CONTENTS

Changes in psychological states of caregivers of patients with moderate or severe Alzheimer's disease following Memantine therapy

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Absract: To assess the psychological states of caregiver of patients with moderate or severe Alzheimer's disease and to explore the effects of memantine therapy for patients on them，40 paitients with moderate or severe Alzheimer's disease and their caregivers were recruited. Patients were treated for 6 months with open-label memantine. Caregivers were assessed at baseline and month 6. Their psychological states were assessed by Symptom Checklist 90，Self-Rating Anxiety Scale (SAS) and Self-rating depression scale(SDS). Difference of their psychological states between different time points and normal Chinese scale were analysed by T-test..Results showes that there are significant difference in depression，anxiety，hostility，paranoia and total SCL-90 scale between baseline and month 6 (all $p<0.05$).When compared to normal SCL-90 scale，there are significant difference in all of items except compulsion，phobophobia and psychosis at baseline wherever no significant difference in all of items between month 6 and normal scale. There are significant difference in SDS and SAS scale of caregivers between baseline，month 6 and Chinese normal scale. In a word,caregivers of patients with moderate to severe Alzheimer's disease may have worse psychological states than normal population and memantine therapy for AD paitents maybe alleviate these problems.

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Key Words Alzheimer's disease; caregiver; psychological states;memantine

1.Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive and functional impairment, and behavioural and psychological symptoms of dementia (BPSD). While BPSD are highly prevalent at all stages of dementia, they are particularly common in severe dementia, with 90% of individuals with severe disease exhibiting one behaviour and 50% having at least four[1]. AD caregivers are often subject to enormous stressors and are at high risk for depression with nearly half of caregivers in some studies meeting formal diagnostic criteria for depression[2]. Caregivers also show increased utilization of health services and psychotropic medications, and one study reported that caregivers who reported distress were 63% more likely than noncaregivers to die within 4 years. Thus, adverse effects of care giving seem to be especially pronounced among dementia caregivers, even after controlling for intensity of care giving involvement[3].

The current study examined the effectiveness of memantine on psychological problems of caregivers of patients with moderate or severe Alzheimer's disease. The objective is to confirm the effectiveness of memantine in the real world setting and to determine whether treatment with memantine would be associated with decreases in nursing burden, caregiver distress and use of as required medications.

2.Subjects and methods

All AD patients come from consecutive AD patients of neurological outpatient clinics and ward from Septmber 2010 to September 2011. Each subject underwent a comprehensive diagnostic screening assessment including physical and psychiatric examinations, as well as a review of his/her medical history. All patients were residing in Zhengzhou, met NINCDS-ADRDA criteria[4] for probable Alzheimer's disease, had moderate to severe AD
as demonstrated by a score of 0–15 on the Mini-Mental State Examination (MMSE). Their caregivers who interested in and agreed to the study were recruited.

Patients meeting entrance criteria were treated for 6 months with open-label memantine. The memantine (Ebixa_) used in this study was provided by Lundbeck Denmark, Inc.. The memantine dose was started at 5mg daily for 1 week and titrated by 5mg/week to 10 mg twice daily for the following weeks. Caregivers were assessed two times: at baseline and month 6. Psychological states were assessed used: ①Symptom Checklist□ 90（SCL-90）[5]. which assesses 10 behaviours occurring in caregivers: somzatization，compulsion，interpersonal

relation，depression，anxiety，hostility，phobophobia，paranoia and psychosis; ②Self-Rating Anxiety Scale (SAS) [5].;③Self-rating depression scale(SDS) [5].

All datas were showed as mean±SD and difference between baseline,month6 and Chinese normal scale were analysed by T-test with SPSS 14.0 software. The p value less than 0.05 was considered to be significantly different.

3.Results

40 AD patients and their caregivers who met the including criteria were enrolled. table 1 summarized their sociodemographic characteristics. The mean age of patients is 73.30±7.54 years and of caregivers is 61.55±5.77 years. Percentage of male in patients is 40.25% whereas in caregivers is 55.00%. For education level,most of patients are less-educted and most of caregivers are moderate-educated. With regard to relation with patients，62.5% of caregivers is spouse of patients and 20% is children of patients.

Table 2 shows the difference in caregivers'SCL-90 scales between baseline and month 6,normal scale. There are significant difference in depression，anxiety，hostility，paranoia and total SCL-90 scale between baseline and month 6. When compared to Chinese normal scale，there are significant difference in all of items except compulsion，phobophobia and psychosis at baseline. There is no significant difference in all of items between month 6 and normal scale.

Table 3 shows the of SAS，SDS scale of caregivers between baseline，month 6 and Chinese normal scale. There are significant difference in depression，anxiety scale of caregivers between baseline，month 6 and Chinese normal scale.

Table 1.Sociodemographic characteristics of AD patients and caregivers

Items	AD patients (n=40)	AD caregivers(n=40)
Age (years)	73.30±7.54	61.55±5.77
Gender		
Male	17 (40.25%)	22 (55.00%)
Education（years）		
≤6	19(47.50%)	13(32.5%)
6~12	12（30.00%）	21(52.5%)
＞12	9 (22.50%)	6（15.00%）
Relation with patients		
Spouse		25 （62.5%）
Sibling		3 （7.5%）
Children		8 （20.0%）
Others		4 （10%）

Table2. Comparison of SCL-90 scale of caregivers between baseline，month 6 and Chinese normal scale

Items	AD caregivers(n=40)				
	baseline	month 6	normal model(n=1388)	t 1	t2
somzatization	1.38±0.58	1.33±0.25	1.37±0.48	-	2.01[b]
compulsion	1.63±0.70	1.61±0.69	1.62±0.58	-	-
interpersonal relation	1.68±0.32	1.64±0.25	1.65±0.51	-	2.03[b]
depression	1.79±0.56	1.53±0.61	1.50±0.59	2.68[a]	2.72[a]
anxiety	1.56±0.24	1.38±0.28	1.39±0.43	2.67[a]	2.65[a]
hostility	1.55±0.39	1.49±0.57	1.48±0.56	2.65[a]	2.05[b]
phobophobia	1.26±0.48	1.24±0.42	1.23±0.41	-	-
paranoia	1.49±0.45	1.42±0.49	1.43±0.57	2.66[a]	2.64[a]
psychosis	1.30±0.48	1.28±0.42	1.29±0.42	-	-
total	138.02±34.12	130.11±30.46	129.96±38.76	2.70[a]	2.69[a]

Note: t1—baseline VS month 6; t2—bseline VS normal model. a—p＜0.05 b-- p＜0.01

Table3. Comparison of SAS，SDS scale of caregivers between baseline，month 6 and Chinese normal scale

Items	AD caregivers(n=40)		normal model(n=1388)	t1	t2
	baseline	month6			
SAS	31.57±5.62	29.12±3.03	29.78±0.46	3.13[a]	5.30[a]
SDS	36.30±6.48	34.14±5.67	33.46±8.55	4.03[a]	4.36[a]

Note : t1—baseline VS month 6;t2—bseline VS normal model. a—p＜0.01

4. Discussion

The current study indicated that caregivers of patients with moderate or severe Alzheimer's disease had worse psychological states than normal population. These problems are: depression，anxiety，hostility and paranoia. Psychiatric and behavioral symptoms occur in the majority of patients with AD over the course of the illness50–52 especially in moderate or severe states， with symptoms of depression among 20%– 50%; patients with AD; agitated or aggressive behaviors appearing in 70%; and delusions or hallucinations in as many as 30%–50% [6].

In fact, depression and psychosis are included as descriptors in Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, criteria for AD. One recent study have shown that the direct influence of patients' cognition on caregiver burden is limited and rather mediated by other disease indicators. Both ADL-abilities and behavioral disturbances are important predictors of perceived caregiver burden, where the latter has the strongest effect [7].Another study[8] indicated that more severe psychiatric and behavioral problems, along with decreased quality of life were all significantly associated with higher levels of burden, and depression among caregivers. The most frequently used pharmacologicaln treatment for Psychiatric and behavioral symptoms is antipsychotics, particularly atypical antipsychotics. Although their use has been supported by evidence from randomized controlled trial (RCT) data, there remain concerns about potential side effects, such as cerebrovascular adverse events,extrapyramidal side effects and metabolic effects[9].

The results of this study showed that memantine can alleviate the worse psychological states of caregivers of patients with moderate or severe AD. The best-studied treatment for moderate to severe AD is the non-competitive NMDA receptor antagonist memantine, which has been shown to be efficacious in RCTs. With regard to its effect on BPSD, a pooled analysis of the effect of memantine treatment in three large 6-month RCTs in moderate to severe AD patients with agitation and aggression or psychosisn showed an advantage for memantine over placebo on the Neuropsychiatric Inventory (NPI) agitation/aggression subscale at week 12 and weeks 24/28[10]. The decreased agitated and aggressive behaviour in institutionalized patients with moderate to severeAD following treatment with memantine was accompanied by improvements in nursing burden and decreased psychotropic use[11].

In a conclusion， this study indicated that caregivers of patients with moderate to severe Alzheimer's disease may have worse psychological states than normal population and memantine therapy for AD paitents maybe alleviate these problems. This conclusion need much more studies to verificated.

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5/2/12

A survey of quality of life and its associated factors in community-living older adults in Qiqihar

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Abstracte: Study the quality of life among the old of empty nest and non empty nest in Qiqihar, and analyze of its influence factors. Method: The investigation objects are divided into two groups, which are at least as old as 60 years old from 6 communities. One has three hundred old people of empty nest, so does the other three hundred with non empty nest. All above were surveyed with self-made questionnaire. Result: There are little differences between cultural level, occupation and medical payment(average $P>0.05$),while great differences between marital status, chronic disease, mental health and somatic pain(average $P<0.05$). Conclusion: The help and support from the whole society to the old of empty nest is very important.
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【Key words】: quality of life; associated factors; community-living

"Empty nest family" means that the family has no children or if have children, but they have grown up to leave the family, leading to the old live alone. With the rising and the aging society of family structure and the core of miniaturization, the number of Chinese empty nest family is increasing [1]. At present, our country has more than 23.4 million old man of empty nest, in the city, the number of empty nest family is at least more than 30%, and nearly a third of the old have no children living together. Social supports are defined as one person get influence with social ties, this kind of influence can help him decrease psychological stress response, relieve mental tension and improve social adaptation ability [2]. This study focus on the old more than 60 people's quality of life, and its impact factors, thereby provide better basic information and evidence for the relevant departments to make relevant policy.

1. Objects and Methods

1.1 Objects

Empty and nonempty nest families in six Qiqihar communities were selected from November to December in 2010, of which the old person was study, using the cluster sampling method. Every one researched in the investigation have no mental illness and memory, no intelligent damage, and voluntary to answer each content during the survey. 630 old men were chosen, including the old in empty nest 300, and in nonempty nest 330.

Requirement: be in empty nest, clear consciousness, normal language ability and have no communication obstacle with the researcher. Willing to cooperate with this study. Age ruled from 60 to 90 years old.

1.2 Method

Use the self-designed questionnaire to design by questionnaires. Put out 670 questionnaires, and take back 630. The effective recover is 94%.

1.3 Statistical Analysis

Apply SPSS10.0 statistical package to analysis. X^2 test was adopted to statistical analysis, and $P<0.05$ has statistic meaning.

2. Results

Table 1: General material comparison of the old from empty and nonempty nest

group	marrage status		occupation		Entertainment activities		Medical payment	
	Yes	No	Yes	No	Yes	No	pay oneself	Medical insurance
In empty nest	140	160	200	100	130	170	63	237
In nonempty nest	200	130	240	90	210	120	55	245
X^2	12.29		2.74		26.07		0.78	
P	<0.05		> 0.05		<0.05		> 0.05	

Table 2: The comparison of factors which influence the old quality of life

Group	Life Enrichment		Negative emotional experience		Chronic disease		Mental health		Body pain	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
In empty nest	130	170	110	190	219	81	137	163	125	175
In nonempty nest	210	120	76	254	181	149	219	111	200	130
X^2	26.07		14.04		22.34		27.39		22.56	
P	<0.05		<0.05		<0.05		<0.05		<0.05	

3. Discussion

3.1 Analyze of the solitary aged who are the quality of life and related element

In this study, there are no remarkable difference in respondents' cultural level, profession and the style of paying a bill for the medical care (those condition $p>0.05$), while there are remarkable difference in marriage condition, chronic disease, emotional health and physical pain (those condition $p<0.05$).

This survey shows that the happiness level and the standard of a full life that divoce or losing spouse'solitary aged have encountered are lower than the aged who are no solitary; It is an important factor affecting the aged psychological health that they experience the stimulation of negative life events (such as losing spouse, children having serious disease etc), and there are highly remarkable positive correlation between the amount of stimulation about negative life events and the aged' depression, anxiety, hostility and terror. Generally speaking, the aged who experience unfortunate marriage (having no spouse) or living alone or too many negative life events can easily become a solitary aged. the solitary aged' daily life depend on taking care of theirselves, feeling alone and helpless in their heart ,and they will appear need satisfaction's problems as soon as they meet precipitant events or crisis in their lives, so the aged who live alone are obviously lower than the aged who live with their children or spouse in respect of the medical care of psychological disease, social function and taking care of daily life, the probability of chronic disease which includes senile inspirital disease and psychological disease (as senile demential, senile depression) that the solitary aged suffer is higher than the aged who is no solitary[3-4].

3.2 Intensify social support to the solitary aged

The solitary aged have a high expectation to improve the quality of their life, and expect to obtain support or aid about mentality and substance from other people and all respect of society. Especiality their children and relations' support are very important, so children should give the aged mental support besides necessary substance.

In addition ,the service innovation in community that the aged spend the rest of their life at home should be continually improved ,and the service innovation (including special medical hygine for the aged, recovery care, recreation and sports of entertainment, information's consultation and senior education etc)should be developed and vigorously promoted, and constracting the survice net in community for the aged, setting up the young volunteer service for the aged, calling on the aged regularly. Encouraging female and the solitary aged who their cultural level is lower to contact society, increasing the chance that the aged contact the society, increasing more entertainment activities, and applying actively the social support.

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Relationships among the occurrence of Obsessive-compulsive Disorder 0f College Students with Personal Coping Style,Family Environment and parental rearing patterns

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Objective To explore the relationships among the occurrence of obsessive-compulsive disorder of college Students with personal coping style,family environment and parental rearing patterns. **Methods** The questionnaires survey was carried out among 94 college studengs with obsessive-compulsive disorder and 102 controls without mental disorder.Using the Trait Coping Style Questionnaire,Family Environment Scale-Chinese Version （FES-CV） and the Egma Minnen av Bardndosnauppforsffan （EMBU） of Chinese Version. **Results** The scores of case group lower than those of controls in positive coping style, intimacy, emotional expression, entertainment, parents emotional warmth and understanding（all $P<$ 0.01），but higher than those of normal controls in contradictions,the father's punishment and strictness（P＜0.01）.Differences were significant. Logistic regression analysis showed that the contradictions of the family environment (OR=1.424)is a risk factor for college students with obsessive-compulsive disorder,positive coping style (OR = 0.672), intimacy (OR=0.601) and emotional expression (OR = 0.608) are protective factors. **Conclusion** Positive coping style, the intimacy of family members,emotional expression and contradictions are impact factors of college students with obsessive-compulsive disorder.
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Key words: College students；Obsessive-compulsive disorder；Coping style；Family environment；parental rearing patterns；Case-control study

OCD (obsessive-compulsive disorder, OCD) is a kind of neurological disease, with forced impulsive, compulsive behavior, obsessive-compulsive symptoms as its main symptoms[1]. It is characterized by forced impulsive and anti-impulsive which cause tremendous anxiety and painfulness for the patients and can not be controlled by themselves. In the United States, OCD is the fourth most common psychiatric disorders, following phobias, substance abuse and severe depression[2]. The prevalence rate of OCD in the general population is 1% to 3% [3], mainly in the 20 to 24 years old, college students are at high risk of OCD [4].The occurrence of OCD may be related to the patient's personality and family environment, but the specific psychological, physiological mechanism is unclear [5]. In this study, several influencing factors such as personal coping style, family environment and parental rearing patterns were investigated to explore the occurrence of OCD, in order to provide guidance in the prevention and treatment of OCD among college students.

METHODS

Patient enrollment

The case was recruited from the outpatients diagnosed with OCD in two psychiatric hospitals of Henan Province, during the period of September 2009 - July 2011, who were university students with nationality of Han, the first onset of the disease, without systemic therapy. Diagnosis confirmed by two chief physician, in line with the Chinese classification of mental disorders diagnostic criteria (CCMD-3) diagnostic criteria for OCD [6]. Physiological diseases caused by obsessive-compulsive symptoms were rule out, schizophrenia and affective disorder and other serious mental illness. 94 eligible patients were recruited, 51 males and 43 females, aged 18 to 23 years,with mean age 19.2 ± 2.3 years old. 102 Control were recruited from Zhengzhou University, 57 males, 45 females, with the mean age of 19.7 ± 1.7 years old. Gender, age and other family information (parents' education level, occupation, residence, etc.) between the two groups showed no statistically significant differences (all $P>$ 0.05).

Case and control groups in the study are willing to sign an informed consent based on understanding the content and meaning of this study.

Research tools

1.Self-made sociological questionnaire of the population including gender, age, parents' educational level, occupation, residence, family income and other general information.

2.Trait Coping Style Questionnaire [7] The questionnaire was prepared by the Jiang qianjin, a total of 20 questions, divided into positive response (PC) and negative response (NC), each including 10 items, from "definitely not" to "definitely "from 1 to 5 points. The Cologne Bach coefficient of positive response (PC) and negative response (NC)were 0.70 and 0.69 respectively, test-retest coefficients were 0.65 and 0.75.

3.Family Environment Scale-Chinese Version (FES-CV) Family Environment Scale (FES) is developed by Moss et al in 1981, translated into Chinese and revised edition in 1991 by Fei Lipeng, revised in 1999, along with the establishment of the Chinese norm, has good reliability and validity. Scale used in this study is the third revision of the Chinese version[7]. Including 90 true-false, 10 subscales, including intimacy, emotional expression, contradiction, independence, success, knowledge, entertainment, moral religion, organization and control, etc., were 10 different evaluation family environment characteristics. Subscales reflect the situation of family environment, the higher the score is, more prominent the factors of family environment.

4.Parenting Style Scale (Egma Minnen av Bardndosnauppforsffan, EMBU)-Chinese version of the EMBU was jointly developed by C. Perris et al in the Department of Psychiatry, Umea University, Sweden in 1980, is used to evaluate the parents' educational attitudes and behavior, the questionnaire used in this study is the Chinese version of these amendments [7] developed by Yue Dongmei. The father parenting is divided into six factors, namely: emotional warmth, understanding (F1), punishment, strictness (F2), excessive interference (F3), preference (F4), refusal, denied (F5), over-protection (F6).Mother parenting styles are divided into five factors, namely: emotional warmth, understanding (M1), excessive interference and protection (M2), refused, denied (M3), punishment, strictness (M4), preference (M5). Subjects were asked for assessing their bring-up styles in early childhood with the scale of never, occasionally, often, always. The higher the factor score is, the more prominent the parenting style that the factor represents is.

Measurement methods

Survey was conducted by trained investigators with same guidance, the respondents truthfully and independently answer all the items in the questionnaire. For those items difficult to understand, specific guidance was provided by trained investigator, without any hint on how to answer the items. It take about 30 minutes to finish the questionnaire, all the questionnaires were collected on the spot.

Statistical analysis

Data and analysis was conducted with SPSS13.0 analysis software, *t* test, χ^2 test and Logistic regression analysis were utilized.

RESULTS

1.The demographic characteristics of the participants

Baseline characteristics were balanced in terms of sex(χ^2=0.01),age *(t* = 0.2), level of education of parents (χ^2=5.4) ,parental occupation (χ^2=3.1) ,residence (χ^2=1.2,*P*> 0.05) .

2.Scores of Trait Coping Style questionnaire between the Case and control group

Table 1 comparison of the coping style between two groups ($\bar{x}$±s)

Subscale	Case group（*n*=94）	control group（*n*=102）	*t*	*P*
Positive response（PC）	31.4±6.7	39.5±5.8	-3.241	＜0.01
Negative response（NC）	32.1±5.3	29.4±6.1	1.817	0.22

Table 1 shows the positive response scores in the case group was higher than that of the control group, the difference was statistically significant; the negative response scores in the case group was lower than that of the control group, the difference was not statistically significant.

3. Family Environment Scale scores between the case and control groups

Table 2 comparison of the family environment between two groups ($\bar{x}$±s)

Subscale	Case group （*n*=94）	The control group（*n*=102）	*t*	*P*
Intimacy	4.73±1.24	7.71±1.44	-5.314	＜0.001
Emotional expression	4.38±1.02	6.10±1.31	-2.743	＜0.001
Contradictions	5.36±1.22	3.44±1.05	3.211	＜0.001
Independence	5.77±1.32	5.61±1.04	0.462	0.755
Success	6.62±1.75	6.73±2.35	-2.413	0.914
Knowledge	5.69±2.01	5.52±2.19	1.504	0.638
Entertainment	3.15±2.16	5.56±2.07	-4.374	0.002
Moral religion	4.89±1.73	5.11±1.92	-2.352	0.089
Organization	5.94±1.32	6.12±2.13	-2.221	0.542
Control	4.11±1.66	3.93±1.25	0.109	0.511

Table 2 shows the intimacy, emotional expression and entertaining score in the case group were lower than that of the control group, the difference was statistically significant; the contradictions of the case group score higher than the control group, the difference was statistically significant; the score difference in terms of the remaining subscale were not statistically significant.

4. parenting style scale scores between the case and control groups

Table 3 comparison of parenting between two groups ($\bar{x}$±s)

Project	Subscale	Case group （*n*=94）	The control group （*n*=102）	*t*	*P*
F1	Emotional warmth, understanding	42.5±7.1	48.9±8.2	-3.724	0.014
F2	Punishment, strictness	19.6±4.0	16.4±4.9	2.655	0.013
F3	Excessive interference	21.8±4.3	18.4±3.7	-0.721	0.201
F4	Preference	9.0±3.2	8.6±2.7	0.880	0.504
F5	Refusal, denied	10.9±2.3	8.0±2.2	-2.314	0.185
F6	Over-protection	11.5±2.3	10.4±2.4	0.913	0.352
M1	Emotional warmth, understanding	44.5±8.1	51.6±9.0	-4.272	0.016
M2	Excessive interference and protection	35.2±4.1	33.1±5.9	-0.723	0.096
M3	Refusal, denied	14.1±3.8	12.5±3.4	-3.144	0.173
M4	Punishment, strictness	12.2±3.2	11.2±3.7	0.564	0.223
M5	Preference	9.4±4.1	8.3±3.2	0.175	0.344

Table 3 shows the score of the parents' emotional warmth, understanding were lower than that of the control group, the score of the father's punishment, strictness subscale was higher, these differences were statistically significant, and the difference in terms of the remaining subscale was not statistically significant.

5. Factor analysis of obsessive-compulsive disorder

When OCD was used as the dependent variable, with demographic characteristics of college students, coping style, family environment and parental rearing patterns as the independent variables, chi-square test was conducted for univariate analysis, showing the difference of F1 (χ^2=13.1 ） ,F2 (χ^2=10.3 ） ,M1 （ χ^2=10.8),PC （ χ^2=58.2), Intimacy (χ^2=10.3 ） , Emotional expression (χ^2=12.1 ） ,Contradiction (χ^2=21.7 ） ,Entertainment (χ^2=15.4) between two groups were statistically significant *(P* <0.05). We take the statistically significant factors as independent variables and a further Logistic regression was conducted, we found that positive response (PC), intimacy, emotional expression and contradiction are four main factors determining whether OCD was present, with the contradiction of family environment a risk factor for OCD (OR = 1.424),and positive response (OR = 0.672), family intimacy (OR = 0.601) and emotional expression (OR = 0.608) protective factors for OCD. Regression results were showed in Table 4:

Table 4 non-conditional Logistic regression of influencing factors of OCD

Factor	β	SE	χ^2	*P*	OR	OR 95% CI
Positive response	-0.481	0.174	6.144	0.009	0.672	0.425～0.890
Intimacy	-0.452	0.171	6.215	0.008	0.601	0.319～0.866
Emotional expression	-0.267	0.125	4.832	0.031	0.608	0.488～0.924
Contradiction	0.616	0.162	6.012	0.014	1.424	1.179～1.984

DISCUSSION

OCD is a highly heterogeneous mental disorders[8] with complex causes, the results of this study showed that college students who had active coping styles are less likely to suffer from OCD risk than those with passive coping styles. This indicates that college students, when encountered with difficulties, can resolve their conflicts and anxieties if they can resort to reasonable assistance and underplay their problems, therefore protect themselves. If, instead, they use such negative emotional coping styles as fantasy, self-blame, avoidance , they may make intensify their conflict with the outside world, which can contribute to the occurrence of obsessive-compulsive symptoms. This results are similar to that of Libin et al [9] who found in their study that patients with obsessive-compulsive disorder are more likely to use immature coping styles rather than the mature coping mechanisms such as sublimation, humor, to deal with their problems.

Individual's mental health and family environment are closely correlated [10]. In the family environment factors, this study showed college students from familys with high intimacy, good emotional expression and entertaining are less likely to suffer from OCD. Whereas college students from families with high-contradiction are at an increased risk of developing OCD. This result was consistent with the views of the experts [11,12]. Contradictions among the family members show anger, agression, and the degree of conflict in high-contradiction families, family members are more prone to fight and attack each other, therefore expose the family member in the state of tension and anxiety. Jin Shenghua [13] argue that there are conflicts in any family, if the family cannot deal with their conflicts in a constructive way, there will be a crisis, and the negative effects of this crisis will be shown on the children who have not developed the ability to live independently, therefore affecting their self-esteem and self-harmony, giving rise to obsessive-compulsive symptoms.

For those families with low family intimacy and poor emotional expression, lack of communication between family members and reluctance to offer help, support and commitment, can lead to the family member being too sensitive, paranoid, result in insecurity and fear of loneliness. For those families with poor provision of entertainment for their members, they cannot provide enough cultural activities and more opportunities for their family members, which can further lead to the low level of individual socialization, poor interpersonal skills. After entering the university, when a person was suddenly encountered with an open environment and a variety of social activities, they tend to become frustrated, withdrawn, unwilling to take the initiative to communicate with others, social interaction function may therefore be inhibited. A study from abroad found that self-restraint of OCD is the psychological basis leading to forced impulsive and anti-impulsive[14]. Therefore, the optimization of the family environment is an important measure to prevent occurrence of OCD, parents are obligated to create a harmonious family atmosphere to enhance the communication and understanding between family members and therefore from their childhood. When it comes to the treatment of students suffering from OCD, their family members should also be involved to accept guidance and training so that they can provide the patients with family support, therefore promoting the rehabilitation of the patients.

For the individual, early environment, particularly his ways of bring-up are very important in the formation of character. In this study the Logistic regression analysis shows, parental styles factors can not enter the final model, this result shows no obvious correlation between the parental styles and OCD. However, emotional warmth of parents, understanding and father's punishment, strictness in the case group, were statistically significant compared with the control group, indicating that individual lack of parental love, understanding, are more prone to feel loneliness and a sense of abandonment; individual whose father is too harsh or use punishment are more likely to do something cautiously, develop rigid thinking, self-blame, regret, fear, anxiety, depression and inferiority. The children are unaware of their status in the hearts of their parents, such as care, love, value, therefore constantly wander between emotional recognition and emotional denial, difficult to integrate these two dramatically opposite sense of self-worth, which often leads to chronic conflict on parent-child relationship, accordingly, perfectionism, and repeated behavior were formed to ensure that self-cuteness and importance in the eyes of their parents [15]. Therefore,

parents need to change their improper way of education, learn to accept, recognize and encourage their children to develop a good character from an early age.

In conclusion, this study shows the individual's coping style and family environment factors are closely associated with the incidence of obsessive-compulsive disorder, positive response, high intimacy and emotional expression helps reduce the incidence of OCD, and high family contradictions will promote the occurrence of OCD among college students. Through analysis of these factors, it should be noted that, aside from genetic factors, we can improve the family environment and increasing social support to optimize individual growth environment, improve the ability of students to actively cope with life, thus reducing the risk of college students suffering from OCD.At the same time, for those college students with OCD more family and social support should be provided to them in order to help control their symptoms and promote their recovery.

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Knowledge about Breast Cancer among Male Medical Students, Jeddah, 2011

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Abstract: **Objectives:** To assess the level of breast cancer knowledge among Saudi Male Medical students, which will help in designing breast cancer awareness education programs for the younger generations. **Methods:** This was a cross sectional study that carried out in the faculties of Medicine at King Abdulaziz University, AlBatarji and Ibnsina, Jeddah Saudi Arabia, between April and June 2011. A questionnaire was distributed to 400 male medical students to assess their knowledge about breast cancer, its risk factors and breast self examination (BSE). Data were collected and analyzed using the Statistical Package for Social Sciences (SPSS).**Results:** About 24% of the participants have a family history of breast cancer and only 17.2% knew what mammogram. The use of oral contraceptive pills (43.5%), exposure to radiation (16%), smoking (25.8%), fatty diet (47%), family history of ovarian cancer (30.3%) and of colonic cancer (28.5%) were recognized by the participants as a risk factor of breast ca ncer. 20% Twenty percent of the students knew what is meant by BSE and 18% knew that it has to be carried out after the monthly period and about 8% of them were very enthusiastic to receive a training course on how the BSE should be done. **Conclusion:** Limited knowledge of breast cancer among male medical students might be an obstacle to screening programs and early diagnosis of breast cancer. Therefore, awareness programs and empowering medical students with knowledge is important area to work on through the medical curriculum development to help in the fight against breast cancer.
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Key words: Knowledge, Breast Cancer, Male Medical Students, Jeddah

1. Introduction

More than a million women, all over the world, are diagnosed with breast cancer every year [1]. In Saudi Arabia the total number of cancer cases among Saudis, in 2006, as reported by the Saudi Cancer Registry (SCR) was 8,054. For the first time, since the establishment of the SCR in 1994, cancer was more among women than men, with a female to male ratio of 1 : 0.94. Cancer of breast, thyroid, colorectal, and Non-Hodgkin's lymphoma, were the most common cancers among women [2].

For primary prevention of breast cancer, women need to be adequately informed of risk factors and risk reduction strategies for breast cancer [3] as it was found that low cancer awareness contributes to delay in presentation for cancer symptoms, and may lead to a delay in cancer diagnosis[4]. Different studies conducted in the kingdom showed that the knowledge of females regarding breast cancer and its screening was low, and exhibited a wide range of prevailing preventive health practices such as breast self-examination (BSE), Clinical Breast Examination (CBE), and Mammography [5–10]. Some of these studies were based on selected groups, like students [7, 9] and teachers [10].

Although there were a lot of studies about knowledge of breast cancer and practice of BSE in female university students [7], there were few studies about the knowledge of breast cancer and practice of BSE in male university especially those in medical colleges either at national or international levels. Hence it was recorded that the adolescent period is a time of rapid change that provides teaching opportunities for shaping health behaviors into adulthood [11]. Therefore, the aim of this study is to assess the breast cancer knowledge level of Saudi Male Medical students, which will help in designing breast cancer awareness education programs for the younger generations.

2. Subjects and Methods:

This study was carried out at the faculty of Medicine of King Abdulaziz (KAU), AlBatarji and Ibnsina, Jeddah Saudi Arabia, between April and June 2011. The research proposal was approved by the ethical committee of the faculty of Medicine,

KAU. A modified version of the questionnaire used by **Sait *et al.,*** to assess the knowledge about cancer breast was used in this study [12]. It is first section included 5 questions on the demographic data of the participant in addition to the family history of breast disease and what is meant by mammogram. The second section of the questionnaire included 6 questions on the risk factors of breast cancer (e.g. oral contraceptive pills, radiation, smoking, fatty foods and family history of ovarian and colonic cancer). The third one included 3 questions on self-breast examination (e.g how and when it should be carried out). Finally, there were 6 general questions including the relationship between breast cancer and breast-feeding, wearing breast brassiere, treatment for breast cancer and diagnosis of breast cancer.

The questionnaires were distributed to 400 male medical students from the three faculties, collected and the data were entered to the computer and analyzed using the Statistical Package for Social Sciences (SPSS) version 15 (SPSS Inc, Chicago, IL, USA). Percentages of the different variables were calculated, risk factors and knowledge about cancer breast was analyzed, and the results were used to build up a base for designing a community educational program.

