distance learning, it is increasingly being accepted as an alternative to traditional classroom learning. Courses can be offered via the Internet, where students are able to interact with instructors and other students without physically being in the same room.

Before considering if distance learning is a viable option for you, there are several questions you should ask yourself:

- What course of study would you pursue?
- Are you interested in pursuing a degree? Brushing up on existing skills?
- Would your course of study require some traditional classroom time (such as laboratory or field work)?
- After obtaining a degree, would you be able to obtain employment that allows for your illness (such as telecommuting or flexible hours)?

### What Is Adult Learning?

Adult learners have a different approach to learning. By the time you reach adulthood, you're most likely responsible for your own success and you're perfectly capable of making your own decisions once you have the information you need.

Adults learn best when learning is focused on them, not the teacher. This is called [andragogy](#), the process of helping adults learn.

Malcolm Knowles, a pioneer in the study of adult learning, observed that adults learn best when:

1. They understand why something is important to know or do.
2. They have the freedom to learn in their own way.
3. Learning is experiential
4. The time is right for them to learn.
5. The process is positive and encouraging.

**Adult characteristics:**

To understand the characteristics of adult learners, their mental and physical condition should be considered in the following referred to some of them.

**Operating speed:**

Slow reaction in adults is natural that necessarily means reducing the logic and practice skills, not due to weakness and increased awareness of natural forces and their skills.

**Consciousness:**

No stimulus and incentives encouraging, despite inhibiting stimuli, slow transfer rate, mental, and weak inhibitors of natural forces (mostly visual and auditory) are factors that slow reaction affect individual mental and cognitive activities, but never able to understand, understanding and learning ability (which varies with the speed of learning) is not relevant.

**Health:**

What is most age, longer duration is necessary to be heard by listening issue. Why is that when elderly people and old could not hear well, their confidence and vulnerable to the possibility that negative beliefs about their find, they are great. Visual abilities can be like other people, usually decreases with age.

**Background of knowledge - skills and beliefs of adults:**

Adults, social experiences, many have already learned different values and beliefs in their pronouns have stabilized, so changes in the new act very cautiously. The idea of such a manner that skill and applying them older and longer life is, Similar resistance to accept new ideas will be more and more severe. Thus, the adult criteria for the built and paid for their ideas and beliefs that are forming. Because of these criteria and the beliefs that they are afraid of failure, Therefore, to prevent it, sometimes against the resistance of new phenomena are only the material taught and its face that make reinforced concrete and tangible interference situation is.

**Choosing a Distance Learning Program:**

Distance learning is one of the fastest-growing components of higher education. Almost 3.5 million students were enrolled in at least one distance learning course in the fall of 2006 and online enrollments are increasing every year. The convenience of taking classes at any time from any location appeals to today's adult learner, especially those who work, have families or live in rural areas.

Today a growing number of paralegal and legal secretarial programs have a distance learning

component (no law schools currently grant credit for distance learning studies). However, not all distance learning programs are of equal quality. Moreover, the increasing popularity of distance learning programs have led to "diploma mills" or "accreditation mills" that offer bogus degrees and certificates. Choosing a distance learning program requires careful research and evaluation. Below are several important factors to consider in choosing a distance learning program.

1. **Accreditation.** Accreditation is a means of ensuring the quality and effectiveness of higher education institutions and programs in the United States. Eight regional accrediting agencies accredit most of the colleges and universities in the United States. A host of national and professional accrediting organizations also exist, including the Distance Education and Training Council (DETC), an organization that identifies and accredits distance learning programs. These twelve questions outlined by the Council for Higher Education Accreditation are helpful in examining a distance learning program's claims of accreditation.

In evaluating distance learning paralegal programs, determine if the school is accredited by one of the regional accrediting bodies and by the American Bar Association (ABA). ABA-approval signifies that the school has met certain standards in terms of academics, facilities and instruction. Graduating from an ABA-approved school may give you an advantage in the legal job market.

2. **Reputation.** The reputation of the distance learning program you attend may hinder or enhance your post-graduate employment prospects. In evaluating the reputation of a distance learning program, you should not solely rely on the school's website or marketing materials. Other ways to investigate the reputation of a distance learning program include:
  - Visiting the school.
  - Talking to alumni (contact the career services department for alumni names and contact information).
  - Researching the distance learning program's record with the Better Business Bureau.
  - Talking to paralegals, attorneys and legal employers about the reputation of the school you are considering.

- Researching the school in print publications, news articles and on the Internet.

1. **Academic Offerings.** When evaluating distance learning programs, it is also important to consider the program's academic offerings. A quality distance learning program offers a comprehensive curriculum with a variety of options, electives and advanced coursework. Talk to professors or an academic dean regarding the content and delivery of courses. The American Association for Paralegal Education (AAfPE) recommends that paralegal instructional content include courses in legal research and writing, litigation, ethics, contracts, business organizations and torts. In addition, courses should develop students' critical thinking, communication, computational, computer and organizational skills, and competency to handle ethical issues, according to the AAfPE.

Legal programs should also offer an experiential learning component such as an internship, practicum, pro bono work or clinical experience. These are great resume-building opportunities and allow you to learn practical skills and gain real-world experience.

2. **Instructional Technologies.** Distance learning courses can be delivered in a variety of ways through a growing array of technological tools including audio tapes, CD or DVD ROM's, e-mail, telephone conferences and web-based delivery systems. Questions to ask include whether the program employs a mix of instructional technology? Is hands-on training and support provided? Can students preview courses online and try out the technologies before enrolling?
3. **Teaching Staff.** The faculty is the backbone of any distance learning program. Are the courses taught by professors or are the courses pre-taped correspondence instruction? If the courses are taught by instructors, what is the background and qualifications of the teaching staff? Are classes taught by paralegals, attorneys or a mix of both?
4. **Career Services.** Another important consideration in any distance learning program is the extent and quality of its career services program. Research indicates that the greater the resources offered by the career services department, the greater the program's job placement success. You might inquire as to what

percentage of graduates find related employment following graduation and whether the career center offers personalized career counseling, job placement assistance, job search seminars, online job boards or resume assistance.

### The World of Distance Education:

Distance education programs are more popular than ever. College and high school students now have hundreds of legitimate distance education schools to choose from. If you're new to the idea of learning through distance education, this article will help you understand the basics.

Distance education is any type of schooling that takes place away from a physical campus. Distance education is also known as:

- distance learning
- virtual learning
- online learning
- e-learning
- online education
- web-based training

There are two types of programs offered by distance education schools: synchronous learning programs and asynchronous learning programs. With synchronous learning, distance education students must log on to the school's website at a set time. Often, they interact with their peers and professors via group chats, web seminars, video conferencing, and phone call-ins. With asynchronous learning, distance education students complete all coursework on their own time. They often learn via assignment sheets, message boards, email, pre-recorded video lectures, mp3s, and traditional mail correspondence. Many students find that distance education courses give them the freedom to complete a degree while meeting their personal and professional obligations. Motivated learners are often able to complete distance education degrees in a fraction of the time often required. Distance education courses also allow students to network with participants from all over the nation. On the downside, distance education courses do not offer the face-to-face interaction found in traditional classrooms. Some students find that they struggle to stay motivated and meet deadlines due to the independent nature of distance education courses.

When searching for a distance education program, the most important factor to consider is accreditation. Make sure the distance education school you choose

is recognized by a regional accreditor or the Distance Education Training Council.

### Conclusion:

In traditional programs that the principles of psychology and curriculum planning, less attention is the form of content presentation ie codification and providing books, original format and have the dominant form, while for adult content that could have valuable experience in addition to writing, other ways also be provided Affect the selection of pictures and images related to the concepts and content produced by including them.

Learning activities such as activities outside the classroom, dialogue, role playing and another type of content is presented. Duties are placed on the learner, a resource for developing knowledge, skills and insights he considered.

Curriculum content only from the training provided to learners or not, but put together their learning through activities that can inform or does, skills and attitude to achieve. In this case, apart from learning that the assays taught learners directly to sustainable and effective learning occurs in his.

Another way of providing content that is educational activities outside the learning environment possible for learning more and better enables adult learners. For example, hits, field trip experiences for learners or transfer is provided, develop knowledge, insight and skills they will.

To ensure that science curriculum and educational aspects, according to community needs and audiences, application form is provided or not, the content selection criteria should be considered. These criteria is being include knowledge, effectiveness, flexibility, diversity, relevance and practical learning.

Some research findings that can be a learning process for the Guidelines for training operations are applied, is given below:

- 1- intrinsic motivation, learning a deeper and make them sustainable. When the need is met directly by the learning itself, what is learned, but is complementary learning. Creating a training activity in adult learning needs, learning ensures stable
- 2- Positive reinforcement (reward) learning to reinforce the negative (punishment) is more effective. Many adults because of negative experiences at the beginning of schooling, are weak and afraid. Feeling of success in adult learning for continuous learning and adult participation is essential.
- 3- Learning, especially regarding skills development, will be added frequently.
- 4 - Duties and meaningful content than meaningless subjects are learned more easily and are later

forgotten. This issue, especially for older adult learners is true. Challenges of adult learning facilitators by the way that content was significantly associated with the experiences and needs of learners is.

5- Passive than active participation in learning activities, learning increases. Adult educators are allowed to participate actively in India, a stable and meaningful learning to help

The task force's policy recommendations are guided by these principles:

- Recognize that adult illiteracy is not an isolated problem but a fundamental barrier to every major challenge facing Kentucky. Without significant improvements in adult literacy the Commonwealth will be unable to make progress on issues such as early childhood education, education reform (elementary/secondary and postsecondary), economic development, and improving the health and well-being of Kentucky's families and communities.
- Shift from top-down implementation of a federal or state program to leading a statewide public campaign that depends fundamentally on a bottom-up commitment of communities, employers, and educational institutions. The campaign must engage all aspects of Kentucky life—all dimensions of state and local government, all education levels, the state's business and civic leaders, voluntary organizations, and all others whose work affects—or is affected by—the problem of adult illiteracy.
- The future of Kentucky depends on narrowing the disparities among counties by improving the adult literacy of the population in all regions of the state.

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### Reference:

1. Fabry, D. L., & Higgs, J. R. (1997). Barriers to the effective use of technology in education: Current status. *Journal of Educational Computing Research*, 17(4), 385-395.
2. Fletcher, W. E., & Deeds, J. P. (1994). Computer anxiety and other factors preventing computer use among United States secondary agricultural educators. *Journal of Agricultural Education*, 35(2), 16-21.

3. Frye, N. (1993). *The Educated Imagination*. Toronto: Canadian Broadcasting Corporation.
4. Ginsburg, L. (1998). Integrating technology into adult learning. In C. Hopey (Ed.), *Technology, basic skills, and adult education: Getting ready and moving forward* (Information Series No. 372, pp. 37- 45). Columbus, OH: Center on Education and Training for Employment. (ERIC Document Reproduction Service No. ED 423 420).
5. Ginsburg, L., & Elmore, J. (2000). *Captured wisdom: Integrating technology into adult literacy instruction*. Naperville, IL: North Central Regional Education Laboratory. (ERIC Document Reproduction Service No. ED 454 408).
6. Glenn, A. D. (1997). Technology and the continuing education of classroom teachers. *Peabody Journal of Education*, 72(1), 122-128.
7. Habermas, Jurgen. (1991). *Knowledge and Human Interests*. Boston: Beacon Press.
8. Hair, J. F., Anderson, R. E., Tatham, R. L., & Black, W. C. (1998). *Multivariate data analysis* (5th ed.). Upper Saddle River, NJ: Prentice Hall.
9. Hardy, Barbara. (1998). *Towards a Poetics of Fiction: An Approach Through Narrative*. *Novel*, 2, 5-14.
10. Hartree, A. (1994). Malcolm Knowles' theory of andragogy: A critique. *International Journal of Lifelong Education*, 3(3), 203-210.
11. Hopey, C. (1999). *Technology and adult education: Rising expectations*. *Adult Learning*, 10(4), 26-29.
12. Isahak Haron & Doraisamy, J. (1992). *Lifelong education in Malaysia: A general survey*. Thesis Mas. UM. 10. 1-13. Kuala Lumpur: Universiti Malaya.
13. Kim K. (2000). *Participation in adult education in the United States, 1998-1999*. U.S. Dept. Of Education, Office of Educational Research and Improvement.
14. King, K. P. (1999). *Unleashing technology in the classroom: What adult basic education teachers and organizations need to know*. *Adult Basic Education*, 9(3), 162-175.
15. Knowles, M. S. (1992). *The modern practice of adult education, andragogy versus pedagogy*. Author of the *Classic Informal Adult Educator*, 3rd Edn. New York: Association Press.
16. Knowles, M. S. (1994). *Andragogy in action: Applying modern principles of adult learning*. San Francisco: Jossey-Bass Inc. Pub.
17. Knowles, M. S. (1999). *The making of adult educator: An autobiographical journey*. 1st Edn. San Francisco: Jossey-Bass Inc. Pub.
18. Kolb, David A. (1993). *Experiential learning: Experience as the source of learning and development*. 1st Edn. United States: FT Press.
19. Krajnc, A. (1999). *Andragogy*. In Collin, J. T. (Ed.), *Lifelong education for adults: An international handbook*. 1st Edn. New York: Pergamon Press.
20. Lang, J. M. (1998). *Technology in adult basic and literacy education: A rationale and framework for planning* (Research report). Cheney: Eastern Washington University, Instructional Media and Technology. Retrieved on November 14, 2003, from <http://cehd.ewu.edu/education/GraduateExamples/JML98Educ601.html>
21. Lawler, P. A., & King, K. P. (2003). *Changes, challenges, and the future*. In K. P. King & P. Lawler (Eds.), *New perspectives on designing and implementing professional development of teachers of adults*. *New directions for adult and continuing education* (Vol. 98, pp. 83-91). San Francisco: Jossey-Bass.
22. Jaffee, L. L. (2001). *Adult literacy programs and the use of technology*. *Adult Basic Education*, 11(2), 109-124.
23. Jordan, W. R., & Follman, J. M. (1993). *Using technology to improve teaching and learning*. *Hot topics: Usable research*. Palatka, FL: Northeast Florida Educational Consortium, Southeastern Regional Vision for Education. (ERIC Document Reproduction Service ED 355 930).
24. Moore, M. G., & Kearsley, G. (1996). *Distance education: A systems view*. Belmont, CA: Wadsworth.
25. Office of Technology Assessment, U.S. Congress. (1993). *Adult literacy and new technologies: Tools for a lifetime* (Final Report No. OTA-SET-550). Washington, DC: Government Printing Office.
26. Neculau, A. (2004). *The adults' education: Romanian experiences*. Iasi, Polirom Publishing House.
27. Păun, E. (1999). *The school: A socio-pedagogical approach*. Iasi, Polirom Publishing House.
27. Norzaini Azman. (2006). *History, trends and significant development of adults education in Malaysia* in *HISTORIA: Journal of Historical Studies*. Vol. VII, No. 2. Bandung: Historia Utama Press.
28. Pratt, D.D. (1993). *Andragogy after twenty-five years: New directions for adult and continuing education*. *Journal Articles*. San Francisco: Jossey-Bass Inc. Pub.
29. Olgren, C. H. (2000). *Learning strategies for learning technologies*. In E. J. Burge (Ed.), *The*



- strategic use of learning technologies. *New directions in adult and continuing education* (Vol. 88, pp. 7-16). San Francisco: Jossey-Bass.
30. Russell, A. (1995). Stages in learning newtechnology: Naive adult email users. *Computers and Technology*, 25(4), 173-178.
  31. Timmermann, S. (1998). The role of information technology in older adult learning. In J. C. Fisher & M. A. Wolf (Eds.), *Using learning to meet the challenges of older adults. New directions for adult and continuing education* (Vol. 77, pp. 61-71). San Francisco: Jossey-Bass.
  32. Sava, S. (2001). Adults' education in Romania: Educational, cultural and social politics. The volume of the first National Conference on Adults' Education, Timisoara, The Almanack of Banat Printing House.
  33. Schifirnet C. (1997). *Changing Adults' Education*. Bucharest, Fiat Lux Printing House.
  34. Sutton-Smith, Brian. (1988). In Search of the Imagination. In K. Egan and D. Nadaner (Eds.), *Imagination and Education*. New York, Teachers College Press.
  35. UNESCO. (1999). *The Hamburg Declaration. Fifth international conference on adult education (Confitea V)*. Paris: UNESCO
  36. Williams, Oscar. (Ed.) (1990). *A Little Treasury of Modern Poetry* (3rd Edition). New York: Charles Scribner's.

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**Assessing Principles of Adult Learning in agricultural education**Arezoo Mirzaei<sup>1</sup> and Mohsen Elini<sup>2</sup><sup>1</sup>Former Graduate Student (M. S), Young Researchers Club, Garmsar Branch, Islamic Azad University, Garmsar, Iran<sup>2</sup>Assistant Professor of Planning Economic and Rural Development Research Institute

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**Abstract:** Good assessment is a collaborative process involving the assessor, learners and others, where appropriate. Your assessment process should be transparent and allow for ongoing feedback from and to the learners. Remember these adult learners want to improve their skills in managing money and are not necessarily interested in formal recognition or being ranked against their peers in the group. Where possible, presenters should emphasize from the start that no-one is going to ‘fail’ the program. Even where students are seeking formal certification of their achievement, presenters can advise that there is no competition between the learners in the group or between an individual and the topic material – it’s all achievable and everyone can make it work for them. Your program should employ methodologies so that your trainers establish a friendly, open atmosphere that shows the participants they will help them learn rather than present as ‘experts’ imparting knowledge. No-one engages well with a trainer/teacher who is just ‘showing off’ what they know. Financial services have a plethora of jargon and complicated ideas that can put many lay people off. Exposing this sort of terminology and explaining it in simple terms – or deciding whether some of it needs exposure at all – is paramount to keeping your learner’s trust and interest.