3. Results:

The study showed that most of the participants were single (92%) , only (6.5%) were married and (1.5%) were divorced. Their ages ranged from (22-25) years with a mean of 23.45 ± 1.75 years. About 24% of the participants admitted that they have a family history of breast cancer, 66% have no family history and 10.3% did not know. On direct questioning on the mammograms, only 17.2% knew what it is, while 82.8% did not know (**Table 1**).

The results of this study summarized the level of knowledge among the medical students about certain risk factors of breast cancer namely; use of oral contraceptive pills (43.5%), exposure to radiation (16%), smoking (25.8%) and fatty diet (47%).

It was found that less than one third of participants recognized a family history of ovarian cancer (30.3%) and of colonic cancer (28.5%) as a risk factor of breast cancer (**Table 2)**.

Regarding the knowledge of breast cancer prevention and treatment it was found that only 20% of the students knew what is meant by breast self-examination (BSE), approximately 18% of them knew that it has to be carried out after the monthly period and not before (as it is recommended) and about 8% of them were very enthusiastic to receive a training course on how the BSE should be done (**Table 3).**

Table 1: The Demographic Data of the participants and their knowledge about the mammogram.

Age	**N**	**%**
Mean ± SD	23.45 ± 1.75	-
Range	(22-25)	-
Marital Status	**N**	**%**
Single	368	92
Married	26	6.5
Divorced	6	1.5
Family History of Breast Cancer	**N**	**%**
Yes	95	23.8
No	264	66
I don't know	41	10.3
Do you know what Mammogram is?	**N**	**%**
Yes	69	17.2
No	331	82.8

Table 2: Knowledge of the participants about Risk Factors of Breast Cancer

Use of Contraceptives	**N**	**%**
Yes	174	43.5
No	81	20.3
I don't know	145	36.3
Exposure to Radiation	**N**	**%**
Yes	64	16
No	32	18
I don't know	304	76
Smoking	**N**	**%**

Yes	103	25.8
No	67	16.8
I don't know	230	57.5
Fatty Diet	**N**	**%**
Yes	188	47
No	146	36.5
I don't know	66	16.5
Family History of Ovarian Cancer	**N**	**%**
Yes	121	30.3
No	261	65.3
I don't know	18	4.5
Family History of Colon Cancer	**N**	**%**
Yes	115	28.5
No	249	62.3
I don't know	36	9

Table 3: Knowledge about Breast Cancer and Self-Examination

Do you know breast self-examination?	**N**	**%**
Yes	80	20
No	320	80
Do you want to be trained on BSE?	**N**	**%**
Yes	31	7.8
No	369	92.2
When BSE should be done?	**N**	**%**
Before menses	31	7.8
During menses	73	18.3
After menses	296	74
Does Breast Feeding Protect from Breast Cancer?	**N**	**%**
Yes	130	32.5
No	43	10.8
I don't know	227	56.8
Does brassiere wearing lead to Cancer?	**N**	**%**
Yes	159	39.8
No	116	29
I don't know	125	31.3
Breast Cancer has treatment	**N**	**%**
Yes	89	22.3
No	41	10.3
I don't know	270	67.5
Breast Cancer leads to death	**N**	**%**
Yes	270	67.5
No	105	26.3
I don't know	25	6.3
Talk to a Cancer Patient	**N**	**%**
Not appropriate	72	18
Benefit	292	73
No difference	36	9
Talk to Students about Cancer	**N**	**%**
Not appropriate	67	16.8
Benefit	302	75.5
No difference	31	7.8

4. Discussion:

It is well known that low cancer awareness contributes to delays in presentation of cancer symptoms and subsequent diagnosis [4], but unfortunately, it seems that younger Saudi generation has limited knowledge about breast cancer [12].

This study showed that the male medical student at the studied faculties have limited knowledge about cancer breast. Approximately 80% of included medical students reported a lack of knowledge regarding breast self-examination. Although the value of BSE is controversial [13], American Cancer Society recommends it as an option for early detection of breast cancer. It benefits women in two ways: women become familiar with both the appearance and the feel of their breasts and detect any changes in their breasts as early as possible [14].

Regarding screening mammography is the only modality proven by randomized clinical trials to allow early detection resulting in overall lower mortality. It is effective not only in women aged 50 years or more, but also in those aged less than 50 years[15] .In spite of this, this study showed that only 17.2% of the male medical students of these faculties knew the mammogram.

Many breast cancer risk factors are detected along many years of epidemiological studies, such e.g. gender, older age, and the older the age the greater the risk of breast cancer, a positive family history of breast cancer, being exposed to large amounts of radiation, such as very frequent spinal x-rays for scoliosis or treatment for Hodgkin's disease at a young age, a personal history of breast or ovarian cancer, being overweight after menopause, or gaining weight as an adult and current or recent use of birth control pills[16-23] .In spite of this, the majority of the participating students did not knew about these risk factors. A similar study was carried out by ***Sait et al.,*** [12] and they concluded that female's students of the high school and college possess limited level of knowledge on breast cancer. However, it also indicated that the students are very enthusiastic to learn about cancer breast and its prevention [12] and it was the case in this study.

In conclusion, the limited knowledge level of breast cancer among male medical students in the studied faculties might be an obstacle to screening programs and early diagnosis of breast cancer. Therefore, awareness programs including lectures, seminars, workshops, and hands on training should be developed. Empowering medical students with knowledge is one important area to work on through the medical curriculum development to help in the fight against breast cancer .This study calls for further ones to demonstrate the areas of deficiencies and the best ways to implement proper strategic plans for the future.

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An Novel Approach for the Assembly of Bio-nanocapsules by Detonation Process

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Abstract: Carbon bio-nanocapsules, a graphitic structure of nanoparticles with a hollow core, have been synthesized via an enhanced detonation process using a Trinitrotoulene (TNT) explosive with parts of toulene as carbon sources and solvent in the presence of titanium dioxide (TiO_2) powder as starting mixtures. Titanium nanoparticles, in stu formed from a detonation-assisted decomposition and rapid reduction of titanium dioxide, show good metal-induced activity for nanocapsule nucleation and for disproportionation reaction of from the TNT detonation. The products of hollow carbon nanocapsules are characterized by XRD, TGA, TEM and EDX techniques. The results shows that surface of hollow carbon bio-nanocapsules displays multilayer wall in structure with 0.35 nm space between the layers and the external diameter of the hollow carbon nanocapsules is 20-90 nm with the thickness of the wall is about 3-10 nm. The method is capable of assembling of the carbon nanocapsules without the participaation of a catalyst. This novel method can be as an alternative technique and may give great potential for the cost-effective ptroduction of hollow carbon nanocapsules.
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Keywords: Synthesis; Nanocapsules; Explosives; Nanoparticles; Detonation; Nanocapsules.

1. Introduction

Nano-structured materials have an area of intense research recently due to novel structure-related physical and chemistry properties as well as variety of significant potential applications [1-3]. Carbon nanocapsules are basically constituted by sp2 C-C covalent bonds as in graphite planes. Their syntheses have been highly successful following various routes, such as laser evaporation or arc-discharge of graphite, catalytic chemical vapor deposition, and decomposition of organic explosives [4;5]. These methods are based on a common key process: the assembly of small carbon species (C*n*) generated at high temperatures. The presence of discontinued defects in the tube structures means that an individual tube could be actually viewed as an assembly of small grapheme sheets and that they could be directly synthesized from the graphene sheets under mild conditions if proper organization technology is available. Although the intrinsic high-energy consumption and intensive hardware of these techniques are mainly responsible for the high cost, the studies on the structures of carbon nanostructures have shown that the practically obtained carbon nanomaterials are highly defective and have a local structure similar to that of turbostratic graphite [6-8]. In the synthesized carbon nanostructured samples, graphitic impurity nanocapsules are always present. Using detonation chemistry for peaceful purposes is an interesting and challenging issue, especially for nanostructure constructions due to simple processing and low production cost. This process has been developed industrially over 15 years to produce flexible graphite for the application of sealing gaskets. In the synthesized carbon nanocapsule samples, graphitic impurity nanoparticles are always present. They seriously hamper the accurate characterization of the bulk properties of nanocapsules and affect their practical applications. To remove these impurities, various purification methods have been developed. Although the graphitic nanoparticles intrinsically contain richer sub-stable nonhexagonal rings and thus are more reactive than carbon nanocapsules, the presence of defects in the tube structures renders the purification difficult. Hence, carbonaceous impurities are also frequently present in the inner voids of tubes. These internal impurities are more resistant and survive even under purification-purposed deep oxidation that causes severe damage to the tubes. How to use these impurity graphitic nanoparticles as a valuable carbon sources is of great interest but, to our knowledge, has not yet been achieved. The synthesis generally employs high-energy explosives and operates at very high loading densities to reach a detonation state with extremely high pressures and temperatures, typically a few tens GPa and thousands degrees [9]. In this paper, the detonation of a TNT explosive was used for the first time to synthesize carbon nanocapsules at HP and HT reaction conditions by introducing metal catalyst (Ti) with some content of carbon source of wax (10 wt %) into the detonation system.

2. Experimental

The detonation of TNT was performed in a sealed stainless steal pressure vessel, induced by rapid heating to its's ignition temperature. TNT/Titanium dioxide /Wax mixture was prepared in desired ratios, serving as catalyst precursor and additional carbon source, respectively. When the detonation occurs, high pressure of shock wave and temperature are produced inside the vessel. After the detonation, the vessel was cooled in air and emptied of gaseous products, and then the solid products were collected. TNT was used as the explosive to generate the high temperature required and to provide part of carbon species for assembling nanocapsules [10]. A small quantity of the as-prepared carbon nanomaterials has been dispersed in ethanol and dispersed onto copper grids in order to perform detailed observations on individual carbon nanostructures by TEM and high resolution TEM (HR-TEM). Energy-dispersive X-ray (EDX) analyses were coupled to TEM observations to determine the nature of the products.

3. Results and Discussion

In this research, the detonation synthetic system (Fig. 1.) can provide a unique environment, which ensures a survival of the pre-fed catalyst and simultaneously a ready generation of the Cn species. This advantage of the detonation system enables us to give an insight into the self-catalysis of carbon nanostructures under the conditions. From the SEM image and TGA results of detonation products using TNT with the mixture of Nickelocene/$C_{14}H_{10}$, it can be seen that copper from the cartridge wall melted and solidified to lumps of different size, ranging from several hundred nanometers to over a micron (Fig. 2).

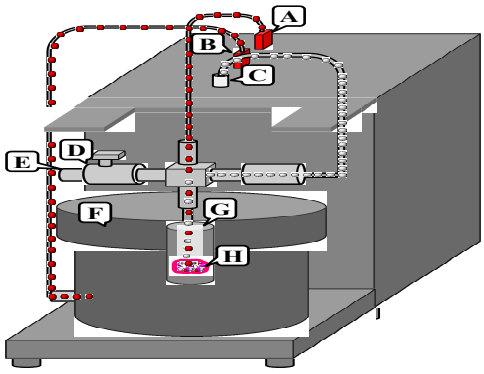


Figure 1. Schematic diagram of catalytic detonation of TNT to form nanomaterials.

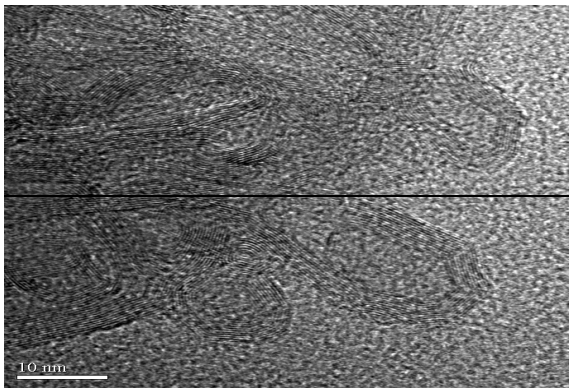


Figure 2. TEM image for the detonation of TNT to synthesis nanocapsules using Ti catalyst.

In addition to the elemental metal catalyst (Ti), agglomerates of carbon nanoparticles can be seen as well. The carbon nanocapsules exhibit outer diameters of 100–120 nm. mixture are virtually stressed that simply mixing TiO_2 with 20% wax. While metal complex reaction is clearly essential, there appears to be pronounced specificity with respect to precursor structure, suggesting perhaps the necessity for certain molecular or packing features conducive to closed shell carbon construction.

Fig. 3 shows a TEM image of the obtained materials with the change of composition to Ti metal, indicating dramatic changes in composition and morphology. Carbonaceous impurities are significantly reduced for both the materials external to the tubes and the materials in the cavities of the tubes. The newly formed tube walls are the consequence of the assembly of the functionalized graphene sheets. The nanostructures are well constructed, with uniform wall thickness along full tube and large interval spaces between the outer and inner tubes. The tube ends are normally open, which facilitates further intuitional observations of the perfect structures. Moreover, TEM image of an individual nanostructures assembly at its open end. Both the outer and inner tube moieties are clearly observable, confirming the encased tubular structures.

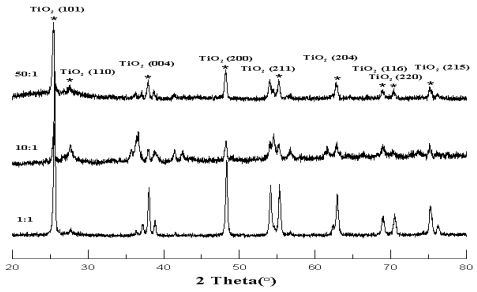


Figure 3. XRD spectrum of the as-synthesized product using TNT/TiO_2/Wax mixture.

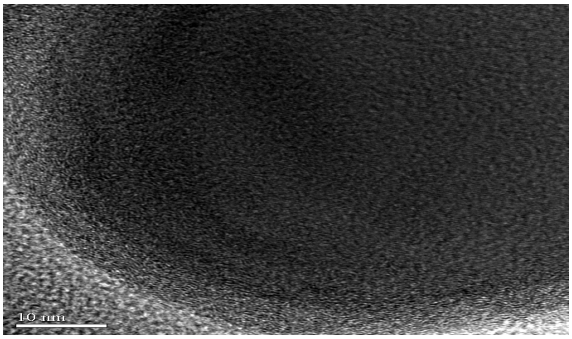
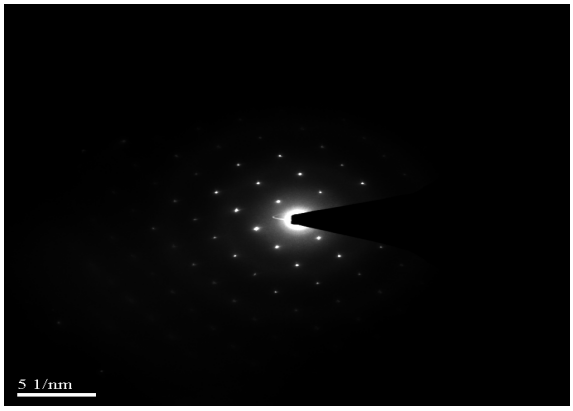


Figure 4. HRTEM image for metallic detonation of TNT to form Ti induced carbon nanostructures.

The high-yield formation of nanostructures other than re-integrated carbon particles indicates that the functionalized grapheme sheets have a preference to assemble in the direction of the pristine tubes. Combined with the uniformity of the wall thickness of the newly formed tube moieties, it also suggests that the graphene layers have very strong self-managing and self-tailoring abilities, even in the used mild wet chemical environment. Fig. 4 presents the high-resolution TEM image of the walls of a tube assembly. The wall of the inner tube shows a fishbone-like graphitic structure with interlayer distances of 0.34 nm, which is similar to the structure of pristine tubes. The outer tube is clearly the newly assembled moiety. It exhibits pre-graphitic short-rangeordered structure, with larger interlayer distances of about 0.35 nm and many discontinued and dislocated defects. Such a structure is a reflection of the soft chemical characteristic of the assembling process involved linkage mode of the small graphene segments. Tailoring the structures into well-ordered graphitic structures is possible by annealing treatments at high temperatures, which could clip off the involved oxygen-containing groups and weld the small graphene sheets together by forming C-C bonds. Fabrication of nanostructures has been accomplished before. The present method is based on a soft chemical technology and it should be easier to rationally control the tubular structures and to produce them. The multisurface and multichannel characteristics of the tube nanostructures should be greatly beneficial for the improvement or tuning of nanocapsule properties and for wide potential applications in catalysis, gas storage and sensing, electrode materials, and so on, like the conventional single-channel nanocapsule.



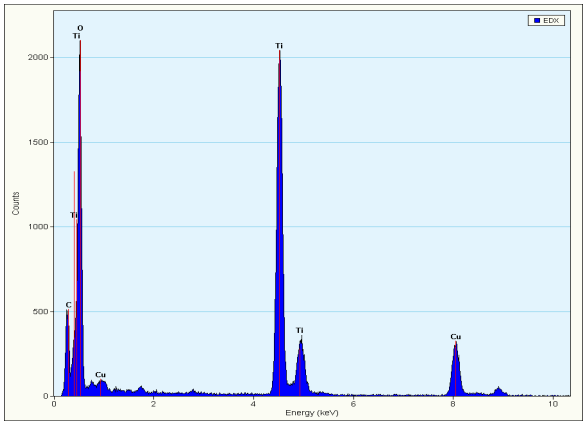


Figure 5. ED patterns and EDX mapping results of the catalytic detonation of TNT to form metallic core-shell nanoparticles

In Fig. 5, a crystalline metal particle can be seen. EDX investigation showed that it is TiO_2 particles containing small amounts of copper (from the ignition wall). Most of the nanocapsules in this sample ended in such spherical crystalline Ti particles which are not transparent to the TEM electron beam. The outer layers are not entirely parallel, indicating some degree of turbostratic disordering. In addition to the metal, the carbon nanomaterials formation was influenced by the experimental set-up, especially the method of sealing. This indicates that the pressure and temperature versus time changes need to be considered in order to fully understand or describe the nanocapsule formation by detonative decomposition techniques. A very high Ti content of nearly 100% of these lumps, was verified using EDX. Additionally, the TEM micrograph shows that the length of the tubes can reach more than a dozen microns and that they are segmented. A detailed and quantitative studies of the influence of nitrogen incorporation on the morphology of carbon nanostructures remains to be conducted in the future. In the experiments without any gasket between the steel plates, a very fast pressure decline can be assumed, as the detonation was loudly audible. The solid carbon yield in these cases was small and no tubes, only nanocapsules were found. The graphitization was not very distinct, as observed by TEM. However, no attempts were made to measure the maximum pressure or its decline as a function of time, and the conclusions of this work hence must remain qualitative.

4. Conclusion

In this paper, a very common CHNO explosive, TNT, is employed with a Ti metal as a catalyst with sufficient carbon sources introduced into the detonation system. Such a catalytic detonation process is chemically much different from that for pure explosives and facilitates practical operation. Compared to the other processes for the syntheses of carbon nanocapsules, the in-stui method used in the current work is characterized by high-density and high-pressure conditions, which experimentally shows that carbon nanostructures can grow in such an environment and provides an alternative process for producing nanostructures and a potential route for carbon bio-nanocapsule formation, especially in high density environments with the existing carbon species with the presence of Ti metal compounds.

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Local Recurrence and Distant Metastases after Breast Conservation Treatment in Women with Triple Negative Breast Cancer Subtype

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Abstract: Introduction: Recently, gene expression studies using DNA microarrays have identified five common subtypes of breast cancer. The triple negative (TN) phenotype, which includes tumors that do not express ER, PR, or HER2 serves as a proxy for the basal-like subtype. At the present time, there is little clinical data evaluating whether a particular breast cancer subtype is associated with increased rates of local and/or distant recurrence after BCT .**Objective:** to evaluate the outcome after breast conservation therapy for triple-negative early-stage invasive breast carcinoma. **Materials and methods:** Between 2000 and 2010, 421 patients with early stage breast cancer patients who treated with BCT were classified as TN (58) if they were negative for all three receptors (ER, PR, and HER2/*neu)* or as non–TN (363) if they were positive for any of the three markers. These patients were evaluated for isolated local and distant recurrence. **Results:** The local relapse rates among the TN group were nearly equal to those of the non–TN. (5.2% vs. 3.9% *P*= 0.63) five-years overall and disease free survival of the TN group were significantly poorer than the other group(62% vs 85 % *p*=0.002) and (39 % vs 75% *p*= 0.00). The isolated local relapse free survival was 90.3% vs 95.7% between the 2 groups. (*P*= 0.365) while the isolated distant metastases free survival was 52% vs 84%. (*P*= 0.00). **Conclusions:** Although women with TN tumors had a higher rate of local failure and a lower rate of freedom from distant metastases compared with women without TN tumors, the absolute difference was relatively small and is not statistically significant and therefore does not preclude BCT for women with TN early-stage invasive breast carcinoma.

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Keywords: Breast Cancer, Hormone Receptor Negative, HER-2/neu Local recurrence.

1. Introduction

Breast cancer encompasses a heterogeneous population of tumors characterized by a variety of clinical, pathological, and molecular features [1-3]. Molecular markers such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) have been used to classify patients into different subtypes. Recently, gene expression studies using DNA microarrays have identified five common subtypes of breast cancer: luminal A (ER or PR positive and HER2 negative), luminal B (ER or PR positive and HER2 positive), HER2 overexpressing (ER and PR negative and HER2 positive), basal-like (ER, PR, and HER2 negative, CK 5/6 positive) and normal breast-like tumor [2,4-6]. HER2 overexpressing and basal-like subtypes are significantly more likely to be grade 3 and are associated with worse recurrence rates and decreased overall survival [3,5-7]. The triple negative (TN) phenotype, which includes tumors that do not express ER, PR, or HER2 on immunohistochemistry (IHC), serves as a proxy for the basal-like subtype with a positive predictive value of approximately 80-97% [8,9]. Although this approach is not complete, several groups have adopted a TN definition for basal-like cancer out of convenience.

Several randomized trials have demonstrated molecular markers were available. The data on ER, PR, and HER2/*neu* were obtained through standard clinical testing, using immunohistochemistry for ER and PR and the HER2/*neu*.

For ER and PR, receptor positivity was based on more than 10% of cells testing positive.

HER2/*neu* scores of 0 and 1 were considered negative, and Positive HER2 was determined using either IHC 3+ staining or amplification on fluorescence *in situ* hybridization (FISH) in patients with IHC 2+.

Patients were classified as triple negative if they were negative for all three receptors or as non–triple negative if they were positive for any of the three markers.

Exclusion criteria for this study included male breast cancer, T3 disease, mastectomy, previous or concurrent malignancy (breast or other site)or patients treated without radiotherapy.

All patients were treated with breast-conserving surgery followed by radiation therapy. The surgical treatment included complete gross excision of the primary tumor. Re-excision of the primary tumor was done if possible if positive margin was proved. Pathologic axillary lymph node staging was performed for all patients. Earlier in the study period, pathologic axillary lymph node staging was generally performed

using a lower axillary lymph node that survival rates following breast-conserving treatment (BCT) were equivalent to those observed with radical mastectomy [10-12]. Given these results, BCT has become an accepted treatment for early stage breast cancer [13]. At the present time, there is little clinical data evaluating whether a particular breast cancer subtype is associated with increased rates of local and/or distant recurrence after BCT [14-17].

The outcome after breast conservation treatment with radiation has not been well described for triple-negative early-stage invasive breast carcinoma. Therefore, the current study was performed to evaluate the outcome after breast conservation treatment with radiation for this subtype of breast carcinoma.

2. Materials and Methods

Between the year 2000 and 2010, 759 patients with American Joint Committee on Cancer stages I-II (18) disease were treated with breast-conserving surgery followed by radiation therapy to the intact breast, with or without systemic therapy, at the Clinical Radiation Oncology Department, Tanta University hospital.

Only those patients in whom ER, PR, and HER2/*neu* status were available were included in the current analysis. This limited the sample to a total of 421 patients in whom all three was defined as clinical evidence of distant disease based on clinical and/or radiographic findings.

All events were calculated from the time of surgery to the time of the event.

For the calculation of overall survival (OS), a death due to any cause was scored as a failure. For the calculation of freedom from distant metastases, a failure was scored at the time of first evidence of distant metastatic disease.

The Kaplan-Meier method was used to calculate OS, freedom from distant metastases, local failure, and distant failure (19) . The time period was defined as the time of surgery of breast carcinoma. The log-rank test was used for statistical comparisons between groups (20) Multivariate analysis was performed using the Cox proportional hazards model (21). Chi - square test was used to compare the characteristics of patients between the 2 groups. All statistical methods was done using SPSS statistical package version 17.

3. Results

In our series, 421 patients were classified according to ER, PR, and HER2 into either TN (58) or non TN (363) patients. TN patients represent about 14 % of the whole study group. Patients, tumor, and treatment characteristics of each group are detailed in table(1). there is a highly significant difference between both groups regarding age with about 45% of triple negative patients less than 40 years compared to 33% of non triple negative patients.

The median age for patients with TN breast carcinoma was 41 years while the median age for patients without a triple negative breast carcinoma was 47 years.

There was no significant difference between patients by T-size, LN status, grade, extensive intraductal component, tumor necrosis, lymphovascular invasion or resection margin. All triple negative patients received chemotherapy compared to 83% of patients in the other group. The most common regimens used were either CMF or FAC or FAC and taxanes . All eligible patients underwent BCS and post operative whole breast irradiation. For the radiation treatment to the whole breast, the median dose was 46 Gy (mean, 45.75 Gy; range, 45-50.4 Gy). The median total dose was 61 Gy (mean, 60.91 Gy; range, 58-66 Gy). Among non triple negative groups, 80,7% of patients received hormone therapy. Few patients with HER 2 overexpression received adjuvant trastuzumab therapy.

During the follow up period, there were 3 (5.2%) and 14 (3.9%) patients in TN and non TN groups respectively who experienced isolated local in breast relapse as the first site of recurrence. the difference was non significant(P= 0.63).

Isolated distant relapse was observed in 19(32.8 %) and 47(12.9 %) patients in TN and non TN groups respectively(p= 0.00).

The 5-years OS and DFS rates of all patients were 82% and 71% respect. Groups respectively (P= 0.00)

By multivariate Cox proportional hazard models, lymph node status, intraductal component, and use of adjuvant systemic treatment were significant prognostic factors affecting OS . Age, tumor size , lymph node status and use of systemic treatment significantly affected DFS (Table2)

4. Discussion

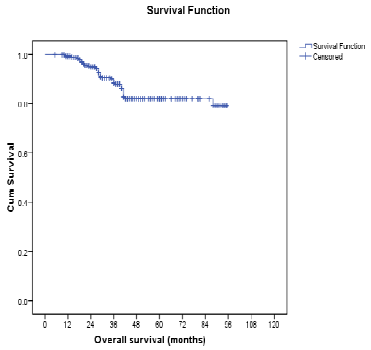
There has been much attention focused on TNBCs since the first identification of basal-like cancers using microarray expression profiling studies in 2005 (22). There is a significant overlap between TNBCs, which are defined by immunohistochemistry, and basaloid like tumors, which are identified from gene expression profiling, such that those two entities are frequently considered synonymous from a clinical perspective.(23-24)

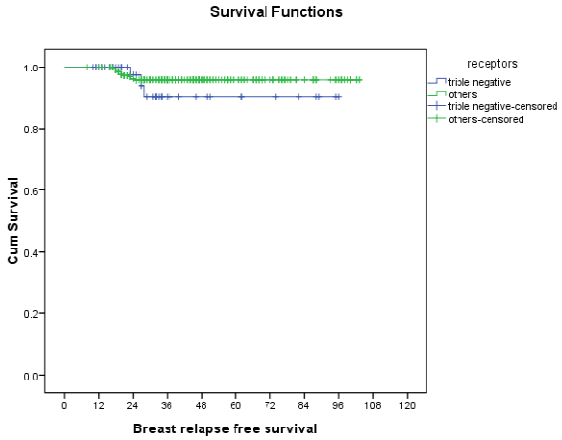
Table (1): Patients characteristics.

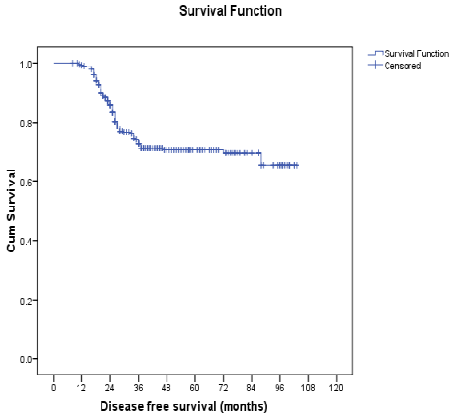
		Receptors							
		Triple negative N=58		Non -triple negative N=363		Total		Chi-square	
		N	%	N	%	N	%	X^2	*P*-value
AGE	<40 y	26	45	121	33.3	155	36.8	13.747	0.000
	>40 y	32	55	242	66. 7	266	63.2		
Tumor size	T1	12	20.69	103	28.37	115	27.32	1. 67	0.43
	T2	46	79.31	260	71.63	306	72.68		
L.N.	N0	12	20.69	95	26.17	107	25.42	1.663	0.435
	N1	24	41.38	159	43.80	183	43.47		
	N2-3	22	37.93	109	30.03	131	31.12		
GRADE	G1	8	13.79	45	12.40	53	12.59	0.111	0.946
	G2	39	67.24	251	69.15	290	68.88		
	G3	11	18.97	67	18.46	78	18.53		
EIC	Positive	18	31	93	25.6	111	26.4	1.295	0.523
	Negative	28	48.3	204	56.2	232	55.1		
	unknown	12	20.7	66	18.2	78	18.5		
Necrosis	yes	6	10.3	33	9.1	39	9.3	0.125	0.939
	no	42	72.4	263	72.5	305	72.4		
	unknown	10	17.2	67	18.5	77	18.3		
LVI	yes	20	34.5	115	31.7	135	34.53	0.503	0.778
	no	33	56.9	223	61.4	256	65.47		
	unknown	5	8.6	25	6.9	30	7.1		
RM	negative	49	84.48	312	85.95	361	85.75	0.341	0.843
	positive	2	3.45	8	2.20	10	2.38		
	close	7	12.07	43	11.85	50	11.88		
Systemic treatment	Chemotherapy alone	58	100.00	70	19.28	128	30.40	153.979	0.000
	Hormonal alone	0	0.00	60	16.53	60	14.25		
	both	0	0.00	233	64.19	233	55.34		
Isolated local recurrence	present	3	5.2	14	3.9	17	4	0.223	0.639
	absent	55	94.8	349	96.1	404	96		
Isolated distant recurrence	present	19	32.8	47	12.9	66	15.7	14.848	0.000
	absent	39	67.2	316	32.8	355	84.3		

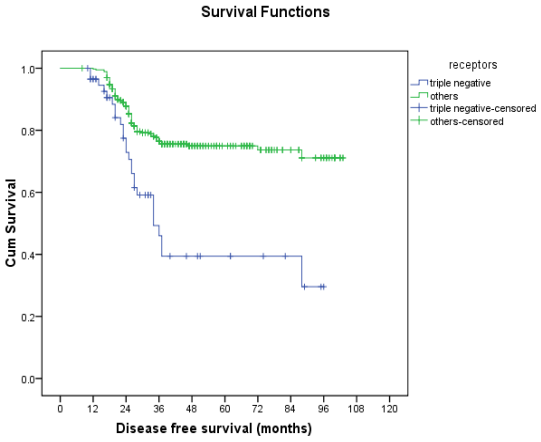
Table (2): Cox proportional hazards multivariate model for overall (OS) and disease-free survival (DFS)

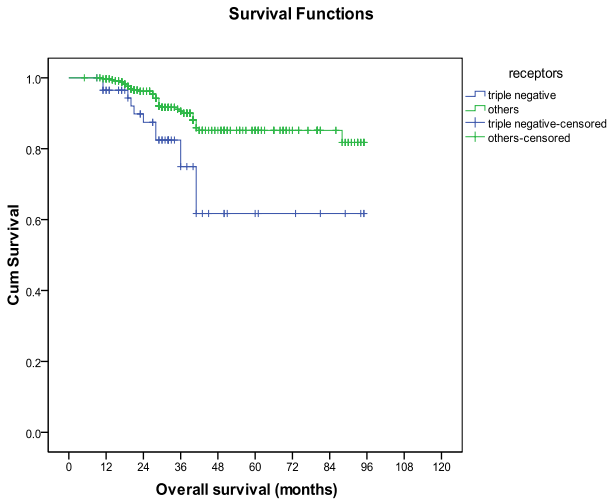
Variable	OS			DFS		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
AGE	.896	.446-1.797	.756	.456	.286-.729	.001
TSIZE	1.927	.527-7.044	.321	2.443	1.138-5.246	.022
LN	2.603	1.263-5.362	.009	2.150	1.406-3.285	.000
grade	.864	.444-1.683	.668	.877	.582-1.322	.531
EIC	.349	.191-.637	.001	.730	.512-1.041	.082
necrosis	.760	.419-1.377	.365	.971	.682-1.382	.869
LVI	.914	.506-1.652	.767	.796	.546-1.162	.238
Tumor subtype	.844	.379-1.876	.677	.935	.552-1.583	.801
Systemic treatment	.681	.470-.987	.043	.533	.419-.678	.000


Survival Function
Survival Function
Censored
Cum Survival
Overall survival (months)


Survival Functions
receptors
triple negative
others
triple negative-censored
others-censored
Cum Survival
Breast relapse free survival


Survival Function
Survival Function
Censored
Cum Survival
Disease free survival (months)


Survival Functions
receptors
triple negative
others
triple negative-censored
others-censored
Cum Survival
Disease free survival (months)


Survival Functions
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triple negative-censored
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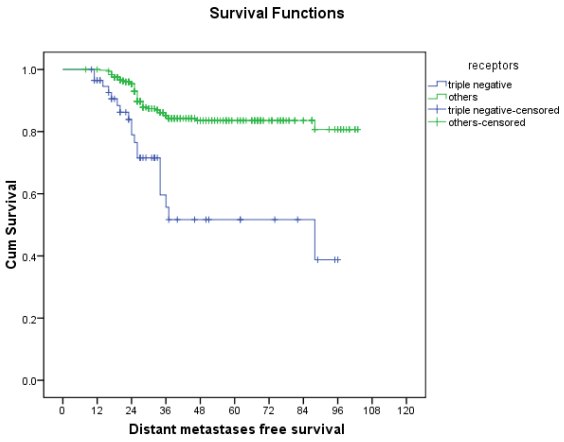
Cum Survival
Overall survival (months)


Survival Functions
receptors
triple negative
others
triple negative-censored
others-censored
Cum Survival
Distant metastases free survival

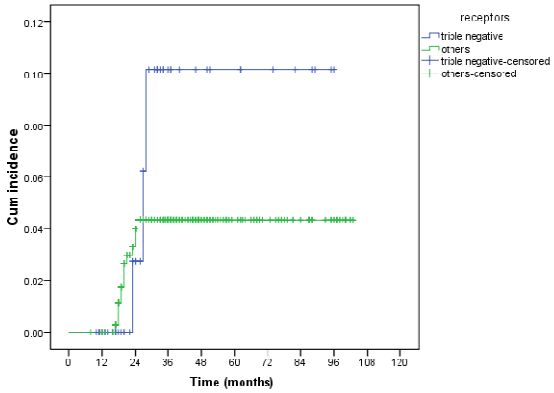


Figure 1. Cumulative incidence of local recurrence as the first event

TNBCs have a poor prognosis and do not respond to therapy directed at known breast cancer growth factor targets, including hormonal therapy and trastuzumab. TNBCs are fast-growing tumors, usually have a high histologic grade, and tend to metastasize earlier compared with breast cancers that express hormone receptors (16,25) . The predominant sites of metastases for TNBCs differ from the luminal subtype. They are more frequently node negative and spread, in70%of patients, to the lung and brain, whereas luminal cancers tend to metastasize, in 70% of patients, to the bone and liver. (16,22.)