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**Keywords:** adult learning, education

**Introduction:**

Adult illiteracy is like a disease that infects virtually every dimension of Kentucky life. Adult illiteracy saps the energy and capability of Kentucky’s people and its economy. Adult illiteracy feeds the state’s unemployment, its welfare rolls, and the correctional institutions. Adult illiteracy severely hinders the life chances of young children, undermines school reform, and limits the opportunities for postsecondary education.

Despite landmark reforms in public schools, too many Kentuckians continue to drop out of school, thereby perpetuating the chronic problem of adult illiteracy. Too many young Kentucky parents are unable to read and lack the basic literacy necessary to provide the necessary stimulating, supportive family environments for young children. It is known that children’s literacy levels are strongly linked to the educational level of their parents and that children of parents who are unemployed and have not completed high school are five times more likely to drop out.

The field of adult education and literacy is plagued by confusion about definitions. Over the years definitions have evolved from provisions in federal law and initiatives of groups advocating particular methodologies or the needs of specific adult populations. The result is that definitions tend to merge statements about the goals to be achieved (e.g., improving the literacy of a particular

population) with a particular means (e.g., adult basic education) to achieve the goal. Therefore, it is helpful to distinguish between at least these dimensions of the issue:

1. “Education attainment” usually refers to the numbers of years of schooling completed or the level of credential (e.g., high school diploma or associate degree) an individual has obtained. Despite concerns about the meaning of credentials, there is a strong correlation between educational attainment and literacy.

2. Other literacy initiatives are defined in terms of a particular educational service, strategy, or means to address a target population’s literacy problems. “Adult basic education” and “family literacy” are examples. These initiatives are often defined in terms of a particular configuration of services for the target population (e.g., assessment and information and counseling services).

Goal six of the National Education Goals illustrates a broadly stated goal that incorporates expectations about both adult literacy and the kinds of policies and services that should be in place to improve literacy. Goal six, “Adult Literacy and Lifelong Learning,” states that, “By the year 2000, every adult will be literate and possess the knowledge and skills necessary to compete in a global economy and exercise the rights and responsibilities of citizenship.”

**Adult characteristics:**

to understand the characteristics of adult learners, their mental and physical condition should be considered in the following referred to some of them.

**Operating speed:**

slow reaction in adults is natural that necessarily means reducing the logic and practice skills, not due to weakness and increased awareness of natural forces and their skills.

**Consciousness:**

no stimulus and incentives encouraging, despite inhibiting stimuli, slow transfer rate, mental, and weak inhibitors of natural forces (mostly visual and auditory) are factors that slow reaction affect individual mental and cognitive activities, but never able to understand, understanding and learning ability (which varies with the speed of learning) is not relevant.

**Health:**

what is most age, longer duration is necessary to be heard by listening issue. Why is that when elderly people and old could not hear well, their confidence and vulnerable to the possibility that negative beliefs about their find, they are great. Visual abilities can be like other people, usually decreases with age.

**Background of knowledge - skills and beliefs of adults:**

adults, social experiences, many have already learned different values and beliefs in their pronouns have stabilized, so changes in the new act very cautiously. The idea of such a manner that skill and applying them older and longer life is, Similar resistance to accept new ideas will be more and more severe. Thus, the adult criteria for the built and paid for their ideas and beliefs that are forming. Because of these criteria and the beliefs that they are afraid of failure, Therefore, to prevent it, sometimes against the resistance of new phenomena are only the material taught and its face that make reinforced concrete and tangible interference situation is.

**Classification of Adult Education**

1. reading and writing literacy level
2. Technical and professional education, people are ready for work
3. in the field of health, behavior and health in the family
4. tutorials political, social, religious. Operating political social goals
5. to satisfy emotional needs and entertainment, like art, literature and the like

**Adult education goals:**

a) Literacy goals:

1. To provide primary education and to allow other adults to learn skills during childhood and youth have been deprived of them.
2. Increase the ability and skills for adults over the executive government and community programs.
3. Preparation of programs and classes that form the adult intellectual development is dedicated to the goal, get a job or degree is better.
4. Increased confidence in adults, through increased awareness and knowledge.
5. raising awareness of adult interest to participate in decision-making
6. to raise awareness of citizens rights, their duties and responsibilities
7. Adults develop abilities to solve problems of personal and social
8. to inform adults the skills and talents.
9. Spread knowledge about their heritage

b) vocational training objectives:

1. Adults equip the skills necessary for subsistence.
2. To provide staffing to promote industry and economy, the third
3. the elimination of class differences and achieve social equality
4. Training of workers with their employment conditions and industrial variables are consistent.

**Principles of Adult Learning****1) PURPOSE**

The Financial Literacy Foundation has prepared this document to provide education materials developers with information on the key principles of adult learning. It is a short summary of a very broad area of research and advice, prepared with the input of Adult Learning Australia, the national peak body representing organisations and individuals in the adult learning field.

**2) NEEDS, WANTS, CONCERNS AND ABILITIES OF YOUR LEARNERS**

Assess the needs, wants, concerns and current abilities of the target learners. Each target group will have their own special needs and probably expect different outcomes from undertaking your training program. Common themes you can prepare for are:

**Why are you here?** - no-one readily admits to not knowing something fundamental that may impact on their life chances. Therefore program material, particularly that designed for adult learners should always treat aspects of why learners are in the

training sensitively. Describe the outcomes expected from the training in positive, enhancing terms and not as redressing a weakness or failure on the part of the learner. For example, "Undertaking this program will improve (rather than redress a failing) the way you manage your money".

**Tell me more** - learners may well enter programs like this with poor past experiences of money matters or at least some trepidation about handling personal finances in the future. Recognise this in the program introduction but individual learners should never be required to expose any of their negative experiences in a group. It might seem a good 'ice-breaker' to ask a new group of learners to share what they expect from the program but resist going too far when asking learners to talk about past problems they may have had with finances. Firstly, they may be uncomfortable doing this in a group and secondly you could start the program in a sea of negative views about financial matters generally. A successful program introduction will focus on where the learners will go rather than dwell too much on where they may have been.

**What do you know?** - Gauge the likely capabilities of your target groups. Overestimating their current skills in dealing with money could mean the program misses fundamental principles and understandings. Underestimating existing knowledge is also not good as plodding through basic material most already are familiar with will bore participants and the full program content will not be assimilated.

**What will I be able to do?** – above all these target groups will want to be hands on and demonstrate to themselves and their peers that that can do something they could not before the training; and do it well. Let them know right at the beginning that they will be able to do things that will be of great benefit to them, not just know more.

**Build on small successes** – if a target group of learners has had limited positives in their life or work experiences its important to provide small and regular 'success' points in the program. Simply exposing the content and assuming everyone is assimilating it, putting it all together holistically and building up their skills is not enough. The beginning of the program should be designed so that a discrete piece of learning that the learners can use right away builds their confidence to move on. The program should be a series of steps where the learners confirm their progress and reinforce one new skill by relating it to another they can already confidently apply.

**Testing!** – many adults and people not regularly engaged in learning fear testing. Many may have had bad experiences of assessment in school and view the practice among peers as stressful. Make sure they understand that what they are in is a life skills

program and no-one can 'fail' as such. In fact each can support others in things they do well that fellow learners may need help with so it's a cooperative not competitive environment that they are learning in. Build in some teamed exercises and assessments to avoid people feeling isolated in their learning and fearful of failure in front of the group.

**Special needs.** You need to consider learners with special needs and those who have English as their second language. Reasonable adjustment should be made depending on each individual learner's particular needs and abilities. Your program material should include advice to the trainer on how to determine the need to make adjustments which, depending on a learner's abilities may include:

- providing interpreters for people who are deaf;
- ensuring access, for example by conducting training and assessment in facilities which have ramps for people using wheelchairs and adjustable desks for people with physical disabilities;
- allowing for access of personal assistants or note takers;
- allowing additional time for assessments;
- allowing oral instead of written responses to questions;
- adaptive technology such as screen readers, speech synthesisers, computer software or hardware; and,
- assistance with managing stress and anxiety.

### 3) HOW DO ADULTS LEARN?

Your program needs to account for:

- Motivation of the learner;
- Reinforcement of the skills and knowledge being developed;
- Retention of key learning; and,
- Transference of what is learnt to new situations.

**Motivation** - Adults learn most effectively when they have an inner motivation to develop a new skill or gain new knowledge. They resist learning material if it is forced on them, or if the only reason given is that the material will, in some vague way, be "good for them to know." Adults need to know why they are being asked to learn something; and they definitely will want to know what the benefits will be before they begin learning. This means the best motivators for adult learners are explicit interest and self benefit. If they can be shown that the program will benefit them pragmatically and practically, they will learn better, and the benefits will be much longer lasting. Typical motivations include a desire for better handling of personal money matters, say in

retirement, wanting a new or first job, promotion, job enrichment, a need to reinforce old skills in say, handling credit or learn new ones, a need to adapt to community changes such as on-line banking and so on. Remember the tone of the program should be motivating. Your program should employ methodologies so that your trainers establish a friendly, open atmosphere that shows the participants they will help them learn rather than present as 'experts' imparting knowledge. No-one engages well with a trainer/teacher who is just 'showing off' what they know. Financial services have a plethora of jargon and complicated ideas that can put many lay people off. Exposing this sort of terminology and explaining it in simple terms – or deciding whether some of it needs exposure at all – is paramount to keeping your learner's trust and interest.

**Appropriate level of difficulty.** The degree of difficulty of your financial literacy program should be set high enough to expose all the essential elements of the topic and challenge learners to succeed, but not so high that they become frustrated by information overload. Too much financial industry terminology strung together can be a complete turn off for people who may already struggle with the fundamentals – is it really a necessary part of the skills they need?

So start with financial information and techniques that relate directly to the learner's own personal needs and wants. Personal budgeting is always useful and less complicated than say, comparing mortgage options. Don't make what could be a lesser used skill so important in the program it de-motivates the learners and loses their interest.

Motivational reward does not necessarily have to be in the monetary sphere; it can be simply a demonstration of social or workplace benefits to be realised from new financial management skills. Older participants could perhaps learn how to help their children with financial decisions. People could be shown how to utilise better financial planning in a club or society they belong to. Its about improving whole of life experiences not just direct monetary reward. The overall thrust of the program should be motivating and, like all good teaching and learning programs, course material should ensure other key adult learning elements are covered.

**Reinforcement.** As we know reinforcement is a very necessary part of any teaching/learning process. Through it, trainers encourage correct modes of behaviour and performance and discourage bad habits. Your program should use both reinforcement techniques throughout. Positive reinforcement is normally used when participants learn new skills. As

implied, positive reinforcement is "good" and reinforces "good" (or positive) behaviour. Negative reinforcement is useful in trying to change bad habits or inappropriate modes of behaviour. The intention is extinction -- that is, the trainer uses negative reinforcement until the "bad" behaviour disappears or the learner understands why past practice is not beneficial to them. Examples could be ensuring participants always compare different rates of interest available to them before signing up for any new debt (a positive reinforcement) and not considering credit purchases that leave them with no income safety net for unforeseen circumstances (negative reinforcement).

**Retention.** Learners must retain what the program delivers to them in order to benefit from the learning. In order for participants to retain the information taught, they must see a meaning or purpose for that information. They must also understand and be able to interpret and apply the information in their own real life contexts. Understanding includes their ability to assign the correct degree of importance to the material and its application in the future. The amount of retention is always directly affected by the degree of original learning. In other words if the learners did not learn the material well initially, they will not retain it well either. Retention by the participants is directly affected by their amount of practice during the learning. After the students demonstrate they can apply new financial skills, they should be urged to practice in their own time and for their own personal needs to retain and maintain the desired performance.

**Transference.** Transfer of learning is the result of training and is simply the ability to use the information taught in your program but in new settings and contexts. As with reinforcement, both types of transfer: positive and negative should be used in the program approach. Positive transference, like positive reinforcement, occurs when the learner uses the skill learnt in your program. It is very important for any learner's orientation to the new skills they develop that they can practice in their own situations. Using knowledge from financial literacy training to work out the best way to use (or not use) credit in their lives is an important tool that many participants could use immediately. Participants can check how much credit debt they have, what interest they are paying and what alternatives there may be. Negative transference, again like negative reinforcement, occurs when the learners applying the skill do not do what they are told not to do. This also results in a positive (desired) outcome. This means it's important to find out what the participants in your program have been using their new skills for. Check

to see if they are applying the techniques properly or whether they have misunderstood a key aspect of the program. Once wrong information is absorbed and used again and again it simply becomes another bad habit that could make financial decision-making worse instead of better.

Transference is most likely to occur in the following situations:

- **Association:** participants can associate the new information with something that they already know. What skills have the learners already mastered that they can bring to bear on better financial planning for example? Perhaps they have a hobby where it is necessary to access information from written materials or the Internet and the same skills could be used to obtain and analyse better financial data to use in their budgeting.

- **Similarity:** the information is similar to material that participants already know; that is, it revisits a logical framework or pattern. Using calendars or electronic planners to plan future holidays, work shifts etc can be transferred to setting up a long-term budget planner for financial payments and income.

- **Critical attribute element:** the information learned contains elements that are extremely beneficial (critical) in personal life or in the workplace. Try to reinforce the importance of aspects of the financial literacy program to the learner's own goals, whether these are in their home life, getting a job or improving their prospects in work they already have. People can even start their own small business ventures if they have the financial skills to work out the costs and benefits first.

#### 4) DELIVERY STRATEGIES

Finally in developing your program consider that adults have different personal and social lives than young people in formal schooling or college. Unlike children and teenagers, adults have many responsibilities that they must balance against the demands of learning. Because of these responsibilities, adults may have barriers against participating in learning. These barriers could include lack of time, money, confidence, or interest, lack of information about opportunities to learn, scheduling problems, "red tape," and problems with child care and transportation. Try to consider these factors when scheduling the program. If it is to be delivered to people in a workplace it should fit around their work times and not require them to come back hours later well after they have completed a hard day's work. Week-ends might seem like good free time to learn but many adult learners are conditioned to week-ends being for family pursuits and are likely to be reluctant to give up hours away from this for financial training. Try to identify groups of learners for each program

that can support each other in transport to where the program is delivered, assistance in minding young children and common interests outside of the formal learning. Groups seeking employment or those soon to retire are obvious examples of participants who will have similar interests and motivations and can help each other to access the training and learn collaboratively to use the new skills.

#### 5) ENGAGEMENT OF THE LEARNER

Good program strategies encourage real learning, where the learner increasingly:

- takes responsibility and ownership of their learning;
- engages in experiential learning;
- partakes in cooperative learning; and,
- engages in reflective learning.

By requiring or encouraging your learners to take a more directive and active role in the program as it is delivered you are encouraging them to engage in the critical processes of:

- making meaning out of the new financial management knowledge they have;
- distilling principles from the program, which will aid their transference of financial skills to new contexts; and,
- practising their financial planning skills and mastering processes to improve their money management.

In your financial literacy program learner directed activities can also encourage greater levels of motivation. The learning is more purposeful, because they have a sense of ownership over what they achieve and identify themselves as the key beneficiaries of the outcomes. An abstract exercise in developing a savings plan for an imaginary person or family may appear to introduce the right principles but it may not resonate with the individuals you are training. Think of your target group. What are their savings goals?

What aspects of their income are available to saving and how can they work this out?

What form of saving is best for them in terms of achievable targets, regular contributions and limited risk?

Teachers and trainers often develop example exercises based on imaginary situations because, frankly, they appear to put everyone on the same testing level and it is easier to assess because there are a common set of 'right' answers. This is not the way to make financial literacy learning work for the target groups. They should be encouraged to work on individual situations entirely relevant to them. This may mean more effort on the part of the trainer in assisting with the work each person is doing and assessing outcomes but the result will be practical

exercises that keep the learners involved and motivated.

#### 6) ASSESSING PROGRESS AND OUTCOMES

Good assessment is a collaborative process involving the assessor, learners and others, where appropriate. Your assessment process should be transparent and allow for ongoing feedback from and to the learners. Remember these adult learners want to improve their skills in managing money and are not necessarily interested in formal recognition or being ranked against their peers in the group. Where possible, presenters should emphasise from the start that no-one is going to 'fail' the program. Even where students are seeking formal certification of their achievement, presenters can advise that there is no competition between the learners in the group or between an individual and the topic material – it's all achievable and everyone can make it work for them. Make sure they understand that they will all leave with better financial skills than they have at the beginning. If someone in the group is somehow 'better' or 'faster' at understanding superannuation than others that is their good fortune but makes no difference to the benefits everyone in the group gains from knowledge and skills in handling this important financial tool. Everyone will improve their life chances through participating in the program and outside of training for formal certification, assessment is to demonstrate this to them and no-one else.

If you want further information on collaboration in the design of assessment materials and the role of learners in the assessment process this can be found in:

- Guide One – Training Package Assessment Materials Kit and Guide Five – Candidate's Kit in the Training Package Assessment Guides; and,
- Learning Circles Resource Manual for Facilitators and Learners (developed by Adult Learning Australia).

#### Conclusion:

In traditional programs that the principles of psychology and curriculum planning, less attention is the form of content presentation ie codification and providing books, original format and have the dominant form, while for adult content that could have valuable experience in addition to writing, other ways also be provided. Affect the selection of pictures and images related to the concepts and content produced by including them.

Learning activities such as activities outside the classroom, dialogue, role playing and ... Another type of content is presented. Duties are placed on the

learner, a resource for developing knowledge, skills and insights he considered.

Curriculum content only from the training provided to learners or not, but put together their learning through activities that can inform or does, skills and attitude to achieve. In this case, apart from learning that the essays taught learners directly to sustainable and effective learning occurs in his.

Another way of providing content that is educational activities outside the learning environment possible for learning more and better enables adult learners. For example, hits, field trip experiences for learners or transfer is provided, develop knowledge, insight and skills they will.

To ensure that science curriculum and educational aspects, according to community needs and audiences, application form is provided or not, the content selection criteria should be considered. These criteria is being include knowledge, effectiveness, flexibility, diversity, relevance and practical learning

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#### Reference:

1. Birzea, C. (2001), The social impact of the continuous professional training. Bucharest, The National Romanian Observer.
2. Brookfield, S. D. (1996). Understanding and Facilitating Adult Learning. San Francisco: Jossey- Bass.
3. Brookfield, S.D. (1997). Developing Critical Thinkers: Challenging Adults to Explore Alternative Ways of Thinking and Acting. San Francisco: Jossey-Bass.
4. Budin, H. (1999). The computer enters the classroom. Teachers College Record, 100, 656-669.
5. Fabry, D. L., & Higgs, J. R. (1997). Barriers to the effective use of technology in education: Current status. Journal of Educational Computing Research, 17(4), 385-395.
6. Fletcher, W. E., & Deeds, J. P. (1994). Computer anxiety and other factors preventing computer use among United States secondary agricultural educators. Journal of Agricultural Education, 35(2), 16-21.
7. Frye, N. (1993). The Educated Imagination. Toronto: Canadian Broadcasting Corporation.
8. Ginsburg, L. (1998). Integrating technology into adult learning. In C. Hopey (Ed.), Technology,

- basic skills, and adult education: Getting ready and moving forward (Information Series No. 372, pp. 37- 45). Columbus, OH: Center on Education and Training for Employment. (ERIC Document Reproduction Service No. ED 423 420).
9. Ginsburg, L., & Elmore, J. (2000). Captured wisdom: Integrating technology into adult literacy instruction. Naperville, IL: North Central Regional Education Laboratory. (ERIC Document Reproduction Service No. ED 454 408).
  10. Glenn, A. D. (1997). Technology and the continuing education of classroom teachers. *Peabody Journal of Education*, 72(1), 122-128.
  11. Habermas, Jurgen. (1991). *Knowledge and Human Interests*. Boston: Beacon Press.
  12. Hair, J. F., Anderson, R. E., Tatham, R. L., & Black, W. C. (1998). *Multivariate data analysis* (5th ed.). Upper Saddle River, NJ: Prentice Hall.
  13. Hanson, Karen. (1988). Prospects for the Good Life: Education and Perceptive Imagination. In K. Egan and D. Nadaner (Eds.), *Imagination and Education*. New York: Teachers College Press.
  14. Knowles, M. S. (1999). *The making of adult educator: An autobiographical journey*. 1st Edn. San Francisco: Jossey-Bass Inc. Pub.
  15. Kolb, David A. (1993). *Experiential learning: Experience as the source of learning and development*. 1st Edn. United States: FT Press.
  16. Kotrlik, J.W., & Smith, M. N. (1999). Computer anxiety levels of vocational agriculture and other vocational teachers. In M. F. Burnett (Ed.), *Proceedings, national agricultural education research meeting* (pp. 1-9). Columbus, OH: American Association for Agricultural Education.
  17. Krajnc, A. (1999). Andragogy. In Collin, J. T. (Ed.), *Lifelong education for adults: An international handbook*. 1st Edn. New York: Pergamon Press.
  18. Lang, J. M. (1998). *Technology in adult basic and literacy education: A rationale and framework for planning* (Research report). Cheney: Eastern Washington University, Instructional Media and Technology. Retrieved on November 14, 2003, from <http://cehd.ewu.edu/education/GraduateExamples/JML98Educ601.html>
  19. Lawler, P. A., & King, K. P. (2003). Changes, challenges, and the future. In K. P. King & P. Lawler (Eds.), *New perspectives on designing and implementing professional development of teachers of adults*. *New directions for adult and continuing education* (Vol. 98, pp. 83-91). San Francisco: Jossey-Bass.
  20. Office of Technology Assessment, U.S. Congress. (1993). *Adult literacy and new technologies: Tools for a lifetime* (Final Report No. OTA-SET-550). Washington, DC: Government Printing Office.
  21. Neculau, A. (2004). *The adults' education: Romanian experiences*. Iasi, Polirom Publishing House. Păun, E. (1999). *The school: A socio-pedagogical approach*. Iasi, Polirom Publishing House.
  22. Norzaini Azman. (2006). History, trends and significant development of adults education in Malaysia in *HISTORIA: Journal of Historical Studies*. Vol. VII, No. 2. Bandung: Historia Utama Press.
  23. Pratt, D.D. (1993). *Andragogy after twenty-five years: New directions for adult and continuing education*. *Journal Articles*. San Francisco: Jossey-Bass Inc. Pub.
  24. Olgren, C. H. (2000). Learning strategies for learning technologies. In E. J. Burge (Ed.), *The strategic use of learning technologies. New directions in adult and continuing education* (Vol. 88, pp. 7-16). San Francisco: Jossey-Bass.
  25. Russell, A. (1995). Stages in learning new technology: Naive adult email users. *Computers and Technology*, 25(4), 173-178.
  26. Timmermann, S. (1998). The role of information technology in older adult learning. In J. C. Fisher & M. A. Wolf (Eds.), *Using learning to meet the challenges of older adults. New directions for adult and continuing education* (Vol. 77, pp. 61-71). San Francisco: Jossey-Bass.
  27. Sava, S. (2001). *Adults' education in Romania: Educational, cultural and social politics. The volume of the first National Conference on Adults' Education*, Timisoara, The Almanack of Banat Printing House.
  28. Schifirnet C. (1997). *Changing Adults' Education*. Bucharest, Fiat Lux Printing House.
  29. Sutton-Smith, Brian. (1988). In Search of the Imagination. In K. Egan and D. Nadaner (Eds.), *Imagination and Education*. New York, Teachers College Press.
  30. UNESCO. (1999). *The Hamburg Declaration. Fifth international conference on adult education* (Confitea V). Paris: UNESCO

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## Cluster Analysis and Dendrogram Mapping of 51 Silkworm Varieties based on Phenotypic Data

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**Abstract:** In different breeding programs, knowledge of inbreeding and genotype similarities and differences of various varieties is necessary. In many breeding projects, hybridization planning and in different crosses, there is a need to exist varieties with far and close genetic characteristics to each other. Based on this, it is necessary to identify the similarities between various breed. This experiment aiming to sort Japanese varieties group of Iran silkworm gene bank and to investigate genetic relationships between them based on individual economic characteristics was designed and planned. Data of the mentioned traits had been recorded based on performance tests. In order to group lines based on several important economic traits, Cluster analysis with UPGMA method performed on the studied genotypes using Ntsys-pc software. For each of concerned economic traits, the matrix analogous indicates Japanese varieties distance to each other had been determined and according this phylogenetic diagram of this varieties that expressing near or far different varieties of Iran Japanese silkworm based on individual economic traits was drawn. According to the results from this research, the studied varieties can locate in different groups, accurately, and express their distance to each other.

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### 1. Introduction

Silkworm is an insect that is important in economic point of view in the world. Farmers are attempting to produce wet cocoon and also increasingly some of them are working in the complementary sectors of silkworm such as drying silk cocoons, silk dyeing, silk weaving, design and texture of silk carpets, traditional silk production, trade carpets, etc. are active.

In recent years, carrying various research projects the phenotypic potential of Iranian Silkworm genetic resources recognized and has been recorded, scientifically. But considering the vast number of genetic stocks is still not an experiment for grouping varieties to individual economic traits separately and attribute this variety to each economic. Genetic relationships between varieties can be identified by exploring characters. Revealing these relationships, the field for such a breeding projects such as appropriate cross and selection is provides.

Cluster analysis is to encompass a set of mathematical methods to find the similarities between the materials used in a set. The goal of cluster analysis is to find the real categories of people and also to reduce the number of data. Also, after the grouping by cluster analysis, the question is whether

a group can separate into sub-groups having significant difference (Frshadfar, 2005).

The main goal of silkworm breeding is gradual improvement of traits which have economic value for animal interests, and increase profits of cocoon producers and other sectors of silkworm industry (Mirhosseini et al, 2005; Sing et al, 1998). Commercial varieties of silkworm in a limited population sizes had been grown and put under breeding programs and high selective pressures. After several generations so that their genetic structure in a result of increased random genetic drift is subject to change. Revealing these relationships, the field for performing breeding projects such as designing appropriate crosses and selection is provides.

Among the animal species, genetic diversity is often a dramatic range among many generations and species. Races are defined as populations within a species whose members can be determine by a series of characteristics about that the race. Although there are many varied definitions, the definition by FAO expressed that in the phenotype of traits, there is a clear boundary between the populations. It may be true in Europe where separating two types of same generations is a specific process that was common by providing cattle husbandry books about two hundred years ago. In other regions such as Africa, a specific

definition of the same generations is not always possible and this depends on the mixing of race between populations. Determine the type of animals for the same generations in these areas can be objective and questionable.

Phylogenetic trees are graphical symbols or designs of the matrix of the distance between populations. As we discovered, trees are not phylogenetic trees, because the difference in effective population size and migration between species may be diverted this image. However, there are different methods for drawing distance matrix trees. Nei (1983) discussed and compared different ways. Most known methods, are UPGMA and NJ (Takazaky and Nei, 1996) that generally have been associated with good results.

In cross systems it is trying to provide desired gene bank for future generations selecting the concerned parents and through the actions of different cross systems, this bank will be brought to the desired level. Currently, the most common methods in breeding is doing in silkworm research and breeding section that ultimately will lead to the synthesis of new lines. About selection systems in the world now, continuous methods, eliminating the independent level and the index is considered which based on this parents choose in proper, accurate and consistent and leads to improve quality and quantity of various performance in breeding processes, in a way that today parameters such as the genetic variance and covariance, correlation coefficient, and heritability and finally economic coefficient traits are helping (Kalpana, 1992; Li, 1996).

In the study conducted by Mirhosseini et al (2005) dendrogram obtained from cluster analysis of silkworm groups based on morphological traits of samples were divided into two groups of indigenous varieties and imported varieties and also each of Chinese and Japanese varieties were located in the two groups related to their. In differentiation in Chinese and Japanese races, mainly morphological traits such as cocoon shape, color eggs, the larval period was considered. In reviews conducted by Balvasi (2003) to compare two groups of Chinese and Japanese varieties different results was obtained. Varieties 32 and 107 in one group, as well as varieties 110 and varieties 31 and 103 in other groups were located and varieties 104 formed a separate group. In the study conducted by Alitalesh (2007) using ten AFLP primer for 90 individuals a total of 208 polymorphism indicator were produced. Results related to the number of positions polymorphism gene variation, the average percentage of polymorphism, the percentage of genetic similarity inter-variety, and the average effective number of alleles was present. But the study by Dalirsefat and

Mirhosseini (1998) using AFLP markers on the indigenous masses, the masses of indigenous native had more inter diversity, while the distance between varieties were small. However, the distances between imported varieties were many. There is little genetic diversity in this research into the varieties. Because these varieties are used to generate hybrid, so their in-group diversity should be reduced a lot.

This experiment aimed at classification the Japanese group varieties of silkworm gene bank of Iran and relations between them based on individual genetic traits were designed and planned. Informed of the potential and relative relationship of genetic reserves of silkworm play an important role in the development of silkworm breeding programs is to improve their performance. The purpose of this study was classification of 51 varieties of Japanese silkworm gene bank of Iran based on the silkworm phenotypic data available in the country Silkworm Research Center. This information may consider as the basis of scientific recognition of existing varieties and design future breeding programs.

## 2. Material and Methods

It is used 51 varieties of Iran silkworm germplasm in this experiment. Fifty one studied varieties included 107-K, 119-K, 113-K, 105, 31, 51, 103, BH-2, B2-09, 1003-4, 1003-5, 1005, M2-6-22-2, M2-6-18(109), M-1-2(5), M2-6-22(107), M2-6-18.3, 307-300-2, 202A-204B, I 20, 101433-9-5, 101433-1-4, 101433-6-6, 1126 (111), 113 (2029), 151 (103×M-1-1), Xihang 2.3, Xihang 3.3, 153 (Xihang-1), 5118×10133-2-2, 5118×10133-3-3, Black-White, 101×F6, F6×101, Kinshu, M-1-1×31, 31×M-1-1, M-1-1×103, 103 Poly Marking, Shaki, 101, T1-J, T5-M, 236, 1524, 1433-15, 1433-9, 7409, N19, White Larvae- Yellow Cocoon, and Black Larvae-White Cocoon.

Also, fifty three studied economical traits included larval weight at 1 day of 5th instar (g), larval weight at 3rd day of 5th instar (g) and larval weight at last day of 5th instar (g), hatchability percentage (%), number of laid eggs, number of fertilized eggs, number of un-fertilized eggs, and number of un-hatched eggs. Larval duration (hr), feeding larval duration (hr), molting larval duration (hr), 1-3 instars larval duration (hr), 1-3 instars feeding larval duration (hr), 1-3 instars molting larval duration (hr), 4-5th instars larval duration (hr), 4-5th instars feeding larval duration (hr), 4-5th instars molting larval duration (hr), 5th instar feeding larval duration (hr), number of total produced cocoons, number of good produced cocoons, number of alive good produced cocoons, number of died good produced cocoons, number of middle produced cocoons, number of alive middle produced cocoons,

number of fertilized cocoons, number of low produced cocoons, number of alive low produced cocoons, number of double produced cocoons, number of alive pupae in double cocoons, number of died pupae in double cocoons, pupae vitality percentage (%), cocoon weight (gr), shell cocoon weight (gr), shell cocoon percentage (%), male cocoon weight (gr), male shell cocoon weight (gr), male shell cocoon percentage (%), female cocoon weight (gr), female shell cocoon weight (gr), female shell cocoon percentage (%), good cocoon weight of 250 larvae (gr), middle cocoon weight of 250 larvae (gr), middle cocoon weight (gr), low cocoon weight of 250 larvae (gr), low cocoon weight (gr), double cocoon weight of 250 larvae (gr), double cocoon weight (gr), total cocoon weight of 250 larvae (gr), total cocoon weight of 10000 4th instar larvae (gr), cocoon number per liter, cocoon weight per liter (gr), pupae weight (gr) and male pupae weight (gr).

Data were collected based on three replications for each variety. It is calculated mean of three replications for each variety using Excel. Meanwhile data was processed using NTSYSpc software (Rolf, 1998) based on economical traits in order to cluster analysis and dendrogram plotting.

### 3. Results

#### The cluster analysis and grouping

To perform cluster analysis and grouping Japanese varieties based on 56 economic traits the method of analysis based of UPGMA cumulative was used. Individuals who are in one group, favor more in-group similarity and less out-group similarity. Therefore, about inbreeding and outbreeding of individual and choice of appropriate and parents together for cross in order to obtain high heterosis, made more accurate and safer decisions. Because the distance of genotypes is greater, in the separated generations, making hybridization will create more diversity and offspring that are more desirable will provide and the possibility of collecting more desirable genes in progeny increases. In general, progenies from genotypes cross which are located in two distinct group will develop more heterosis than genotypes in one group.

#### Grouping with the UPGMA method by the NTSYSpc software

In this study, NTSYSpc software (Ralph, 1998) was used to grouping 51 varieties. Its benefits were the ability of grouping with various methods with different distance measures.

#### Larval weight at 1 day of 5th instar

Phylogenetic tree using the UPGMA method was formed for attribute larval weight at 1 day of 5th instar instars. In cross section 1.83 phylogenetic tree was divided into two main clusters. Main clusters

contain a number of varieties (1, 39, 40, 49, 48, 3, 51, 14, 35, 27, 38 and 50) and other varieties were located at the other major clusters. Another major cluster subdivided into two smaller clusters. The first cluster contains varieties 2, 11, 18, 4, 20, 32 and the second cluster contain the other varieties.

#### Larval weight at 3rd day of 5th instar

Grouping was performed according to age, of larval weight at 3rd day of 5th instar UPGMA method separately with the above method. This test showed that in cutting 4.11 varieties were divided into two clusters. In a single cluster only variety (24) was used. Other varieties were placed in other clusters. In cutting 1.7, varieties subdivided into two smaller clusters. One cluster contains varieties 2, 8, 19, 9, 24 and 29 and another cluster was containing other varieties.

#### Larval weight at last day of 5th instar

Grouping using the UPGMA method was performed for attribute larval weight at last day of 5th instar between Japanese varieties of silkworm gene bank. In cutting 2.89, varieties were divided into two main clusters. The first cluster contains varieties 28, 41, 10, 51 and 39. The second cluster contained the other varieties. A variety interval in the second major cluster was relatively low (about 0.72). Also this cluster was divided into two smaller clusters.

#### Hatchability percentage

By grouping with varieties of silkworm gene bank of Iran in Hatchability percentage, it became clear that varieties (28, 41, 10, 51 and 39) were located in a major cluster. Another major cluster in cutting about 0.8 was divided into two smaller clusters. One of the two clusters containing varieties (1, 24, 8, 31, 36, 30, 40, 38.7, 49 and 50) and another cluster containing the other varieties.

#### Number of laid eggs

Grouping with the UPGMA method for attribute number of laid eggs was performed between Japanese varieties of silkworm gene bank. In cutting 2.86, varieties were divided into two main clusters. The first cluster contains variety 14. Between 1.55 distances second cluster was divided into two clusters. A variety interval in the second major cluster was relatively low. Also this cluster was divided into smaller clusters.

#### Number of fertilized eggs

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of number of fertilized eggs in cutting (Euclid distance) 1.73 two main clusters was formed. One of these clusters in cutting 1.35 was divided into two smaller clusters. A cluster was consists of varieties (14, 44 and 49) and another including 16 varieties. In cutting 1.07 the tree divided into two smaller cluster

contain varieties (25, 28 and 51) on one side and other varieties on the other hand.

#### **Number of un-fertilized eggs**

Result from grouping varieties of Japanese silkworm of Iran gene bank showed that for the number of un-fertilized eggs in cutting 2.14 two main clusters were formed. One cluster contains varieties 3, 48, 49, 39, 8, 46, 31, 38, 10 and 29. Another cluster was divided into two main clusters, and so into smaller clusters that contain more varieties.

#### **Number of un-hatched eggs**

Phylogenetic tree using the UPGMA method was formed for the number of un-hatched eggs. In cutting 2.60 phylogenetic tree was divided into two main clusters. Main clusters contain a number of varieties (28, 41, 10, 39 and 51) and other varieties were the other major clusters. Other major clusters in cutting 1 were divided into two smaller clusters. These clusters are also were divided into clusters with several different intervals.