TNBC tumors seem to be more sensitive to chemotherapy, and although there is a sharp decrease in survival early after diagnosis, survival plateaus after 8 to 10 years (16) .

In the current study, we evaluated 421 patients with conservatively treated breast cancer in whom all three markers were available to validate the prognostic utility of this classification scheme and to determine whether triple negative breast cancers have a more aggressive locoregional and distant relapse rate.

Among all patients included, the triple negative cohort still had a poorer prognosis than the non–triple negative cohort, but the two cohorts had a similar local relapse-free survival (90.3 vs 95.7 in TN and non TN groups respectively, *P*= 0.365).

In fact, the local relapse rates among the triple negative cohort were nearly equal to those of the non–triple negative cohort (5.2% and 3.9% of patients in TN and non TN groups *P*= 0.63).

This is of particular importance given the fact that triple negative patients are significantly younger, and younger women have been shown to have a higher rate of local relapse compared with older women(26-31), therefore, those patients should not be considered poor candidates for breast-conserving therapy.

The fact that these women with triple negative tumors had similar local relapse rates indicates that these tumors are not radiation resistant. This is consistent with the fact that these basal-like tumors are theoretically responsive to DNA-damaging agents and should, therefore, be relatively radiation sensitive. Larger patient cohorts with a longer follow-up will be required to further validate these data.

Only limited data are available for local control after breast conservation treatment with radiation relative to biologic subtype.

Haffty et al reported that there was no significant difference in local failure at 5 years for the triple-negative subgroup versus the non–triple-negative subgroup (17% vs. 17%, respectively; *P* = .82) (14) .

Nguyen *et al.,* reported the outcome for patients grouped according to ER status, PgR status, and HER2 status as a surrogate for biologic subtype of disease (15). The 5-year rate of local recurrence was 7.1% for the basal-like (triple-negative) subgroup, 8.4% for the HER2 subgroup, 1.5% for the luminal B subgroup, and 0.8% for the luminal A subgroup. In comparison with the luminal A subgroup, there was an increased rate of local failure for the basal-like (triple-negative) subgroup (*P* = .0099) and the HER2 subgroup (*P* = .012).

In a study evaluating HER2 as a single factor, **Harris *et al.,*** reported no difference in local failure at 5 years for the HER2-positive group compared with the HER2-negativegroup (0% vs. 2%, respectively; *P* = .15)[32]. **Parikh *et al.,*** reported an increased rate of local recurrence for premenopausal patients with CK19-negative tumors compared with CK19-positive tumors (relative risk, 3.54; *P* < .01) [33] .

For 3038 patients treated with either breast conservation treatment or mastectomy, **Cheang *et al.,*** used a panel of tumor markers to define biologic subtype of disease [34] . The 10-year rate of local relapse was 7% for the luminal A subgroup, 11% for the luminal B subgroup, 15% for the luminal/HER2-positive subgroup, 15% for the basal-like subgroup, and 18% for the HER2-positive subgroup.

For 1601 patients treated with either mastectomy or breast conservation, **Dent *et al.,*** reported no significant difference in local failure for the triple-negative group compared with the non–triple-negative group (HR, 0.8; *P* = .38) [16] .

There are emerging data for using genomic factors to predict the risk of local recurrence after breast conservation treatment. Using a 21-gene recurrence score assay, Mamounas et al evaluated the risk of local-regional recurrence for patients treated in the National Surgical Adjuvant Breast and Bowel Project B-14 and B-20 trials for patients with node-negative, ER-positive breast cancer[35] . After breast

conservation treatment with radiation for 390 patients, there was a strong interaction of local-regional failure with age. For patients aged < 50 years, the 10-year rate of local-regional failure was 12.5% for tumors with a low-risk recurrence score, 27.7% for tumors with an intermediate-risk recurrence score, and 26.5% for tumors with a high-risk recurrence score (P = .057). However, the 10-year rates of local-regional recurrence were relatively low for patients aged ≥ 50 years, regardless of the results of the recurrence score assay (3.6% vs. 3.7% vs. 4.8%, respectively; P = .663).

Nuyten *et al.,* reported that the wound response signature obtained by gene expression profiling was able to segregate the patients after breast conservation treatment into a high risk versus a low risk of local recurrence at 10 years (29% vs. 5%, respectively; P = .0008) [36] . The wound signature profile remained a significant predictor for local recurrence on multivariate analysis (P = .01)

Freedman *et al.,* [17] defined three groups: ER or PR (+), HER2, and TN. Patients in the TN and HER2 groups were more likely to have T2 and grade 3 diseases. The median age of the TN and HER2 groups was both 54 years, which was also older than that of the present study. They also reported that there was no overall difference in total locoregional recurrence rates between the three groups (p=0.13). Additionally, a higher rate of distant metastases in the HER2 group was observed (11.9%, p=0.009) which translated into a lower recurrence-free survival rate (84%, p=0.005).

It is also notable that, despite the use of adjuvant systemic chemotherapy, triple negative patients seemed to have a poor prognosis, with a distant metastasis-free survival rate of only 52% at 5 years. The interpretation of these findings remains debatable given the relatively small number of patients and the retrospective nature of this study, and further studies evaluating the impact of adjuvant systemic therapy in triple negative patients is warranted.

By definition of their lack of receptors for ER, PR, and HER2/ *neu*, patients with triple negative tumors are not candidates for adjuvant hormonal therapy or trastuzumab[37]. Basal-like tumors have been shown to overexpress HER1 (epidermal growth factor), and patients with these tumors may be candidates for prospective studies evaluating targeted antibodies against epidermal growth factor that are already in clinical use [5,38] .

Clearly, additional prospective and retrospective studies are warranted to further refine prognosis and optimize treatment in patients with triple negative breast cancers. We have demonstrated here, using the simple commonly available markers of ER, PR, and HER2/*neu*, that patients with triple negative breast cancers have a relatively poor prognosis, with a poorer distant metastasis-free, disease-free, and Overall survival.

The current study has several potential limitations. Firstly, because HER2 testing has evolved over time, there was not uniform testing for all of the breast carcinomas in the current study.

Second, the relatively small number of patients in the triple-negative subset limited the power to detect small, but potentially statistically significant, differences. Whether alternative forms of chemotherapy for these breast cancer patients, with or without biologic modifiers, will prove superior can only be addressed by well-designed prospective studies. Third, the groups were differentiated based on tumor markers, not gene profiling. Nonetheless, segregating patients into prognostic groups on the basis of tumor markers is clinically relevant, particularly in view of targeted therapies currently available.

Another potential weakness of the study is unavoidable selection biases in a retrospective series such as this. For the current study, only patients who had available ER, PR, and HER2/*neu* data were included.

Finally, the patients were treated largely before the routine use of trastuzumab and lapatinib. Thus, the results in the current study, particularly for the HER2-positive patients, could be substantially different for patients treated using newer targeted therapies.

Conclusion

Although women with triple-negative tumors had a higher rate of local failure and a lower rate of freedom from distant metastases compared with women without triple-negative tumors, the absolute difference was relatively small and not statistically significant and therefore does not preclude breast conservation treatment with radiation for women with triple-negative early-stage invasive breast carcinoma.

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5/6/2012

5-HT_{2c} receptors modulate the discharge activities of inspiratory neurons in the medial region of Nucleus Retrofacialis of neonatal rats in vitro

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Abstract: Objective To investigate whether 5-HT_{2C} receptors modulate the discharge activities of inspiratory neurons in the medial region of Nucleus Retrofacialis (mNRF) of neonatal rats. **Methods** Experiments were performed in in vitro brainstem slice preparations from neonatal rats. These preparations included the mNRF with the hypoglossal nerve (XII nerve) rootlets retained. The rhythmic discharge activities of the inspiratory neurons (I neurons) and respiratory-related rhythmic discharge activities (RRDA) were simultaneously recorded by using microelectrodes in the mNRF and suction electrodes at the XII nerve rootlets, respectively. Roles of 5-HT_{2C} receptors on the discharge activities of I neurons were investigated by administration of the 5-HT_{2C} receptor agonist 2-Chloro-6-(1-piperazinyl)-pyrazine hydrochloride, 6-Chloro-2-(1-piperazinyl)pyrazine hydrochloride (MK212), and its specific antagonist 4-dionehydrochloridehydrate,8-[5-(2,4-Dimethoxy-5-(4-trifluoromethylphenylsulphonamido)phenyl-5-oxopentyl]-1, 3,8-triazaspiro[4.5]decane-2 (RS102221) dissolved in modified Kreb's solution for perfused slices. **Results** MK212 prolonged inspiratory time (TI), shortened respiratory cycle (RC), enhanced integral amplitude (IA) and the spike frequency (SF) of I neurons. By contrast, RS102221 produced opposite effects. **Conclusions** 5-HT_{2C} receptors take part in modulate the discharge activities of I neurons in mNRF of neonatal rat.
[QIAN Zhibin, SONG Xiaorong, JI Mingli, LIU Chunxia, LIU Xiaoli. **5-HT_{2c} receptors modulate the discharging activities of inspiratory neurons in the medial region of Nucleus Retrofacialis of neonatal rats in vitro.** Life Sci J 2012;9(3):183-186]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 27

Key words: the medial region of Nucleus Retrofacialis; 5-HT_{2C} receptors; inspiratory neuron; brainstem slices

Introduction

Neuronal networks in the medulla oblongata generate respiratory rhythm in mammals. The previous experiments have demonstrated that the medial region of Nucleus Retrofacialis (mNRF) is the site of respiratory rhythmogenesis[1,2]. The mNRF overlaps the preBötzinger complex partly, a region proposed to be the core site for respiratory rhythm generation[3].

In rats, serotonergic projections from the caudal raphe nuclei to the hypoglossal nucleus to regulate respiration[4]. 5-HT receptors have been found in the ventral respiratory group (VRG) from neonatal mice and rat where they are essential for the modulation of respiratory rhythm[5]. 5-HT receptors are involved in generation and modulation of basic respiratory rhythm in mammals[6,7]. The 5-HT membrane receptors include seven subtypes, besides 5-HT_3 is ligand-gated ion channel receptor, others are G-protein coupled receptors[8]. To investigate the effects of 5-HT_{2C} receptors on the inspiratory neurons (I neurons) and to further elucidate the role of the 5-HT_{2C} receptors are involved in the respiratory network, the present study was designed and performed.

Materials and methods

Materials Neonatal Sprague–Dawley rats, both sexes, 0-3 days, n=7, were supplied by Experimental Animal Center of Xinxiang Medical University. MK212 and RS102221 were bought from Sigma.

Methods Rats were deeply anesthetized with ether and quickly decapitated at the C3-C4 spinal level. The brainstem was dissected in ice-cold modified Kreb's solution (MKS in mmol/L: NaCl 124, KCl 5, $CaCl_2$ 2.4, $MgSO_4$ 1.3, $NaHCO_3$ 26, Glucose 30 and pH 7.35–7.45) that was equilibrated with carbogen (95% O_2 and 5% CO_2). This operation must be finished within 3 mins. The brainstem was glued rostral end up onto an agar block, mounted into a vibratome and serially sliced until the rostral boundary of the mNRF was identified by anatomical landmarks such as disappearance of the facial nucleus and appearance of the inferior olive, the nucleus ambiguus, and the hypoglossal nucleus. A 750μm transverse slice was cut, which contained the mNRF with the hypoglossal nerve rootlets retained. The brainstem slice was transferred to a recording chamber (3ml) and continuously perfused with oxygen-saturated MKS at a rate of 7–9 ml/min at 25–27℃. The activities of I neurons in mNRF and the discharge of hypoglossal nerve (XII nerve) rootlets were simultaneously recorded by using

microelectrodes and suction electrodes, respectively. The RRDA were amplified by a DC preamplifier; the activities of I neurons were amplified by a microelectrode amplifier. Signals were band-pass filtered (0.1–3.3 kHz), data were sampled (5 kHz) and stored in the computer via BL-420F biological signal processing system after being amplified.

MK212 and RS102221 were dissolved in DMSO and diluted to 10μmol/L, keeping the concentration of DMSO less than 0.1%. In this concentration DMSO has no effects on the discharge of inspiratory neuron[9,10].

Statistical analysis

All data were expressed as means±S.E.M, and repeated measure was used to compare the values obtained before and after drug application. Differences were considered statistically significant as $p < 0.05$.

Results

7 I neurons were recorded, respiratory related neurons were identified by correlating their on-going activities with the hypoglossal rhythmic discharges[2,7]. As shown in Tab.1 and Fig.1, application of MK212 significantly prolonged TI by 15.94%, shortened RC by 17.86%, enhanced IA and SF by 32.79% and 41.31% of I neurons, respectively. After washout of MK212, the discharge activities of I neuron were recovered to the control level. By comparison with MK212, RS10221 produced opposite effects, it shortened TI by 20.22%, prolonged RC by 23.46%, decreased IA and SF by 14.51% and 30.05% of I neurons.

Tab.1 Effect of MK212 and RS102221 on the discharge activities of I neurons

Group	TI(s)	IA(μV.s)	RC(s)	SF(Hz)
Control	0.69±0.06	674.27±60.21	18.54±2.85	16.34±2.61
MK212	0.80±0.05 **	895.33±70.21 **	15.73±2.52 **	23.09±3.07 **
Washout	0.67±0.06	694.57±57.03	17.11±2.66	17.22±3.11
RS102221	0.55±0.04 $^{**\#\#}$	576.44±34.67 $^{**\#\#}$	22.89±3.14 $^{**\#\#}$	11.43 ±2.48 $^{**\#\#}$
F	12.593	33.205	15.123	3.746
P	0.001	0.000	0.000	0.000

$^{**}p < 0.01$ vs control group $^{\#\#}p < 0.01$ vs MK212 group

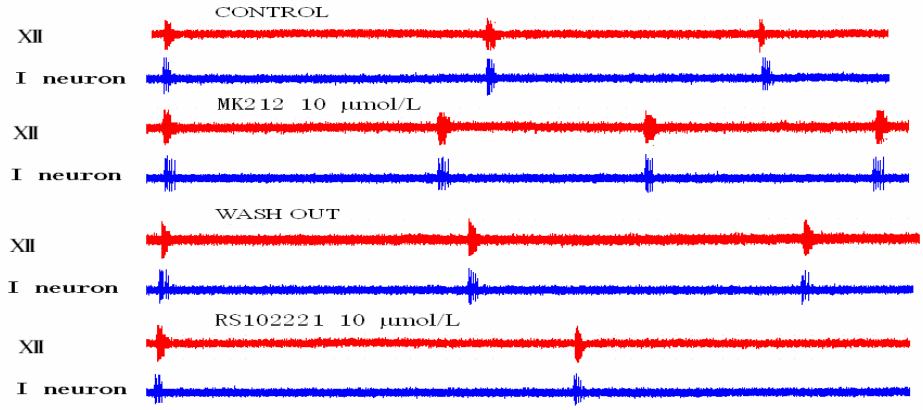

Fig.1 Effect of DOI and ketanserine on the discharge activity of I neurons

Discussions

This study was performed in in vitro brainstem slices containing the neurons critical for integration of respiratory drive. The respiratory frequency of this preparation was markedly slower than that in vivo due to the isolation of nervous system from mechanosensory afferent inputs and the removal of vagal mechanosensory afferent inputs[11]. However, the discharge patterns of respiratory motor neurons in vitro were similar to that in the intact mammal but different from gasping[12,13]. The I neurons, which appear to be fundamental components of the inspiratory pattern generation, have been proposed to be responsible for respiratory rhythm.

Our experiment found that RS102221 significantly shortened TI, prolonged RC, decreased

IA and SF of I neurons, confirming that there are serotonergic neuron releasing endogenous 5-HT which modulate the discharge activities of I neurons in slice. Blocking 5-HT_{2C} receptors by RS102221 depress the excitability of I neuron. On the other hand, MK212 prolonged TI, shortened RC, enhanced IA and SF suggesting that there are 5-HT_{2C} receptors in membrane of I neuron. 5-HT_{2C} receptors modulate the excitability of I neurons, RRDA was changed to follow I neurons discharge activity. RC was shortened significantly by MK212 which indicated that activating 5-HT_{2C} receptors increased interneuronal activities and inhibit expiratory neurons, further suggesting that 5-HT_{2C} receptors are involved in the phase-switching between expiration and inspiration.

After being activated, 5-HT_{2C} receptors activate phospholipase C, it catalyzed phosphatidylinositol diphosphate transforming into inositol triphosphate and diacylglycerol[14]. Phosphatidylinositol diphosphate increase [Ca^{2+}] of cytolymph from endocytoplasmic reticulum[15]; protein kinase C was actived by diacylglycerol[16]. Protein kinase C increased open probability of Na^+ channel through phosphorylation and also increased concentration of reactive oxygen species[17,18]. Reactive oxygen species can increase open probability of Na^+ channel too[2,19]. The increasing open probability of Na^+ channel in turn increased the excitability of inspiratory neurons and respiratory center. We presume this is the mechanism 5-HT_{2C} receptors effect on inspiratory neurons.

In conclusion, the present study suggests that in medullary respiratory center, 5-HT_{2C} receptors modulate the basic rhythmic respiration through modulate the excitability of medullary respiratory neurons.

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5/2/2012

Effect of Allium Sativum Extract on Serum Lipid and Antioxidant Status in hypercholesterolemic Rabbits

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Abstract: The present study was conducted to investigate the lipid-lowering and antioxidative activities of aqueous garlic extract (AGE, 1 ml/Kg, orally, corresponding to 500 mg/Kg/ day) in heart and liver tissues of rabbits fed with high-cholesterol diet. Twenty-eight male white New Zealand rabbits were divided into four groups, 7 rabbits each. The first one, group 1, served as a control, group 2 (hypercholesterolemic group), rabbits fed (2% cholesterol enriched diet) for 4 weeks, group 3, (aqueous garlic extract AGE), rabbits were given orally aqueous garlic extract (500mg/Kg b.w/ day) for 4 weeks, group 4, rabbits fed 2% cholesterol- enriched diet in conjunction with AGE (500 mg/Kg b.w / day) orally for the same period. At the end of the feeding period, rabbits were fasted over night and slaughtered and blood and tissue samples were taken for biochemical and histopathological studies. Obtained results showed that AGE suppressed the high levels of serum lipid profile including total cholesterol, low density lipoprotein cholesterol, and triglycerides, while it increased the concentration of high density lipoprotein cholesterol. The high serum activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase as well as creatin kinase and lactate dehydrogenase significantly decreased in high-cholesterol rabbits treated with AGE. AGE lowered the high level of cardiac and hepatic lipid peroxidation and raised the low activities of catalase, superoxide dismutase and glutathione peroxidase in both the cardiac and hepatic tissues. Histopathological examinations revealed that AGE preserved myocardial and hepatic tissues. It can be concluded that bioactive compounds containing in aqueous garlic extracts might be responsible for both lipid- lowering and antioxidative actions to protect the heart and liver from hypercholesterolemia.

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Key words: Garlic; enzymes; hypercholesterolemia; antioxidant activity; rabbits**;** histopathology.

1. Introduction

Cardiovascular diseases, particularly coronary heart disease have become a growing problem, especially in developing countries. Hypercholesterolemia is widely known as a dominant risk factor for the development of cardiovascular diseases **[1,2]**. It has been reported that oxidative stress induced by reactive oxygen species, plays an important role in the etiology of several diseases including atherosclerosis and coronary heart disease **[3].** Hyperlipidemia has also been found to induce oxidative stress in various organs such as the liver, heart, and kidney **[4,5]**. To lower high blood cholesterol, a number of lifestyle changes are recommended including smoking cessation, limiting alcohol consumption, increased physical activity and diet control **[6].** However, most people could not successfully control their blood cholesterol because of the modern life style.Therefore, medication is considered their last choice which have been reported to have serious adverse effects, particularly liver damage **[7].** Moreover, they lack several desirable properties such as efficacy and safety on long-term use, cost, and simplicity of administration. These factors do not fulfill conditions for patients'compliance. So, attention is being directed to the medicines of natural origin with hypolipidemic activity. There are several kinds of medicinal plants that contain antioxidant and lipid-lowering effect since they are enriched of bioactive compounds that might be effective therapy, safe, and cheap. Among them garlic (*Allium sativum*, family: *Lilliaceae*).

Garlic is well known for its medicinal benefits, especially in helping to prevent cancer and cardiovascular diseases **[8].** Alliins (S-alk(en)yl-L-cysteine sulfoxides) are sources of major active compounds in allium plants. Allicin (diallythiosulfinate) is the main biologically active component of aqueous garlic extract **[9].** Garlic and its compounds which have been reported to have diverse biological activities such as regulating plasma lipid levels, anticarcinogenic, lead and mercury detoxification, antithrombotic, antibacterial, antioxidant, antihypertensive, antidiabetic, and various other biological actions **[10-12].**

Its variety of preparations are widely used as therapeutically effective medicament for cardiovascular diseases. Consumption of garlic and cardiovascular disease progressions is inversely correlated **[13].** Furthermore, garlic has significant antiarrhythmic effect in both ventricular and supraventricular arrhythmias **[14]**. It is reported that chronic use of garlic in moderate doses augments the

endogenous antioxidants activities and depletes the oxidative damaging effects by either increasing the synthesis of endogenous antioxidants or decreasing the generation of oxidants like oxygen free radicals **[15]**. Therefore, the present study aimed to investigate lipid-lowering and antioxidative activities of aqueous garlic extract in heart and liver tissues of rabbits fed a diet rich in cholesterol.

2. Materials and Methods:

1 -Chemicals

Cholesterol (extra pure, powdered; merck,Darmstadt, Germany). Garlic (A. *Sativum*, family: *Lilliaceae*) bulbs were purchased from the local vegetable market (Cairo, Egypt). The cloves were peeled .Peeled garlic (30g) was crushed with distilled water in a mortar. The crushed material was carefully decanted by pressing and 60 ml of aqueous extract was extracted. One milliliter of aqueous extract contained 500 mg of garlic materials **[16].**

All other chemicals that required for the biochemicals assays were of highest purity and analytical grade and purchased from Sigma –Aldrich Chemic (Deisenhofen , Germany).

2- Experimental animals

Twenty-eight male white New Zealand rabbits of about 1000 -1200 g body weight, bred in the Animal House Colony of The National Research Centre, Dokki, Cairo, Egypt. Animals were allowed 7 days for acclimatization at 24ºC with 12 hr light – dark cycle and fed standared laboratory diet and water *ad libitum* before the experiment. Animal procedures were performed in accordance with Guidelines for Ethical Conduct in the Care and Use of Animals.

3-Experimental design

After one week of acclimation, animals were then divided into four groups of rabbits each of seven **Group1** (control group): rabbits fed a plain chow diet. **Group 2**(hypercholesterolemic group): rabbits fed (2 % cholesterol enriched diet) for 4 weeks. **Group 3 :**(Aqueous garlic extract AGE) : rabbits were given orally aqueous garlic extract (500 mg /Kg b.w) daily by stomach tube for 4 weeks, **[16]. Group 4:** rabbits were fed 2% cholesterol – enriched diet in conjunction with AGE (500 mg /Kg b.w / day) orally for 4 weeks.

4- Blood collection and tissue homogenate

At the end of experimental feeding, food was withheld for 16- 18 hr. Blood samples were then withdrawn from marginal ear viens and serum was separated by centrifugation at 3000 rpm. The serum was used for estimation of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides (TG). Also, serum was used for determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and creatin kinase (CK). After that, the animals were slaughtered and their livers and hearts were dissected out immediately and weighted, portions of them were preserved in 10% formalin (pH 7.2) and subjected to histopathological examination. The remaining parts of livers and hearts immediately homogenized in 50 mM ice-cold phosphate buffer (pH 7.4) to give 20% homogenate (w/v) (Lin et al., 1998). The homogenates were centrifuged at 1700 rpm and 4°C and the supernatants (20%) were used for the determination of cardiac and hepatic lipid peroxidation. Further diluted with phosphate buffer solution were done for the determination of cardiac and hepatic catalase (0.5%), superoxide dismutase (0.5%) and glutathione peroxidase (2%) activities.

Biochemical analysis:

Serum TC, HDL-C, LDL-C and TG were estimated spectrophotometerically by the methods of **[17-20],** respectively using commercially available kits obtained from Stanbio Laboratory(USA). Serum ALT and AST activities were measured by the dinitophenylhydrazene (DNPH) method according to **[21]** using commercial kits from Stanbio Laboratory (USA). Alkaline phosphatase, CK and LDH activities in serum were estimated by the method of **[22-24],** respectively using commercial kits from Stanbio Laboratory (USA). Cardiac and hepatic lipid peroxidation were assayed by the measurement of malondialdehyde (MDA) by spectrophotometric method **[25]** using commercial kits from Roche Diagnostics Kits (Germany). The level of lipid peroxidation was expressed as nmol/g.tissue. Cardiac and hepatic catalase (CAT) activities were carried out spectrophotometerically by the modified method of **[26]** using 50 ul diluted cardiac and hepatic homogenates. Cardiac and hepatic superoxide dismutase (SOD) activities were determined spectrophotometerically as the ability of the enzyme to inhibit the phenazine methosulphate- mediated reduction of nitroblue tetrazolium dye by the method of **[27]** using 50 µl diluted cardiac and hepatic homogenates. Cardiac and hepatic glutathione peroxidase (GPx) activities were assayed by spectrophotometric method using reduced glutathione and cumene hydroperoxide as substrate using 20µl diluted cardiac and hepatic homogenates by the modified method of **[28].** Reagent kits for determination of cardiac and hepatic CAT,SOD and GPx activities were purchased from Roche Diagnostics kits (Germany). The specific activities of cardiac and hepatic catalase, superoxide dismutase and glutathione peroxidase were expressed as units/g heart or liver tissue.

Assessment o f heart and liver damage:

Samples of the heart and liver from all animals were fixed in 10% neutral formalin and embedded in

paraffin blocks. Sections (4 µm thickness) were stained with hematoxylin and eosin (H&E) and examined microscopically for detection of histopathological alteration **[29]**.

Statistical analysis:

Data were expressed as mean ±S.E. The data were analyzed by an analysis of variance **(ANOVA)** and the level of significance was determined by Ducan's multiple range tests **[30],** to clarify the significant between the individual groups. *P* values less than 0.05 were considered significant. Results were processed by the computer programs.

3. Results:

As shown in **table (1);** two percent cholesterol diet supplementation for four weeks resulted in a significant elevation ($P<0.001$) of serum TC, HDL-C, LDL-C and TG by (1479, 192, 4031.5 and 329.9 % respectively) compared to the control group. Meanwhile, the combined treatment with (AGE+ cholesterol) resulted in lowering the concentration ($P<0.05$ and $P<0.01$) of serum TC, LDL-C and TG by (52.3, 52.8 and 38.02%) and elevating the concentration ($P<0.05$) of HDL-C by 15.8% compared to the hypercholesterolemic rabbits group. Moreover, our results showed that groups treated with AGE only revealed non- significant changes ($P>0.05$) in these parameters compared to control group.

As shown in **table (2);** there was a significant increase ($P<0.001$) in serum CK and LDH activities by (246.1 and 230%) compared to the control group indicating the severity of cardiac injury caused by cholesterol enriched diet. Treatment with (AGE+ cholesterol) significantly attenuated elevation ($P<0.05$ and $P<0.01$) of these enzymes compared to the hypercholesterolemic group. However, there were no changes in the activity of heart enzymes in rabbits treated with AGE alone compared to the control group ($P>0.05$).

As shown in **table (3);** the activity of AST, ALT and ALP were significantly ($P<0.01$) increased by (23.45, 50 and 22% respectively) in cholesterol group compared to the control group indicating the severity of hepatic injury caused by cholesterol enriched diet. Treatment with AGE alone resulted in no change in the activities of liver enzymes compared to the control group. The activity of the above enzymes were significantly ($P< 0.01$) reversed in groups treated with (AGE+cholesterol) compared to the cholesterol group.

Heart and liver stress and antioxidant enzymes activity:

Malondialdehyde (MDA) level is widely used as a marker of free radical mediated lipid peroxidation injury. As shown in **table (4) and (5);** cardiac and hepatic MDA levels in cholesterol groups were significantly ($P<0.001$) higher by 99.2 and 118.5 respectively, than those in the control group. Treatment with AGE alone resulted in no changes in the levels of cardiac and hepatic MDA compared to the control group. The levels of MDA in different tissues in the groups treated by (AGE + cholesterol) were significantly lower by 42.5 and 57.5 ($P<0.01$) than those in the cholesterol groups.

Cardiac and hepatic CAT, SOD and GPx activities were measured as an index of antioxidant status of cardiac and hepatic tissues. **Tables (4 and 5)** illustrated that in comparison with the control group, there was significant decrease ($P<0.01$) in all antioxidant enzyme activities in both heart and liver in cholesterol groups. The activity of CAT decreased by 52.3 and 42.9 %; SOD activity decreased by 37.7 and 43.6 % ; GPx decreased by 53.2 and 32.3 % in both heart and liver tissues respectively. While there were significant increase ($P<0.05$, $P < 0.01$) in cardiac and hepatic CAT, SOD and GPx activities in the groups treated by (AGE+ cholesterol) compared to the hypercholesterolemic groups. Treatment with AGE alone resulted in insignificant increase in cardiac and hepatic CAT, SOD and GPx activities compared to the control group.