#### **Larval duration**

Analysis of silkworm strains for larval duration performed under UPGMA approach. Cluster divided varieties into two sub-group in distance of 2.8. One of clusters was including variety of 41. Other varieties were in second main cluster. Varieties divided into two sub-group in distance of 2. First group was including (2, 8, 43, 28, 24, 50, 26, 48, 7 and 3) varieties and another cluster were including remained varieties.

#### **Feeding larval duration**

Hierarchical agglomerative clustering performed based on UPGMA method for trait of feeding larval duration. The variety of number 1 located in distance of 9.99 from others. One of main clusters divided into four sub-groups. One of clusters was including variety of 10.

#### **Molting larval duration**

Analysis of silkworm strains for molting larval duration performed under UPGMA approach separately. Varieties divided into two sub-group In distance of 5.75. Other varieties were in two main clusters. Fewer clusters were including varieties of 47 and 49.

#### **1-3 instars larval duration**

Hierarchical agglomerative clustering performed based on UPGMA method for 1-3 instars larval duration. In distance of 3.46 the phylogenetic tree was divided into two main clusters. In a single cluster only variety (40) was used. Other varieties were placed in other clusters. Another major cluster subdivided into two smaller clusters in distance of 1.73.

#### **1-3 instars feeding larval duration**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of 1-3 instars feeding larval duration in cutting (Euclid distance) 1.90 two main clusters was formed. A cluster consists of varieties (1, 3, 16, 18, 20, 51, 8, 28, 15, 19 and 49) and another including another varieties. One of these clusters in cutting 1.3 was divided into two smaller clusters. These clusters are also were divided into clusters with several different intervals.

#### **1-3 instars molting larval duration**

Result from grouping varieties of Japanese silkworm of Iran gene bank showed that for the 1-3 instars molting larval duration in cutting 5.02 two main clusters were formed. One cluster contains variety of 49. Another cluster was divided into two main clusters including variety of 1 and another cluster that contain more varieties.

#### **4-5th instars larval duration**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of 4-5th instars larval duration in cutting (Euclid distance) 3.17 two main clusters was formed. One of these clusters consists of variety of 3 and another cluster that contain more varieties. In cutting 1.58 the tree divided into two smaller clusters contain varieties. These clusters are also were divided into clusters with several different intervals.

#### **4-5th instars feeding larval duration**

Phylogenetic tree using the UPGMA method was formed for attribute 4-5th instars feeding larval duration. In cross section 2.55 phylogenetic tree was divided into two main clusters. One of these clusters consists of varieties (47 and 49) and other varieties were located at the other major cluster. In distance of 1.6 Another major cluster subdivided into two smaller clusters. The first cluster contains varieties 2, 40, 8, 50, 28, 26, 48, 30, 44 and 3 and the second cluster contains the other varieties.

#### **4-5th instars molting larval duration**

Phylogenetic tree using the UPGMA method was formed for attribute 4-5th instars molting larval duration. In cross section 2.45 phylogenetic tree was divided into two main clusters. Main clusters contain a number of varieties (12, 41 and 51) and other varieties were located at the other major clusters. Another major cluster subdivided into two smaller clusters. These clusters are also were divided into clusters with several different intervals.

#### **5th instar feeding larval duration**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of 5th instar feeding larval duration cutting (Euclid distance) 2.55 two main clusters was formed. One of these clusters consists of varieties of 3,

50 and 26 and another cluster that contain more varieties. In cutting 1.65 the tree divided into two smaller clusters contain varieties. The first cluster contains varieties 9, 16, 21, 15, 42, 45, 24, 3, 49, 50 and 26 and the second cluster contain the other varieties.

#### **Number of total produced cocoons**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of number of total produced cocoons (Euclid distance) 1.4 two main clusters was formed. One of these clusters was consists of variety of 38 and another cluster that contain more varieties

#### **Number of good produced cocoons**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of number of good produced cocoons (Euclid distance) 2.68 two main clusters was formed. One of these clusters was consists of variety of 38 and another cluster that contain more varieties.

#### **Number of alive middle produced cocoons**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of number of alive middle produced cocoons cutting (Euclid distance) 1.72 two main clusters was formed. One of these clusters was consists of varieties of 8, 46, 25, 29 and 38 and another cluster that contain more varieties.

#### **Number of alive good produced cocoons**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of number of alive good produced cocoons cutting (Euclid distance) 1.72 two main clusters was formed. One of these clusters was consists of varieties of 10, 12 and 29 and another cluster that contains varieties 8, 46, 25, 29 and 38 and the second cluster contain the other varieties.

#### **Number of died good produced cocoons**

Phylogenetic tree using the UPGMA method was formed for attribute number of died good produced cocoons. In cross section 2.62 phylogenetic trees was divided into two main clusters. Main clusters contain a number of varieties (10, 11, 12, 30 and 42) and other varieties were located at the other major clusters. In distance of 1.1. Other major cluster subdivided into two smaller clusters.

#### **Number of middle produced cocoons**

Phylogenetic tree using the UPGMA method was formed for number of middle produced cocoons. In cross section 2.82 phylogenetic tree was divided into two main clusters. Main clusters contain a number of varieties (2, 6 and 9) and other varieties were located at the other major clusters with close distances.

#### **Number of alive middle produced cocoons**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a

trait of number of alive middle produced cocoons cutting (Euclid distance) 3.75 two main clusters was formed. One of these clusters was consists of variety of 2 and another cluster In cross section 1.5 phylogenetic tree was divided into two main clusters.

#### **Number of fertilized cocoons**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of number of fertilized cocoons cutting (Euclid distance) 3.47 two main clusters was formed. One of these clusters was consists of variety of 6 and another cluster In cross section 1.6 phylogenetic tree was divided into two main clusters.

#### **Number of low produced cocoons**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of number of low produced cocoons cutting (Euclid distance) 4.51 two main clusters was formed. One of these clusters was consists of variety of 30 and another cluster In cross section 1.3 phylogenetic tree was divided into two main clusters.

#### **Number of alive low produced cocoons**

Grouping using the UPGMA method was performed for number of alive low produced cocoons between Japanese varieties of silkworm gene bank. In cutting 4.53, varieties were divided into two main clusters. The first cluster contains variety (30). another cluster In cross section 1.3 phylogenetic tree was divided into two main clusters.

#### **Number of double produced cocoons**

Phylogenetic tree using the UPGMA method was formed for number of double produced cocoons. In cross section 2.24 phylogenetic tree was divided into two main clusters. Main clusters contain a number of varieties (40, 14, 40, 26, 39, 50 and 48) and other varieties were located at the other major cluster. Another cluster In cross section 0.85 phylogenetic tree was divided into two main clusters.

#### **Number of alive pupae in double cocoons**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of number of alive pupae in double cocoons cutting (Euclid distance) 2.25 two main clusters was formed. Main clusters contain a number of varieties (8, 9, 14, 12, 50, 26, 39, 40 and 48) and another cluster In cross section 0.9 phylogenetic tree was divided into two less clusters. Other varieties were located at the other major clusters with close distances.

#### **Number of died pupae in double cocoons**

Phylogenetic tree using the UPGMA method was formed for number of died pupae in double cocoons. In cross section 3.31 phylogenetic tree was divided into two main clusters. Main clusters contained tow varieties (3 and 14) and other varieties were located at the other major cluster. Another

cluster in cross section 0.85 phylogenetic tree was divided into two main clusters.

#### **Pupae vitality percentage**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of pupae vitality percentage divided to two main clusters. Main clusters contain a number of varieties (10, 30, 42, 11, 45 and 12). Another cluster in cross section 1.19 phylogenetic tree was divided into two less clusters. Other varieties were located at the other major clusters with close distances.

#### **Cocoon weight**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of cocoon weight in cross section 7.11 divided to two main clusters. The first cluster contains variety (13). Other varieties were located at the other major clusters with close distances.

#### **Shell cocoon weight**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait shell cocoon weight represented a single main cluster. It represents close genetic distances between varieties for this trait.

#### **Shell cocoon percentage**

Phylogenetic tree using the UPGMA method was formed for shell cocoon percentage. In cross section 3.28 phylogenetic tree was divided into two main clusters. Main clusters contain two varieties (19 and 20) and other varieties were located at the other major cluster.

#### **Male cocoon weight**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of male cocoon weight in cross section 7.14 divided to two main clusters. The first cluster contains variety (8). Other varieties were located at the other major clusters with close distances.

#### **Male shell cocoon weight**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of male shell cocoon weight cutting (Euclid distance) 1.1 two main clusters was formed. One of these clusters was consists of varieties (3,40,19,7,34,18,39,20,31,50,38 and 48) and another cluster was divided into two main clusters.

#### **Male shell cocoon percentage**

Phylogenetic tree using the UPGMA method was formed for male shell cocoon percentage. In cross section 2.24 phylogenetic tree was divided into two main clusters. One of these clusters was consists of varieties (8, 9, 40, 12, 14, 26, 50, 29 and 48) and other varieties were located at the other major cluster.

#### **Female cocoon weight**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a

trait of female cocoon weight cutting (Euclid distance) 7.07 two main clusters was formed. One of these clusters was consists of variety of 42 and another cluster was divided into two main clusters.

#### **Female shell cocoon weight**

Phylogenetic tree using the UPGMA method was formed for female shell cocoon weight. In cross section 5.30 phylogenetic tree was divided into two main clusters. One of these clusters was consists of variety of 18 and other varieties including varieties 18, 19, 24, 15, 29 and 11 were located at the other major cluster.

#### **Female shell cocoon percentage**

Hierarchical agglomerative clustering performed based on UPGMA method for trait of female shell cocoon percentage. The variety of 19 was separated from others. One of main clusters divided into two sub-group including varieties 2, 40, 18, 50, 42 and 20 and other varieties.

#### **Good cocoon weight of 250 larvae**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of good cocoon weight of 250 larvae cutting (Euclid distance) 2.39 two main clusters was formed. One of these clusters was consists of varieties of 48 and 39 and another cluster was divided into a number main clusters.

#### **Middle cocoon weight of 250 larvae**

Phylogenetic tree using the UPGMA method was formed for middle cocoon weight of 250 larvae. In cross section 4.30 phylogenetic tree was divided into two main clusters. One of these clusters was consists of variety of 2 and other varieties were including other varieties. In cross section 2.14 phylogenetic tree was divided into two less clusters including (2 and 8) and other major cluster.

#### **Middle cocoon weight**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of middle cocoon weight cutting (Euclid distance) 1.70 two main clusters was formed. One of these clusters was consists of 18 varieties and another cluster was divided into a number main clusters.

#### **Low cocoon weight of 250 larvae**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of low cocoon weight of 250 larvae cutting (Euclid distance) 5.01 two main clusters was formed. One of these clusters was consists of variety of 30 and another cluster was divided into two less clusters.

#### **Low cocoon weight**

Phylogenetic tree using the UPGMA method was formed for low cocoon weight. In cross section 2.98 phylogenetic trees was divided into two main clusters. One of these clusters was consists of

varieties of 4, 5, 10, 16, 8, 11, 13, 22, 39, 24 and 47 and other clusters were including other varieties.

#### **Double cocoon weight of 250 larvae**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of double cocoon weight of 250 larvae cutting (Euclid distance) 2.24 two main clusters was formed. One of these clusters was consists of varieties of 8, 9, 14, 50, 12, 26, 40, 39, 24 and 49. Other clusters were including other varieties.

#### **Double cocoon weight**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of double cocoon weight cutting (Euclid distance) 2.31 two main clusters was formed. One of these clusters was consists of varieties of 30, 34, 41, 35, 41, 35, 42, 46, and 43. Other clusters were including other varieties.

#### **Total cocoon weight of 250 larvae**

Phylogenic tree using the UPGMA method was formed for total cocoon weight of 250 larvae. In cross section 1.98 phylogenic trees was divided into two main clusters. One of these clusters was consists of varieties of 1, 48, 39, 3, 51, 33, 41 and 35 and other clusters were including other varieties.

#### **Total cocoon weight of 10000 4th instar larvae**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of total cocoon weight of 10000 4th instar larvae cutting (Euclid distance) 1.81 two main clusters was formed. One of these clusters was consists of varieties of 8, 11, 24, 15, 19, 20, 26, 18, 32, 25 and 29. Other clusters were including other varieties.

#### **Cocoon number per liter**

Phylogenic tree using the UPGMA method was formed for Cocoon number per liter. In cross section 1.67 phylogenic trees was divided into two main clusters. One of these clusters was consists of varieties of 16, 44, 9, 16 and 6 and other clusters were including other varieties. One of these clusters was consists of varieties of 1, 7, 19, 18, 3, 49, 11, 21, 48, 33, 38 and 42.

#### **Cocoon weight per liter**

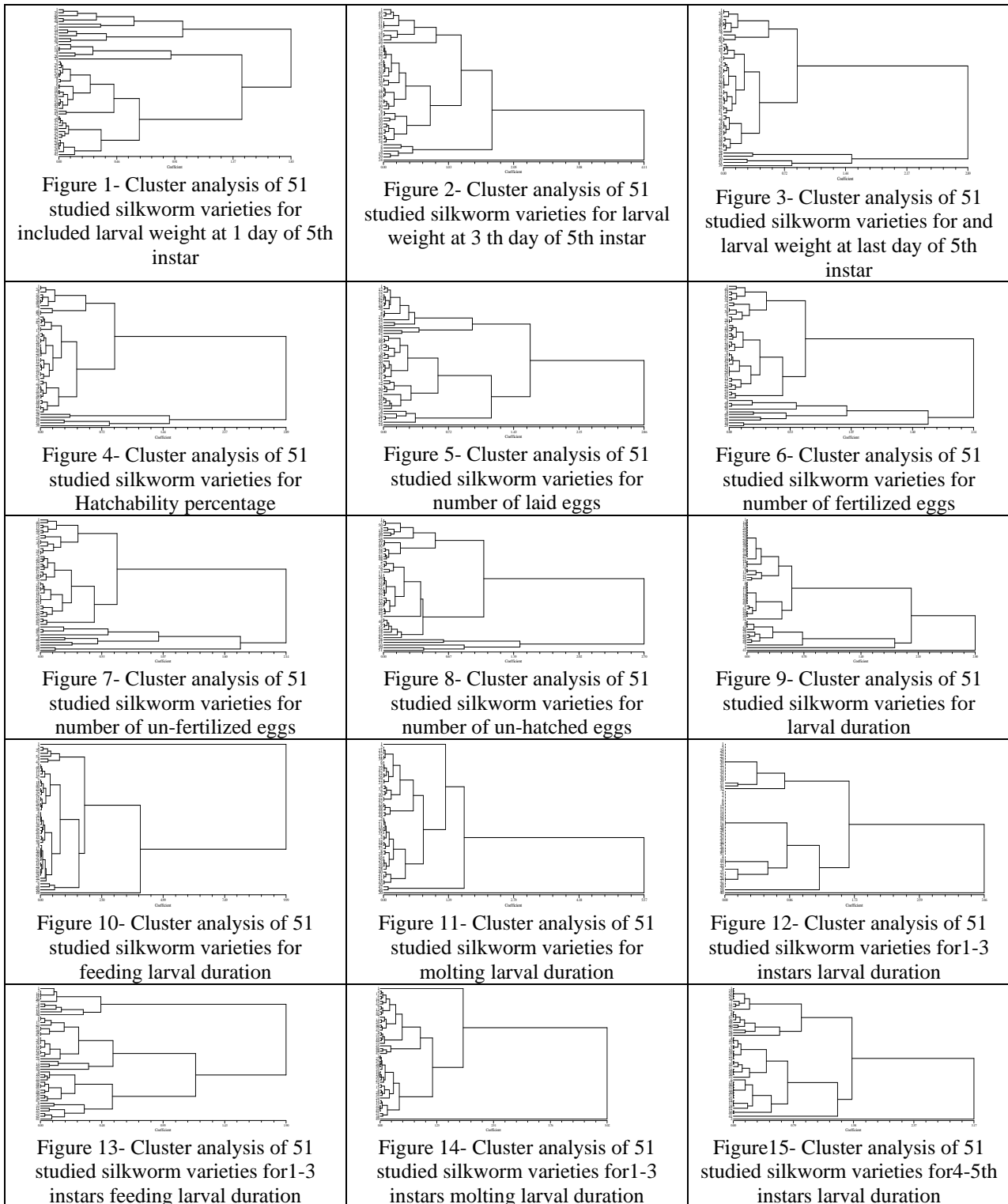
Analysis of silkworm strains for Cocoon weight per liter performed under UPGMA approach. Cluster divided varieties into tow sub-group in distance of 2.8. One of clusters was including varieties of 7 and 32. Varieties divided into tow sub-group in distance of 2. First group was including (2, 18, 19, 20 and 42) varieties and another cluster was including remained varieties.

#### **Pupae weight**

Phylogenic tree using the UPGMA method was formed for Cocoon number per liter. In cross section 2.24 phylogenic tree was divided into two main clusters. One of these clusters was consists of varieties of 2, 42, 19, 18 and 20 and other clusters were including other varieties. One of these clusters was consists of varieties of 1, 31, and 29.

#### **Male pupae weight**

The test results showed that in grouping the Japanese varieties of Iran silkworm gene bank with a trait of male pupae weight cutting (Euclid distance) 2.24 two main clusters was formed. One of these clusters was consists of varieties of 2, 48, 38, 12, 31, 19 and 29. Other clusters were including other varieties.