Table (1): Effect of aqueous garlic extract (AGE) and cholesterol on serum lipid in male rabbits

Parameters / Groups	Total cholesterol (mg/dl)	High density lipoprotein cholesterol (mg/dl)	Low density lipoprotein cholesterol (mg/dl)	Triglycerides (mg/dl)
Control	60.48±26.20^{a}	16.3±3.40^{a}	5.4±3.10^{a}	77.6±21.01^{a}
AGE	64.12± 18.10^{a}	17.5±5.10^{a}	6.7±1.20^{a}	73.9±18.90^{a}
Cholesterol	955.30±85.60^{b***}	47.6±12.60^{b***}	223.1±15.4^{b**}	333.6±55.61^{b**}
AGE + cholesterol	455.30±63.10^{c**}	55.1±23.50^{c*}	105.2±4.10^{c**}	206.81±62.07^{c*}

Each value represent the mean ± S.E (n = 7).

Within each column, means superscript with the same letter are not significantly different. *P < 0.05, **P < 0.01 and ***P < 0.001

(b) Significantly different from control group, (c) Significantly different from cholesterol group.

Table (2): Effect of aqueous garlic extract (AGE) and cholesterol on serum enzymes activities of heart in male rabbits

Parameters / Groups	Creatin kinase (U/L)	Lactate dehydrogenase (U/L)
Control	115.41 ± 11.02^{a}	165.75 ± 25.45^{a}
AGE	122.13 ± 12.33^{a}	171.45 ± 10.24^{a}
Cholesterol	399.51 ± 120.16^{b***}	548.08 ± 150.73^{b***}
AGE + cholesterol	175.42 ± 20.55^{c*}	230.27 ± 23.78^{c**}

Each value represent the mean ± S.E (n = 7).
Within each column, means superscript with the same letter are not significantly different. *P < 0.05, **P < 0.01 and ***P < 0.001
(b) Significantly different from control group, (c) Significantly different from cholesterol group.

Table (3): Effect of aqueous garlic extract (AGE) and cholesterol on serum enzymes activities of liver in male rabbits

Parameters / Groups	Aspartate aminotransferase (U/ml)	Alanine aminotransferase (U/ml)	Alkaline phosphatase (U/ml)
Control	30.75 ± 4.95[a]	29.61 ± 6.15[a]	121.3±4.72[a]
AGE	34.12 ± 5.22[a]	33.11 ± 7.20[a]	123.4±2.63[a]
Cholesterol	92.51 ± 12.24[b***]	81.46 ± 6.15[b***]	311.1±12.4[b***]
AGE + cholesterol	46.44 ± 7.58[c**]	53.62 ± 2.39[c**]	269.3±8.21[c**]

Each value represent the mean ± S.E (n = 7).
Within each column, means superscript with the same letter are not significantly different. **P < 0.01 and ***P < 0.001
(b) Significantly different from control group, (c) Significantly different from cholesterol group**.**

Table (4): Effect of aqueous garlic extract (AGE) and cholesterol on some antioxidant / oxidative markers in cardiac tissue of male rabbits .

Parameters / Groups	Malondialdehyde (nmol/g tissue)	Catalase (U/g tissue)	Superoxide dismutase (U/g tissue)	Glutathione peroxidase (U/g tissue)
Control	63.11 ± 1.40[a]	1052 ± 50.2[a]	204.12 ± 21.30[a]	66.4 ± 3.02[a]
AGE	61.33 ± 2.34[a]	1080± 30.11[a]	213.14 ± 18.20[a]	79.1 ± 6.35[a]
Cholesterol	125.72 ± 1.81[b***]	501.70 ± 15.30[b**]	127. ± 10.50[b**]	31.15 ± 1.61[b**]
AGE + cholesterol	72.22 ± 1.23[c**]	520.15 ± 9.04[c*]	164.55+8.30[c*]	45.11 ± 3.18[c*]

Each value represent the mean ± S.E (n = 7).
Within each column, means superscript with the same letter are not significantly different. *P < 0.05, **P < 0.01 and ***P < 0.001
(b) Significantly different from control group, (c) Significantly different from cholesterol group**.**

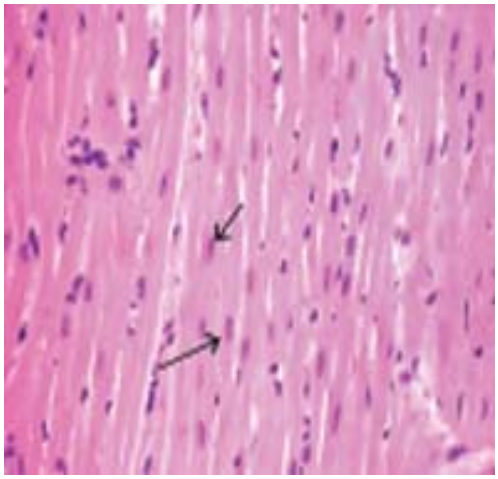
Table (5): Effect of aqueous garlic extract (AGE) and cholesterol on some antioxidant / oxidative markers in hepatic tissue of male rabbits

Parameters / Groups	Malondialdehyde (nmonl/g tissue)	Catalase (U/g tissue)	Superoxide dismutas (U/g tissue)	Glutathione peroxidas (U/g tissue)
Control	21.01 ± 2.03[a]	2014 ± 81.3[a]	380 ± 25.1[a]	82.04 ± 5.20[a]
AGE	23.9 ± 1.52[a]	2094 ± 97.1[a]	400 ± 16.7[a]	95.59 ± 8.83[a]
Cholesterol	45.9 ± 7.18[b***]	1150 ± 70.40[b**]	214 ± 11.6[b**]	55.52 ± 4.51[b**]
AGE+cholestrol	19.51 ± 1.02[c**]	1431 ± 60.8[c*]	341 ± 13.4[c**]	63.48 ± 7.97[c*]

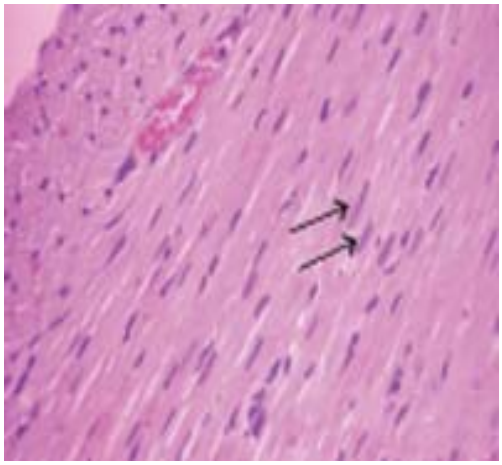
Each value represent the mean ± S.E (n = 7).
Within each column, means superscript with the same letter are not significantly different. *P < 0.05, **P < 0.01 and ***P < 0.001
(b) Significantly different from control group, (c) Significantly different from cholesterol group**.**

Histopathological examination:

The histological examination of rabbit heart sections as shown in **Fig.1**.(A): Heart of a control rabbit showing normal striated muscles.Oval elongate nucleus centrally (arrows) and homogeneous cytoplasm in normal myocardial cells. (B): Heart sections of rabbit treated with AGE (500 mg/Kg b.wt) orally once daily for four weeks showing structure as seen in control. (C,D): Heart sections of a rabbit fed with high – cholesterol diet (2% cholesterol) demonstrating multi-focal vacuolar degeneration (arrows) and necrosis of myocardial cells as well as separation of cardiac muscle bundles. (E): Heart of a rabbit treated with AGE plus cholesterol showing better- preserved appearance of myocardial cell morphology with oval – elongate nucleus centrally (arrows) and homogeneous cytoplasm. Also, the histological examination of rabbit liver sections as shown in **Fig.2**. (A): Liver of a control rabbit showing normal hepatocytes architecture. Hepatocyte had the round nucleus centrally (arrows) the flat endothelial cells are around the central vien (CV). (B): Liver of rabbit treated with AGE (500 mg /Kg b. wt) orally once daily for four weeks, showing normal hepatocytes architecture. (C): Liver section of rabbit fed with high-cholesterol diet (2% cholesterol) demonstrating diffuse vacuolar degeneration fat vacuoles (arrows),necrosis of hepatocytes and markedly focal fibrosis, leading to disintegration of hepatic cords. (D): Liver of a rabbit treated with AGE plus cholesterol showing less injury of central vein and less fat vacuole(arrows) comparing to high cholesterol rabbit.



(A)



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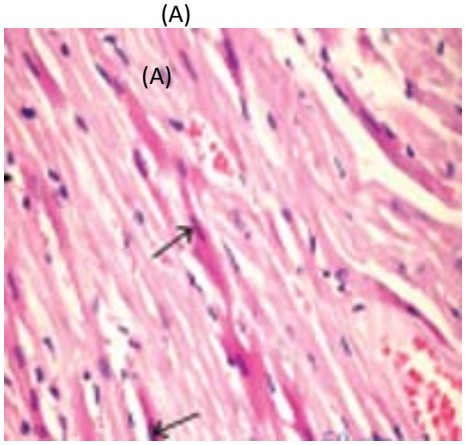
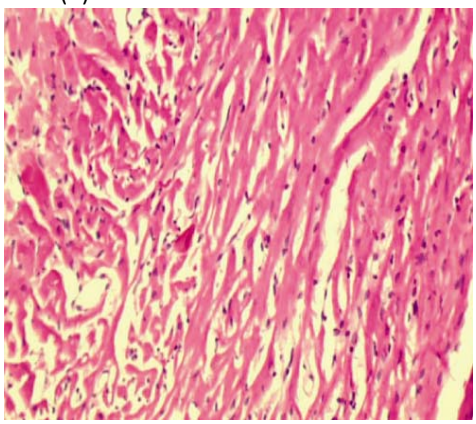
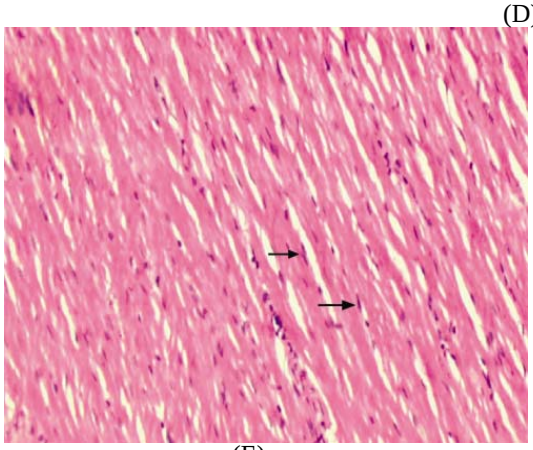


(C)

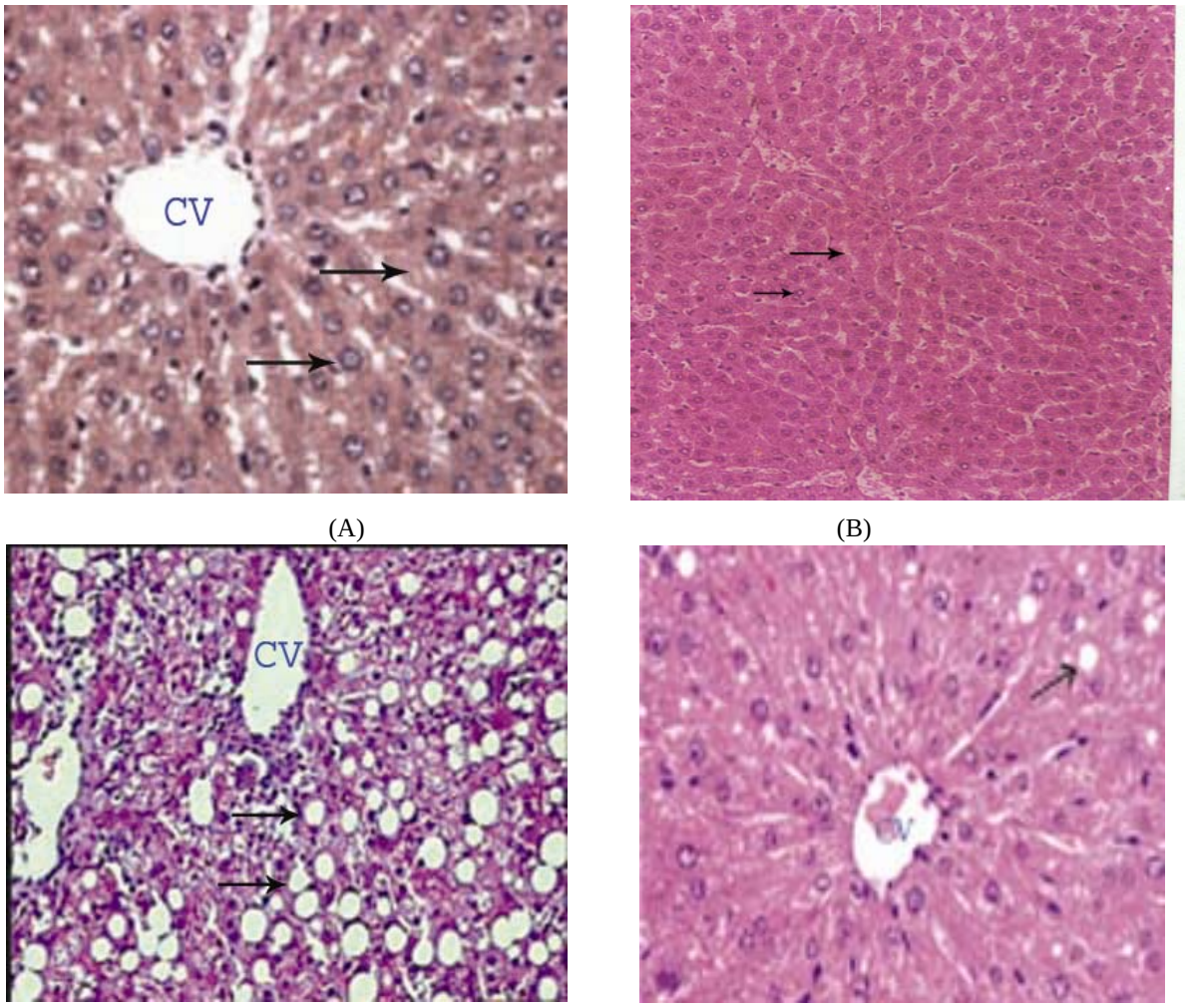


(D)



(E)

Fig. 1: Photomicrograph of the heart section of (A): control rabbit heart showing normal striated muscles.Oval elongate nucleus centrally and homogeneous cytoplasm in normal myocardial cells. (B): heart sections of rabbit treated with AGE showing structure as seen in control. (C,D): heart sections of a rabbit fed with high –cholesterol diet demonstrating multi- focal vacuolar degeneration and necrosis of myocardial cells as well as separation of cardiac muscle bundles.(E): heart of a rabbit treated with AGE plus cholesterol showing better- preserved appearance of myocardial cell morphology with oval –elongate nucleus centrally and homogeneous cytoplasm.(H & E X 400).



(A) (B)

(C) (D)

Fig.2: Photomicrograph of the liver section of (A): control rabbit showing normal hepatocytes architecture. hepatocyte had the round nucleus centrally,the flat endothelial cells are around the central vien (CV).(B): liver of rabbit treated with AGE showing normal hepatocytes architecture.(C): liver section of rabbit fed with high-cholesterol diet demonstrating diffuse vacuolar degeneration fat vacuoles , necrosis of hepatocytes and markedly focal fibrosis, leading to disintegration of hepatic cords.(D): liver of a rabbit treated with AGE plus cholesterol showing less injury of central vein and less fat vacuole comparing to high cholesterol rabbit. (H& E X 400).

4. Discussion:

It has been widely known that elevation of serum cholesterol can lead to atherosclerosis; blood supply to the organs gradually diminishes until organ function becomes impaired. Several lines of evidence show that the improvement and incidence of atherosclerosis and coronary heart disease are associated with a lowering of serum cholesterol level **[1,6].** The consumption of excessive calories and diet containing fatty acids and cholesterol leads to hypercholesterolemia for human or animals **[6].** Hypercholesterolemia induces oxidative stress, which is known to have adverse effects on the integrity of cells **[31,32].**

Our results show that, significant increase in serum TC, TG, LDL-C and HDL-C levels which used as indicators of hypercholesterolemia resulted from feeding rabbits cholesterol supplemented diet. These findings were in the same line as with those results reported by **[33-35].**The significant increase in LDL levels in the hypercholesterolemic rabbits represents the effect of hypercholesterolemia on the oxidative modification of LDL, probably via increased susceptibility to oxidation **[36].** Oxidation of LDL is a lipid peroxidation process resulting in formation of a wide range of biologically active products, including peroxides and malondialdehyde. The oxidatively

modified lipids and their degradation products are belived to have proinflammatory, immunogenic, and cytotoxic properties which contribute to both the initiation and progression of atherosclerotic lesions **[37]**.

In animals treated orally with AGE there was significant decrease in serum TC,TG and LDL-C levels while, there was an elevation in the concentration of HDL-C as compared to hypercholesterolemic group indicating that AGE have hypolipidemic activity **[38,39]** and also, it could be effective to alleviate atherosclerosis which then eventually prevents the occurrence of cardiovascular diseases **[8].**

Our findings were in line with **[40]** who found that treatment rabbits with garlic extract (1.5 ml/kg/ day) orally for 4 months caused significant decrease in the serum total- cholesterol and triglyceride. Also, **[38]** revealed that (1% garlic powder supplement) for 12 weeks resulted in lowering the concentration of TC, TG ,LDL-C and elevation of the concentration of HDL-C, which has a protective function in the prevention of oxidation reactions and consumption of antioxidant potency. **[41]** found that allicin (diallyl disulfide) which an active constituent of garlic can lower the serum lipid profile in hyperlipidemic rabbits.

Also, it was found that aqueous garlic extracts showed higher antioxidant activity than those extracted with different polarity solvents.Aqueous extracts were used as a supplementation to diet-treated animals. This verifies other studies showing that the water- soluble S-allylcysteine reduces the extent of lipid peroxidation and significantly enhances antioxidant activities in vitro and in vivo **[42,43].** **[44]** reported that, garlic consumption decreased serum total cholesterol and TG but increased HDL cholesterol in hyperlipidemic individuals and animals. Also, some studies **[33,45,46]** suggest that the use of garlic can prevent the formation of atherosclerotic lesions in animals fed a high –fat diet. The active ingredients of garlic, allicin, and other sulfur compounds may act as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors reducing the production of cholesterol in the liver **[40]** and reduce platelet aggrication and clotting **[47].**

Antioxidants and hypolipidemic agents suppress the development of hypercholesterolemic atherosclerosis and induce regression of atherosclerosis **[48].** Suppresses the development of atherosclerosis is associated with decreases in oxidative stress and serum lipids **[49].** Also, the increase in HDL-C levels represents a protective factor and are associated to decreased risk for atherosclerosis **[35].**

It is widely known that the heart and liver are primary organs at risk from hypercholesterolemia. The present results showed that cholesterol fed rabbits produced heart injury as indicated by marked elevation of serum CK, LHD activities as well as markedly pathological changes of myocardiocytes including multi-focal vacuolar degeneration and early necrosis of myocardial cells as well as separation of cardiac muscle bundles. These changes were associated with a decrease in the antioxidant defense as manifested by the significant increase in lipid peroxidation of cardiac tissue and a significant decrease in the activities of antioxidant enzymes activity of SOD, CAT, GPx compared with control. This increase in serum CK and LDH might indicate the leakage of these enzymes through the membranes which are widely used as paramaters for the diagnosis of cardiac dysfunction and the increase of CK level in serum and myocytes culture media as a result of possible cell damage is occurring in concert with the decrease of CK activity in cardiac tissue **[50].** Our results also revealed that cholesterol-fed rabbits produced liver injury as indicated by marked elevation in serum hepatic enzyme levels AST, ALT and ALP associated with markedly histological changes consisted of diffuse vacuolar degeneration; fat vacuoles and necrosis of hepatocytes and markedly focal fibrosis as well as a marked decrease in the antioxidant defense system as manifested by the significant increase in lipid peroxidation and a significant decrease in the activity of antioxidant enzyme activity of SOD, CAT, GPx. These results are in agreement with **[40,51]** who reported that cholesterol-fed rabbits increased serum hepatic enzyme levels AST, ALT and ALP due to increased production of free radicals, which initiate lipid peroxidation leading to cellular damage as a result of induction of cytochrom P-450 in the liver producing highly reactive trichloromethyl free radical which in turn, in the presence of oxygen generated by metabolic leakage from mitochondria causing lipid peroxidation of membrane lipid leading to loss of integrity of cell membranes and damage of hepatic tissue

Animals treated with AGE along with cholesterol showed significant decrease in serum CK and LDH as well as significant decrease in serum hepatic enzymes as compared to cholesterol-enriched diet group indicating that AGE had a free radical scavenging activity which probably provides organs protection from hypercholesterolemia **[44].** So, the current results suggest that garlic exerts hypocholesterolemic or antiatherogenic.

[38] observed that, garlic supplemented lowered the aortic and hepatic cholesterol and triglycerides of rabbits. Moreover, the study of **[47]** revealed that garlic has antioxidant activity and inhibits platelet aggregation and lowering of arterial blood pressure, which are the important events in myocardial infarction. Also, **[40]** reported that rabbits treatment with AGE may contribute to significant amelioration in hepatic steatosis and peroxidation process. Also, **[52]** demonsterated that aqueous GE with its potent free radical scavenging and antioxidant properties seems to

be a highly promising agent in protecting hepatic tissue against oxidative damage.

Rabbits fed cholesterol –enriched diet showed significant decrease in the activities of cardiac and hepatic SOD, CAT and GPx accompanied by significant increase in both cardiac and hepatic lipid oxidation (as measured by malondialdehyde (MDA) content) in heart and liver of rabbits. These results are in accordance with **[53]** who reported that oxidation of cholesterol fractions (in particular, LDL) has been accepted as playing an important role in the atherosclerotic process, and because lipid peroxidation is a radical process implicated in this formation. Also with **[40] who** observed that cholesterol-induced steatosis leads to a weakened antioxidant defence system and caused peroxidation in the hepatic tissue.In addition, oxidative stress disrupts the equilibrium between prooxidants and antioxidants in biological systems and leads to lipid peroxidation and free radical generation **[32].** The level of malondialdehyde (MDA) is considered as a biomarker of lipid peroxidation **[54]**. **[47]** observed that, during myocardial infarction, superoxide radicals generated at the site of damage, modulates SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages myocardium. Obtained results revealed that treatment of rabbits with AGE leads to significant increase in cardiac and hepatic CAT, SOD and GPx activities accompanied with significant decrease in cardiac and hepatic MDA level. The water soluble S-allylcysteine sulfoxide (alliin), a bioactive compound of garlic, reduces the extent of lipid peroxidation and significantly enhanced antioxidant activities in vitro and in vivo **[42, 33, 55].** It is known that SOD catalyses superoxide anions to hydrogen peroxide, which is broken down by CAT and GPx, and then prevents further generation of free radicals. Garlic pretreatment increased the activity of SOD and CAT and it scavenges superoxide radicals and reduced myocardial damage caused by free radicals. **[53, 33]**.

Also, garlic extracts increased superoxide dismutase (SOD) and catalase(CAT) activities in vascular cultured cells. S-allylcysteine sulfoxide (alliin), a bioactive compound of garlic, prevented the reduction of hepatic SOD and CAT activities in diabetic rats **[33].** Also, it has been reported that garlic significantly lowered plasma and erythrocyte MDA levels while increasing antioxidant enzyme activity in elderly subjects **[55].**

The present study revealed that, AGE was not only able to lower the serum lipid profile but also suppress the high serum levels of CK, LDH, AST, ALT,and ALP. Moreover, lipid peroxidation was markedly suppressed, whereas the activities of antioxidant enzymes increased in both the cardiac and hepatic tissues of rabbits. In addition, AGE had a free radical scavenging activity which probably provides organs protection from hypercholesterolemia which were supported by histological examination of myocardiocytes and hepatocytes. There for it could be concluded that AGE may be of therapeutic importance, not only as a lipid-lowering agent in serum but also as a cytoprotective agent to protect the heart and liver from hypercholesterolemia.

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5/2/2012

Comparison of Inferotemporal Approach and the Medial Canthus Approach with Short Needle Length in Regional Ophthalmic Anesthesia

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Abstract: Background: The usage of short needle in ophthalmic anesthesia is getting more popular with the inferotemporal approach being the most common site tested by the anesthetists. In this study, we compared the efficacy of using short needle (12 mm) in peribulbar anesthesia in two different approaches; inferotemporal approach and the medial canthus approach. **Methods:** 110 patients undergoing elective cataract surgery under local anesthesia were enrolled in this study. They received single injection peribulbar anesthesia with a 12 mm needle. The needle was inserted either into the inferotemporal area or in the medial canthus. Ocular akinesia was assessed 10 minutes after the block using the simple akinesia score. A score of 3 or less was accepted to provide adequate akinesia for the surgical procedure to be performed. If the block was inadequate for surgery after 10 minutes, supplementary anesthesia was provided using the same needle. **Results:** There was high statistically significant difference with respect to the volume injected, being higher in group 1 compared to group 2 (7.91±0.92 and 7.350.±97 respectively). No significant differences were noted between groups with respect to supplementation, akinesia and complications. **Conclusion:** peribulbar blockade performed either in the inferotemporal area or in the medial canthus using a short 12 mm needle is comparable.
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Keywords: inferotemporal- medial canthus- regional- ophthalmic anesthesia.

1. Introduction

Regional anesthesia is commonly performed for ophthalmic procedures. A variety of local anesthetic techniques have been developed and refined[1]. Peribulbar anesthesia remains a popular choice for patients undergoing cataract surgery[2]. Both non-akinetic and akinetic methods are widely used[3,4]. Non-akinetic methods include topical, subconjunctival, deep fornix anesthesia and lidocaine gel[4]. Althoug akinesia is not essential for modern cataract surgery, some ophthalmic surgeons may prefer to operate on immobile eyes. A study suggests that patients also prefer an akinetic regional ophthalmic block[5]. Single-injection medial canthus periocular anesthesia is a promising technique of ophthalmic regional anesthesia[6]. The aim of this study was to compare inferotemporal approach to medial canthus approach with short needle length (12 mm) in regional ophthalmic anesthesia regarding akinesia and analgesia.

2.Patients and Methods:

After approval of the Ethical Committee of The Research Institute Of Ophthalmology and informed patient written consent, 110 patients of ASA I and II physical status undergoing elective cataract surgery were enrolled in this study. Patients were allocated into two groups (1 and 2); 55 patients each. The two groups received single injection peribulbar block via a 12 mm needle. Group 1 received the injection by the medial canthus approach. Group 2 received the injection by the inferotemporal approach. In the perioperative area, a 22-guage cannula was inserted intravenously in all patients. ECG, pulse oximetry and non-invasive blood pressure monitoring were applied to all patients in the study. The anesthetic mixture used was: in the ratio of 1:1 0.5% bupivacaine and 2% lidocaine to which hyaluronidase 10 units/ml was added. In group 1, the needle was inserted in the most medial part of the medial canthus and introduced forward without piercing the medial check ligament. In group 2, the needle was inserted through the lower eyelid as far lateral as possible in the inferotemporal quadrant until its hub reached the orbital rim. Digital pressure was applied by the thumb and index finger around the needle hub during anesthetic injection. In the two groups, after negative aspiration was done, a volume of 6-8 ml of local anesthetic solution was injected until achieving total drop and fullness of the upper eyelid. Digital compression was applied, using the index and middle fingers of both hands above each other, in the two groups for five minutes. Ocular akinesia was assessed ten minutes after the block using the simple akinesia score. Ocular movements were scored for each direction of gaze in the superior, inferior, medial and lateral directions with a maximum score for each direction of 3 points and a possible total maximum of 12 points (3 = full movement, 2 = moderate movement,

1 = flicker, 0 = no movement). A score of 3 or less was accepted to provide adequate analgesia for the surgical procedure to be performed. If the block was inadequate for surgery after 10 minutes, supplementary anesthesia was provided using the same needle and technique according to the group. Axial lengths were recorded. The volume injected and the needs for supplementary local anesthetic were also recorded as well as any complications occurring during the block.

Statistical Analysis:

Results were expressed as means ± standard deviation (SD) or number (%). Comparison between the mean values different parameters of the two studied groups was performed using unpaired student t test. Comparison between categorical data was performed using Chi square test. The data were considered significant if p values was ≤ 0.05 and highly significant if $p < 0.01$. Statistical analysis was performed with the aid of the SPSS computer program (version 12 windows).

3. Results:

There were 110 patients in this study, 55 in each group. Group 1 received medial canthus injection and group 2 received inferotemporal injection. All patient data were included in the statistical analysis where no patients were excluded. Patient's demographic data in the two groups were similar and no significant differences were detected. (Table 1). The axial length, measured in cms, was similar in both groups (24.39±2.39 in group 1 and 24.55±2.43 cm in group 2) with no statistical significance. The volume injected in both groups showed high statistical significance (7.91±0.92 in group 1 and 7.35± 0.97 in group 2). No statistical significance was detected regarding the need for supplementation of local anesthetic in the two groups where 83.3% in group 1 did not need supplementation in comparison to 76.4% in group 2. No complications were detected in both groups, (Table 2).

Table 1: Demographic Data

Characteristics	Group 1 Medial canthus (n= 55)	Group 2 Inferotemporal (n= 55)	*P* value
Age (yrs)			
Range	30-81	26-78	
Mean ± SD	58.06 ± 11.64	56.44 ± 11.79	0.472 NS
Sex (female/male)	30/25 (55.6%/44.4%)	25/30 (45.5%/54.5%)	0.292 NS
ASA (I/II)	18/37 (33.3%/66.7%)	23/32 (41.8%/58.2%)	0.361 NS

Data are expressed as mean ± standard deviation or number (%).
$p > 0.05$= NS= not significant.

Table 2: Volumes of local anesthetic initially injected, need for supplementation and complications

Characteristics	Group 1 Medial canthus (n= 55)	Group 2 Inferotemporal (n= 55)	*P* value
Axial length	24.39 ± 2.39	24.55 ± 2.43	0.723 NS
Volume injected	7.91 ± 0.92	7.35 ± 0.97	0.002**
Efficiency			
Do not need supplementation	45 (83.3%)	42 (76.4%)	0.365 NS
Need supplementation	10 (16.7%)	13 (23.6%)	0.365 NS
Complication (yes)	0 (0%)	0 (0%)	1.00 NS

Data are expressed as mean ± standard deviation or number (%).
p> 0.05= NS= not significant. **p< 0.01= highly significant.

4. Discussion:

Our results demonstrated that both approaches, the medial canthus and the inferotemporal, were similar in terms of needs for supplementation, akinesia and complications. Also, the study revealed that the use of a 12 mm needle in both groups with digital pressure in group 2 gave a satisfactory degree of akinesia. The satisfactory akinesia produced by either technique can be partly explained by anatomical details. The spread of local anesthetic after periocular injection outside the muscle cone is greatly affected by the connective tissue septa of the orbit[7] . The so-called muscle cone not a

well defined anatomical entity, but is instead composed of a complicated network of muscles and intermuscular connective tissue septa with communicating fat compartments[7]. The only statistically significant difference detected in our study was regarding the volume of local anesthetic initially injected. The volume injected was found to be higher in group 1 (medial canthus group) compared to group 2 (inferotemporal group) (7.91+-0.92 and 7.35+-0.97 respectively). The fascial sheath of the eye ball extends to the rectus muscle sheaths[6]. This explains why a large volume of local anesthetic was preferentially guided to those muscle sheaths to produce good akinesia. In the present study none of the techniques were associated with any major complication. The most frequent side effect was conjunctival chemosis, which did not affect the surgical procedure. All the techniques were associated with acceptable patient satisfaction; additional analgesics during surgery were rarely needed and almost all the patients said they would have their second eye operated upon under regional anesthesia.

In conclusion, the data of the present study suggested that the medial canthus approach and the inferotemporal approach were comparable, with the exception of the volume of local anesthetic initially injected which was found to be higher in the medial canthus group.

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The role of the Chief Knowledge Officer (CKO) in knowledge management implementation (Case study in private banks in Iran)

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Abstract: Many researchers will learn that the knowledge is power. knowledge is an important resource for preserving valuable heritage, Learning new things and new, Solve problems, Create competitive advantage and Establish new positions For the individual and the organization now and for the future. In recent decades has been widely investigated management and knowledge management. At the same time, a wide field of academic research and practical applications has been created. Knowledge management is the process for the flows of knowledge among the people. And it means for achieving innovation in processes, products and services and effective decisions and adapt to the dynamic and competitive market environment. This paper examines the knowledge management and The role of the Chief Knowledge Officer (CKO)in knowledge management implementation Case study in private banks in Iran. The chief task of Chief Knowledge Officer (CKO) that has been made in this study are:1. Create motivations for employees to share their knowledge with others. 2. Create solidarity among the organization members 3. Understanding and appropriate use of technology 4. Creating a learning organization 5. Creation of strategic thinking 6. Create opportunities for sharing and applying knowledge to employees in the organization. Managers of knowledge regarding these components can be efficiently and effectively implement their knowledge management.
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Keywords: knowledge management, Chief Knowledge Officer (CKO), knowledge sharing

1- Introduction

Before we start to explore and understand the details of what knowledge management is, and how to implement knowledge management projects and initiatives, we need to first ask ourselves why we want to consider knowledge management in the first place? What are the real benefits that can be gained from effective knowledge management for the individual, the team, the entire organization, the community, the nation, or even the entire planet Earth Knowledge management is far reaching. Maybe you are considering developing your own personal knowledge management competencies, to become a more effective player in the global knowledge economy, or becoming a more competitive knowledge leader and knowledge driven organization. Maybe you wish to develop and apply knowledge management strategies to government, military operations, global poverty eradication, international disaster management and even, now, knowledge management for global climate change. The list is endless. Knowledge management is applied today across the world, in all industry sectors, public and private organizations and humanitarian institutions and international charities. Most importantly, effective knowledge management is now recognized to be 'the key driver of new knowledge and new ideas' to the innovation process, to new innovative products, services and solutions. Once we can understand the value and benefits to be gained, we will then become far more motivated to look further at the implementation of knowledge management. Doing 'knowledge management' for knowledge management's sake is likely to produce a failure, or mediocre results at very best. Knowledge management, as a discipline, must result in better achieving, or even exceeding, your objectives. The purpose of knowledge management must not be to just become more knowledgeable, but to be able to create, transfer and apply knowledge with the purpose of better achieving objectives.

1. Knowledge management

Knowledge management is the management of the organization towards the continuous renewal of the organizational knowledge base - this means e.g. creation of supportive organizational structures, facilitation of organizational members, putting IT-instruments with emphasis on teamwork and diffusion of knowledge (as e.g. groupware) into

place. Knowledge Management (KM): This is, as the word implies, the ability to manage "knowledge". We are all familiar with the term Information Management. This term came about when people realized that information is a resource that can and needs to be managed to be useful in an organization. From this, the ideas of Information Analysis and Information Planning came about. Organizations are now starting to look at "knowledge" as a resource as well. This means that we need ways for managing the knowledge in an organization. We can use techniques and methods that were developed as part of Knowledge Technology to analyze the knowledge sources in an organization. Using these techniques we can perform Knowledge Analysis and Knowledge Planning. [1]

Knowledge management is an audit of "intellectual assets" that highlights unique sources, critical functions and potential bottlenecks which hinder knowledge flows to the point of use. It protects intellectual assets from decay, seeks opportunities to enhance decisions, services and products through adding intelligence, increasing value and providing flexibility.

Knowledge management complements and enhances other organizational initiatives such as total quality management (TQM), business process re-engineering (BPR) and organizational learning, providing a new and urgent focus to sustain competitive position.

2. The Importance of Knowledge Management

one of the most significant keys to value-creation comes from placing emphasis on producing knowledge. The production of knowledge needs to be a major part of the overall production strategy. One of the biggest challenges behind knowledge management is the dissemination of knowledge. People with the highest knowledge have the potential for high levels of value creation. But this knowledge can only create value if it's placed in the hands of those who must execute on it. Knowledge is usually difficult to access – it leaves when the knowledge professional resigns .The only irreplaceable capital an organization possesses is the knowledge and ability of its people. The productivity of that capital depends on how effectively people share their competence with those who can use it." – Andrew Carnegie Therefore, knowledge management is often about managing relationships within the organization. Collaborative tools (intranets, balanced scorecards, data warehouses, customer relations management, expert systems, etc.) are often used to establish these relationships. Some companies have developed knowledge maps, identifying what must be shared, where can we find it, what information is needed to support an activity, etc. Knowledge maps codify information so that it becomes real knowledge; i.e. from data to intelligence.[2]

For example, AT&T's knowledge management system provides instant access for customer service representatives, allowing them to solve a customer's problem in a matter of minutes. Monsanto uses a network of experts to spread the knowledge around. Employees can look up a knowledge expert from the Yellow Page Directory of knowledge experts. In the book Value Based Knowledge Management, the authors advocate that every organization should strive to have six capabilities working together:

1. Produce : Apply the right combination of knowledge and systems so that you produce a knowledge based environment.
2. Respond : Constantly monitor and respond to the marketplace through an empowered workforce within a decentralized structure.
3. Anticipate : Become pro-active by anticipating events and issues based on this new decentralized knowledge based system.
4. Attract : Attract people who have a thirst for knowledge, people who clearly demonstrate that they love to learn and share their knowledge opening with others. These so-called knowledge professionals are one of the most significant components of your intellectual capital.
5. Create : Provide a strong learning environment for the thirsty knowledge worker. Allow everyone to learn through experiences with customers, competition, etc.
6. Last : Secure long-term commitments from knowledge professionals. These people are key drivers behind your organization. If they leave, there goes the knowledge.[3]

Knowledge professionals will become the dominant force behind the new economy, not unlike the farmer was once the key player behind the agricultural age. By the year 2010, one-third of the workforce in the United States will be comprised of knowledge professionals. It is incumbent upon all organizations to embrace this need for managing knowledge. Just take a look at those organizations that seem to create value against the competition. You will invariably find a strong emphasis on knowledge management.[4]

4. Knowledge Management Cycle

Early research suggested that a successful KM effort needs to convert internalized tacit knowledge

into explicit knowledge in order to share it, but the same effort must also permit individuals to internalize and make personally meaningful any codified knowledge retrieved from the KM effort. Subsequent research into KM suggested that a distinction between tacit knowledge and explicit knowledge represented an oversimplification and that the notion of explicit knowledge is self-contradictory. Specifically, for knowledge to be made explicit, it must be translated into information (i.e., symbols outside of our heads. Later on, Ikujiro Nonaka proposed a model (SECI for Socialization, Externalization, Combination, Internalization) which considers a spiraling knowledge process interaction between explicit knowledge and tacit knowledge. In this model, knowledge follows a cycle in which implicit knowledge is 'extracted' to become explicit knowledge, and explicit knowledge is 're-internalized' into implicit knowledge. More recently, together with Georg von Krogh, Nonaka returned to his earlier work in an attempt to move the debate about knowledge conversion forwards[5].

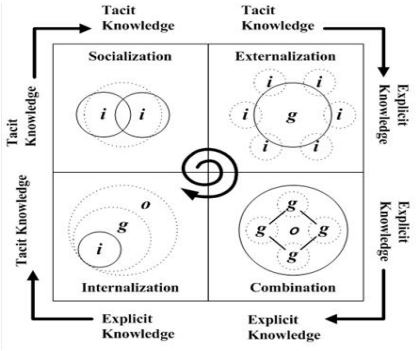
A second proposed framework for categorizing the dimensions of knowledge distinguishes between embedded knowledge of a system outside of a human individual (e.g., an information system may have knowledge embedded into its design) and embodied knowledge representing a learned capability of a human body's nervous and endocrine systems.[6]

A third proposed framework for categorizing the dimensions of knowledge distinguishes between the exploratory creation of "new knowledge" (i.e., innovation) vs. the transfer or exploitation of "established knowledge" within a group, organization, or community. Collaborative environments such as communities of practice or the use of social computing tools can be used for both knowledge creation and transfer. The theory of organizational knowledge creation developed by Nonaka and his colleagues.[7]

The creation of knowledge is a continuous process of dynamic interactions between tacit and explicit knowledge. The four modes of knowledge conversion interact in the spiral of knowledge creation. The spiral becomes larger in scale as it moves up through organizational levels, and can trigger new spirals of knowledge creation.[8]

Socialization. Sharing tacit knowledge through face-to-face communication or shared experience. An example is an apprenticeship.

Externalization. Developing concepts, which embed the combined tacit knowledge. And which enable its communication.



Fig(1): Knowledge Management Cycle

Combination. Combination of various elements of explicit knowledge: building a prototype is an example.

Internalization. Closely linked to learning by doing, the explicit knowledge becomes part of the individual's knowledge base (e.g. mental model) and becomes an asset for the organization.[9]

5. Chief Knowledge Officer (CKO)

CKO is a corporate title for the person responsible for overseeing knowledge management within an organization. The CKO position is related to, but broader than, the chief information officer (CIO) position. The CKO's job is to ensure that the company profits from the effective use of knowledge resources. Investments in knowledge may include employees, processes and intellectual property; a CKO can help an organization maximize the return on investment (ROI) on those investments.[10]

It was Thomas H. Davenport, one of the „founding fathers" of Knowledge Management who has successfully introduced the concept and described the „activity portfolio" of the Chief Knowledge Officer (CKO), fertilizing the discussion about the "knowledge leadership" of an organization (Davenport, 1994). Michael J. Earl and Ian I. Scott created a well-itemized tipology of the CKO's, as integrator and synchronizations of all the relevant aspects of the corporate knowledge flow, building and maintaining a network from knowledge champions, knowledge sponsors, knowledge partners and knowledge skeptics [11]. The expression itself became very popular, but the appearance of CKO's in a corporate leadership hierarchy was very limited in the last decade. Conversely, the sweep of Knowledge Governance could bring the "big time" for the new generation CKO's.[12]

The role of the CKOs is so immature that there is no job specification. Different corporations are likely

to have different expectations of it. So CKOs have had first to work out an agenda for themselves .and they commonly refer to the rapid learning involved[9]This is mainly because their mission or mandate is not clear. everybody here, me included, is on s vertical learning curve about knowledge management Admitted one CKOs [13]. Almost invariably, CKO are appointed by the CEO. One CEO said:"at the time, appointing a CKO was much more of a gut feeling than anything else". In other words, CEOs have appointed CKOs more through intuition and instinct than through analysis or strategic logic.[14]

The CKOs we studied thus had to discover and develop the CEOs implicit vision of how knowledge management would make a difference. On the one hand, the CEOs were thinking boldly;. On the other, they were not thinking in detail. Their goals, however were fairly clear. Usually concerned with correcting one or more of these perceived corporate deficiencies:

- Inattention on the explicit or formal management of knowledge in ongoing operation.
- Failure to leverage the hidden value of corporate knowledge in business development.
- Inability to learn from past failures and successes in strategic decision machining.
- Not creating value or "making money" from knowledge embedded in products or held by employees.[15]

6. The CKOs Network

CKOs spend a lot of time "walking around the organization". In particular, they interact with four type of managers. They look for those who are excited about a particular knowledge management idea or project and thus have identified where improvement is possible and are likely to want to try something now. These are their *knowledge champions*. They also seek to identify from the senior executive cadre those who are enthused by knowledge management. Identify with the concept, and make public statement about it. These are *potential knowledge sponsors* who will invest in and support knowledge management projects.[8]

Surprisingly, several CKOs we studied also spend time identifying executives who are hostile to knowledge management and or the appointment of a CKO. They sense that in a new and as yet ill-defined corporate initiative. Especially one with the CEO's personal support, there will be doubters and reactionaries who must be converted to the cause or avoided for now. These are the *knowledge skeptics*. Finally the CKO, once he or she initiated a project of any substance, will need allies in implementation. Typically, is executives and HR professionals, these are the *knowledge partner*. [16]

Figure (2): The CKOs Network

7. Duties of Chief Knowledge Officer (CKO)

Chief Knowledge Officers have the skills to be able to perform their duties in the organization. Chief Knowledge Officer tasks are:

1. Create motivations for employees to share their knowledge with others.
2. Create correlation between organization members.
3. Understanding and appropriate use of technology.
4. Creating a learning organization.
5. Creation of strategic thinking.
6. Create opportunities for sharing and applying knowledge to employees in the organization.
7. Encourage individual learning and innovative thinking
8. Implement reward plans and incentives
9. Determine what technology is needed for the knowledge management effort and implement these technologies.
10. Put processes in place in order to facilitate the creation of organizational learning.
11. Measure the impact of knowledge management on the business.[17]

8. Method

This research has been done to develop knowledge on the role of CKOs. The purpose of this research is an applied research. How to collect data from the research project, is a descriptive Survey. It aims to assess the

awareness about knowledge management and CKOs.

9. Sample

Statistical population is private sector employees of bank that Knowledge management projects are being implemented in banks and in these organizations CKOs are trying to more effectively and efficiently to implement knowledge management. Sample for this research is knowledge management staff of private banks in Tehran and Semnan city that Including those existences are somehow familiar with this process in the bank that are 42 bachelor and 15 master.

10. Data collection tool

Using data collection tools in the investigation is different, Because the data collection tool to the subject, purpose and research design depends. Basis points in the method of research tools are such as: interviews, library studies and questionnaires were used for data collection. A questionnaire to identify the duties of chief knowledge officer is designed with Likert Scale that the Likert Scale is a five point scale that by SPSS software has been analyzed.

11. Validity of questionnaires

Validity means that we are measuring what we want to measure. There are a number of types of validity including:

- Face Validity - whether at face value, the questions appear to be measuring the construct. This is largely a "common-sense" assessment, but also relies on knowledge of the way people respond to survey questions and common pitfalls in questionnaire design;
- Content Validity - whether all important aspects of the construct are covered. Clear definitions of the construct and its components come in useful here;
- Criterion Validity/Predictive Validity - whether scores on the questionnaire successfully predict a specific criterion. For example, does the questionnaire used in selecting executives predict the success of those executives once they have been appointed; and
- Concurrent Validity - whether results of a new questionnaire are consistent with results of established measures.

To increase the validity of research were reviewed the research literature from the library of theses and research papers and several books. After interviews with managers and experts, research variables are identified and questionnaire was prepared. Finally questionnaire was reformed with faculty advisors consultation.we Ensure that respondents understand the questions in the questionnaire does not have a problem with the final questionnaire it was distributed.

12. Reliability of estimates of questionnaire

Reliability means the consistency or repeatability of the measure. This is especially important if the measure is to be used on an on-going basis to detect change. There are several forms of reliability, including:

- Test-retest reliability - whether repeating the test/questionnaire under the same conditions produces the same results; and
- Reliability within a scale - that all the questions designed to measure a particular trait are indeed measuring the same trait. Questionnaire reliability is measured using

Cronbach's alpha. value 0.89 has acceptable.

13. Analysis of data

13.1. Data analysis tool

In this study, has been used statistical analysis of a specialized SPSS software And the One-Sample Test was used to check Hypothesis.

13.2. Evaluation hypothesis

Evaluation The first hypothesis

Table (1): One-Sample Test To test the role of CKO in Create Motivation

	Test Value = 3					
	t	df	Sig	Mean Difference	95% Confidence	
					Lower	Upper
motivation	6.572	56	.033	.825	.57	1.08

According to the analysis that in Table (1) is shown, the test sig(P-Value) is 0.033 and is smaller than 0.05 and can be concluded that the motivation of a staff is important for duty CKO for the efficiency and effectiveness of knowledge management programs.

Evaluation The second hypothesis

Table (2): One-Sample Test To test the role of CKO in Create correlation

	Test Value = 3					
	t	df	Sig.	Mean Difference	95% Confidence	
					Lower	Upper
associate	3.38	56	.001	.518	.21	.82

According to the analysis that in Table (2) is shown, the test sig(P-Value) is 0.001 and is smaller than 0.05 and can be concluded that the Create correlation is important for duty CKO for the efficiency and effectiveness of knowledge management programs.

Evaluation The third hypothesis

Table (3): One-Sample Test To test the role of CKO in using information technology

	Test Value = 3					
	t	df	Sig.	Mean Difference	95% Confidence	
					Lower	Upper
technology	8.3	56	.030	1.000	.76	1.24

According to the analysis that in Table (3) is shown, the test sig(P-Value) is 0.030 and is smaller than 0.05 and can be concluded that using information technology is important for duty CKO for the efficiency and effectiveness of knowledge management programs.

Evaluation The fourth hypothesis

Table (4): One-Sample Test To test the role of CKO in Creating a learning organization

	Test Value = 3					
	t	df	Sig.	Mean Difference	95% Confidence	
					Lower	Upper
learning	11.99	56	.020	1.140	.95	1.33

According to the analysis that in Table (4) is shown, the test sig(P-Value) is 0.02 and is smaller than 0.05 and can be concluded that Creating a learning organization is important for duty CKO for the efficiency and effectiveness of knowledge management programs

Evaluation The Fifth hypothesis

Table (5): One-Sample Test To test the role of CKO in Creation of strategic thinking

	Test Value = 3					
	t	df	Sig.	Mean Difference	95% Confidence	
					Lower	Upper
strategic	12.543	56	.015	1.193	1.00	1.38

According to the analysis that in Table (5) is shown, the test sig(P-Value) is 0.015 and is smaller than 0.05 and can be concluded that Creation of strategic thinking is important for duty CKO for the efficiency and effectiveness of knowledge management programs.

Evaluation The sixth hypothesis

Table (6): One-Sample Test To test the role of CKO in Creating Development opportunities

	Test Value = 3					
	t	df	Sig.	Mean Difference	95% Confidence	
					Lower	Upper
opportunity	18.0	56	.043	1.421	1.26	1.58

According to the analysis that in Table (6) is shown, the test sig (P-Value) is 0.043 and is smaller than 0.05 and can be concluded that Creation of strategic thinking is important for duty CKO for the efficiency and effectiveness of knowledge management programs.

Conclusion

Organizations are realizing that intellectual capital or corporate knowledge is a valuable asset that can be managed as effectively as physical assets in order to improve performance. The focus of knowledge management is connecting people, processes and technology for the purpose of leveraging corporate knowledge. The database professionals of today are the Knowledge Managers of the future, and they will play an integral role in making these connections possible.

Based on the obtained results can be concluded that knowledge is a public good and is not Assets to maintain a superiority over another, and has overflowed its positive effects. Knowledge management is a new branch of management that emphasizes the knowledge.Material and physical assets to knowledge assets will shift. KM collects all information and knowledge around an organization systematically and analyzes Knowledge to achieves goals.that has been made in this study are:1. Create motivations for employees to share their knowledge with others. 2. Create solidarity among the organization members 3. Understanding and appropriate use of technology 4. Creating a learning organization 5. Creation of strategic thinking 6. Create opportunities for sharing and applying knowledge to employees in the organization. Managers of knowledge regarding these components can be efficiently and effectively implement their knowledge management.

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The Influence of an Eight-Week Whirling-Kung Training Course on the Heart Rate Variability

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Abstract: The purpose of this study is to explore the influence of an eight-week whirling-kung training course on the heart rate variability (HRV) in order to know whether or not practicing whirling-kung continuously for 5 to 15 minutes three times a week is helpful to physical and mental health, indicated by the HRV indicators of the subjects. Results from paired-samples t tests show that in the whirling-kung group and the walking group, the HRV components such as SDNN, TP and HF did not increase significantly as hypothesized, while LF did not decrease significantly as hypothesized. Perhaps the relatively small sample sizes and the insufficient training courses have led to these consequences. However, the SDNN and TP in the control group has a statistically significant drop ($p<0.05$), compared to their rise in both the treatment group 1 and 2, though not statistically significant, which might suggest, like the walking group (treatment group 2), whirling might have avoided the dropping of the SDNN and TP.
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Keywords: heart rate variability, whirling-kung, whirling-kung training course

1. Introduction

The "whirling-kung" in this study originates from Sufi Whirling[1], which was the major spiritual practice of Mawlawiyah, a Muslim group founded by Rumi al-Balkhi, Maulana Jalauddin[2], who died in 1273 B.C. People who practice Sufi Whirling are called Whirling Dervishes [1].

Since it was introduced to Taiwan a few years ago[3], Sufi Whirling has developed from a religion-related activity into a kind of exercise to promote body-mind balance, healing and health, just as Tai-chi has developed from a type of martial art to an activity for body training, healing, and even self-cultivation. To avoid religious connotation, we use the term "whirling-kung" to differentiate it from Sufi Whirling. The root "kung" is taken from the word kung-fu, to express its efficacy of promoting body-mind fitness through whirling. This research gave up using the term "whirling chikung" because the term chi-kung (or qi-gong) has long been overly applied and become too ambiguous in meaning.

Compared to many other activities, whirling-kung is easy to practice without complicated procedures and no equipment is needed. While practicing whirling-kung, the whirler stands upright and spins counterclockwise or clockwise on their own feet continuously for a certain period of time. The minimum time required for whirling is not our current concern and is in need of further study in the future.

What really concerns us is the influence of whirling-kung on human health, since whirling-kung is considered as an exercise to promote body-mind balance, healing and health. However, from our literature review, we did not find any rigorous research to tap into this issue. There are only numerous witnesses and self-reports by those who practice Sufi-whirling or whirling-kung. Therefore, in this research, the researchers intended to explore this issue through rigorous research.

To achieve this end, after literature review, we decided to use heart rate variability (HRV) as a simple measure of health. The Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (TFESC & NASPE) [4] claimed that 20 years before, the world has seen the recognition of a significant relationship between the autonomic nervous system and cardiovascular mortality. Evidence found in experiments for relationship between a propensity for lethal arrhythmias and signs of either increased sympathetic or reduced vagal activity has caused the development of quantitative markers of autonomic activity, of all which heart rate variability (HRV) is one of the most promising ones.

There are several ways to perform the evaluation of HRV, the easiest of which are perhaps the time domain measures. In a continuous electrocardio-graphic (ECG) records, the researcher can detect each QRS complex (as shown in Figure 1), and determine the normal-to-normal (NN) intervals (i.e. all intervals between adjacent QRS complexes), or the instantaneous heart rate. Among others, the simplest variable to calculate is the standard deviation of the NN interval (SDNN).

However, the durations of the recordings used to determine SDNN values, as well as other HRV measures, should be standardized and short-term 5-minute recordings and nominal 24 hour long-term recordings appear to be appropriate options.

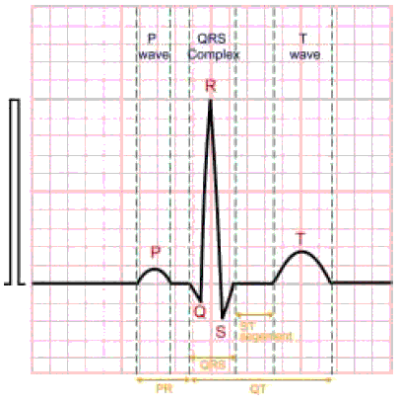

Figure 1 QRS complex: from the start of Q wave to the end of S wave (from: DailyCare BioMedical Inc.)

TFESC & NASPE [1] has summarized the variety of time–domain measures of HRV in Table 1 and has also listed selected frequency–domain measures in Table 2.

2.The HRV indexes used in this research

World Health Organization [5] defines healthy as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity." According to Merriam-Webster [6] it is "the condition of being sound in body, mind, or spirit; especially: freedom from physical disease or pain." It has been found that, in poor health conditions such as aging, septicemia, chronic obstructive pulmonary disease, chronic renal failure, diabetes, hypertension, coronary heart disease, myocardial infarction or heart failure, HRV decreases. A lot of researchers have also suggested that HRV serves as an index of the autonomic activity, especially that of parasympathetic nervous systems. Autonomic, or involuntary, system regulates the visceral activity of the body, and it is composed of sympathetic and parasympathetic nervous systems [7]. The sympathetic nervous system is always on the alert for emergencies, while parasympathetic nervous system mainly reserves the energy of the body. Some researchers also came to similar conclusion [8]: Using all measures, HRV of healthy subjects declines with aging, with measure-dependent patterns. Using the SDNN index, rMSSD and pNN50, HRV of healthy subjects, particularly those >65 years old, may decrease to below levels associated with increased risk of mortality. In other words, the extent of decrease of parasympathetic activity correlates with aging or the seriousness of bad health.

In order to assess whether whirling-kung training would improve human health, we found that there are many researches that use HRV as measures to assess the influence of certain type of practices on HRV. In a typical example of this research genre [9], the researchers investigate changes in autonomic nervous function through Qi-training. The power spectrum of heart rate variability (HRV) was examined in 20 sedentary healthy subjects and 20 Qi-trainees. It was found that Qi-training in healthy young subjects during controlled respiration increases the high frequency (HF) power and decreases the low frequency / high frequency (LF/HF) power ratio of HRV. These results support the hypothesis that Qi-training increases cardiac parasympathetic tone. In addition, Qi-trainees were found to have higher parasympathetic heart modulation compared with their age-matched, sedentary counterparts. This augmented HRV in Qi-trainees provides further support for long-term Qi-training as a possible non-pharmacological cardio-protective maneuver. In conclusion, Qi-training may stabilize the autonomic nervous system by modulating the parasympathetic nervous system. In another research [10], the researcher has found that Tai-chi has a short-term effect of raising HF and dropping LF and suggested older people do Tai-chi to become healthy.

Given the similarity between whirling-kung training and the above research genre, the researchers have inferred that whirling-kung may be able to increase HRV (SDNN and TP) and parasympathetic activity (HF), and at the same time decrease sympathetic activity (LF), all of which seem to contribute to better physical and mental health. That's also why the above four indexes of HRV (SDNN, TP, LF and HF), are adopted in this research. RMSSD and pNN50 correlate between themselves and with HF power (r = 0.96) [11].

Table 3 summarizes why this research used SDNN, TP, LF, and HF as the Autonomic nervous activity indexes.

The purpose of this research is to develop an eight-week whirling-kung training course and to test its influence on the heath of the mind and the body by evaluating some of the HRV measures (SDNN, TP, LF and HF). If the efficacy is significant, the researchers will continue to design different courses for different ages so as to provide the busy modern people with one more pastime which is good for the body and the mind, and at the same, is easy to take up.

3.The research framework and hypotheses

This research includes three groups of subjects, in which the treatment group 1 undergoes an eight-week whirling-kung training course, the treatment group 2 undergoes the same length of time of walking, and the control group just live a normal life. Whirling-kung was assigned as the independent variables.

Table 1 Selected time-domain measures of HRV

Variable	Units	Description
SDNN	ms	Standard deviation of all NN intervals
SDANN	ms	Standard deviation of the averages of NN intervals in all 5-minute segments of the entire recording
RMSSD	ms	Square root of the mean of the sum of the squares of differences between adjacent NN interval
SDNN index	ms	Mean of the standard deviations of all NN intervals for all 5 min segments of the entire recording.
SDSD	ms	Standard deviation of differences between adjacent NN intervals
NN50 count		Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording. Three variants are possible counting all such NN intervals pairs or only pairs in which the first or the second interval is longer.
pNN50	%	Percent of difference between adjacent NN intervals that are greater than 50 ms

Given the variety of HRV indicators, the researchers have chosen four of them that are most likely connected to practicing whirling-kung: SDNN, LF, HF, and TP.

As shown in Figure 2, there are three hypotheses in this research, as follows:

Table 2 Selected frequency domain measures of HRV

Variable	Units	Description Analysis of short-term recordings (5 min)	Frequency range
5 min total power	ms^2	The variance of NN intervals over the temporal segment	approximately≤0·4 Hz
VLF	ms^2	Power in very low frequency range	≤0·04 Hz
LF	ms^2	Power in low frequency range	0.04-0.15Hz
LF norm	n. u.	LF power in normalized units LF/(Total Power–VLF) ×100	
HF	ms^2	Power in high frequency range	0.15-0.4Hz
HF norm	n. u.	HF power in normalized units HF/(Total Power–VLF) ×100	
LF/HF		Ratio LF [ms^2]/HF [ms^2]	

Table 3 The autonomic nervous activity indexes adopted in this research

Autonomic Nervous Activity Indexes	Description
(1) SDNN	A global index of HRV and reflects all the long-term components and circadian rhythms responsible for variability in the recording period
(2) TP	The total variance and corresponds to the sum of the four spectral bands, LF, HF, ULF and VLF
(3) LF	An increase of LF has been generally considered to be a consequence of sympathetic activity
(4) HF	Generally defined as a marker of vagal modulation. This component is respiration-mediated and thus determined by the frequency of breathing

(1) After a consecutive eight-week whirling-kung training, the SDNN, HF, and TP in the treatment group 1 will rise significantly.
(2) After a consecutive eight-week whirling-kung session, the LF in the treatment group 1 will drop significantly.
(3) After a consecutive eight-week whirling-kung session, there are statistically significant differences among the post-test scores of the SDNN, HF, LF, and TP of the treatment group 1, the treatment group 2 and the control group.

4. Research methods

4.1 The Subjects

The subjects in this study are 36 senior high school freshmen or juniors from Hualien, Taiwan. To exclude the variable of gender, all of the subjects are female. In this research, there are 24 voluntary

subjects randomly assigned as the treatment group 1 (practicing whirling-kung) and the treatment group 2 (walking), each with 12 subjects. In addition, the researchers have also recruited another 12 voluntary subjects and assigned them as the control group (with no experimental intervention). The subjects who are in the eight-week whirling-kung group have to open their eyes while whirling, rotate counterclockwise on their own axis, instead of revolving around a center, in order to avoid being similar to the control group.

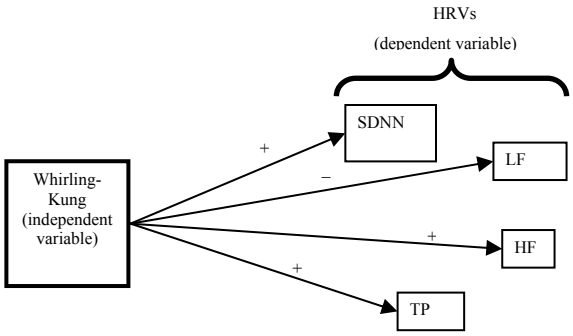

Figure 2 The Research Framework

4.2 Data Collection Method

The researchers used CheckMyHeart by Daily Care BioMedical, Inc. to measure and analyze HRV.

4.3 The Design of experiments

Twenty four voluntary subjects were randomly assigned as the whirling group (the treatment group 1, shown as X in Figure 3) and the walking group (the treatment group 2, shown as Z in Figure 3). Voluntary subjects were manually assigned as the normal living group (the control group, shown as C in Figure 3). Besides, in the treatment group 1, the duration of whirling was increased gradually from 5 minutes per session, 10 minutes per session, up to 15 minutes per session in three different stages respectively, in order to help some of the subjects gradually overcome their fear for whirling. In the treatment group 2, the duration of walking was also increased correspondingly.

The duration of the training course (i.e. the treatment) was 8 weeks, with three sessions per week. As a result, there were 24 sessions, which was divided into three stages (with 8 sessions per stage), as shown in Figure 3.

4.3.1 The Treatment Group 1 (The Whirling-kung Group)

The subjects may take turn spinning in the following three fashions. This research has no intent to probe the difference among different ways of whirling:

1. Use the left foot as the axis and move the right foot forward.
2. Use the right foot as the axis and move the left foot backward.
3. Use the body as the axis and move the left foot backward and the right foot forward simultaneously.
4. Control the whirling velocity according to the background music for whirling.
5. Move or position the arms at will.

Before the whirling session ends, the subjects may slow down gradually in accordance with the music rhythm until a full stop, or keep whirling and stop suddenly and completely when the music ends.

X	M	X1	M	X2
	M	X3	M	
Z	M	Z1	M	Z2
	M	Z3	M	
C	M		M	
	M		M	

X1: Whirling 5 minutes per session
X2: Whirling 10 minutes per session
X3: Whirling 15 minutes per session
Z1: Walking 5 minutes per session
Z2: Walking 10 minutes per session
Z3: Walking 15 minutes per session
M: Measurement of HRV

Figure 3: The design of experiments

4.3.2 The Treatment Group 2 (The Walking Group)

The subjects in the treatment group 2 perform an eight-week consecutive walking sessions in the same place as the treatment group 1 in the following manner:

1. While walking, the subjects have to walk in a large circle counterclockwise around the center of the place, in a normal "walking" fashion.
2. While walking, both feet are not allowed to lose contact with the floor at the same time.
3. While walking, the subjects are not allowed to rotate on their own axis in order to avoid being similar to the treatment group 1.
4. While walking, the subjects are required to walk in accordance with the tempo of the accompanying music to control the speed.
5. Before the walking session ends, the subjects may slow down gradually in accordance with the music rhythm until a full stop, or keep walking and stand still

suddenly and completely when the music ends.

4.3.3 The Control Group (The Normally Living Group)

The subjects only have to lead a normal life, with neither whirling nor walking consecutively. However, during the eight-week research session, they are required to avoid spinning continuously like doing whirling-kung for more than five minutes.

4.3.4 Controlling Interference Variables

1. All the subjects are not allowed to run or jog for a certain amount of time and distance during the eight-week research.
2. During the eight-week research, no subjects are allowed to take part in any other new physical activity or similar program.
3. The treatment group 1 and the treatment group 2 have to carry out the research plan simultaneously in the same place for the same length of time.
4. Each time when the experiment starts, the treatment group 1 and the treatment group 2 have to move to the music. When the experiment ends, all the subjects are allowed to stay where they are and take a rest.