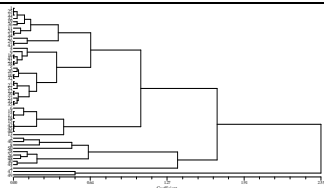


Figure 16- Cluster analysis of 51 studied silkworm varieties for 4-5th instars feeding larval duration

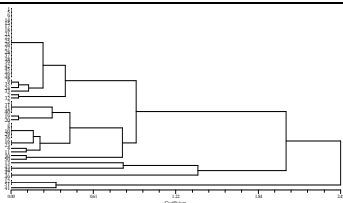


Figure 17- Cluster analysis of 51 studied silkworm varieties for 4-5th instars molting larval duration

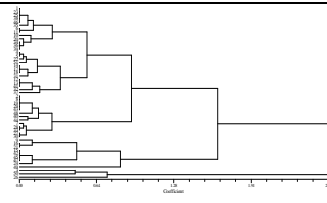


Figure 18- Cluster analysis of 51 studied silkworm varieties for 5th instar feeding larval duration

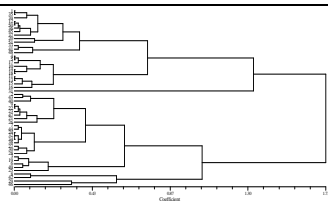


Figure 19- Cluster analysis of 51 studied silkworm varieties for number of total produced cocoons

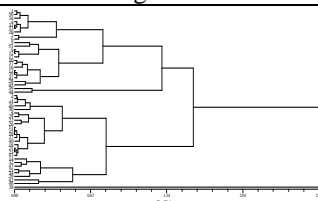


Figure 20- Cluster analysis of 51 studied silkworm varieties for number of good produced cocoons

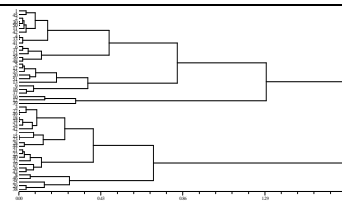


Figure 21- Cluster analysis of 51 studied silkworm varieties for number of alive good produced cocoons

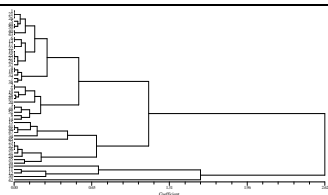


Figure 22- Cluster analysis of 51 studied silkworm varieties for number of alive good produced cocoons

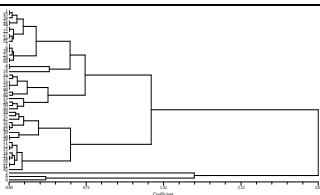


Figure 23- Cluster analysis of 51 studied silkworm varieties for number of died good produced cocoons

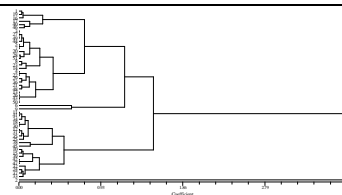


Figure 24- Cluster analysis of 51 studied silkworm varieties for, number of middle produced cocoons

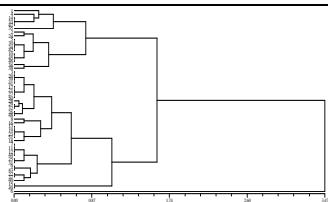


Figure 25- Cluster analysis of 51 studied silkworm varieties for, number of alive middle produced cocoons

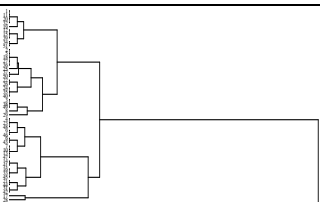


Figure 26- Cluster analysis of 51 studied silkworm varieties for number of died middle produced cocoons

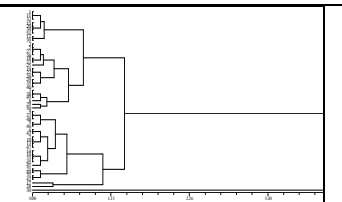


Figure 27- Cluster analysis of 51 studied silkworm varieties for number of low produced cocoons

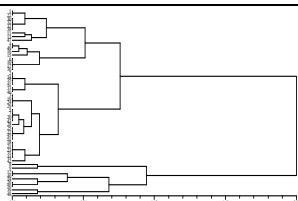


Figure 28- Cluster analysis of 51 studied silkworm varieties for number of double produced cocoons

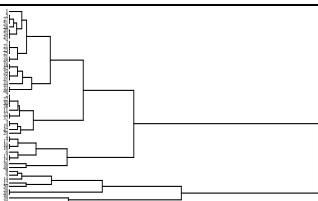


Figure 29- Cluster analysis of 51 studied silkworm varieties for number of alive pupae in double cocoons

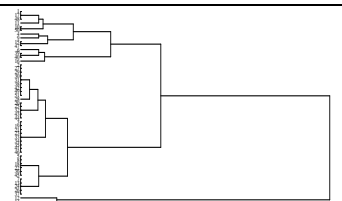


Figure 30- Cluster analysis of 51 studied silkworm varieties for number of died pupae in double cocoons

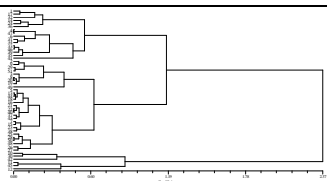


Figure 31- Cluster analysis of 51 studied silkworm varieties for number of died pupae in double cocoons

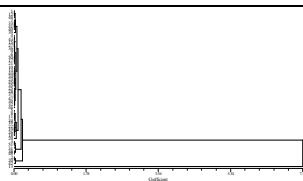


Figure 32- Cluster analysis of 51 studied silkworm varieties for male shell cocoon weight

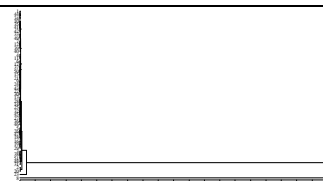


Figure 33- Cluster analysis of 51 studied silkworm varieties for male shell cocoon percentage

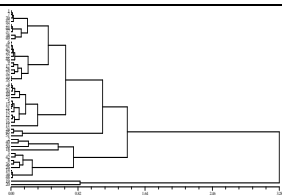


Figure 34- Cluster analysis of 51 studied silkworm varieties for female cocoon weight

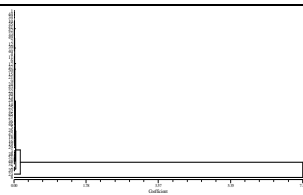


Figure 35- Cluster analysis of 51 studied silkworm varieties for female shell cocoon weight

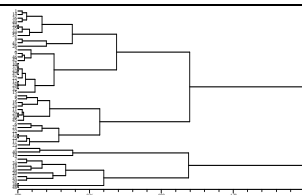


Figure 36- Cluster analysis of 51 studied silkworm varieties for female shell cocoon percentage

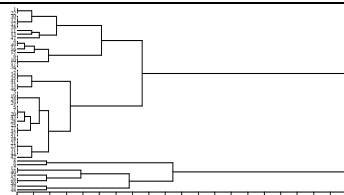


Figure 37- Cluster analysis of 51 studied silkworm varieties for cocoon weight of 250 larvae

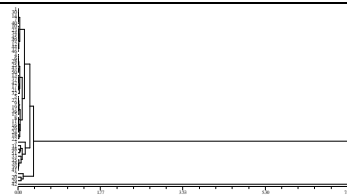


Figure 38- Cluster analysis of 51 studied silkworm varieties for middle cocoon weight of 250 larvae

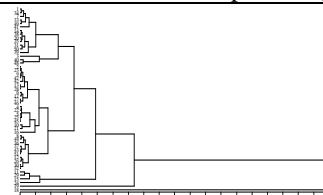


Figure 39- Cluster analysis of 51 studied silkworm varieties for middle cocoon percentage

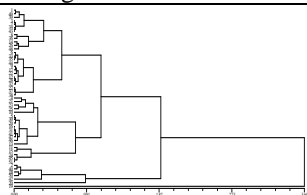


Figure 40- Cluster analysis of 51 studied silkworm varieties for low cocoon weight of 250 larvae

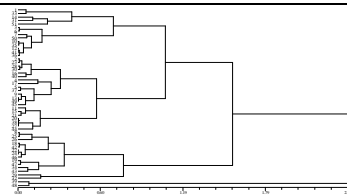


Figure 41- Cluster analysis of 51 studied silkworm varieties for low cocoon percentage

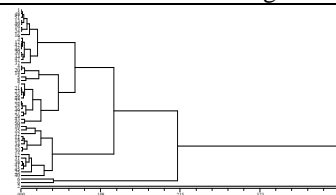


Figure 42- Cluster analysis of 51 studied silkworm varieties for double cocoon weight of 250 larvae

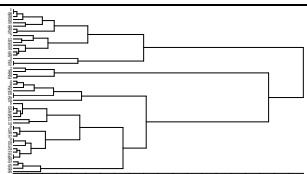


Figure 43- Cluster analysis of 51 studied silkworm varieties for double cocoon weight

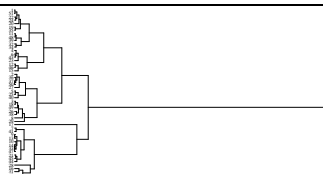


Figure 44- Cluster analysis of 51 studied silkworm varieties for total cocoon weight of 250 larvae

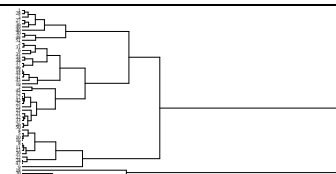
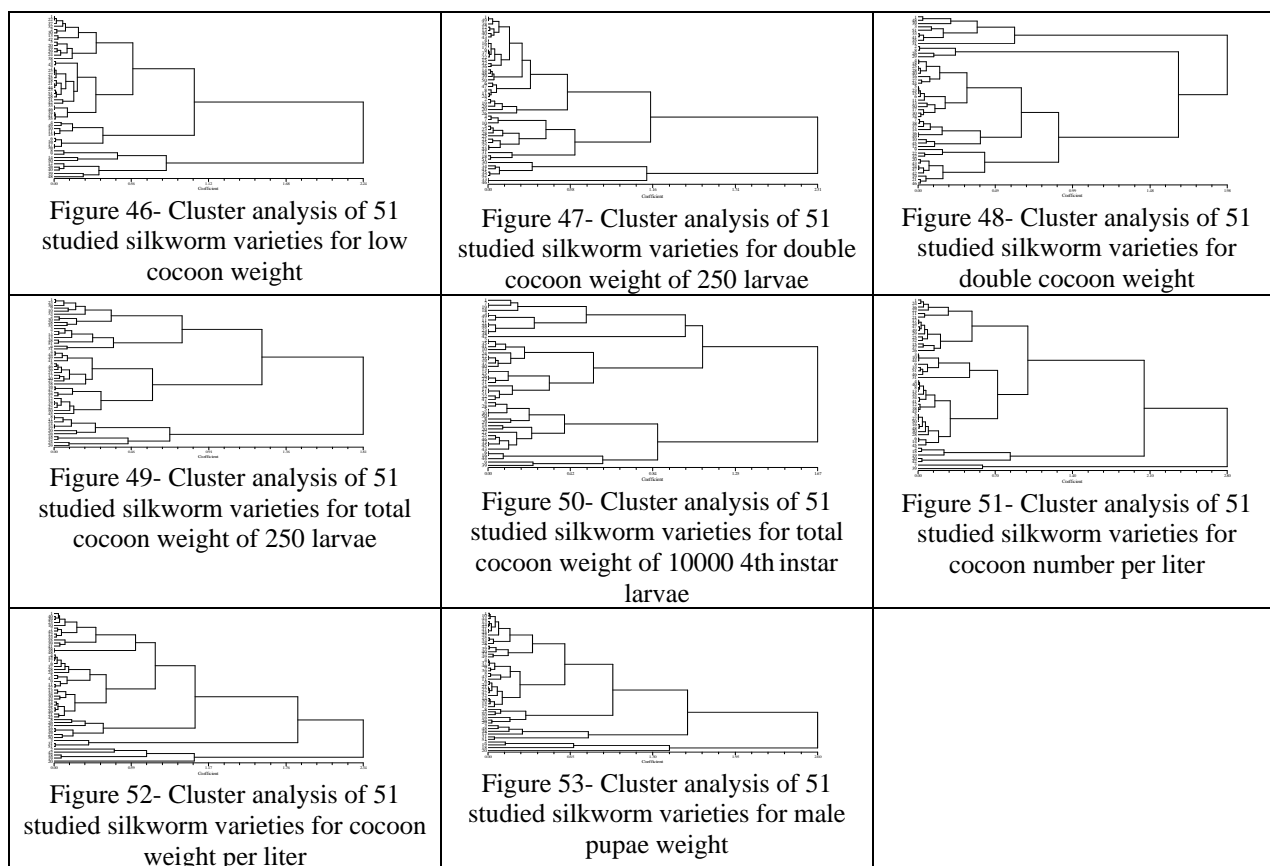


Figure 45- Cluster analysis of 51 studied silkworm varieties for total cocoon weight of 10000 4th instar larvae



#### 4. Discussion

This study examines the 51 Japanese varieties of silkworm gene bank in terms of genetic relationships. For this purpose, data Silkworm Research Institute of the country were used. Excel software were used to transfer data, calculate the mean records and data standardization. Also Ntsys software was used for clustered analyzing of data with UPGMA procedure. Software (NTSYSpc) was used for grouping with the UPGMA procedures. The purpose of this experiment was to assess the in-breeding ties of varieties to reduce silkworm gene bank size with making candid some of them for merging with each other. Also separate groupings were done for individual traits, and finally 53 phylogenetic trees were obtained.

Genetic potential of each variety is different according to various genetic traits. For example, when certain groups based on traits such as reproductive traits are analyzed, and grouping relevant dendrogram and grouping done by the UPGMA method with different characteristics, is different. This has caused to observe different grouping for separate traits.

Many varieties have a common origin and affinity. Various dendrogram showed that some varieties in many of these grouping which conducted based on different characteristics were in one cluster. This relationship led to place some varieties with a low

distance within one cluster together with the large distances with other varieties between clusters.

Scientific knowledge of the studied varieties can be a potential for future research work and use their genetic ability in future breeding programs in the Research Center. The large number of varieties had been recorded in this center. Therefore it is necessary with identify their abilities, the breeding programs for improved production traits and genetic advances should be used. The research has provided suitable area for this study.

Due to the large Japanese silkworm varieties of the countries, costs, problems of management, maintenance and breeding problems in them is high. Grouping can be done based on clusters of small candidates to be merged together in terms of reduced costs and better manage and also can be work on mixed varieties in breeding point of view. Because if we want to arrange some crosses without consider to the relationship between varieties a series of  $51 \times 51$  crosses is necessary which according to the three replications for each around 8000 crosses should be done. This can cause operational and administrative costs. The higher number of to higher updated cross also the experimental error is inevitable. However, if parents have candidate a smaller number of varieties

this work take place easier and the more accurate results will be obtained. (Bizhannia and Seidavi, 2008).

UPGMA is a grouping method that was used in this study. Nevertheless, research conducted by Dalirsefat and Mirhosseini (2007), to assess genetic diversity at least thirty individuals for each varieties and 50 marker as gene locus was used to estimate genetic diversity. In addition, UPGMA method was used for grouping (Nei, 1983). Tree dendrogram of genetic distance index was drawn by this method. It is obvious that, the AFLP markers which used in this study had enough the ability to detect Khorasan native breed from Japanese commercial breed. Separate grouping of Khorasani and Japanese race reflected, geographic origin and morphological quality and quantity characteristics, which are differ in that two race. Lemon and orange Khorasani races has been located in a classification and located separately with purple which were in accordance with grouping based on morphological characteristics (Mirhosseini, 1998).

For heterosis, those varieties should be selected that have a maximum distance and lower in-breeding coefficient. For this purpose information from their out-breeding and inbreeding (according to mentioned grouping) is important. Because the maximum heterosis occurs in the farthest varieties which have the most distance to each other and the least in-breeding.

In some target cases, breeding is in some traits or particular traits and other traits are not considered for the grower. For this purpose, clusters could identify varieties distance for each attribute (Nei, 1983).

This study had been examined the 51 varieties of Japanese silkworm. Origin of many Chinese and Japanese varieties may be the same. Study the genetic relationships of Chinese silkworm varieties of Iran can help us with superior genetic characteristics of each of these two groups, have attempted to hybridization as well as to prevent from the occurrence of the loss from it. Having this information can also maximize heterosis between the two groups. Silkworm races around the world have emerged, and through some Changes in Phenotype and genotype in a long period, different races had been generated. With the assumption that all races over a long period derived Chinese varieties with an annual laying period (Chatterjee and Datta, 1992).

Iranian native silkworm varieties have some useful and valuable features. For example, genes related to resistance and environmental compatibility which had been institutionalized as a result of long periods of natural selection and evolution in native silkworm. With proper design of crosses between indigenous varieties and those of Japanese varieties that have high production potential, but are weak in terms of disease resistance, hybrids consistence with environment and commercial can be obtained.

This study examines 53 economic traits of silkworm payment. Also, a review conducted with smaller number of economic characteristics of silkworm. Including silk cocoon weight (SGW), larval weight (LBW), larval length (LBL), cocoon length (CL), cocoon weight (CW), width of cocoon (CWD), shell weight (SW) and the percentage of raw silk (RSP), which plays an important role in the selection races and have a continuity with the traits of silkworm cocoons and silkworm net.

According to the field for more research on silkworm genetic resources, there are high potential for complementary research. Therefore, it is suggested to perform similar studies on the other genetic stocks of the country silkworm varieties such as Chinese silkworm gene bank in the future. On the other hand it is possible to use molecular techniques such as using genetic markers for the study of silkworm genetic resources on the country. In this study, 56 economic traits of silkworm were investigated. It is recommended to invest on the varieties K-119 and BH-2 that in this study along with two other varieties, gained the highest rank. That is, the obstacles facing genetic varieties such as general and specific combining ability to be remove as well as needed reviews in climate accommodation and resistance to disease to introduce these varieties as the varieties. Also, it is suggested to perform similar studies in the context of other traits, such as biochemical characteristics. We hope that this study provide appropriate context for other studies and breeding programs such as the establishment different mating systems, selection based on varieties performance and so.

#### Acknowledgements:

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#### References

1. Farshadfar A. Multivariate Statistical Principles and Methods, Taghebostan Press 2005;489.
2. Mirhosseini SZ, Ghanipour M, Shadparvar A, Etebari K. Selection indices for cocoon traits in six commercial silkworm (*Bombyx mori* L.) lines. Phi. Agr. Sci 2005;88:328-336.