4.3.5 The Duration of the Experiment

The experiment was conducted from approximately 12:45 to 13:15 on Mondays, Wednesdays, and Fridays within an eight-week period between March and May in 2011. The first to the eighth sessions lasted 5 minutes, and the ninth to the sixteenth sessions lasted 10 minutes, and the seventeenth to the twenty-fourth sessions lasted 15 minutes.

5. DATA PROCESSING AND STATISTICAL ANALYSIS

The data acquired from the study have been processed by SPSS17.0 to test and analyze the following:

1. Paired-samples t tests for the three groups, to see whether there are statistically significant differences between their pre-tests and post-tests.

2. ANCOVA, using pre-tests as covariances to find out whether there are statistically significant differences among the three groups.

3. Set α=0.05.

5.1 Paired-samples t tests for the treatment group 1 (whirling)

The paired-samples t tests for treatment group 1 are as follows:

For SDNN of the treatment group 1, $t(11) = -0.597$, $p > .05$, so there is no statistically significant difference between the pre-test and the post-test. For LF, $t(11) = 0.201$, $p > .05$, so there is no statistically significant difference between the pre-test and the post-test. For HF, $t(11) = -1.240$, $p > .05$, so there is no statistically significant difference between the pre-test and the post-test. For T, $t(11) = -0.703$, $p > .05$, so there is no statistically significant difference between the pre-test and the post-test.

5.2Paired-samples t tests for the treatment group 2 (walking)

During the experiment, one of the subjects in the group dropped out, so the sample size became 11. The paired-samples t tests for the treatment group 2 are as following:

For SDNN of the treatment group 2, $t(10) = -0.605$, $p > .05$, so there is no statistically significant difference between the pre-test and the post-test. For LF, $t(10) = -1.338$, $p > .05$, so there is no statistically significant difference between the pre-test and the post-test. For HF, $t(10) = 0.409$, $p > .05$, so there is no statistically significant difference between the pre-test and the post-test. For T, $t(10) = -0.691$, $p > .05$, so there is no statistically significant difference between the pre-test and the post-test.

5.3Paired-samples t tests for the control group (normal)

During the research, one of the subjects in the group dropped out, and HRV data of two of them had been unable to be measured, so the sample size became 9. The paired-samples t tests for the control group are as following:

For SDNN of the control group, $t(9) = 2.640$, $p = .030 < .05$, so there is a statistically significant difference between the pre-test and the post-test. For LF, $t(9) = 1.855$, $p = .101 > .05$, so there is no statistically significant difference between the pre-test and the post-test. For HF, $t(9) = 1.997$, $p = .081 > .05$, so there is no statistically significant difference between the pre-test and the post-test. For T, $t(9) = .042 < .05$, so there is a statistically significant difference between the pre-test and the post-test.

5.4 ANCOVA

Analysis of covariances (ANCOVA) is applied to test whether there are any statistically significant differences among the post-tests of HRV from the three groups. First, using “group” as dependent variable, the pre-tests as fixed factors, and the post-tests as covariates, the researchers used Univariate Analysis of Variance and got the following tables:
Before doing ANCOVA, the test of homogeneity of variance is needed.

Table 4 Comparison of pre-test and post-test from the treatment group 1

	Source	N	M	SD	df	t
SDNN	Pre-test	12	51.15	22.54	11	-0.597
	Post-test	12	55.27	443.55		
LF	Pre-test	12	447.83	509.72	11	0.201
	Post-test	12	423.50	443.55		
HF	Pre-test	12	456.42	374.76	11	-1.240
	Post-test	12	716.58	860.90		
TP	Pre-test	12	1511.42	1321.81	11	-0.703
	Post-test	12	1853.75	2071.80		

α=0.05

Table 5 Comparison of pre-test and post-test from the treatment group 2

	Source	N	M	SD	df	t
SDNN	Pre-test	11	51.99	15.34	10	-0.605
	Post-test	11	55.62	22.10		
LF	Pre-test	11	432.91	384.31	10	-1.338
	Post-test	11	656.27	713.56		
HF	Pre-test	11	417.91	232.30	10	0.409
	Post-test	11	383.09	277.33		
TP	Pre-test	11	1362.45	847.00	10	-0.691
	Post-test	11	1624.18	1297.13		

α=0.05

Table 6 Comparison of pre-test and post-test from the control group

	Source	N	M	SD	df	t
SDNN	Pre-test	9	64.97	14.40	8	2.640*
	Post-test	9	47.72	10.74		
LF	Pre-test	9	576.78	307.09	8	1.855
	Post-test	9	330.56	255.54		
HF	Pre-test	9	691.67	377.27	8	1.997
	Post-test	9	414.78	345.62		
TP	Pre-test	9	2043.11	927.24	8	2.420*
	Post-test	9	1105.22	525.54		

α=0.05

Table 7 Test of homogeneity of variance within groups:

Source	F	Description
Group * SDNN Pre-test	1.076	F=1.076, p>.05, not statistically significant
Group * LF Pre-test	2.064	F=2.064, p>.05, not statistically significant
Group * HF Pre-test	1.182	F=1.182, p>.05, not statistically significant
Group * TP Pre-test	1.497	F=1.497, p>.05, not statistically significant

p <.05

From the Table 7, the homogeneity of variance within groups is not violated, so ANCOVA can be done.

Table 8 Analysis of Covariance

	Source	SS	df	MS	F
SDNN	Group	385.736	2	192.868	.503
	Error	10735.785	28	383.421	
	Total	11121.521	30		
LF	Group	580882.096	2	290441.048	1.443
	Error	5637091.507	28	201324.697	
	Total	6217973.603	30		
HF	Group	769294.494	2	384647.247	1.372
	Error	7851727.635	28	280418.844	
	Total	8621022.129	30		
TP	Group	2941591.433	2	1470795.717	.774
	Error	53239131.070	28	1901397.538	
	Total	56180722.500	30		

$p < .05$

For SDNN, $F(2, 30)=.503$, for LF, $F(2,30)=1.443$, for HF, $F(2,30)= .372$, and for TP, $F(2,30)= .774$, there are therefore no differences among HRV post-tests of the three groups. The following is a summary of ANCOVA.

Table 9 The means, standard deviation, and adjusted mean of the pre-tests, post-tests of the three groups:

Dependent variables	Groups	Pre-test			Post-test		
		N	Mean	SD	Mean	SD	Adjusted Mean
SDNN	Treatment 1	12	51.15	22.54	55.27	25.54	57.29
	Treatment 2	11	51.99	15.34	55.62	22.10	57.24
	Control	9	64.97	14.40	47.72	10.74	43.06
LF	Treatment 1	12	447.83	509.72	423.50	443.55	443.70
	Treatment 2	11	432.91	384.31	656.27	713.56	686.15
	Control	9	576.78	307.09	330.56	255.54	267.12
HF	Treatment 1	12	456.42	374.76	716.58	860.90	758.56
	Treatment 2	11	417.91	232.30	383.09	277.33	455.60
	Control	9	691.67	377.27	414.78	345.62	270.21
TP	Treatment 1	12	1511.42	1321.81	1853.75	2071.80	1915.25
	Treatment 2	11	1362.45	847.00	1624.18	1297.13	1778.83
	Control	9	2043.11	927.24	1105.22	525.54	834.21

6. Conclusions

According to the statistical results, after an eight-week whirling-kung program, neither of the HRV data from the treatment group 1 and 2 has shown significant differences. Perhaps the relatively small sample sizes have led to these consequences, for it's quite hard to make potential voluntary subjects conquer the fear for whirling. The statistical results show that, in either the treatment group 1 or 2, the SDNN, TP, and HF haven't risen significantly, and the LF in them hasn't dropped significantly, either. However, the SDNN and TP in the control group has a statistically significant drop, ($p<0.05$), compared to their rise in both the treatment group 1 and 2, though not statistically significant, which might suggest, like the walking group (treatment group 2), whirling might have avoid the dropping of SDNN and TP.

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"It Might Have Been a Slip of Tongue": Iranian EFL Teachers' Reaction to their Colleagues' Linguistic Goofs

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Abstract: This research sought to investigate Iranian EFL teachers' reaction to their colleagues' mistakes and the probable factors influencing their response. Moreover, it was momentous to the researchers to figure out whether teaching experience and gender play any significant role in the way the participants respond or not. Therefore, 144 teachers were selected with different teaching experiences. The participants were provided with a Discourse Completion Task (DCT) in which they were asked to imagine themselves in a situation where a colleague makes a mistake, and to respond how they would react with the aid of 7 options and a blank space to write a comment or an answer, which was not included. At the end, their responses were first transformed into tables and bar graphs illustrating the frequency and percentage of each option, and then were deeply analyzed. It was concluded that gender and teaching experience do not have a profound effect on the applied correction method and teachers' speech act of correction and the way they react to their colleagues' mistakes is more culture-bound than being related to experience and gender. [Reza Pishghadam, Paria Norouz Kermanshahi. **"It Might Have Been a Slip of Tongue": Iranian EFL Teachers' Reaction to their Colleagues' Linguistic Goofs.** Life Sci J 2012;9(3):215-220] (ISSN:1097-8135). http://www.lifesciencesite.com. 32

Keywords: Corrective behavior, Power status, Correction, Feedback, Teacher

1. Introduction

Granted the fact that students` mistakes or errors are integral parts of each language learning class (Brown, 2007), it seems that correcting any linguistic misbehavior of students is a crucial issue, which should be done carefully. Moreover, correcting mistakes or errors seems to be a cross-cultural notion, which is handled differently in different cultures (Takahashi & Beebe, 1993). Corrective behavior and power relations are evidently intertwined, in a way that different people from different power status group provide a wide range of feedback.

Generally research has focused on the way teachers correct students` errors, or students correct their teachers, disregarding other types of error-correction In fact, numerous studies have been conducted to examine teachers' corrective behavior towards learners or vice versa (e.g. Pishghadam & Norouz Kermanshahi, 2011; Takahashi & Beebe, 1993).

However, to the knowledge of the researchers, no studies are available shedding light on teachers' corrective behavior towards other teachers, i.e. the same power status group. In the current research, it is hypothesized that factors such as gender and teaching experience might affect the way teachers react to their colleagues' mistakes.

2. Theoretical background

2.1. Corrective behavior

Before dealing with the issue of correction, it is quite necessary to clarify the significant difference between an *error* and a *mistake*. According to Brown (2007, p. 257), while errors are manifestations of learners' competence, mistakes are "performance lapses" as a result of a "failure to utilize a known system correctly". Errors and mistakes also differ with regard to the issue of correction. As Brown (2001) contends, *mistakes* rarely call for treatment, while *errors* demand teacher response.

There are two different outlooks to learners' errors (Scrivener, 1994, p. 109):

a) They prove that students are not learning.
b) They prove that learning is in progress.

Nowadays, there is a tendency to the second outlook, believing that when learners make an error, they are in fact "experimenting with the language, trying out ideas, attempting to communicate and making progress". According to Long (1983), in learning a first language error correction may be of no use; on the contrary, it is beneficial to second language learners, both adults and children (as cited in Celce-Murcia, 2001).

There is compelling evidence that error correction is quite a critical issue to which teachers need to be sensitive; since an "insensitive correction" may lead to sapping of learners' confidence (Harmer, 1998). In a similar vein, Chastain (1988, p. 290) argues that second language learners desire to speak the language, but the fear of "public failure" and "groping for words" may act as an obstacle which hinders the efforts. Therefore, scholars put forward some hints to make the best out of correction:

a) Teachers had better start with what learners "can do right" rather than what they "can do incorrectly" (Chastain, 1988, p. 290).
b) Teachers must not single out a particular learner for criticism; rather mention errors without saying who made them (Harmer, 1998, p. 94).
c) Regular correction may lead to the "inhibition of the already taciturn" students; hence, teachers had better not interrupt learners continuously unless they signal for help (McDonough & Shaw, 2003, p. 152).
d) Teachers should make an effort to resist the tendency to correct every single error. They must bear in mind that their purpose is neither to show off their knowledge nor to make an error-free speech; they are there to create a positive climate which encourages learners to talk (Chastain, 1988).
e) Praising students for their success is just as important as correcting them after failure. So error correction can be accompanied by some expressions and encouraging words such as good, well-done, etc. (Harmer, 1998).
f) Chastain (1988) believes that teachers can react to learners' errors in the same way that native speakers do to nonnative speakers. Based on research (Chun, A. E., Chenoweth, N. A., Day, R. R., & Luppescue, S., 1982, as cited in Chastain, 1988), native speakers correct merely 8.9% of nonnative speakers' errors since the focus is on communication rather than language.

To know how teachers treat learners' errors is of paramount importance. Piles of studies are available which investigate teachers' corrective feedback either to find a relationship between learners' errors and teachers' response or to pinpoint a correlation between error correction and accuracy, motivation or acquisition. For instance, observing patterns of error treatment in ESL classrooms, Panova and Lyster (2002) tried to find a relationship between feedback type and learners' response. Many researchers highlighted the type of correction favored by teachers and learners and concluded that teachers prefer indirect correction (Ellis, Basturkmen & Loewen, 2001). Some other researchers examined correction in writing such as Vickers and Ene (2006) who concluded that self-correction is the best in writing since it leads to greater grammatical accuracy.

In nearly all studies done on correction or corrective feedback, it is brought into focus that error correction has a social dimension, which means any criticism or praise will be public (Allwright, 2005). Thus according to Szesztay (2004, p. 133), teachers need to think deeply when they correct a learner in order not to make them feel "absolutely stupid" and to "maintain rapport".

Among the few studies on correction, we can refer to Takahashi and Beebe (1993) who examined American and Japanese performance of the speech act of correction with unequal power status. They studied the use of positive remarks and softeners to make each speech act less face-threatening and to make communication smoother. In fact, they were about to observe the effect of power and distance of addresses on subjects' choice of expression, and to compare them in two different languages. They carried the research in two different power statuses 1) higher to lower, and 2) lower to higher; that is, 1) teachers correcting learners, and 2) learners correcting teachers. They concluded that: first, Japanese who use English transfer some style shifting patterns from their L1. Second, using a positive remark when correcting someone of lower status is an American pattern and Japanese rarely use it, so it is clear that their acquisition is not complete.

Gao and LIU Shao-zhong (2009) conducted a similar study on Chinese individuals. What they concluded for the first situation was that "providing no correction was a frequently employed pattern in higher to lower status" by Chinese participants (p. 34) which was not the same as what Takahashi and Beebe concluded about Americans and Japanese. For the second situation, Gao and LIU shao-zhong (2009, p. 34) claimed that Chinese EFL learners preferred to "take typical linguistic formula 'it seems…' or 'as if…' before correction and they preferred to use softeners, mitigation devices and questions, etc. to save the higher-status people's face" and to show their uncertainty.

To our knowledge, there are merely three studies on the speech act of correction in Iran (Pishghadam & Norouz Kermanshahi, 2011(a) & 2011(b); Pishghadam, Hashemi & Norouz Kermanshahi, 2011) focusing on how learners correct teachers, peers and themselves and one study on how teachers correct learners (Pishghadam & Norouz Kermanshahi, 2011), this research seems to be quite significant with the chief purpose of finding out about the corrective behavior that EFL teachers adopt towards other teachers, considering factors such as teaching experience and gender.

3. Methodology

3.1. *Setting and participants*

This study was carried out in language institutes of Mashhad, Iran, and two groups of participants took part who were all EFL teachers. The first group consisted of 144 individuals with different teaching

experiences ranging from 0-5 years (N=48), 5-10 years (N=48) and above 10 years (N=48). The second group contained 180 EFL teachers of both genders, male (N=90) and female (N=90).

3.2. Instrument

In order to gather large amount of data at full pelt and include more participants in the research, Discourse Completion Task (DCT) was selected to be the instrument for data collection. A questionnaire was devised based on the guidelines laid down by Takahashi and Beebe (1993) that put forward an imaginary situation and teachers should respond how they would react. The content validity of the questionnaire was substantiated through a pilot study in which 60 EFL teachers took part. On the recommendations of an expert in this field and based on the feedback received from participants, questions were revised and ambiguities were removed. Seven options were available in the DCT for the respondents to check off plus one apace to add any comments or response (see the Appendix)

3.3. Procedure

The process of data collection took about three months, starting in January 2011 and ending in March. The devised questionnaire was distributed among EFL teachers randomly selected, and took about 5 minute. The participants of the first group were divided into three categories based on their teaching experience and the second group according to their gender. The participants' responses were initially transformed into tables and graphs displaying the frequency of each answer to each option and were later analyzed.

4. Results

The proposed situation deals with ***teacher correcting teacher*** and 7 choices are available plus one to pen down what they would say if it were not included in the previous eight options. The number of responses to each option and the group to which the respondent belongs are delineated below:

Situation- You are an English teacher. During a workshop, one colleague is explaining how to teach a point, but he/she makes a mistake in his/her speech. Instead of 'adopt a child' he/she has said 'adapt a child'. What would you say/how would you react?

4.1. Teachers' responses considering gender

Option A- I would probably say nothing.

Ignoring the mistake made by another colleague is the most favored reaction based on the frequencies displayed in Table 1. More than half of the teachers preferred to keep silent. From the explanations that they provided, it could be inferred that remaining silent in this situation was due to being at the same level with the addressee or considering it just as a slip of tongue not actually an error. As the results demonstrate, it is obvious that in this situation there is not much difference between males and females.

Table 1. Frequency and percentage of teachers'answers to each option considering gender

Variable / Options	A		B		C		D		E		F		G	
Male	58	32.2%	4	2.2%	0	0%	1	0.5%	18	10%	9	5%	1	0.5%
Female	62	34.4%	6	3.3%	0	0%	2	1.1%	21	11.6%	2	1.1%	4	2.2%
Total	120	66.6%	10	5.5%	0	0%	3	1.6%	39	21.6%	11	6.1%	5	2.7%

Option B- I would wait for other colleagues to correct, and if they didn't, I'd correct.

According to table 4.12., 5.5% of teachers opted for correcting their colleague but that depended on the reaction of others; that is, if other teachers did not correct the person he/she would do that. According to Table 1 more females (3.3%) tended to be tolerant of the mistakes.

Option C- *I'm sorry…?*

Interestingly, no single teacher preferred to stop his/her colleague to ask for clarification or repetition.

Option D- *Adapt a child?!*

Repeating the erroneous sentence with a questioning intonation might have two different purposes; it may simply serve as a question for clarification or might sarcastically tease the teacher. Males and females differ slightly in this regard, as 0.5% of males and 1.1% of females have opted for this option.

Option E- *You mean 'adopt'?*

Among the teachers, 21.6% preferred to correct their colleagues by providing the correct form in a question. This may also function as an aid to the addressee or in some contexts a method for teasing. More females (11.6%) tended to select this option than males (10%).

Option F- *Your speech was perfect, but you made a small mistake, you should say 'adopt a child' not 'adapt a child'.*

According to Table 1, 5% of males prefer to praise the addressee before correcting him/her which is more than females (1.1%).

Option G- *Thank you, but you should say 'adopt'.*

Whether or not to praise the person before correcting him/her has long been a controversial issue and greatly depends on the power status of the interlocutors. In this situation, both the speaker and

the hearer are teachers and therefore of the same power status; it might be considered inappropriate in some cultures to thank the person before correction. Comparing males and females, females tend to go for this option more than males. Figure 1 displays all details about gender differences in a graphic way.

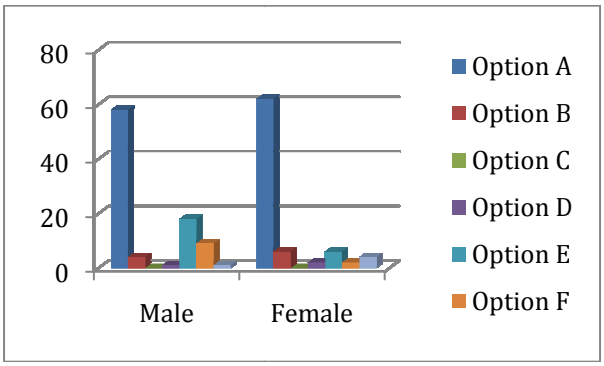

Figure 1.
Teachers' responses considering gender

4.2. Teachers' responses considering experience

Table 2. Frequency and percentage of teachers' answers to each option considering teaching experience

Variable / Options	A		B		C		D		E		F		G	
0-5 years	31	21.5%	2	1.3%	0	0%	1	0.6%	10	6.9%	3	2.08%	1	0.6%
5-10 years	32	22.2%	2	1.3%	0	0%	1	0.6%	11	7.6%	2	1.3%	1	0.6%
Above 10 years	34	23.6%	3	2.08%	0	0%	1	0.6%	11	7.6%	4	2.7%	1	0.6%
Total	97	67.3%	7	4.8%	0	0%	3	2.08%	32	22.2%	11	6.2%	3	2.08%

Table 2 displays the differences of error correction with respect to experience.

Option A- I would probably say nothing.
Whether or not to correct a colleague who is of the same power status with the other teachers who aim to correct is a controversial issue. The most favored option among all is option A and the frequency of responses to that is considerably greater than the other ones (67.3%). Though not notably different, teachers of Group 1, Group 2 and Group 3 react differently; that is, as the amount of teaching experience increases the ignorance of the mistake becomes more probable.

Option B- I would wait for other colleagues to correct, and if they didn't, I'd correct.

Respondents to this option were the ones who did not prefer to keep silent and did not act immediately either. They seem to have been more tolerant as they waited for other colleagues to take action first. According to Table 2, more experienced teachers (Group C = 2.08%) tend to be error-tolerant.

Option C- *I'm sorry…?*

Asking for clarification seems not to be considered as an appropriate method of correction in the current situation since it is not selected even by one teacher.

Option D- *Adapt a child?!*

As mentioned in the previous section, repeating the ill-formed utterance in a question form might serve the purpose of asking for clarification or taunting the teacher. In each group of teachers with different experiences, merely 1 respondent selected this option.

Option E- *You mean 'adopt'?*

Teaching experience does not seem to have a profound effect on the use of this specific speech act of correction due to the fact that almost the same number of respondents went for this option (about 7%).

Option F- *Your speech was perfect but you made a small mistake, you should say 'adopt a child' not 'adapt a child'.*

Among three groups of teachers with different experiences, the third group –experienced ones- opted for this option more than the others (2.7%), but still not a considerable percentage in comparison with option 1.

Option G- *Thank you, but you should say 'adopt'.*

It might be due to the fact that thanking a person of equal status before correcting him/her is considered inappropriate in some cultures to that very few teachers opted for this option- 0.6% of each group and 2.08% in total.

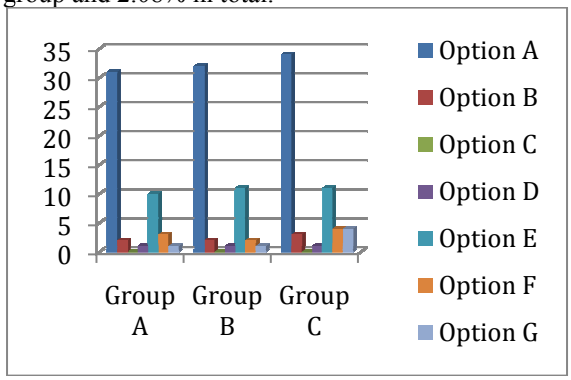

Figure 2. Teachers' responses considering teaching experience

5. Discussion

This study was conducted to examine teachers' corrective behavior towards their colleague's mistakes; that is, to find out whether in the context of Iran, teachers would correct someone of the same power status and what strategies they might adopt to carry out the speech act of correction. Moreover, it attempted to investigate whether or not teaching experience and gender would play any role in teachers' corrective behavior.

As the outcomes of this study revealed, less than half of the teachers tended to correct their colleagues when making a mistake. And among the ones who preferred to correct, a few ones preferred to wait for other colleagues to take action and if not they would do that, especially females and experienced teachers. In the part, which was left blank for the participants to write their comments almost all the ones who had opted for option 'A' claimed that it is due to the fact that they consider it just as 'a slip of tongue' and not actually a mistake.

What is of paramount importance is that in Iran, a hierarchical system is dominant in which group harmony is highlighted and social order is quite significant; however, some other cultures opt for assertiveness and egalitarianism (Shang-chao, 2008; Scollon & Scollon, 2001). Moreover, as opposed to American culture where equal social status is presiding and individuals have equal rights, in Iranian culture, people accept themselves as being in different social positions and therefore are expected to know where to 'speak up' and where to 'speak down', considering the concept of 'ehteram' (respecting others). Considering all these, it seems that Iranian teachers prefer not to correct each other as to show respect and to preserve equal power status.

Except for the second and last option, gender and teaching experience did not seem to have a profound effect on the applied correction method and teachers' speech act of correction. Thus it can be inferred that this is more culture-bound rather than being related to experience and gender.

According to Hofstede (1980, 1991 as cited in Samovar, Porter & Stefani, 2011), there are some value dimensions in each culture, which greatly influence the way people behave, one of which is 'individualism-collectivism'. In collective societies, such as Iran, the concept of 'we' is brought to focus which influences the way people communicate. As the teachers in Iran, regardless of their gender and experience, prefer not to threaten their colleagues' face, it can be concluded that due to being in a collective society and to show 'ehteram' (respect), teachers remain silent in encountering a colleague's mistake and even if it is an error, they consider it to be a mistake.

Whether or not to praise the addressee before correcting him/her was a question previously raised by Takahashi and Beebe (1993); they claimed that when the person who corrects is of a higher power status, he/she mostly adds a positive remark; nevertheless, when the corrector is of a lower power status, it is totally inappropriate to praise the addressee. The latter situation seems to be more similar to the current situation where teachers are of approximately same power status. However, among those who preferred to correct their colleague, about 6% tended to praise their coworker before correcting him/her.

Moreover, according to the obtained results, more males and experienced teachers preferred the speech act through which they directly mention that someone has made a mistake and provided the correct form, though not a considerable percentage.

The results obtained from this research can be discussed in terms of two important implications. First, it will be of great importance to cross-cultural studies which aim to compare different cultures and figure out the sources of cross-cultural miscommunication or failure. Moreover, the results will pave the way for those who essay to design materials containing speech acts and in ELT classrooms.

Thus far, studies have been carried out to examine learners' corrective behavior in different situations and teachers' corrective behavior towards learners and colleagues. It is also of interest to explore how teachers react to their own mistakes, whether or not they accept their mistakes and confess to that, so further research is called for to fill this gap. Moreover, another study can be carried out to examine the psychological effects of error correction on individuals.

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Appendix

Situation: You are an English teacher. During a workshop, one colleague is explaining how to teach a point, but he/she makes a mistake in his/her speech. Instead of 'adopt a child' he/she has said 'adapt a child'. What would you say/how would you react?

A) I would probably say nothing. ☐
B) I would wait for other colleagues to correct, and if they didn't, I'd correct. ☐
C) *I'm sorry…?*☐
D) *Adapt a child?!* ☐
E) *You mean 'adopt'?* ☐
F) *Your speech was perfect but you made a small mistake, you should say 'adopt a child' not 'adapt a child'.* ☐
G) *Thank you, but you should say 'adopt'.* ☐

☐ Something else

4/29/2012

Determination of immediate and long term effects of Earthquake-2005 on Tarbela Dam, Pakistan

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Abstract: The catastrophic earthquake of 7.6 magnitude on Richter scale hit Northern Pakistan on October 8th 2005 at 084955 PST. The yellow earthquake drill was performed immediately after the earthquake to investigate the effects of this earthquake on the different structures of the Tarbela dam .The earthquake drill comprising of hydrographic survey, monitoring of all the instruments, physical inspection of the major structures, movement survey, and comparisons of seepage. The data indicates changes in the seepage pattern and pore pressures before and after earthquake. The pore pressures rise upstream as well as downstream of grout curtain was observed. The maximum seepage increase up to 1.11 cfs in RDA-22 and drainage adit discharge from l827 to 1913.64 gallons per minute were recorded. No significant change, movement or settlement of expansion joints were observed, however displacement of a retaining wall 13 to 14 mm at the top of" a retaining wall at construction joint was recorded. Minor movement of top set slope and deposition of sediments in front of tunnels have also been found. Long term monitoring to study the effects in depth such as micro fracturing of the structures, seepage at right abutment and allied problems is suggested to ensure the safety of the dam.

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Introduction

The earthquake of October 8, 2005 has caused a catastrophic damage to life and property in Northern areas of Pakistan. The earthquake has occurred due to motion in MBT (main boundary thrust) and Kashmir fault. This earthquake has greatly changed and affected the geomorphology of the area by development of large number of landslides which caused catastrophe. Mushtaq and Haq (2006) reported more than 2500 aftershocks recorded by seismic station at Tarbela. Many researchers have studied the earthquake of 2005 to assess the damages and future protection (Mahdi, & Siddique, 2006; Sharif et al 2006; Dunning et al 2007).

Quittmeyer, et al 1979 and NESPAK, 1995 studied the seismicity of Pakistan with reference to Tarbela Dam. According to Mahdi et al (2005),"The earth response to a particular reservoir depends upon regional tectonics stresses, on the porosities and permeabilities of the geological formations under laying the reservoirs, on the presence of the faults in the vicinity and on the depth/volume of water in the reservoir". Mushtaq & Haq (2006) carried out a study on dam performance and seismicity after 2005 earthquake with their focus on the two large dams, Tarbela and Mangla and concluded," that all the structures have contributed to perform satisfactorily and that there was no abnormality of any cause for concern". Ghumman and Masood (2006) studied the slope stability analysis of earth dam against earthquake loading and highlighted the role of soil properties," a soil having good shear strength will contribute better safety against slope failure than a soil having low cohesion and angle of internal friction of soil". Keeping in view of importance of the large dams, it is a standard practice that after every earthquake of 5 and above magnitude special dam safety monitoring exercise is performed to find out any effects on large dams. Hence a special monitoring yellow drill was performed after the earthquake at Tarbela Dam.

Tarbela Dam is one of the greatest water resources development projects of the world built on Indus River. The project consists of the 9000 feet long and 479 feet high earth and rock filled embankment across the entire width of the river with two spillways and two auxiliary embankment dams, located on the left bank valley. Four tunnels through right abutment have been constructed for irrigation releases and power generation. The dam has developed a lake of 97 km long, spread over an area of 260 square km with a gross storage of 11.62 MAF.

This study is performed to evaluate the effects of the catastrophic earthquake on the Tarbela Dam of Pakistan. Data of seepage for various structures at similar levels have been included for seven consecutive years after earthquake i.e., 2005- 2011 to access long term affects of the earthquake on seepage behavior.

Materials and methods

Data have been collected for seepage, delta movement and, settlement and pore pressures changes through extensive measurements carried out immediately after the earthquake by the project authority. To investigate the effects of earthquake, following methodology has been adopted.

1 Physical inspection of all major structures
2 Hydrographic survey
3 Monitoring/comparison of instrumental seepages.
4 Movement survey
5 Comparison of seepage data at similar reservoir level from 2005-2011.

Results and Discussions

The physical inspection indicated that no significant effects were observed immediately after the earthquake except reported by Qureshi (2005),"a retaining wall around the top of gate shaft structure near the rock slope has been displaced in order of 13-14 mm at the top of wall at construction joint. Two expansion joints on the upstream wall have opened up to 1 mm due to earthquake. The radial expansion joint of penstock unit 4 has further expanded after earthquake".

Hydrographic survey of the reservoir from, range line No 1-18 was carried out on October 8, 2005 by the project authority of Tarbela Dam. The results are given in Table 1. The comparison with hydrographic survey performed during September 2005, indicates that shifting of sediments up to two feet from Range No 12.75 to 13.33 and 13.75 to 15 on top set slope and deposition of 2.2 feet from Main Embankment Dam to Range line No 12 took place due to earthquake. The hydro-graphic survey of intake area for tunnels T-1 to T – 4 was carried out on October 9, 2005. The field record shows that I feet sediments.

The pore pressure rise in a number of piezometer took place as a result of shaking of earthquake. Monitoring data of these instruments (Table-2) shows that the effects are more prominent at right abutment near KGA-4 and especially upstream as well as downstream of FGA-3. Although pressure rise was nominal and recorded upstream as well as downstream of RGA-5 grout curtain and RDA-2 this was retained for sometime.

Table 1 Results of hydrographic survey conducted on Oct. 8, 2005 showing the movement of delta due to earthquake

Range Line No	Elevation on Sept., 2005	After Earthquake on Oct 8, 2005	Move-ment
1.	1202.5	1203.6	+1.1
2.	1203.0	1203.6	+0.6
3	1205.0	1204.6	–0.4
4	1208.7	1208.6	–0.1
5	1210.0	1209.6	–0.4
6	1216.0	1215.6	–0.4
7	1222.3	1221.6	–0.7
8	1229.4	1228.6	–0.8
9	1239.4	1238.6	0.8
10	1248.0	1247.6	–0.4
11	1258.4	1260.6	–0.2
12	1275.4	1277.6	+2.2
13	1356.4	1357.6	+1.2
14	1373.6	1371.6	–2.0
15	1377.5	1377.6	+0.1
16	1380.0.	1379.6	–0.4
17	1377.0	1375.6	–1.4
18	1379.5	1377.6	–1.9

deposition in front of Tunnel No T-1 and T-4.(Qureshi,2005)

Comparison of seepage from different important structures has been made which indicates that a change in the seepage pattern has been induced by the earthquake. Measurements made at different weirs before and after earthquake are given in Table-3. It has been observed that in a number of drain holes turbid flow has started just after earthquake which is attributed due to movement in the sediments deposits of the lake. The hydrographic survey confirmed that there is erosion of the upper two feet layer of the sediment deposit in the lake. Measurements of suspended fines from various outlets of the dam are given in Table-4. The survey conducted immediately after the earthquake show that maximum settlement of 0.055 feet has occurred at station 71+00 at crest of main embankment dam.

Long term monitoring data given in Table-5 shows that seepage at critical locations returned to original values during the subsequent years after earthquake.

Table 2: Comparison of Pore Pressure Changes before and after Earthquake at Right Abutment

Piezo No	Location	Before E/quake	After Earthquake						
Date→		03.10.2005	08.10.2005	09.10.2005	10.10.2005	11.10.2005	13.10.2005	15.10.2005	17.10.2005
B-273(1)	RDA-2	1316.06	1327.76	1344.49	1344.49	1344.49	1342.81	1342.81	1342.81
B-275	-do-	1350.31	1360.34	1362.01	1302.01	1362.01	1360.34	1360.34	1360.34
B-277(1)	-do-	1286.69	1300.07	1300.07	1300.07	1300.07	1300.07	1300.07	1300.07
B-277(2)	-do-	1286.89	1300.07	1300.07	1300.07	1300.07	1300.07	1300.07	1300.07
B-287(1)	-do-	1352.47	1370.5	1361.17	1301.17	1361.17	1360.5	1359.83	1359.83
B-288(1)	RGA-3	1354.49	1362.52	1363.19	1360.51	1363.91	1362.52	1361.85	1361.85
B-579	RGA-5	1360.7	1365.71	1365.71	1365.71	1365.71	1365.71	1365.71	1365.71
B-665	RGA-4	1445.44	1450.45	1452.13	-	1452.13	1452.13	1456.13	1452.13
B-609	-do-	1349.69	1354.71	1356.38	1356.36	1358.38	1358.38	1356.38	1356.38
B-560	RGA-8	1363.09	1366.43	1371.46	-	1369.7Sf	1369.78	1369.78	1369.78
B-562	-do-	1388.51	1413.6	1396.88	-	1403.56	1410.25	1410.25	1410.25
563	-do-	1416.17	1431.22	1432.9	-	1432.9	1431.22	1431.22	1431.22
612	-do-	1382.62	1387.64	1409.38	-	1407.71	1406.04	1406.04	1406.04
613	-do-	1388.42	1388.49	1394.85	-	1396.53	1396.53	1396.53	1396.53
652A	-do-	1391.96	1401.99	1432.1	-	1408.68	1408.68	1408.68	1408.68
654A	-do-	1387.2	1398.73	1407.09	-	1407.09	1405.42	1405.42	1405.42
655	-do-	1415.23	1430.28	1435.3	-	1436.97	1433.63	1433.63	1433.63
657	-do-	1416.36	1429.74	1433.09	-	1433.09	1431.41	1431.41	-
658	-do-	1417.06	1430.44	-1430.44	-	1430.44	1430.44	1430.44	-
P8-1	Debris Dam	1441.72	1445.55	--	-	-	-	-	1442.88
P8-2	-do-	1439.91	1445.41	--	-	-	-	-	1442.82
B-728	RGA-5 u/s	1471.07	1476.43	--	-	-	-	-	1472.75
B-729	RGA-5 d/s	1407,23	1409.24	--	-	-	-	-	1405.56
B-730	RGA-3 u/s	1416.4	1418.07	-	-	1418.07	1416.4	-	-
B-742	RGA-8	1311.24	1314.58	1314.58	-	1314.58	1314.58	1314.68	-
769(1)	Con tunnel	1428.33	1429.75	-	1430.83	-	-	-	1431.67
769(3)	-do-	1327.33	1428.42	-	1428.75	-	-	-	1429.25

Table 3: Comparison of the Adit Seepage (cfs) before and after Earthquake)

Adits	Before E/quake	After Earthquake 8.10.2005 to 22.10.2005															
	1.10.05 Morning	8 Morn	8 Even	9 Morn	9 Even	10 Morn	10 Even	11 Morn	12 Morn	12 Even	13 Morn	14 Morn	15 Morn	17 Morn	18 Morn	19 Morn	20 Morn
	RL→1525.7	1525.25		1525.38		1525.31		1525.25	1528.06		1624.76	1624.31	1524.55	1524.38	1524.88	1524.94	1525.26
	1.10.05	9.10.05	9.10.05	9.10.05	9.10.05	10.10.05	10.10.05	11.10.05	12.10.05	12.10.05	13.10.05	14.10.05	15.10.05	17.10.05	18.10.05	19.10.05	20.10.05
RAA15-1	0.32	0.23	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.27	0.24	0.23	0.23	0.23	0.23	0.23

RAA-2	2.25	2.25	2.31	2.37	2.37	2.37	2.37	2.37	2.37	2.37	2.37	2.37	2.37	2.37	2.37	2.37	2.37
RDA-22	4.44	4.44	4.53	5.08	5.03	5.17	5.21	5.25	5.27	5.29	5.27	5.36	5.27	5.55	5.55	5.55	5.55
LLA-1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
LLA-2	2.48	2.18	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.48	2.44	2.38	2.38	2.38	2.38
SSDA-1	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
SSDA-2	0.22	0.22	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26
ADA-2	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
ASDA-3	1.58	1.58	1.62	1.58	1.58	1.58	1.58	1.58	1.53	1.58	1.58	1.58	1.58	1.58	1.58	1.58	1.58
LDA-3	0.94	0.94	0.94	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92

Table 4 Results of suspended fines collected from various outlets of the dam.

Outlet		U-1		U-6		TR-1		U-10	
Date	RL	Time	Fine mg/l	Time	Fines mg/l	Time	Fines mg/l	Time	Fines mg/l
8-10-2005	1525.05	1245	554	1250	514	1705	199	1651	366
		1330	634	1335	626	2245	113	2240	71
		1645	248	1655	352	-	-	-	-
		2235	115	2238	74	-	-	-	-
10-10-2005	1525.35	1218	34	1216	41	1210	25	2218	43
		1600	27	-	-	2210	30	-	-
		2214	32	-	-	-	-	-	-
11-10-2005	1525.23	-	-	-	-	1105	154	1810	575
		-	-	-	-	1335	70	-	-

Table.5 Comparison of Adit seepage (wiers) in cfs at similar reservoir levels from 2005 to 2011 at rising leg.

Adit No	Year	2005	2006	2007	2008	2009	2010	2011
	Reservoir Level	1525.05		1525.10	1525.82	1526.40	1525.34	1525.31
RAA-2		2.115		1.6512	1.5944	1.4279	1.3738	1.3140
RDA-22		4.71		4.477	4.3345	4.3878	4.1235	4.019
LLA-2		1.58		1.179	1.219	1.439	1.280	1.266
ASDA-2		0.36		0.618	0.584	0.580	0.563	0.537
ASDA-3		0.81		1.335	1.285	1.335	1.285	1.219

Conclusions and recommendations

The investigations indicate that the performance of various structures of the dam remained satisfactory during the earthquake. The increase in seepage was temporary and returned to original level during the post earthquake period.

The existing seismic network is required to be further strengthened by providing additional seismic instruments in the northern parts of the country for the periodic monitoring and prediction of seismicity in Pakistan..

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The oncogenicity change and effect on tumor of HL-60 cells with silent nucleostemin gene in nude mice

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Abstract: Objecttive. To investigate the oncogenicity change of HL-60 cells with silent nucleostemin gene in nude mice and the role of Nucleostemin（NS）specific short hairpin RNA （NS-shRNA）for the anti-leukemia effect in nude mice xenograft tumor model. Methods. HL-60 cells were taken as the model, and were directly transfected with one of Nucleostemin short hairpin RNA（NS-shRNA） which its effect of silencing NS gene is remarkable . In addition, negative control group and blank group were set up. The progress of tumors was observed regularly. Tissues of tumor in every group were handled with pathological section and dyed with HE. Determine the NS protein by immunocytochemisty. In addition, the heterogenic nude mice xenograft tumor models of high-oncogenic HL-60 leukemia cells were established. NS-shRNA was synthesized in vitro to prepare lipid inclusion, and was intraperitoneal inoculated into the mice. The volume and weight of the tumor bodys were measured, the slices of xenograft tumor were stained by HE dye, the NS protein inhibiting effect was detected by immunocytochemistry, and the apoptotic cells of HL-60 in the tumor body were examined by Tunel technique. Results. Different groups need different time to progress the tumor. The experimental group need longer than control group, and the tumor was smaller. The final tumor volume of mouse in experimental group was different significantly with other two groups（$P<0.05$）. But the difference between negative control group and blank group was not significant（$P>0.05$）. Under microscope, it showed that interstitial connective tissue and blood vessels were fewer than other two groups, and the cells arranged becomes loosely. HL-60 cells were not uniform. The cells with karyorrhexis and small nucleus increased. Pykno-levels of nuclear chromatin were not uniform and tumor giant cells decreased. All mice in our study were successfully transplanted by high-oncogenic HL-60 leukemia cells, and the volume of the tumors was even smaller. After treated with NS-shRNA lipid inclusion for 13 days, the tumor volume，weight and NS protein in the tumor cells were statistically lower than control groups. Large areas of patchy destroyed of tumor tissue and "apoptosis character" changes appeared in treated group. A great deal of apoptotic cells appeared in tumor tissue after therapy, detected by Tunel technique. Conclusion. The oncogenicity of HL-60 cells with silent nucleostemin gene was decreased. It is likely related to the change of cells′ biological characters. The anti-leukemia effect of NS-shRNA in nude mice xenograft tumor model is significant; one of the mechanisms probably induce the apoptosis of leukemia cells by the down-regulation of NS expression.

[FU Shuzhen, SUN Xiaoli, Abdallah Dlykan, JIA Yu, WANG Yangyuan, LIU Shuai, YU Lina, ZHANG Hui, YUE Baohong. **The oncogenicity change and effect on tumor of HL-60 cells with silent nucleostemin gene in nude mice** . Life Sci J 2012;9(3):226-232] (ISSN:1097-8135). http://www.lifesciencesite.com. 34

Keywords: Nucleostemin; short hairpin RNA; nude mice xenograft tumor; leukemia; HL-60 cell; oncogenicity

1. Introduction

Neucleostemin(NS), which was discovered in 2002,is a p53-binding protein predominantly exists in the nucleus of the stem cells, but not in terminally differentiated cells. The ensuing research found that nucleostemin was highly expressed in some kinds of human solid tumor or cancer cells [1, 2, 3]. The previous study of our team revealed that NS was also expressed continuously and highly in the leukemia cell lines HL-60 and K562, as well as the leukemia cells derived from patients who suffered from myeloid, monocytic and lymphoid leukemia. The further study found NS-specific short hairpin RNA (NS-shRNA) could interfere NS-mRNA and NS protein expression of the leukemia cells HL-60, and then affect its biological characteristics including proliferation, differentiation and apoptosis[4,5,6]. Based on the series of researches above, this study will change the researches focusing

on NS from in vitro to in vivo and from cellular level to histological level. We inoculated the NS-shRNA-treated HL-60 cells into the nude mice, and then observed its oncogenicity and tumorigenesis. In addition, we transfect NS-shRNA into nude mice xenograft tumor models as Lipid inclusions to study the effects to the growth and the indicator change of the tumor, and to probe into the antileukemic action of NS-shRNA in nude mice. This work would form the basis of understanding further the function of NS and changing the properties of tumor cells via inhibiting NS.

2. Materials and Methods

2.1 Materials and reagents

The human leukemia cell lines HL-60 cells were provided by Shanghai Institutes for Cell; Balb/c nude mice (SPF grade, male, 5~6w old and 18~22g) were purchased from the Slack experimental animals Co. Ltd. in Shanghai. Transwell was provided by Coster Co, Polycarbonate microporous membrane filter was provided by Watllman Co. Control sequence of siRNA was provided by shanghai Gema genepharma Co.ltd. immunocytochemistry kit was provided by zhongshan goldenbridge biotechnolocy Co. Tunel kit was provided by huamei biotechnolocy Co.ltd.

2.2 Experimental animals

Balb/c nude mice (SPF grade, male, 5~6w old and 18~22g) were purchased from the Slack experimental animals Co. Ltd. in Shanghai. The animals were kept in the laminar flow room of "isolated cage with independent air supply" in common sterilizing room. Enviromental conditions were controlled at 24~26℃ for temperature and 35%~45% for humidity. The cages, padding, water and fodder were all disinfected. The experimenter disinfected hands before conducting aseptic manipulation.

2.3 The sequences of NS-shRNA

According to the principles of shRNA designing, two NS specific shRNA which each contains a 9bp loop [aaguucucu] were finally determined. After annealing, the NS-shRNA could fold to the hairpin structure naturally. Preliminary experiments were carried out in order to select a more effective NS-shRNA for the follow-on experiments.

NS-shRNA- 1:

5′-GCUGAGCUAAGGAAACAGAucucuu

gaaUCUGUUUCCUUAGCUCAGCUU-3′

NS-shRNA-2:

5′-GCCUAGGAAAGACCCAGGAaaguuc

ucuUCCUGGGUCUUUCCUAGGCUU-3′

2.4 The sequences of negative control siRNA oligo

The sense strand:

5′ -UUCUCCGAACGUGUCACGUTT-3′

The antisense strand:

5′ -ACGUGACACGUUCGGAGAATT-3′

The negative control siRNA sequence was unrelated with NS, and had no homologous coding sequence with mammals. The 21bp sense strand contained a 19bp sequence which had nothing to do with NS-mRNA and was ended in a 2-thymidine 3′-overhang. The 19bp sequence of the sense strand and the antisense strand were complementary. This siRNA oligo sequence was synthesized by Shanghai Gema genepharma Co.ltd.

2.5 Proceeding preliminary experiment to select NS-shRNA

HL-60 cells were cultured routinely and harvested in logarithmic growth phase. Then the cells were adjusted to the density of 4×10^5/ml with whole medium and aliquoted into 6-well plates for 2.5ml per well. These cells were divided randomly into the transfected groups (R1 and R2) and the control groups (C1 and C2). The steps of transfection:

7.5μl Code Break siRNA transfection reagent was added to 625μl non-serum medium. R1 group was added NS-shRNA-1,and R2 group was added NS-shRNA-2, the final concentration was adjusted to 10 nmol/L. C1 group was added transfection reagent only as blank control, and C2 was added non-related siRNA sequence as negative control, the final concentration was also 10 nmol/L. 48 hours after transfection, the total RNA of each group were extracted by TRIzol reagent for NS gene amplification by RT-PCR, in order to detect the blocking effect of NS-mRNA.

2.6 Preparation for inoculation

24 hours before inoculation, the closed laminar flow chambers of the "independent air supply isolation cages" were placed under the ^{60}Co radiation therapeutic machine, giving a total dose of 4.5Gy exposure to the mice inside the cage. HL-60 cells were cultured in 200ml culture flask and then were transfected. These cells were divided into 3 groups:

The transfected group: Select the more effective NS-shRNA to transfect HL-60 cells and then inoculate.

The negative group: Transfect the HL-60 cells with non-related siRNA and then inoculate.

The blank group: Add the transfection reagent only.

The experimental mice were grouped randomly, and each group had 8 mice.

2.7 The mice were inoculated by the transfected HL-60 cells

24 hours after transfection, each group cells were harvested and centrifuged at 500rpm for 10min. Then the cells were collected to a sterile 40ml centrifuge tube and washed with sterile saline. The cells were

resuspended for cell counting and the cell concentration was adjusted to 1×10^8/ml, then the cells were inoculated subcutaneously into the front leg of nude mice at a dose of 0.25ml (containing 2.5×10^7 cells). Aseptic operation was followed in the whole procedure.The nude mice were observed continuously for 5 days and the tumor formation was recorded. 5 weeks later, the nude mice were sacrificed by stretching neck method and dissected, then the tumor tissues were removed and fixed by paraformaldehyde(PFA) for pathological section and HE staining. The C1 group and C2 group were inoculated and handled with the same methods as above.

2.8 Determination of NS protein-positive cells

The cells were identified through immunocytochemistry staining under optical microscope. The cells which showed brown-yellow or brown blocky materials in the nuclei or nucleolus were positive.

2.9 Establish the heterogeneic athymic mice xenograft tumor models of high-oncogenic HL-60 leukemia cells

Collect HL-60 cells in doubling generation time and adjust the concentration, then inject hypodermically the cells into dorsal part of foreleg on mice which was exposed to ^{60}Co with 4.5GY. After the tumor grow to enough big, cut off the cells and culture the cells in vitro to fifth generation. Add 0.5ml liquid supernatant of HL-60 cells cultured without serum for 24h into TCPS, and add 25ul suspension cells into inside Transwell, adjusting the cells to density to 2×105/ml and culture for 12h. With giemsa staining, select 5 fields of view presently by microscope to count the cells and the mean value representing the cell's oncogenicity. Inject the cells into 30 mices and divide them into 3 groups presently when the tumors grow to 400mm^3.

2.10 Preparing the lipid inclusions of NS-shRNA and control siRNA

Inject 125μl cultured medium without serum and antibiotic into 1.5ml centrifuge tube, and mixing after adding 3.0ul transfection Reagent. Then culture at room temperature for 15~20min.

2.11 Inoculating the NS-shRNA and control siRNA into mice

Every mouse of treatment group was inoculated lipid inclusions of NS-shRNA with 3μg NS-shRNA every 3 days for 4 times. Every mouse of negative control group was inoculated control siRNA with the same method. Every mouse of blank control was inoculated normal saline. After 13 days, kill the mice and get the tumor of every group to weigh and measure the volume of the tumor.

2.12 Observation of the tumor formation

The tumor formation in nude mice was observed regularly. When the tumor grew out, the tumor volume was measured by vernier caliper every 3 days, including the maximum diameter (Max) and minimum diameter (Min) of the tumor. Then the tumor volume was calculated and the tumor growth curve was mapped. The formula for calculating the tumor volume is as follows:

Tumor volume(V) =Maximum diameter(Max)×Minimum diameter square(Min ^{2})/2[7].

2.13 Observation of the histological sections

Sections and regions which were dyed well and uniformly were selected to observe the cell size, the connective tissue, the distribution of blood vessels, the nuclei size and the chromatin.

2.14 Text the apoptosis of tumor by Tunel

The tumor with embedded in paraffin and serial section was cultured with 0.3%H_2O_2 carbinol for 30min, washed by water three times and then ice-bathed with connect fully liquid for 2min, according to the Tunel kit.

2.15 Statistical analysis

All data were processed by SPSS 16.0 and reported as $\bar{x} \pm s$. Statistical significance was set P<0.05, and P<0.01 means high significance.

3. Results

3.1 Detection of blocking effect of the synthetic NS-shRNA

HL-60 cells were harvested for NS gene amplification by RT-PCR after incubated with the two synthetic NS-shRNA repectively. According to scanning gray scale analysis, the related score is 0.826 in C1 group, 0.809 in C2 group, 0.503 in R1(NS-shRNA-1) group and 0.207 in R2(NS-shRNA-2) group. Thus, the synthetic NS-shRNA-1 and NS-shRNA-2 could block the expression of NS-mRNA, with the inhibiting rates of 39.10 % and 74.94 %. It showed that NS-shRNA-2 was more effective, so it was selected for further experiments (Figure 1).

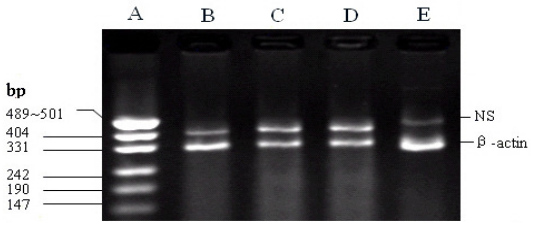

Fig 1 RT-PCR result of human leukemia HL-60 cells treated 48 h by NS-shRNA

A：DNA Mark B： NS-shRNA-1

C：blank control D： negative control

E： NS-shRNA-2

3.2 The tumor formation of HL-60 cells transfected with NS-shRNA-2 in nude mice

The mice in C1 group and C2 group began to grow tumors at about 14~15 day after inoculated with HL-60 cells, but the tumor formation of the mice in R2 group began at about 18~19 day after inoculation. The time of tumor formation in experimental group (R2 group) was longer than the control groups (C1 and C2 group). Finally, there were 4 mice in each of the three groups (4/8) formed the tumors.

3.3 The change of NS protein in HL-60 cells after transfection

72 hours after HL-60 cells incubated with NS-shRNA-2, the immunocytochemistry staining showed that NS expression rate of the cells in C1 group and C2 group were（94.77±2.69）% and（93.55±2.84）%, and the positive score were 180.22±16.41 and 177.33±13.34,respectively. NS protein expression of the HL-60 cells in R2 group were significantly decreased, the expression rate was（46.33±2.67）% and the score was 51.33±6.23. The difference of the score between the two control groups(C1 and C2) was not significant(P>0.05), but comparing R2 group with the two control groups(C1 and C2), it was significant. The results showed that the synthetic NS-shRNA-2 in vitro could obviously inhibit the expression of NS protein in HL-60 cells, and the inhibition rate reached to 71.5 %(figure 2 and 3). It indicated that after NS-shRNA-2, which entered into HL-60 cells by transfection, inhibited part of the expression of NS-mRNA, the amount of NS protein could decrease.

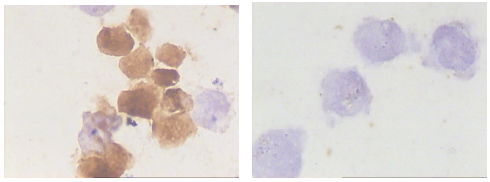

Fig 2 Detecting NS protein by immunocytochemistry in HL-60 cells transfected

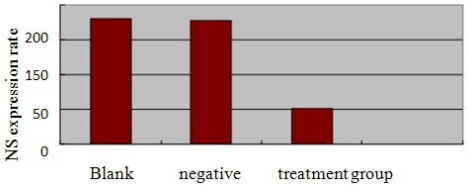

Fig 3 The expression of NS protein in HL-60 cells by immunocytochemistry after transfected

3.4 The tumor growth after the HL-60 cells tranfected with NS-shRNA were implanted into the nude mice

After implantation, the tumor growth was observed regularly, and the tumor size was measured every 3 days. 30 days later, the final size of the tumor was calculated, and the tumor was weighed. In appearance, the tumor size of the R2 group was obviously smaller than the two control groups (C1 and C2). The stripped tumor was consistent with the tumor in vivo (figure 4,5). Comparing the R2 group with the C1 and C2 group, the tumor size and weight were significantly light(P<0.05); There were no significant differences between the C1 and the C2 group(P>0.05)（table 1, figure 6）.

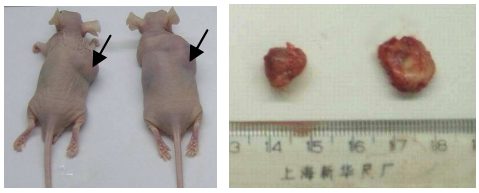

Fig 4 the growth of tumor in NS-shRNA-2 group nude mice and tumor bodies
（The arrows indicate the tumors）

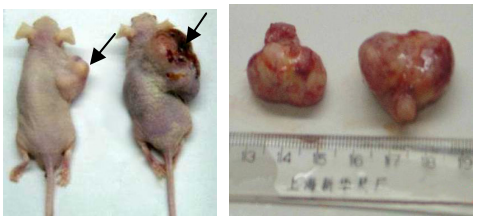

Fig 5 the growth of tumor in control group nude mice and tumor bodies
（The arrows indicate the tumors）

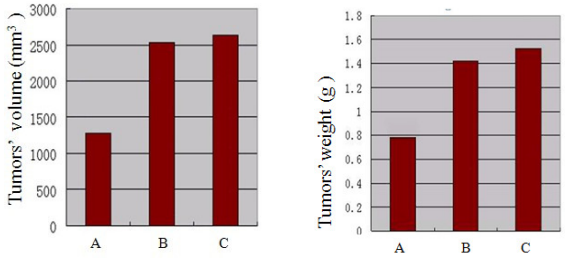

Fig 6 the tumors volume and weight
A: the transfected group B: the negative control group C: the blank control group

3.5 The histological observation of the leukemia xenotransplantation tumors after transfection with NS-shRNA

30 days later, the nude mice were all sacrificed for dissected examination, and no metastatic lesions were found in lymph nodes or organs. The tumor tissues derived from each group were embedded in paraffin for making pathological sections and then stained with HE. Under the microscope, it was observed that the cells in tumor tissues obtained from the three groups were all grew focally. The cells of the C1 and C2 group were similar, they were arranged tightly with a lot of connective tissues and vessels; the HL-60 cells in the

tumor tissues were varied in size, the chromatins were condensed and heave stained, the tumor giant cells were seen often, and the pleomorphism was marked, the apoptotic cells were occasionally found. The connective tissues and vessels of the R2 group were less than the C1 and C2 group, the cells in the tumor tissues were arranged loose, leaving much space; the sizes of HL-60 cells varied much greatly, nuclear fragmentations and small nuclei cells increased, the density of the chromatin was inhomogeneous, and the tumor giant cells were less seen (figure 7).

Table 1. The growth of tumors after transfected by NS-shRNA-2 ($\bar{x} \pm s$)

group	Treated	Negative control	Blank control
Inoculation num (n)	8	8	8
Tumor num (n)	4	4	4
rate	50%	50%	50%
Volume (mm^3)	1282.6±434.1#	2533.3±683.1	2632.5±621.8
weight(g)	0.79±0.29*	1.42±0.4 1	1.53±0.37

#Compared to other two groups: t=3.090、3.560, both P<0.05
Compared between two control group: t=0.304, P>0.05
* Compared to other two groups: t=2.509、3.148, both P<0.05
Compared between two control group: t=0.398, P>0.05

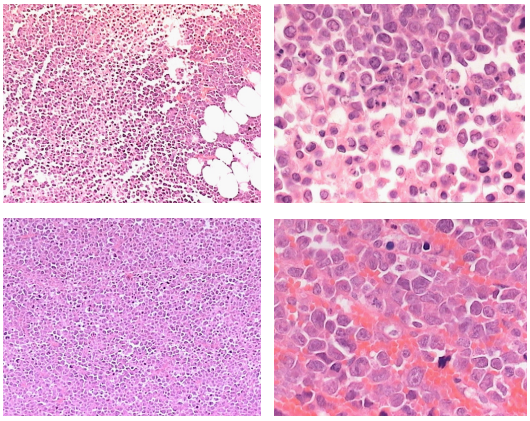

Fig 7 The tumor tissue of transfected group nude mice(up) and contral group nude mice(down) HE staining Left：200× Right：400×

3.6 Establish the nude mice xenograft tumor models of high-oncogenic HL-60 leukemia cells

The mice exposed to ^{60}Co appeared tumor after 7 days by inoculated with HL-60 cells. The ratio of oncogenicity is 70%. Get the tumor and washed to collect the cells after 10 days. After 5 passage, testing the cell's nvasiveness by Transwell test indicate that there have obvious differention about the cell's nvasiveness between the treated with NS-shRNA and not. Cells can cross the membrane is 216±12.3, and the cells can't cross is 159±20.7. P<0.05 by t test. 30 mice was inoculated by HL-60 cells for the second time and appeared tumor with same volume. After 15 days, the trement group is （407.8±45.3）mm^3，the negative control group is （415.7±61.0）$mm^3$and the blank control group is （413.2±75.3）mm^3. The difference among the three groups was not obvious processed by Statistics.

3.7 The antileukemie effection of NS-shRNA in mice

Injected with HL-60 cells 15 days after, inoculated NS-shRNA-2 into treatment groups. After 13 days, the tumor of this group grows with rate of 207.1%. While the negative control group is 497.1% and the blank control group is 569.6%. The tumor's volume of the three groups as the table 2.

Table 2. Stsatistic results of tumors volume and weight

Group	Treatment	Negative contrc	Blank control
Volume before treatment（mm^3）	407.8±45.3#	415.7±61.0	413.2±75.3
Volume after treatmer（mm^3）	1252.4±348.7	2472.0±279.5	2766.7±369.2
Volume increase（%）	207.6	494.7	570.0
Weight（g）	1.18±0.27*	2.04±0.73	2.35±0.41

vs negative control, blank control respectively, both P>0.05; & vs negative control, blank control respectively, both P<0.01; * vs blank control, negative control respectively, both P<0.01; And P>0.05 compared with negative control and blank control.

3.8 The result of histotomy and HE staining

Observed by microscope, the cells in tumor of two control groups arrayed in order, and there have much more connective tissues and vessels in interstitial than the trentment group. The cells in treatment group's cells become different in volume and form. Many cells have some characteristic of apoptosis, such as become smaller, shrinking, nuclear chromatin Agglutination and break into rounded granule like apoptotic body (Figure 8). The tumors have many broken areas.

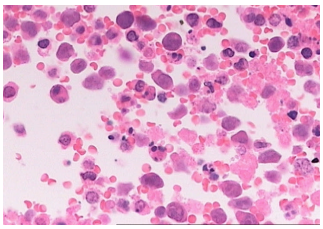

Figure 8. Tumor tissue treated by HE staining（400×）

3.9 The result of immunocytochemistry of the NS protein in cells

The positive rate of NS protein in HL-60 cells of the tumor of blank control group is （97.33±1.76）%, the scores is 220.93±16.54, while the negative control group is（90.72±1.47）% and 195.11±9.71. the positive of NS protein of treatment group is lower than other two groups with (71.59±1.80）% and 110.26±13.46. it indicated that there is obvious difference between the treatment and other two groups($P<0.01$). The two control groups have no obvious difference($P>0.05$). The restrain rate of NS protein in treatment group is 43.5% and 50.1%. (Figure 9)

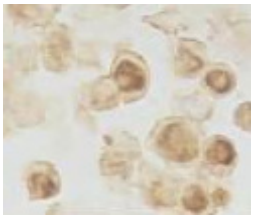

Figure 9. Immunohistochemistry results of NS protein in treated tumor tissues（1000×）

3.10 The result of tunel test

There are many apoptotic cells in the tumor of treatment, while the tumor in control groups have little apoptotic cells. (Figure 10)

A B C

A：Treated group； B：Negative control group； C：Blank control group

Figure 10. Tunel results（400×）

4. Discussion

Nucleostemin is a kind of protein that is necessary for maintaining the proliferation of cells and makes the cells stop differentiation [8]. NS protein may be a specific regulatory factor responsible for stem cells and cancer cells crossing G2/M checkpoint [9]. It has been demonstrated that the down-regulation of NS expression could result in cell cycle arrest and cell differentiation. At present the consensus made by domestic and international experts is that nucleostemin is highly expressed in stem cells and cancer cells. It is considered that NS is related to the regulation of stem cells and cancer cells proliferation, and it could reflect the situation of cell proliferation [10~12]. Some scholars have seen NS as a marker of stem cells and a target of anticancer drugs [11, 12]. Previous studies of our research group have demonstrated that NS-shRNA could interfere the expression of NS protein, and then influence the biological characteristics of HL-60 cells including proliferation, differentiation and apoptosis [6, 13].

Silencing NS gene could obviously weak the oncogenicity of HL-60 cells. The tumor formation rate from the point of view, the time of transfected group is longer than the control group, and the tumor size and weight are all less than the control group. There are also significant differences in stromal connective tissues, blood vessels and HL-60 cells between the transfected group and the control group, this indicates that silencing the NS gene by NS-shRNA-2 could weaken the oncogenicity of HL-60 cells.

The direct transfection RNAi without building the expression vector is a transient response. Along with the extending of time, the interfering effect of NS-shRNA will gradually disappear. At the initial stage of HL -60 cells are inoculated into the nude mice, the interfering effect of NS-shRNA-2 weakens the cell proliferation and reduces the number of cells which could form the tumor, further studies need to be done to determine whether cell differentiation and apoptosis are accompanied in this stage, so we consider that's why the transfected cells grew smaller tumors and need longer time.