3. Singh T, Chandrasekharaiah M, Samson MV. Correlation and heritability analysis in the silkworm, *Bombyx mori* L. *Sericologia* 1998;38:1-13.
4. Nei M. The theory and estimation of genetic distance. *Genetic Structure of populations*, N.E. Morton University Press of Hawaii 1983;256.
5. Takezaki N, Nei M. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* 1996;144: 389-399.
6. Kalpana GV. Breeding of superior races of silkworm *Bombyx mori* L. for tropical climates. Thesis submitted to the University of Mysore, for the degree of doctor of philosophy. 1992; 285.
7. Li CC, Weeks DE, Chakravarti A. Similarity of DNA fingerprints due to chance and relatedness. *Hum. Here* 1993; 43:45-52.
8. Balvasi A. Study of Genetic diversitie of Iranian silkworm using ISSR Markers. Master Thesis. Tehran University. Faculty of Agriculture 2003; 139.
9. Alitalesh L. Study of Genetic diversitie of three chainese silkworm strains using AFLP Markers. Master Thesis. Guilan Universitie. Faculty of Agriculture. 2007; 132.
10. Mirhosseini SZ. Study of Genetic diversitie of Iranian silkworm using DNA Markers. PhD Thesis. Tarbiat Modares Unieiversitie. Faculty of Agriculture 1998;169.
11. Rohlf FJ. NTSYSpc: numerical taxonomy and multivariate system, version 2.02e. Exeter Software, New York 1998.
12. Bizhannia AR, Seidavi AR Principles and Methods of Silkworm Breeding. Haghshenas Press 2008; 146.
13. Dalirsefat SB, Mirhoseini SZ. Assessing genetic diversity in Iranian native silk-worm (*Bombyx mori*) strains and Japanese commercial lines using AFLP markers. *Iran J. Biot* 2007; 5(1).
14. Chatterjee SN, Datta RK. Hierarchical clustering of 54 races and strains of the mulberry silkworm, *Bombyx mori* L.: significance of biochemical parameters. *Theor. Appl. Gen* 1992;85(4):394-402.

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## Preliminary Study in diagnosis and early prediction of Preeclampsia by Using FTIR Spectroscopy Technique

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**Abstract:** Background: Preeclampsia is a heterogeneous condition, potentially involving several separate pathophysiological pathways; currently no clinical screening test is useful for prediction of preeclampsia development. Fourier-transform infrared spectroscopy (FT-IR) holds great promise for clinical chemistry measurements. Methods: FT-IR spectra of serum samples from pregnant women -14 patients and 31 normotensive were obtained. Absorbance ratios, second derivative spectra, ANOVA test and personal correlation statistical analysis were tacking in comparison studies. The parameters studied were proteins and lipids. Results: Different absorbance ratios for specific bands were calculated and plotted versus the patient samples. These ratios yielded statistically significant increase/decrease among the groups under investigation. The results showed that among the normotensive control group three subjects later developed preeclampsia. The results obtained from the IR-measured (amide A/amide B) ratio of serum confirmed, with 92.9 % confidence level, the effectiveness of this technique for the diagnosis of preeclampsia. Normotensive pregnant women who developed preeclampsia were considered as subjects at high risk. Conclusion: This study suggests, for the first time that FT-IR spectroscopy can be successfully used as an accurate and rapid test, for diagnosis and early prediction of preeclampsia, starting from 20 week of gestation.

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**Key words:** Fourier Transform Infrared Spectroscopy (FTIR); Oxidative Stress; Dyslipidemia; Preeclampsia; Serum  
**Abbreviations:** Fourier transform infrared spectroscopy (FTIR);

### 1. Introduction:

Preeclampsia, which affects 3% to 10% of pregnancies <sup>[1]</sup>, is a pregnancy-specific disorder characterized by hypertension, proteinuria and edema. The efforts to develop screening tests for potential use in clinical practice have yielded disappointing results <sup>[2]</sup>. Markers were generally chosen on the basis of specific pathophysiological abnormalities that have been reported in association with preeclampsia. Maternal concentrations of these biomarkers have been reported to be either increased or reduced early in gestation before the onset of preeclampsia. Given that preeclampsia is likely to be a heterogeneous condition, potentially involving several separate pathophysiological pathways, it is not surprising that simple clinical indicators are ineffective in identifying women who would benefit from pathway-specific treatment <sup>[3]</sup>. A variety of substances indicative of endothelial dysfunction are increased in the blood or urine of women with preeclampsia<sup>[3,4-5]</sup>. Many of these substances are elevated weeks before (as well as during) clinically evident preeclampsia <sup>[6,7]</sup>. It has been suggested that preeclampsia is a disease of antioxidant inadequacy

appearing when the normal antioxidant balance is upset <sup>[8]</sup>.

During the last decade, Fourier transform infrared (FTIR) spectroscopy has proven and accepted to be a powerful tool for the study of biological samples. The primary reason for this is that common biomolecules such as proteins, nucleic acids, and lipids, have characteristic functional groups having unique molecular vibrational modes (vibrational fingerprints) corresponding to specific infrared light frequencies <sup>[9,10]</sup>. The composition and structure of molecular functional groups can be determined by analyzing the position, width, and intensity of infrared light absorption<sup>[12-16]</sup>.

In this study we have tested FTIR spectroscopy as a potential specific accurate diagnostic tool for identifying normal pregnancy and preeclampsia, The second objective of this study was to define a new biophysical marker that is simple, valid and rapid, with potentially no limitation in clinical practice for early prediction of women – that are at high risk- who might later develop preeclampsia.

### 2. Materials and Methods

This study was approved by the Bioethical and Research Committee at the Faculty of Medicine, King Abdulaziz University (KAU). An oral voluntary consent was obtained from all the participating subjects.

The main focus of this work was to conduct prospective or cross sectional studies aimed at evaluating the feasibility of using a clinical and biophysical test, performed during pregnancy, before the development of preeclampsia.

#### **Inclusion Criteria:**

Cases eligible for inclusion in this study were normotensive pregnant women that have no evidence of proteinuria (control group) and patient group either with mild or severe preeclampsia. Preeclampsia was defined as hypertension (systolic blood pressure  $\geq 140$  mmHg and diastolic blood pressure  $\geq 90$  mmHg after 20 weeks' gestation) and proteinuria ( $\geq 300$ mg in a 24 hr urine collection or one dipstick measurement of  $\geq 1+$ ) according to the Committee of Terminology of American College of Obstetricians and Gynecologists (ACOG) definition [17]. Severe preeclampsia was diagnosed on the basis of diastolic blood pressure  $\geq 110$  mmHg or significant proteinuria (dipstick measurement of  $\geq 2+$ ) or the presence of severity evidences such as headache, visual disturbances, upper abdominal pain, oliguria, convulsion, elevated serum creatinine, thrombocytopenia, marked liver enzyme elevation, and pulmonary edema.

#### **Exclusion criteria:**

Included fetal anomalies, chronic heart disease and inflammatory disorders.

#### **2.1. Selection of women:**

- **Age:** The age of 45 women participating in this study was from 16 to 49 years ( $32.0 \pm 7.6$  years).

- **Week of gestation:** All the subjects were in second and third trimester. The gestational age was between 20 to 42 weeks. Data taken from the medical record of subjects are given in Table 1.

- **Study groups:** 31 normotensive pregnant women were taken as control group, and 14 patients diagnosed with preeclampsia were taken as the patients group. All subjects received obstetrical care at KAU hospital.

#### **2.2. Blood Collection and serum separation**

Three ml of non-fasting venous blood were collected from the subjects and drawn into plain tubes. Blood samples were immediately centrifuged at 4000 rpm for 10 min to separate serum. The serum samples were then removed and stored at  $-80^{\circ}\text{C}$ .

All the samples were lyophilized prior to FT-IR measurements.

#### **2.3. Infrared Measurement**

The lyophilized samples were dispersed in potassium bromide (KBr) by gently mixing with a pestle in an agate mortar to obtain a homogenous mixture as described by Paul and Robert [14] but with 1% concentration. The mixture was then pressed in a die at 5 metric tons force for 3 s, creating a 1.1 cm diameter transparent disc with imbedded samples. For each sample, the absorbance of three different FT-IR spectra were recorded at room temperature ( $26^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) in the mid infrared range ( $4000\text{--}400\text{ cm}^{-1}$ ) using a Shimadzu FTIR-8400s spectrophotometer with continuous nitrogen purge. Those three spectra were then coadded. Typically, 20 scans were single-averaged for a single spectrum and at spectral resolution of  $4\text{ cm}^{-1}$ . To minimize the difficulties arising from unavoidable shifts, baseline correction was applied. Each spectrum was normalized as normalization produces a spectrum in which maximum value of absorbance becomes 2 and minimum value 0. Other normalization methods such as normalization to amide II band had also been tested and gave negligible changes in the results. The parameter studied was proteins and lipids. The absorbance ratios were taken from the raw spectra.

#### **2.4. Statistical analysis**

Different absorbance ratios for specific bands were calculated and plotted versus the patient samples. An analysis of variance (ANOVA) and personal correlation was conducted to confirm the results obtained from IR measurements.

### **3. Results**

#### **3.1. IR spectral features and assignments**

The infrared spectra were obtained for 45 lyophilized serum samples from pregnant women, including 31 samples from women clinically and laboratory assessed as healthy normotensive and 14 samples from patients already diagnosed as preeclampsia. Each spectrum was normalized and base line corrected. Careful examination of the FTIR spectrum obtained from each sample revealed differences in the intensities of the absorption bands in relation to each other, and band shifts were observed among the groups under investigation. Some spectral features in certain control samples resembled those found in the patient spectra. Careful inspection of the FTIR spectrum obtained from each sample (Fig 1) revealed that the amide B band centered at  $3074\text{ cm}^{-1}$  for control samples (15-32) shifted to  $3076\text{ cm}^{-1}$  for control samples (33-45) and to  $3080\text{ cm}^{-1}$  in patient group. The symmetric

stretching mode of vibration of the CH<sub>3</sub> band centered at 2871 cm<sup>-1</sup> for control samples (15-32) shifted to 2873 for control samples (33-45) and to 2869 cm<sup>-1</sup> in the patient group. Moreover, it is also observed from the figure that the intensity of the weak shoulder at 1740 cm<sup>-1</sup> for the control samples (15-32) turned to a strong shoulder for the majority of patient and control samples (33-45) spectra (Fig. 1B).

Accordingly, to facilitate data interpretation, the control group was divided into two groups: control-1 (samples from 15-32) and control-2 (samples from 33-45), from subjects whose spectra behaved like patient spectra. According to the medical history of most of the control-2 subjects (Table 1) we may consider this group as subjects at high risk although they are normotensive.

For clarity the sum, of equal numbers, of coadded spectra from control-1 samples, the sum of control-2 spectra and the sum of patient spectra are overlaid and shown in (Fig.1). These spectra were dominated by protein, lipids, phospholipids and carbohydrates bands. The major absorbance bands and their assignments are given in Table 2.

### 3.2. Ratios measurements

The band maxima of the absorption bands around the frequencies 3301, 3074, 2927, 2873, 1652, 1541, 1398, 1244 cm<sup>-1</sup> were determined after the entire spectrum for each sample has been normalized and base line corrected for subsequent statistical analysis. The following absorbance ratios (**I**) were calculated and represented graphically.

$$\text{Amide A/Amide B} = [I_{(3301\text{cm}^{-1})}/I_{(3072\text{cm}^{-1})}], \text{ RI} = [I_{(1652\text{cm}^{-1})}/I_{(2927\text{cm}^{-1})}], \text{ RII} = [I_{(1541\text{cm}^{-1})}/I_{(2927\text{cm}^{-1})}]$$

The Amide A/Amide B ratio from all serum samples is represented graphically in Fig (2). It appears from the Figure that the change in the relative intensity of the amide A and B of NH stretching bands (A3301/A3072 absorbance ratio) has the highest value (<1.6 ±0.12) for patient (except patient number 6) and control-2 groups compared to control-1 group. The arrows in the figure are pointed to those control-2 samples which were clinically considered as normal at the time of sample collection and, after following their files and medical record, at the end of their pregnancy they all developed preeclampsia (samples number 34,35 & 39).

The same results are obtained in case of RI and RII ratios (Fig. not shown). For patient and control-2-group the RI and RII values are always greater than 1.6 and 1.4, respectively, compared to the control-1 value which shows much lower values for these ratios. For phosphate/amide II ratio (Fig. not shown)

the values of this ratio are lower than 0.4 for patient and control-2 group relative to the control-1-group.

To determine whether the three groups were statistically different from each other, ANOVA test was performed. In the above mentioned IR results, the hypothesis that one of the two means is bigger than the other is assumed before doing the test. The ANOVA results (Table 2) indicated that for all examined ratios there are highly significant differences between patient and control-1 groups. There is no significant difference between patient and control-2 groups in all examined ratios, except in RI. The variation between control-1 & 2 groups is highly significant in all the above tested ratios.

Also personal correlation (Table 3) and linear regression were done between Amide A/Amide B ratio (the golden standard method to discriminate between patient, normotensive control-1, and normotensive control-2 subjects those at high risk to develop preeclampsia), age, week of gestation, body mass index (BMI) and blood group. The results revealed that there is a strong relation between BMI and the elevated Amide A/Amide B ratio. This is true, as overweight increases the incidence of preeclampsia. Moreover, from linear regression analysis the blood group O<sup>+</sup> seems to play a role and increases the risk of this disease (Fig. 3), although this observation should be studied further more with large number of patients and risk groups.

### 3.3 Second Derivative Analysis

Since eclampsia/preeclampsia involves the modification of existing serum albumin proteins, as well as lipids profile we analyzed the changes of protein structure between the examined groups in more detail. Fig. (4) shows the mean spectra of control-1, control-2 groups together with the patient group in the regions of the esterified lipid C=O stretching band (1750-1700 cm<sup>-1</sup>), the amide I (1600-1700 cm<sup>-1</sup>) and amide II (1500-1600 cm<sup>-1</sup>) bands. Spectra are shown as second derivative. The second derivative of the original spectra offers a direct way to identify the peak frequencies of characteristic components and thus permits much more detailed qualitative and, eventually, quantitative studies. It is obvious from the figure that there are marked shifts in the position and the intensity of both amide I and amide II bands, which strongly affect the overall serum proteins secondary structure.

These amide I frequencies are compatible with the fact that overall protein structure in the control-1-group consists primarily of  $\alpha$ -pleated sheets,  $\beta$ -helix and random coil, as the amide I bands centered at 1690, 1652 and 1641 cm<sup>-1</sup> respectively, whereas in patient group and control-2-group serum samples have a relatively high proportion of  $\beta$ -helix,



random coil and  $\beta$ -turns, as the increase intensities of bands at 1652, 1641 and 1688  $\text{cm}^{-1}$  respectively.

For the esterified lipid C=O band, the second derivative spectrum of control-2 displayed a strong sharp band centered at 1741  $\text{cm}^{-1}$  together with a weak shoulder at 1721  $\text{cm}^{-1}$ , while the control-1 spectrum exhibited only a strong broader band at 1741  $\text{cm}^{-1}$ . The patient spectrum showed a more intense strong sharp band at 1744  $\text{cm}^{-1}$ , and a weak band at 1712  $\text{cm}^{-1}$ .

### 3.4 Difference Spectrum

In order to further assess and identify the changes in proteins and lipids in serum samples, typical IR marker bands for proteins and lipids were evaluated by creating a 'difference spectrum' in the range of 1800-1400  $\text{cm}^{-1}$  (Fig. 5).

Fig. (5A) shows the difference spectrum between patient and control-1 groups, which showed

a significant increase in lipid content, triglycerides, fatty acids and cholesterol in patients group, indicated by the positive bands of ester (1737  $\text{cm}^{-1}$ ) and the positive bands in region 1400-1480  $\text{cm}^{-1}$ .

One can also clearly see that the protein secondary structure of control-1 group has lower percentage of  $\beta$ -helix and  $\beta$ -turn than the patient group indicated by the negative amide I band and shifts.

The difference IR spectrum between control-2 and patient groups is shown in (Fig. 5B). It is obvious from the figure that the protein secondary structure of control-2 group has a higher proportion of  $\beta$ -helix structure than patient group, as indicated by the positive band at 1658  $\text{cm}^{-1}$ . Meanwhile, the control-2 group has a lower amount of lipid ester C=O, as indicated by the negative band at 1744  $\text{cm}^{-1}$ .

**Table 1: General information for subjects given from medical records in KAU hospital.**

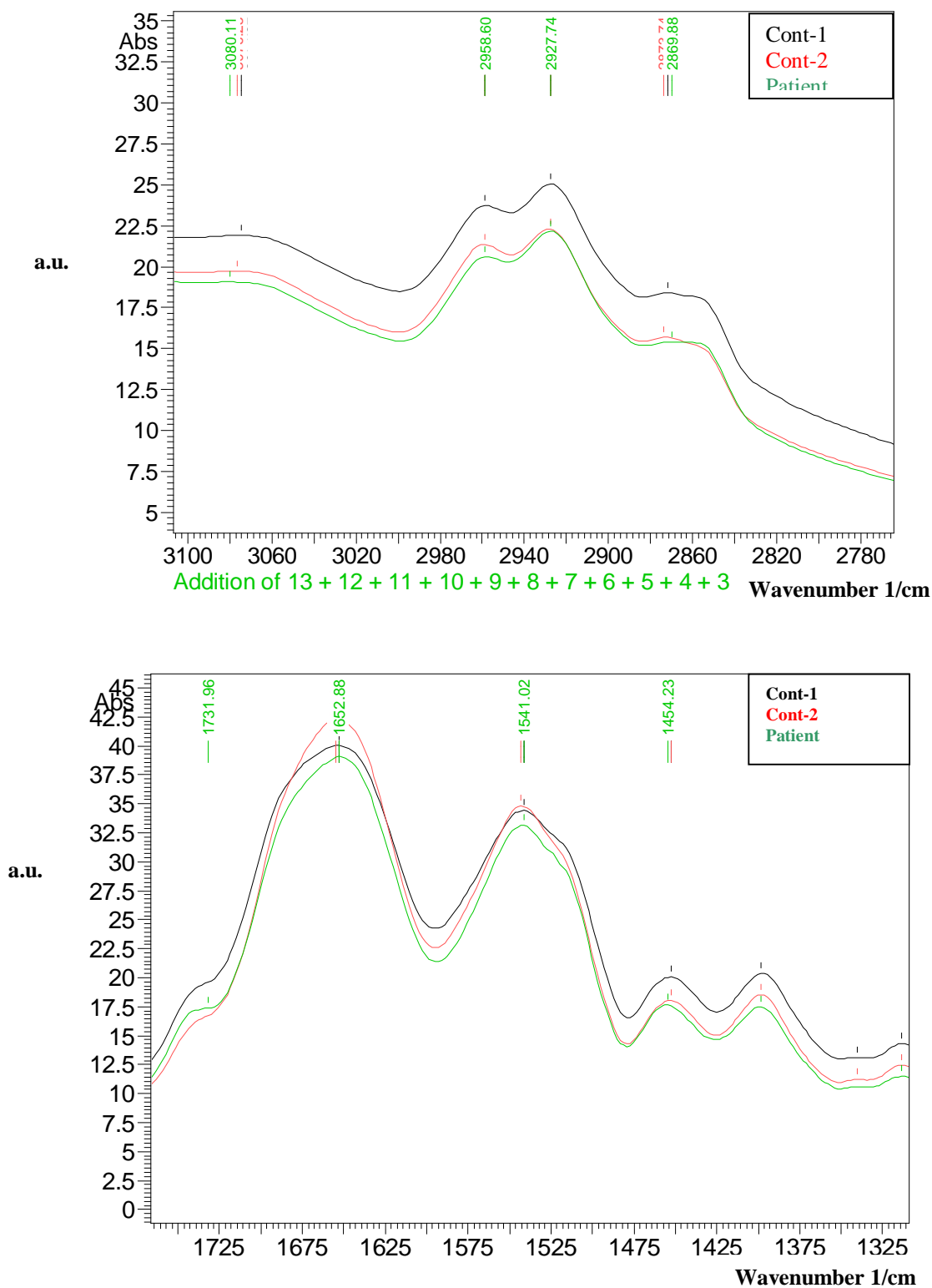
Sample No	Gestation Age	Age	Blood Group	BMI	History	
Patients	1	42	30	O+	31.3	Mild PET, BP=147/89, protein= 0.12, SVD, fibrinogen, ALP, Neutrophils <b>medication</b> =aldomate 500 mg, adulate 20 mg.
	2	40	26	B+	30.0	Mild PET, BP=155/90,SVD, protein= 1.6, fibrinogen, ALP, Neutrophils, history preeclampsia, BP <b>medication</b> = adalate 20 mg.
	3	38	38	A+	35.0	Mild PET, BP= 139/84, GDM, hypertension, +ve protein urea, CS (trans lie verse baby).
	4	37	22	O+	24.3	Protein=0.162, BP=140/85, BP, fibrinogen, ALP, Neutrophils, PIH, <b>medication</b> =aldomate 250 mg.
	5	37	43	O+	43.2	BP, FDM, CS (brain aneurysm), LDL, cholesterol, protein= 0.15, ALP, Neutrophils, history preeclampsia, <b>medication</b> =omeprazole, glucophage 75 mg, omperazol, Insulin.
	6	37	43	O+	35.9	Mild PET, BP= 146/90, SVD, fibrinogen, ALP, LDH, history preeclampsia.
	7	37	26	A+	33.9	Mild PET, protein=0.399, BP= 147/85, protein=trace, fibrinogen, ALP, Neutrophils, history preeclampsia <b>medication</b> =omperazol 10 mg, aldomate 250 mg.
	8	36	31	A+	41.2	Mild PET, BP=144/80, 151/76, protein= 0.2, CS, TSH, fibrinogen, LDH, Eosinophils, history preeclampsia <b>medication</b> = thyroxin (hypothyroidism).
	9	35	37	B+	32.9	Mild PET, BP=150/90, protein=0.2, CS, previous PET, fibrinogen, ALP, AST, Neutrophils, <b>medication</b> =adalat 20 mg.
	10	34	27	A+	39.8	previous PET,CS, BP, GDM, <b>medication</b> = aldomat 250 mg.
	11	34	24	B+	36.0	BP= 149/90, 168/105, CS, fibrinogen, ALP, LDH, Neutrophils, <b>medication</b> = heparin, aldomt 500 mg.
	12	34	16	O+	23.9	Mild PET, BP= 146/84, protein= +2, SVD, wince, fibrinogen, ALP, LDH, AST, APTT, <b>medications</b> = adalat, ampecillin1g.
	13	32	26	O+	-	SVD, Neutrophils, fibrinogen, <b>medications</b> = thyroxin.
	14	31	40	A+	36.0	Mild PET, BP= 148/65, protein= 0.28, fibrinogen, ALP, Neutrophils, <b>medications</b> = aldomat 250 mg.
Controls	15	37	37	B+	30.7	BP=115/67, SVD.
	16	37	46	B+	-	BP= 132/76, SVD, fibrinogen, ALP <b>medication</b> = ampecillin1g.
	17	35	25	O+	-	BP= 104/48, CS.
	18	34	24	B+	-	BP= 95/52.
	19	33	29	A+	-	BP= 132/58.

20	32	38	O+	38.1	BP= 97/63, CS.
21	32	35	A+	29.8	BP= 117/55.
22	30	20	A+	28.8	BP= 101/50.
23	25	28	B+	34.8	BP= 92/56, SVD medication= thyroxin 25 mg (Hypothyroidism).
24	25	36	O <sup>-</sup>	47.2	BP= 155/77, BP= 132/85, SVD.
25	25	35	B+	-	BP= 107/57, CS.
26	25	27	O+	42.8	BP= 102/53, SVD.
27	24	27	O+	-	BP= 97/63, CS, Neutrophils.
28	22	29	O+	28.2	BP= 117/55, twins, CS.
29	22	22	A+	-	BP= 101/50.
30	20	38	O+	-	BP= 92/56, SVD.
31	20	36	O+	-	BP= 118/59, SVD.
32	20	28	B+	-	BP= 100/63, CS.
33	38	43	O+	27.2	BP= 121/76, SVD, anemia during pregnancy, Neutrophils, ALP medications= Erythromycin 500 mg.
34	22	21	O+	16.7	BP= 110/56, SVD. TSH, glucose, AST, Neutrophils, Basophils.
35	20	40	A+	33.4	BP= 121/82, CS, Neutrophils, Eosinophils, Fibrinogen, APTT.
36	20	27	O+	24.7	BP= 90/55, Neutrophils.
37	35	32	B+	-	BP= 113/60, CS.
38	35	33	O+	-	BP= 135/87, GDM, SVD, BP, history with disease.
39	35	38	A+	27.4	BP= 109/59, kidney disease, total protein, posterior vaginal repair.
40	33	38	O+	28.0	BP= 122/78, anemia during pregnancy, SVD.
41	25	28	B+	37.1	BP= 118/74, CS, Eosinophils.
42	25	42	O+	-	BP= 113/75, SVD, hyperglycemia.
43	24	49	O+	-	BP= 139/67, diabetes, history preeclampsia, BP, CS, medications= aldomat.
44	24	31	O+	27.3	BP= 113/66, ALP.
45	21	27	A+	-	BP= 96/63, kidney disease, Neutrophils, Eosinophils, Fibrinogen, APTT.

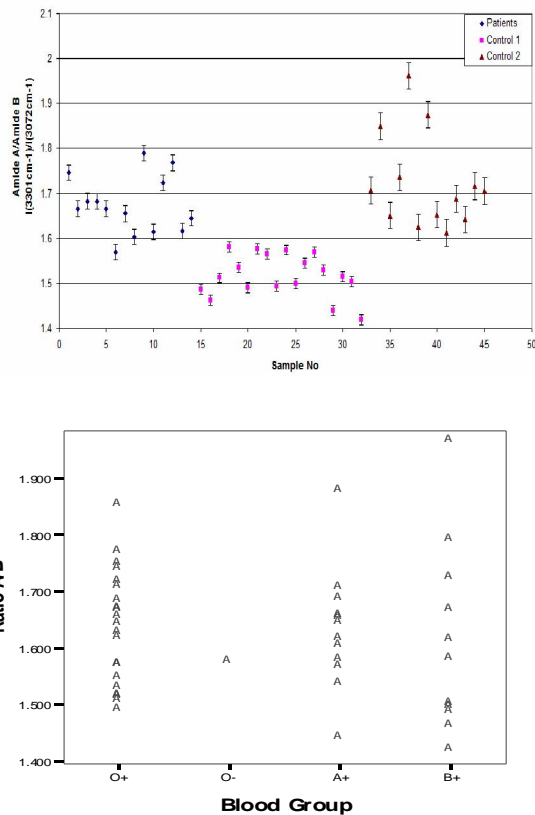
**Table 2: The results of ANOVA test and the p-values among control-1, control-2 and patient groups**  
Multiple Comparisons

LSD

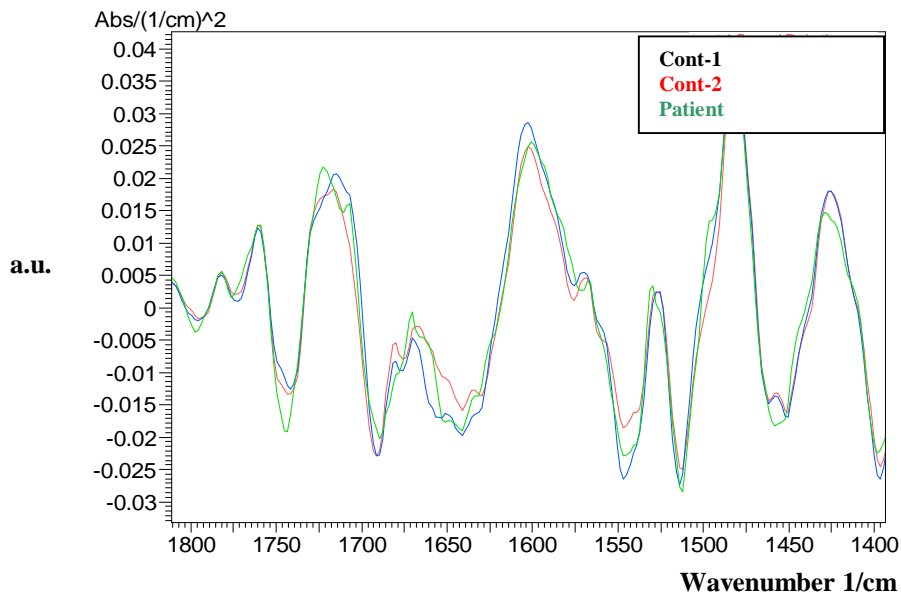
Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.
RI	Patients	Control 1	.173383*	.044620	.000
		Control 2	-.124244*	.048918	.015
	Control 1	Patients	-.173383*	.044620	.000
		Control 2	-.297627*	.043534	.000
	Control 2	Patients	.124244*	.048918	.015
		Control 1	.297627*	.043534	.000
RII	Patients	Control 1	.112800*	.036778	.004
		Control 2	-.060827	.040321	.139
	Control 1	Patients	-.112800*	.036778	.004
		Control 2	-.173627*	.035883	.000
	Control 2	Patients	.060827	.040321	.139
		Control 1	.173627*	.035883	.000
Amide A / Amide B	Patients	Control 1	.152450*	.028148	.000
		Control 2	-.042808	.030859	.173
	Control 1	Patients	-.152450*	.028148	.000
		Control 2	-.195258*	.027463	.000
	Control 2	Patients	.042808	.030859	.173
		Control 1	.195258*	.027463	.000



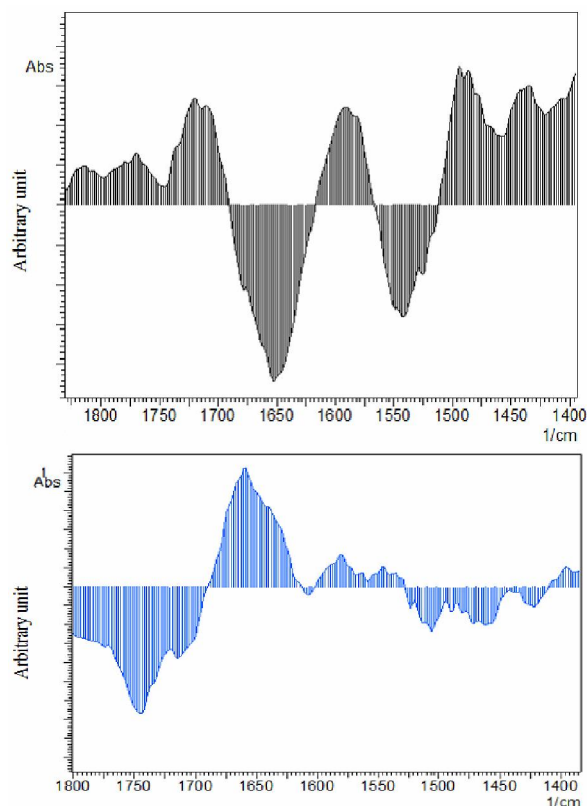
**Fig. 1.** FTIR spectra represent control-1, control-2 and patient groups in region (3100-2800 cm<sup>-1</sup>), the arrows pointed to the band shifts in the amide B and CH<sub>3</sub> bands (A). FTIR spectra in region (1700-1300 cm<sup>-1</sup>), the arrows pointed to the shifts in amide I band together with the variation in the ester band intensity at 1737cm<sup>-1</sup>(B).



**Fig. 3** Linear regression method the figure shows the relation between Amide A/ Amide B ratio and the blood group of all subjects. All ratios over 1.6 are for patient and control-2 groups.



**Fig. 4.** IR second derivative spectra of serum samples from control-1, control-2 and patient groups in range (1800-1400  $\text{cm}^{-1}$ ). The arrows pointed to the shift in the band positions and the changes in band intensities for esterified C=O(1750 -1700  $\text{cm}^{-1}$ ) and amide I bands(1700-1600  $\text{cm}^{-1}$ ).



**Fig. 5(A)** The difference spectrum between patient and control-1 groups showed a significant increase in lipid content, triglycerides, fatty acids, cholesterol and the decreased protein content in patient group than the control-1. **(B)** The difference IR spectrum between patient and control-2 showed higher proportion of protein  $\alpha$ -helix structure than patient group indicated by the positive band at  $1658\text{ cm}^{-1}$  and lower content of lipids in control-2 group compared with patient group.

**Table 3: Personal correlation between control-1, control-2 and patient samples and the blood group.**

		Correlations				
		Gestation Weeks	Age	Blood Group	Ratio A/B	Weight
Gestation Weeks	Pearson Correlation	1.000	.082	.139	.258	.335*
	Sig. (2-tailed)		.594	.361	.087	.024
	N	45.000	45	45	45	45
Age	Pearson Correlation	.082	1.000	-.078	-.060	.257
	Sig. (2-tailed)	.594		.612	.696	.088
	N	45	45.000	45	45	45
Blood Group	Pearson Correlation	.139	-.078	1.000	-.080	.178
	Sig. (2-tailed)	.361	.612		.599	.242
	N	45	45	45.000	45	45
Ratio A/B	Pearson Correlation	.258	-.060	-.080	1.000	-.074
	Sig. (2-tailed)	.087	.696	.599		.628
	N	45	45	45	45.000	45
Weight	Pearson Correlation	.335*	.257	.178	-.074	1.000
	Sig. (2-tailed)	.024	.088	.242	.628	
	N	45	45	45	45	45.000

\*. Correlation is significant at the 0.05 level (2-tailed).

#### 4. Discussion

There is currently no clinically useful screening test to predict the development of preeclampsia<sup>[29]</sup>. The results obtained from this study will be discussed according to the changes which occurred in the protein secondary structure and the alteration in lipid profiles as a consequence of endothelial dysfunction and oxidative stress which accompany the manifestation of preeclampsia.

A variety of substances indicative of endothelial dysfunction are increased in the blood or urine of women with preeclampsia<sup>[4,5]</sup>. Many of these substances are elevated weeks before (as well as during) clinically evident preeclampsia<sup>[1,6-7]</sup>.

It has been suggested that preeclampsia is a disease of antioxidant inadequacy appearing when the normal antioxidant balance is upset<sup>[8]</sup>. Oxidative damage by free radicals or reactive oxygen species (ROS) can result in lipid peroxidation and protein modification<sup>[30]</sup>, causing changes in membrane properties and cell dysfunction<sup>[31]</sup>. The highly reactive primary products of lipid peroxidation, lipid hydroperoxides, are formed when free radicals attack polyunsaturated fatty acids or cholesterol in membrane and lipoproteins. Lipid hydroperoxides function in normal physiology by regulating enzymes and redox-sensitive genes<sup>[32,33]</sup>. However, uncontrolled lipid peroxidation, such as that occurring in case of preeclampsia, can result in cellular dysfunction and damage<sup>[1,34,35]</sup>.

Such damage, known as oxidative stress, is normally prevented by an extensive and multilayered antioxidant system consisting of both low and high molecular weight components<sup>[30]</sup>.

Albumin is the most abundant serum protein. Among the variety of biological mechanisms which have been proposed to explain the beneficial effects of higher albumin concentrations. A direct protective effect of the albumin molecule and the inverse relationship between serum albumin level and mortality risk has been suggested<sup>[36-38]</sup>.

Albumin may represent the major and predominant circulating antioxidant in plasma, which is known to be exposed to continuous oxidative stress<sup>[39]</sup>. Alterations in the structure of albumin may result in impairments of its biological properties<sup>[40]</sup>. These modifications could occur in preeclamptic patients and insulin dependent diabetes mellitus patients, which are reasons of the pathological conditions associated with early occurrence of vascular complications, together with functional alterations of albumin.

On the bases of the forgoing consideration, one can explain the significant alteration in the amide I band intensities, together with the observed band shifts in the raw and second derivative infrared

spectra of serum samples for control-2 and patient groups compared to the control-1 group. These band shifts are associated with the changes in the conformational structure of proteins. Thus, a dramatic change in proteins secondary structure takes place which, in turn, impair the antioxidant effect of serum albumin and blood circulating antioxidant. These changes occurred earlier and were more pronounced in the women who later developed preeclampsia. Our results are also agreed with results obtained by Fraile et al.,<sup>[41]</sup>. They observed that the Amide II/ Amide I ratio is higher for pure albumin as compared with Albumin-lipid systems. Their results revealed that lipids destabilize albumin native structure.

IR spectroscopy support at least modification of secondary structure in albumin upon addition of lipids<sup>[41]</sup>. The variations in amide A/amide B ratio (the golden standard marker) can be used for diagnostic purposes to differentiate normal pregnancies from preeclamptic patients. For all patients and control-2 this ratio is always higher than 1.6. Moreover, it can be used to predict women who have the potential to develop preeclampsia starting from 20 week of gestation, since the results exhibited a similar behavior between the patient and control-2 group (group at high risk) compared with control-1 group. Meanwhile, the IR second derivative spectra are also capable of differentiating all groups under investigation.

Two out of three samples (samples 34, 35 and 39), who were at 20, 22 weeks of gestation, later developed preeclampsia.

The observed decrease in the intensities of protein bands in our results was also observed by Serge et al.,<sup>[42]</sup> when incubated bovine serum albumin with glucose, revealing structural modifications in the protein. Cohen et al.,<sup>[43]</sup> proved that glycated albumin increases oxidative stress. Our results are consistent with the above mentioned data. Thus, the decreased protein band intensities and the increased glucose band centered at 1033 cm<sup>-1</sup> were observed for all patient samples. Hence pregnancy is a state of physiological insulin resistance. This state reaches maximal in the third trimester, and is exacerbated in preeclampsia<sup>[44,45]</sup>.

The most outstanding results from this study is that by using Amide A/ Amide B ratio we could early predict preeclampsia, from the thirty one control subjects, in samples number 34, 35 and 39 two of whom were 20, 22 week of gestation. It has been accepted that preeclampsia affects only about 3-10 percent of the all pregnancies. In this study we demonstrated 9.6 % control subjects that had subsequently developed preeclampsia from

normotensive pregnant women. This ratio is more or less comparable with that reported in the literature.

There are scores of reports that lipid peroxidation products are increased in plasma/sera of women with preeclampsia<sup>[34, 35]</sup>. However, most lipid peroxidation assays have sensitivity and specificity problems<sup>[46, 47]</sup>. Morris et al., 1998<sup>[48]</sup> found no evidence that circulating lipid peroxidation products (8-iso-PGF<sub>2</sub>, lipid hydroperoxides, and malondialdehyde) are elevated in preeclampsia once appropriate precautions were taken, including addition of antioxidants, to prevent in vitro oxidation. These findings disagreed with our results, given that even preeclamptic women under medication their IR spectra gave the observed proatherogenic changes in lipid profile (esterified band), significant changes in the amide A/Amide B, RI & RII ratios.

Lipoprotein abnormalities may be involved in the pathogenesis of preeclampsia. Several studies have demonstrated proatherogenic lipid profiles in women months before clinical signs of preeclampsia<sup>[49]</sup>. Triglyceride levels are elevated, high density lipoprotein (HDL) levels tend to be lower, and small dense low-density lipoprotein (LDL) particles are higher in preeclampsia compared with normal pregnancies<sup>[50-53]</sup> this shift in LDL particle size to smaller and denser subfractions is thought to be particularly important, as these are highly susceptible to oxidation and may play a critical role in the endothelial dysfunction seen in preeclampsia<sup>[3, 50]</sup>. All of these proatherogenic changes in the lipid profile are also found in cardiovascular disease and diabetic subjects and represent those at high risk for coronary artery disease<sup>[54]</sup>.

The results obtained in this study support the previous work which suggests that there was a positive correlation between preeclampsia and lipid parameters, the significant increased triglycerides and decreased HDL-cholesterol during preeclampsia provide evidence of abnormal lipid metabolism<sup>[55]</sup>.

The present study has shown that the region of ester C=O stretch (1750-1700cm<sup>-1</sup>) can be used as a marker for characterizing triglycerides (TG) and cholesterols, which are the main components for VLDL and LDL, respectively. The C=O stretching bands for unsaturated TG and unsaturated Cholesterols exhibit a band at about 1746 and 1738 cm<sup>-1</sup>, respectively<sup>[56]</sup>.

The peaks at 1745(cholesterol and triglycerides ester C=O), 1710(carbonyl C-O stretch), and 1621cm<sup>-1</sup>(peptide C=O stretch) positively correlated with LDL oxidation<sup>[57]</sup>.

According to the above mentioned data, the significant shift and increase in the intensity of the band at 1744cm<sup>-1</sup> and the appearance of a weak band at 1712cm<sup>-1</sup> in patient second derivative spectra

compared to control-1& 2 can be attributed to the oxidation of LDL during preeclampsia.

## 5. Conclusion:

FTIR spectroscopy provide a new accurate method in recording changes of serum protein secondary structure and concentration during normal pregnancy and preeclampsia. It also permit the follow up of dyslipidemia that takes place in serum samples starting from 20 week of gestation. The novel golden standard biophysical marker is the amide A/ amide B ratio obtained from the original spectrum and it can give us a screening test in diagnosis and prediction of women who are at great risk to develop preeclampsia. The results obtained from the IR measured ratio Amide A/Amide B of serum revealed, with 92.9 % confidence limits, the possibility of accurate diagnosis of preeclampsia, as well as 100% differentiation between the control-1 group and the risk (control-2) group. These results are to be evaluated taking into consideration the accuracy level of the highest useful method currently used for prediction and diagnosis of preeclampsia (Doppler ultrasonography), which ranges from 20-60 %, with positive predictive value of 6-40%.

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## References

- [1] Carl A, Oxidative stress in the Pathogenesis of preeclampsia. 1999; 222-235
- [2] Francois A, Screening for pre-eclampsia: the quest for the holy grail? *The Lancet* 2005; 365(9468) : 1367-1369.
- [3] Villar J, Say L, Shennan A, Lindheimer M, Duley L, Conde-Agudelo A, Merialdi M, Methodological and technical issues related to the diagnosis, screening, prevention, and treatment of preeclampsia and eclampsia. *Int. J. Gynecol. Obstet.* 85 Suppl.1 2004; S28-S41.
- [4] Taylor R.N, Roberts JM, Endothelial cell dysfunction. In: Lindheimer MD., Roberts JM, Cunningham FG, EGs. *Chesley's Hypertensive Disorders in Pregnancy* (2<sup>nd</sup> ed.) Stamford, CT: Appleton & Lange, pp395-429, 1999.

- [5] Roberts J, Endothelial dysfunction in preeclampsia. *Sem Report Endocrinol.* 1998;16: 5-15.
- [6] Krauss T, Juhn W, Lakoma C, Augustin HG, Circulating endothelial cell adhesion molecules as diagnostic markers for the early identification of pregnant women at risk for development of preeclampsia. *Am. J. Obstet. Gynecol.* 1997; 177: 443-449.
- [7] Taylor RN, Crombleholme WR, Friedman SA, Jones LA., Casal DC, Roberts JM, High plasma cellular fibronectin levels correlate with biochemical and clinical features of preeclampsia but cannot be attributed to hypertension alone. *Am. J. Obstet. Gynecol.* 1991; 165: 895-901.
- [8] Stark J, Preeclampsia and cytokine induced oxidative stress. *Br. J. Obstet. Gynecol.* 1993; 100: 105-9.
- [9] Carmona P, Rodriguez-Casado A, Alvarez I, de Miguel E, Toledano A, FTIR microspectroscopic analysis of the effect of certain drugs on oxidative stress and brain structure. *Biopolymer* 2008;89:548-554.
- [10] Dumas P and Miller J, The use of synchrotron infrared microspectroscopy in biological and biomedical investigations. *Vib. Spec.* 2003;32:3-21.
- [11] Griffiths PR, and de Haseth JA, Fourier transform infrared spectrometry. John Wiley and Sons, New York, 45, 1986.
- [12] Jakson M and Mantsch HH, In Mantsch H.H., Chapman D. (eds.), *Infrared spectroscopy of biomolecules*, Wiley-Liss, Toronto, 311, 1996.
- [13] Baker MJ, Gazi E, Brown MD, Shanks JH, Gardner P, and Clarke NW, FTIR-based spectroscopic analysis in the identification of clinically aggressive prostate cancer. *British J. Cancer* 2008; 99: 1859-1866.
- [14] Paul GL, Robert DS, Cancer grading by Fourier transform infrared spectroscopy. 1998;4: 37-46.
- [15] Jackson M, Sowa MG, Mantsch HH, Infrared spectroscopy: a new frontier in medicine. *Biophys. Chem.* 1997; 68: 109-125.
- [16] Diem M, Boydston-White S, & Chiriboga L, Infrared spectroscopy of cells and tissues: shining light on a novel subject. *Appl. Spectrosc.* 1999; 53: 148A-161A.
- [17] Cuningham FG, Gant NF, Leveno KJ, Gilstrap III LC, Hauth JC, Wenstrom KD, Williams Obstetrics. 21 st Ed. McGraw-Hill 2001; 568-9.
- [18] Wong PT, Wong RK, Caputo TA, Godwin TA, Rigas B, Infrared spectroscopy of exfoliated human cervical cells: evidence of extensive structural changes during carcinogenesis. *Proc. Natl. Acad. Sci. USA* 88, 10988-10992 (1991).
- [19] Liu K-Z, Li Jia, Kelsey SM, Newland AC, Mantsch HH, Quantitative determination of apoptosis on leukemia cells by infrared spectroscopy. *Apoptosis* 2001;6: 269-278.
- [20] Mantsh HH, Apoptosis-induced structural changes in leukemia cells identified by IR spectroscopy J. *Mol. Str.* 2001; 565-566: 299-304.
- [21] Deleris G, Petibios C, Application of FT-IR spectrometry to plasma contents analysis and monitoring. *Vib. Spectrosc.* 2003; 32: 129.
- [22] Cakmak G, Togan I, & Severcan F. 17 -Estradiol induced compositional, structural and functional changes in rainbow trout liver, revealed by FT-IR spectroscopy: A comparative study with nonylphenol. *Aquatic Toxicology* 2006; 77:53-63.
- [23] Jackson M, Ramjiawan B, Hewko M, Mantsch H, Infrared microscopic functional group mapping and spectral clustering analysis of hypercholesterolemic rabbit liver, *Cell. Mol. Biol.* 1998;44: 89-98.
- [24] Toyran N, Lasch P, Naumann D, Turan B, & Severcan F. Early alterations in myocardia and vessels of the diabetic rat heart: An FTIR spectroscopic study. *Biochem. J.* 2006; 397:427-436.
- [25] Toyran N, Zorlu F, Donmez G, Oge K, & Severcan F. Chronic hypoperfusion alters the content and structure of proteins and lipids of rat brain homogenates: A Fourier transform infrared spectroscopy study. *Eur. Biophys. J. Biophys. Let.* 2004; 33: 549-554.
- [26] Garidel P. Mid-FTIR-microspectroscopy of stratum corneum single cells and stratum corneum tissue. *Physic. Chem. Chem. Phys.* 2002; 4:5671-5677.
- [27] Hendlar RW, Barnett SM, Dracheva S, Bose S, & Levin IW. Purple membrane lipid control of bacteriorhodopsin conformational flexibility and photocycle activity-An infrared spectroscopic study. *Eur. J. Biochem.* 2003; 270:1920-1925.
- [28] Mendelsohn R, & Mantsch HH. Fourier transform infrared studies of lipid-protein interaction. In A. Watts & J.J. H. H. M. De Pont (Eds). *Progress in protein-lipid interactions* (vol. 2. pp. 103-147). Amsterdam: Elsevier Science Publishers.
- [29] Conde- Agudelo, Agustín MD, MPH; Villar, Jase MD, MPH, Lindheimer, Mushal MD. World Health Organization Systematic Review of Screening Tests for Preeclampsia. *Obstet. Gynecol.* 2004; 104(6): 1367-1391.
- [30] Sinclair A, Barnett A, Lunec J, Free radicals and antioxidant systems in health and disease. *Br. J. Hosp. Med.* 1990; 43: 334-344.
- [31] Corinne M, John R, Ewen W, Rhoda W, James J, James Mc, Erythrocyte glutathione balance and membrane stability during preeclampsia. *Free Radical Biol. Med.* (1998); 24(6): 1049-1055.
- [32] Smith WL, Marnett LJ, DeWitt DL, Prostaglandin and thromboxane biosynthesis. *Pharmacol Ther.* 1991; 49: 153-179.
- [33] Sen CK, Packer L, Antioxidant and redox regulation of gene transcription. *FASEB J* 1996;10: 709-720.



- [34] Hubel CA, Roberts JM, Taylor RN, Musci TJ, Rogers GM, McLaughlin MK, Lipid peroxidation in pregnancy: New perspectives on preeclampsia. *Am. J. Obstet. Gynecol.* 1989; 161:1025-1034.
- [35] Walsh SW, Maternal-placental interactions of oxidative stress and antioxidants in preeclampsia. *Sem Reprod Endocrinol.* 1998; 16: 93-104.
- [36] Goldwasser P, Feldman J, Association of serum albumin and mortality risk, *J. Clin. Epidemiol.* 1997; 50: 693-703.
- [37] Philips A, Shaper A, Whincup P, Serum proteins and mortality, *Lancet* 1990; 335: 858.
- [38] Halliwell B, How to characterize a biological antioxidant, *Free Radic. Res. Commun.* 1990; 9: 1-32.
- [39] Soriani M, Pietraforte D, Minetti M, Antioxidant potential of anaerobic human plasma: role of serum albumin and thiols as scavengers of carbon radicals, *Arch. Biochem. Biophys.* 1994;312: 180-188.
- [40] Terawaki H, Yoshimura K, Hasegawa T, Matsuyama Y, Negawa T, Yamada K, Matsushima M et al, Oxidative stress is enhanced in correlation with renal dysfunction: examination with the redox state of albumin, *Kidney Int.* 2004;66: 1988-1993.
- [41] Fraile M, Blanco-Melgar, Martinez R, Lopse G, Gallego J, Carmona P, Structure and interactions of albumin- Lipid Systems as studied by infrared spectroscopy. *J. M, Strac.* 2003; 651-653: 231-236.
- [42] Serge C, Philippe R, Sergio A, Emmanuel B, Effect of Oxidative modifications induced by the glycation of bovine serum albumin on its structure and on cultured adipose cells. *Biochimie.* 2006; 88(10): 1467-1477.
- [43] Cohen M, Elizabeth S, Sheldon C, Clyde W, Glycated albumin increases oxidative stress, activates NF-kB and extracellular regulated kinase (ERK), and stimulates ERK-dependent transforming growth factor-1 production in macrophage RAW cells. *J. Lab. Clin. Med.* 2003; 141(4): 242.
- [44] Catalano PM, et al. Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. *AM J Obstet Gynecol.* 1999;180: 903-16.
- [45] Kuhl C, Etiology and pathogenesis of gestational diabetes. *Diabetes Care* 1998; 21(Suppl 2): B19-26.
- [46] Payor WA, Godber SS, Noninvasive measure of oxidative stress status in humans. *Free Radic. Biol. Med.* 1991; 10: 177-184.
- [47] Puhl H, Waeg G., Esterbauer H, Methods to determine oxidation of low-density lipoproteins In: Packer L, Ed. *Methods in Enzymology.* San Diego: Academic Press, pp425-441, 1994.
- [48] Morris JM, Gopaul NK, Endresen MJ, Knight M, Linton EA, Dhir S, Anggard EE, Redman CW, Circulating markers of oxidative stress are raised in normal pregnancy and preeclampsia. *Br. J. Obstet. Gynaecol.* 1998; 105:1195-1199.
- [49] Arthur M, Richard L, Kevin L, Sina H, and Boggess K, Maternal serum dyslipidemia occurs early in pregnancy in women with mild but not severe preeclampsia. *Am. J. Obstet. Gynecol.* 2009 ;201:293.
- [50] Sattar N, Bedomir A, Berry C, Shepherd J, Greer IA, Packard C, Lipoprotein subfraction concentrations in preeclampsia: pathogenic parallels to atherosclerosis. *Obstet. Gynecol.* 1997; 89: 403-8.
- [51] Lorentzen B, Endresen M, Clausen T, Henriksen T, Fasting serum free fatty acids and triglycerides are increased before 20 weeks of gestation in women who later develop preeclampsia. *Hypertens Pregnancy* 1994; 13: 103-9.
- [52] Chappell L, Seed P, Briley A, et al., A longitudinal study of biochemical variables in women at risk of preeclampsia. *Am. J. Obstet. Gynecol.* 2002; 187:127-36.
- [53] Gratacos E, Casals E, Sanllehy C, Cararach V, Alonso P, Fortuny A, Variation in lipid levels during pregnancy in women with different types of hypertension. *Acta Obstet. Gynecol. Scand.* 1996; 75: 896-901.
- [54] Carmena R, Duriez P, Fruchart J, Atherogenic lipoprotein particles in atherosclerosis. *Circulation* 109 (suppl 1) 1112-1117. View Record in Scopus Cited By in Scopus (87).
- [55] Rubina A, Tabassum M, Pre-eclampsia and lipid profile. *Pak J. Med Sci.* 2007; 23(5): 751-754.
- [56] Masayuki N, Mitsuyo O, Hiroyuki K, Infrared study of human serum very low-density and low-density lipoproteins. Implication of esterified lipid C=O stretching bands for characterizing lipoproteins. *Chem. Phys. Lipids* 2002; 117(1-2): 1-6.
- [57] Henry SL, Andrew P, John N, Manford D, Grady W, Quantitative determination of low density lipoprotein oxidation by FTIR and chemometric analysis. *Lipids* 2004; 39(7): 687.

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