The success rate of tumorigenesis of various types of leukemia cells in nude mice is very low. The reason is that the NK cell activity in thymus defecting nude mice is high; however, most leukemia cells are sensitive to NK cells [14]. We gave the nude mice a total dose of 4.5Gy exposure to $^{60}C_O$ radiation before inoculating the leukemia cells, in order to reduce the immune response of nude mice and increase the success rate of transplanted tumors. NS is a p53-binding protein, its biological functions needs the mediation of p53. The HL-60 cells used in this study are p53-null, so we speculate that NS should have another signal pathway except p53, which is the next target of our research.

In this study, the synthesis of the NS- shRNA have been transferred in the form of lipid inclusions in tumorigenic nude mice , and effectively blocked the NS protein expression of the HL-60 leukemia cells in the tumor tissue .Transplanted tumor volume and weight were significantly less than the two control groups. Histological observation of structural damage

to the tumor tissue, cell lyses, more cells have the characteristics of “apoptosis". Tunel confirmed that apoptotic cells of the treatment groups were significantly increased. Therefore, this study suggests that the NS -shRNA played an anti-leukemia effect in nude mice, this effect may be achieved through regulate NS protein expression of the leukemia cells .Tsai and Liu et al used solid tumor cells and found that the NS product's inhibition accompanied by diminishing of cell proliferation capacity, and part of the cancer cells exit the cell cycle [15]. We previously using synthetic NS - shRNA in vitro studies acted on leukemia cells, it also have similar results, which suggests that the diminished capacity of cell proliferation in this case may be related to enhancement of cell apoptosis. Therefore induced apoptosis making leukemia cell proliferation diminish may be an important way of vivo anti- leukemia mitigation in animal model of nude mice. It laid a theoretical foundation for the NS gene as candidate genes for cancer treatment, Nikpour P recently get results similar to the experimental and perspectives [16].

Stephanie Filleur studied siRNA as a therapeutic drug into the animals, and the result showed that intraperitoneal injection is the best way [17]. This study also confirmed the intraperitoneal injection is effective, and intraperitoneal injection as a treatment model , especially used in people malignant tumors and diffusion growth of malignant tumors, is more easily accepted .However, it is most worth considering that whether this treatment model is also effective in the case of the perfect human body immune mechanism. If it can be proved to be the same or similar effective, it will play a tremendous role in RNAi-based gene therapy.

On the mechanism of NS expression inducing leukemia cell apoptosis, the signal transduction pathway in this process, shRNA in nude mice is how to move to the tumor tissue, and a series of questions also require further study.

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Response of Acid and Alkaline Phosphatase Activities to Copper Exposure and Recovery in Freshwater Fish *Carassius auratus gibelio var*

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Abstract: Phosphatase is known to be sensitive to metal exposures and can be used to predict metal toxicity. In this study, freshwater fish *Carassius auratus gibelio var* were exposed to different concentrations (0.1, 0.2, 0.5, 1.0 and 2.0mg/L) of copper for 96 h, and the group of 2.0 mg/L exposure was then transferred to clean water for different days (1, 4, 8 and 12d) to assess recovery profile. Responses of acid phosphatase (ACP) and alkaline phosphatase (ALP) activities from kidney, liver, gill, spleen, muscle and brain to copper exposure and recovery were investigated. As shown from the results, after a 96-h copper exposure, ACP and ALP activities in different organs/tissues appeared to be different. At the highest copper concentration (2.0 mg/L), compared with the control, ACP activity decreased significantly in kidney, liver, gill and spleen, but increased significantly in muscle and brain. ALP activity decreased significantly in kidney, liver, gill, spleen and brain. However, after removing 2.0 mg/L copper exposure, ACP and ALP activities in different organs/tissues all normalized within 12 days. The observed data suggest that ACP and ALP in spleen of *Carassius auratus gibelio var* are most sensitive to copper stress and might be used as suitable biomarkers for copper contamination in aquatic environment.
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Keywords: Copper exposure;acid phosphatase;alkaline phosphatase;recovery;*Carassius auratus gibelio var*

1. Introduction

The increasing industrial, agricultural and anthropogenic activities have caused a rise of metal emissions and aquatic environments have been continuously contaminated by heavy metals. In particular, environmental concentration of copper has been increasing in aquatic environments recently because the wide application of $CuSO_4$ as a pesticide in agricultural practices and as an algicide, fungicide and bactericide in the control of algae and pathogens in aquaculture. Although copper, as a trace element, is essential to perform the functions of specific proteins and enzymes, it becomes toxic and has a potential hazard to aquatic organisms when beyond the normal level (Cerqueira and Fernandes, 2002; Li. et al., 2006). The toxicity of copper to fish has been widely studied in recent years and a wide range of effects on biotransformation, histology, haematology, osmoregulation, immunological modulations and behaviour of fish have been reported (Shariff et al., 2001; Cerqueira and Fernandes, 2002; Oliveira et al., 2004; Liu. et al., 2010). Besides, the knowledge of effects of contaminants on enzymatic activities in fish is also very important to describe the health of fish status and to understand the ecological impacts (Radhaiah et al., 1987). There are some studies that focussed on copper induced the changes of enzymatic activities have highlighted the importance of using enzymatic activities in biomonitoring programs as an Early warning system reflecting the copper pollution in aquatic environments (Antognelli et al., 2003; Carvalho and Fernandes, 2008; Atli et al., 2006). These studies involved in different enzymes and fish species, however, none of the reports focused on the effect of copper on phosphatase activities in different organs/ tissues of freshwater fish *Carassius auratus gibelio var*.

Phosphatase is a hydrolytic enzyme, leading to the release of *ortho*-phosphate from phosphorus compound and based on the optimum pH of action environment, classified into acid phosphatase (ACP, EC 3.1.3.2, optimum pH≤6.0) and alkaline phosphatase (ALP, EC 3.1.3.1, optimum pH≥8.0) (Jansson et al., 1988). Acid phosphatase is identified as a marker enzyme for the detection of lysosomes in cell fraction (Cajaraville et al., 2000) and alkaline phosphatase is a intrinsic plasma membrane enzyme found in almost all animal cells (Mazorra et al., 2002). Both enzymes are metalloenzyme, involved in various metabolic processes, such as permeability, growth and cell differentiation, protein synthesis, absorption and transport of nutrients, and gonadal maturation (Ram and Sathayanesan, 1985). Any change in acid and alkaline phosphatase activities can affect the metabolism of the fish. In fisheries sciences, changes in phosphatase activities have been regarded as indices of growth, illness and spawning of fish (Goldemberg et al., 1987; Matusiewicz and Dabrowski, 1996). And

in the assessment of ecotoxicology, these enzymes have also been used as bioindicators of heavy metals intoxication because of their sensitivity to metal pollution (Anan et al., 2002; Mora et al., 2004).

Carassius auratus gibelio var, a triploid freshwater fish with natural gynogenesis, being close relationship to the crucian carp, is only distributed in Qihe river of Henan province, China. The fish is famous for its delicious taste, rich nutrition and high commercial value. In recent years, this fish has been widely cultured throughout Henan province of China, but the copper pollution of farming water reduced its quality and affect the health of consumers through the food chain. At present, the studying of physiology, growth and reproduction of this fish has become a important task because of its economic importance. However, little research has been carried out on the relationship of this fish and heavy metal pollution for the protection of the aquaculture environment and consumer health.

In this study, the responses of acid and alkaline phosphatase activities to copper exposure in kidney, liver, gill, spleen, muscle and brain of *Carassius auratus gibelio var* were investigated. Furthermore, after removed the copper exposure, recovery process of ACP and ALP activities was evaluated in different periods. The recovery research from copper stress in fish has been investigated by some authors in recent years (Cerqueira and Fernandes, 2002; Shaw and Handy, 2006; Zahner et al., 2006), but these studies all focused on the histopathological and physiological changes in recovery process, the recovery of enzyme activity was not mentioned. Therefore, the phosphatase recovery phase of this experiment will extend our knowledge of the reversibility of enzyme activity in fish. In short, the main objective of this study was to assess the toxicity of copper to *Carassius auratus gibelio var*, investigate the reversibility of phosphatase activity after removal from copper exposure, evaluate the effectiveness of ACP and ALP as early biomarkers to monitor copper pollution in aquatic ecosystems and provide the useful database for healthy breeding of *Carassius auratus gibelio var*.

2. Material and Methods

Fish collection and care

Experimental fish (*Carassius auratus gibelio var*) were obtained from aquatic farm of Henan Normal University (Xinxiang, China), with body lenghth 12.36±1.54 cm and body weight 100.54±1.25 g, and then were transferred to laboratory and acclimatized for 14 days before copper exposure. The fish were reared in experimental tanks sized 100×50×40 cm, each containing 10 fish in 100 L of test solution or tap water only for controls. The tap water used for the experiments had a pH value of 7.9±0.20, conductivity of 578.8±17.8 μs/cm, total hardness of 305.2±19.3 mg $CaCO_3$/L and alkalinity of 140.1±12.5 mg $CaCO_3$/L. Supplemental aeration was provided to maintain dissolved oxygen levels near saturation, the temperature was kept at 25±1 ℃ and the photoperiod controlled (12D: 12L). The fish were fed with commercial fish pellet once a day during acclimation, and without food during experimentation.

Experimental protocol

According to 96 h LC_{50} value (6.02 mg/L) of copper for *Carassius auratus gibelio var* obtained by the acute toxicity test, in this study, sublethal copper concentrations were assigned as 0, 0.1, 0.2, 0.5, 1.0 and 2.0 mg/L (corresponding to 0%, 1/60, 1/30, 1/12, 1/6 and 1/3 of 96 h LC_{50}). Copper was added to the exposure tanks through diluting the stored solution of $CuSO_4$, prepared by analytical grade $CuSO_4 \cdot 5H_2O$ (from Chemical Reagent Company of China). The tap water without adding copper ions was considered as 0 mg/L of copper concentration (the control). In pre experiment, fish exposed to 2.0 mg/L of copper concentration（1/3 96h LC_{50}）for 96h did not show acute and subacute copper intoxication symptoms. So, experimental fish (n=100) were randomly divided into six groups (the control group and 2.0 mg Cu^{2+} /L group each had 30 fish, and the other four groups each had 10 fish.) and exposed to the different copper concentrations for 96h. Then, 5 fish of each group were sampled to be used to biochemical analysis. The surplus fish exposed to 2.0 mg Cu^{2+} /L for 96h were transferred to the clean water to conduct the recovery experiment, and then 5 fish in the recovery group and the control group were taken out randomly and sampled on Day 1, 4, 8, 12 respectively. The experiments were carried out using a static-renewal regimen, 50% of the experimental solution was replaced daily to ensure the relative stabilization of copper concentrations.

Sample prepration

The experimental fish were dissected carefully in ice, and the kidney, liver, gill, spleen, muscle and brain tissues were sampled immediately. The tissues were homogenized in ice-cold physiological salt water (1:9, w/v). Homogenates were centrifuged at 10000 g for 10 min at 4 ℃ in a Universal 30RF centrifuge (Hettich, Tuttlingen, Germany). the supernatant was collected and stored at -80 ℃ until biochemical analysis. All the above operations were carried out below 4 ℃.

Determination of the enzyme activity

Acid phosphatase (ACP) was analyzed according to the methods of Pennington (1961). Alkaline phosphatase (ALP) was measured based on

the method as described by Bretaudiere et al. (1977). The protein content of enzyme crude extract was determined using Coomassie Brilliant Blue (G-250) method repoted by Bradford (1976) and bovine g-globuline (BSA, purchased from Amresco) was used as the standard. The optical density was measured at 405 nm (ACP) and 400 nm (ALP) using a UV–VIS spectrophotometer (TU-1810APC, China) respectively. Enzyme activity unit was expressed as nmol/mg protein per minute.

Statistical analysis

Experimental data are presented as Mean± Standard Deviation (Mean±SD). Statistical analysis was implemented in SPSS statistical package programs. One-way ANOVA was used to compare variables among the different groups. An unpaired two-tailed Student's *t*-test was used to analyze significant differences. Significant level was assigned at $P = 0.05$ (significant difference) and $P = 0.01$ (highly significant difference).

3. Results

Response of ACP and ALP activities to copper exposure and recovery in kidney

After a 96-h copper exposure, compared with the control, ACP activity in kidney significantly decreased at 0.5, 1.0 and 2.0 mg Cu^{2+}/L exposures ($P<0.01$), with the inhibition rate at 5%, 9% and 12%, respectively. The lowest value of ACP activity appeared at 2.0 mg Cu^{2+}/L exposure (Figure 1a). ALP activity increased at lower copper concentration, and decreased with the elevated copper concentration, and reached the peak value at 0.2 mg Cu^{2+}/L. Compared with the control, ALP activity significantly increased by 31% at 0.2 mg Cu^{2+}/L exposure ($P<0.01$), but significantly decreased by 38% and 39% at 1.0 and 2.0 mg Cu^{2+}/L, respectively ($P<0.01$) (Figure 1c).

During the recovery period, compared with the control, ACP activity in kidney was significantly lower than that in the control on Day 1 and 4 ($P<0.05$). On Day 8 and 12, ACP activities were approximate to the control value ($P>0.05$), and it was suggested that ACP activity was restored to the normal physiological level on the 8th day (Figure 1b). ALP activity in kidney was significantly lower than that in the control on Day 1, 4 and 8 ($P<0.05$ or $P<0.01$), however, compared with the 96-h exposure, ALP activity increased continuously from Day 1 to Day 12 and was significantly higher on Day 8 and 12 ($P<0.01$). On day 12, ALP activity was similar to the control ($P>0.05$), which indicated that ALP activity returned to normal level (Figure 1d).

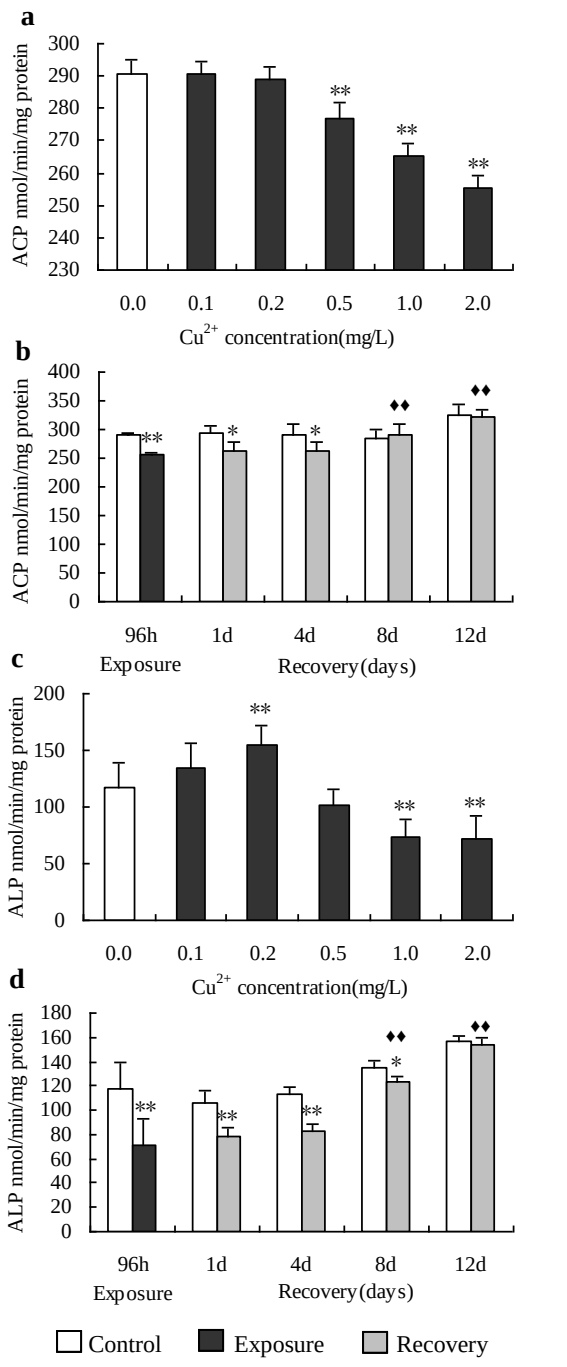

Figure 1. Changes of ACP and ALP activities in kidney of *Carassius auratus gibelio var* after a 96-h copper exposure and recovery of enzyme activity after removed 2.0 mg/L exposure. The values are expressed as mean±SD (n=5). Compared with the control, "*" respresents the significant difference (p <0.05), "**" respresents the highly significant difference (p <0.01); Compared with the 96-h copper exposure, "♦" respresents the significant difference (p <0.05), "♦♦" respresents the highly significant difference (p <0.01).

Response of ACP and ALP activities to copper exposure and recovery in liver

Figure 2a and Figure 2c illustrates the changes in ACP and ALP activities in liver after a 96-h copper exposure. With the increase of copper concentration, both ACP and ALP activities increased firstly and reached the peak values at 0.2 mg Cu^{2+}/L exposure and then decreased. Compared with the control, ACP activity significantly increased by 5% and 4% at 0.2 and 0.5 mg Cu^{2+}/L exposures, respectively (P<0.01), but significantly decreased by 3% at 2.0 mg Cu^{2+}/L exposure (P<0.05). ALP activity significantly increased by 54% and 138% at 0.1 and 0.2 mg Cu^{2+}/L exposures, respectively (P<0.01), but significantly decreased by 53% at 2.0 mg Cu^{2+}/L exposure (P<0.05).

During the recovery, after removed 2.0 mg/L copper exposure, ACP activity in liver was significantly lower than that in the control on Day 1, 4 and 8 (P<0.05 or P<0.01). Compared with the 96-h exposure, ACP activity decreased continuously and remarkably from Day 1 to Day 4 (P<0.05 or P<0.01). On Day 12, ACP activity showed no significant difference compared with the control (P>0.05), which indicated that ACP activity recovered to the normal level (Figure 2b). ALP activity in liver was significantly lower than that in the control on Day 1 and 4 (P<0.01). On Day 8 and 12, ALP activity was very close to the control (P>0.05), it was demonstrated that ALP activity in liver recovered to normal level on the 8th day. Compared with the 96-h exposure, ALP activity increased significantly on Day 4, 8 and 12 (P<0.01) (Figure 2d).

Responses of ACP and ALP activities to copper exposure and recovery in gill

After a 96-h copper exposure, ACP activity in gill decreased gradually with increased copper concentration and reached the least value at 2.0 mg Cu^{2+}/L exposure. 0.5, 1.0 and 2.0 mg Cu^{2+}/L exposures caused significant decreases in ACP activity in gill compared with the control (P<0.01), with the inhibition rate at 7%, 17% and 19%, respectively (Figure 3a). With the increased copper concentration, ALP activity gradually increased firstly and reached the peak at 0.5 mg Cu^{2+}/L. ALP activity significantly increased by 50% and 92% at 0.2 and 0.5 mg Cu^{2+}/L exposures, respectively (P<0.01), but significantly decreased by 34% at 2.0 mg Cu^{2+}/L exposure (P<0.01) (Figure 3c).

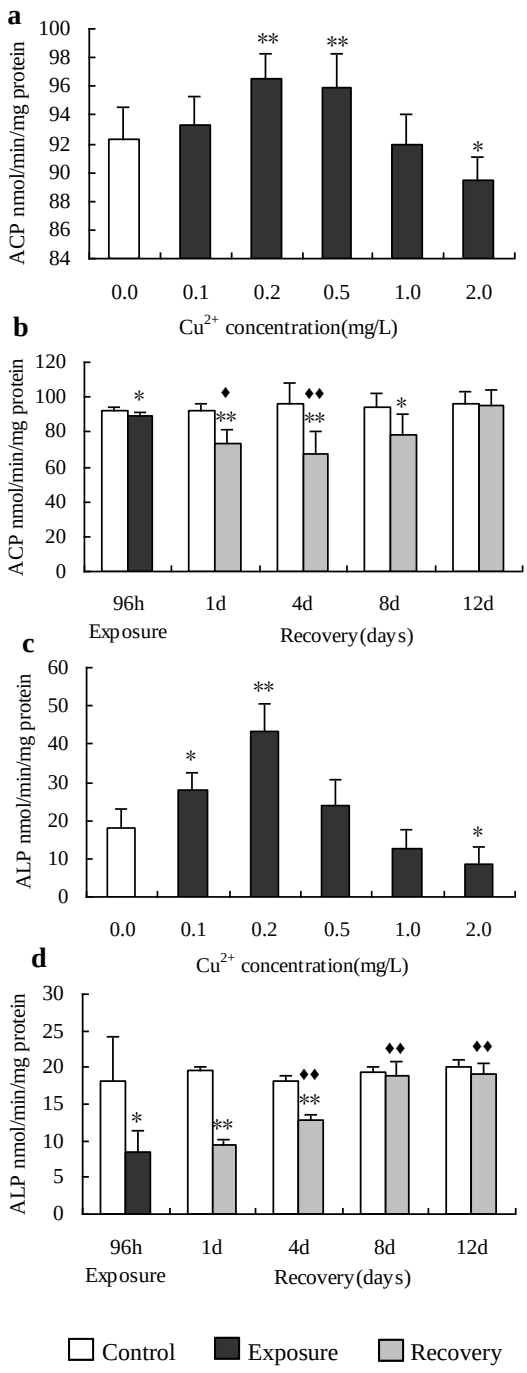

Figure 2. Changes of ACP and ALP activities in liver of *Carassius auratus gibelio var* after a 96-h copper exposure and recovery of enzyme activity after removed 2.0 mg/L exposure. The values are expressed as mean ±SD (n=5). Compared with the control, "*" respresents the significant difference (p <0.05), "**" respresents the highly significant difference (p <0.01); Compared with the 96-h copper exposure, "♦" respresents the significant difference (p <0.05), "♦♦" respresents the highly significant difference (p <0.01).

As shown from Figure 3b and Figure 3d, after 2.0 mg/L copper exposure was removed, ACP activity in gill showed no significant changes on Day 1, 4, 8 and 12, in contrast with the control (P>0.05). It was suggested that ACP activity in gill returned to normal level on the first day. ALP activity was lower significantly than that in the control on Day 1 and 4 (P<0.05 or P<0.01). Compared with the 96-h exposure, ALP activity slightly decreased on Day 1 (P>0.05), but significantly increased on Day 4 (P<0.05). On Day 8 and 12, ALP activity reached the control level (P>0.05), which showed that ALP activity in gill recovered to the normal level on the 8th day.

Response of ACP and ALP activities to copper exposure and recovery in spleen

As seen in Figure 4a and Figure 4c, after a 96-h copper exposure, ACP and ALP activities in spleen decreased gradually with the increasing copper concentration and reached the minimum value at 2.0 mg Cu^{2+}/L exposure. Compared with the control, ACP activity significantly decreased by 2%, 5%, 6%, 7% and 11% at 0.1, 0.2, 0.5, 1.0 and 2.0 mg Cu^{2+}/L exposures, respectively (P<0.01). It was demonstrated to have a significant negative correlation between the ACP activities (A) and the exposure concentrations (X). The regress equation is $A = -6.1513X + 293.26$ (R^2=0.9748). Compared with the control, ALP activity significantly decreased by 18%, 35%, 50%, 60% and 62% at 0.1, 0.2, 0.5, 1.0 and 2.0 mg Cu^{2+}/L exposures, respectively (P<0.05 or P<0.01). A significant negative correlation was also found between ALP activities (Y) and the exposure concentrations (X), and the regress equation is $Y = -8.5151X + 70.113$ (R^2=0.9447).

After 2.0 mg/L exposure was free, ACP activity in spleen was still significantly lower than that in the control on Day 1 and 4 (P<0.01). Compared with the 96-h exposure, ACP activity decreased continuously from Day 1 to Day 4, and appeared to be significantly lower on Day 4 (P<0.05). But on Day 8 and 12, ACP activity indicated no significant difference compared with the control (P>0.05), and it was shown that ACP activity in spleen recovered to normal level on the 8th day (Figure 4b). ALP activity was significantly lower than that in the control on Day 1 (P<0.01), but significantly higher than that in 96-h exposure group (P<0.01). On Day 4, 8 and 12, ALP activity was close to the control (P>0.05), and it was suggested that the normal level of ALP activity in spleen was restored on the 4th day (Figure 4d).

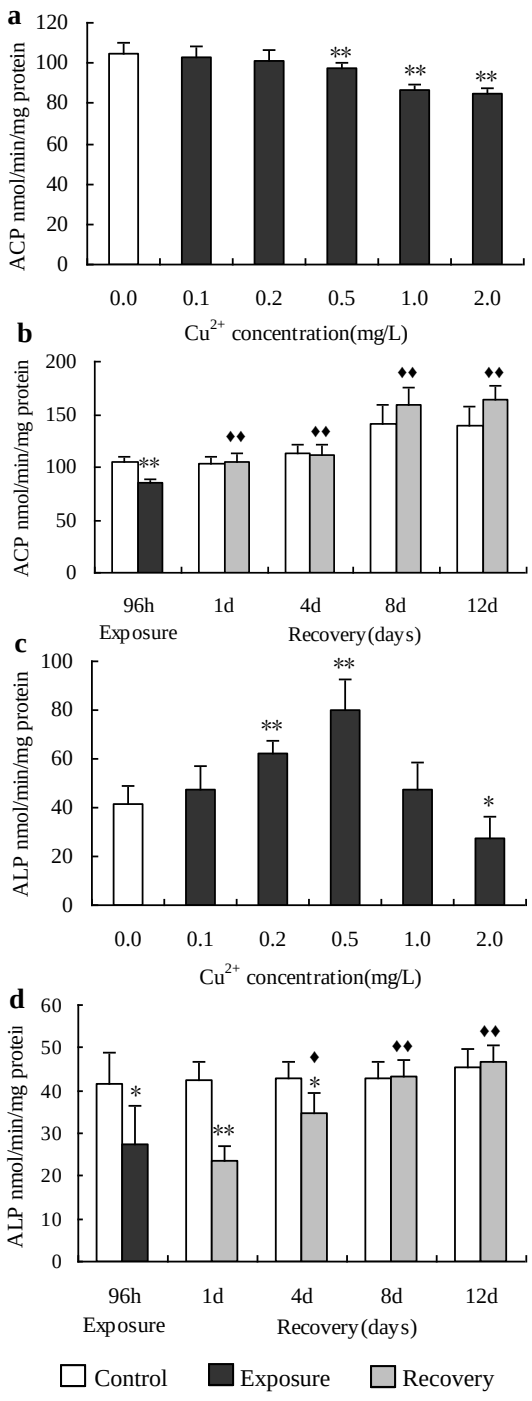

Figure 3. Changes of ACP and ALP activities in gill of *Carassius auratus gibelio var* after a 96-h copper exposure and recovery of enzyme activity after removed 2.0 mg/L exposure. The values are expressed as mean ±SD (n=5). Compared with the control, "*" respresents the significant difference (p <0.05), "**" respresents the highly significant difference (p <0.01); Compared with the 96-h copper exposure, "♦" respresents the significant difference (p <0.05), "♦♦" respresents the highly significant difference (p <0.01).

Response of ACP and ALP activities to copper exposure and recovery in muscle

After a 96-h copper exposure, compared with the control, ACP activity in muscle showed no significant changes at 0.1, 0.2, 0.5 and 1.0 mg Cu^{2+}/L exposures (P>0.05), but significantly increased by 14% at 2.0 mg Cu^{2+}/L exposure (P<0.05) (Figure 5a). ALP activity indicated no significant difference between the exposure and the control (P>0.05) (Figure 5c).

During the recovery span, in 2.0 mg/L exposure group, ACP activity in muscle was significantly higher than that in the control on Day 1, 4 and 8 (P<0.01). Compared with the 96-h exposure, ACP activity increased continuously from Day 1 to Day 8, while on Day 12, ACP activity got to the control level (P>0.05). It was suggested that ACP activity in muscle normalized on the 12th day (Figure 5b). Compared with the control, there were no significant change in ALP activity during the recovery period (Figure 5d).

Response of ACP and ALP activities to copper exposure and recovery in brain

Changes in ACP and ALP activities in brain after a 96-h copper exposure are depicted in Figure 6a and Figure 6c. ACP activity in brain elevated gradually with the increase of copper concentration and reached the maximum value at 2.0 mg Cu^{2+}/L exposure. 1.0 and 2.0 mg Cu^{2+}/L exposures caused significant increases in ACP activity in brain compared with the control (P<0.01), with activation rate at 8% and 11%, respectively. Compared with the control, ALP activity slightly decreased at 0.1, 0.2 and 0.5 mg Cu^{2+}/L exposures (P>0.05), and obviously decreased by 18% and 32% at 1.0 and 2.0 mg Cu^{2+}/L exposures, respectively (P<0.01), and reached the lowest value at 2.0 mg Cu^{2+}/L exposure.

After 2.0 mg/L copper exposure was removed, compared with the control, ACP activity in brain significantly increased on Day 1, 4 and 8 (P<0.01). Compared with the 96-h exposure, ACP activity increased continuously from Day 1 to Day 8 and was significantly higher than that in 96-h exposure group (P<0.05 or P<0.01). However, on the day 12, ACP activity was restored to the control level (P>0.05) (Figure 6b). Compared with the control, there was no significant change in ALP activity in brain on Day 1, 4, 8 and 12 (P>0.05), and it was indicated that ALP activity in brain normalized on the first day (Figure 6d).

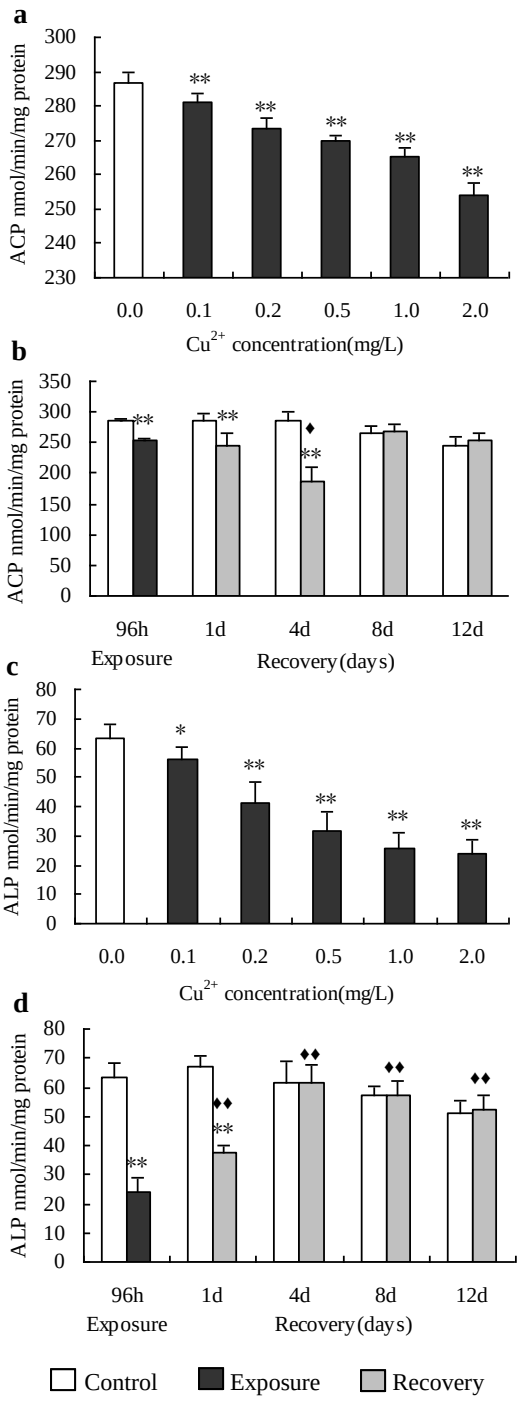

Figure 4. Changes of ACP and ALP activities in spleen of *Carassius auratus gibelio var* after a 96-h copper exposure and recovery of enzyme activity after removed 2.0 mg/L exposure. The values are expressed as mean ±SD (n=5). Compared with the control, "*" respresents the significant difference (p <0.05), "**" respresents the highly significant difference (p <0.01); Compared with the 96-h copper exposure, "♦" respresents the significant difference (p <0.05), "♦♦" respresents the highly significant difference (p <0.01)

4. Discussions

In toxicological studies, ACP and ALP are important biochemical enzymes to be used to detect the alteration of physiological metabolism of animal induced by metal exposure (Reddy and Bhagyalakshmi, 1994; Oruc and Uner, 1999). ACP is a lysosomal enzyme, many environmental contaminates including heavy metals could be sequestered in lysosomes of eukaryotic cells, and some metals could alter the structure, permeability and integrity of lysosomal membranes and result in enzyme diffusion into cytosol (Hedayati et al., 2010). In this study, ACP activity increased significantly at 0.2 and 0.5 mg Cu^{2+}/L exposures in liver, at 2.0 mg Cu^{2+}/L exposure in muscle and at 1.0 and 2.0 mg Cu^{2+}/L exposures in brain. This elevation might be due to the activation of the enzyme which was kept in a latent state inside the membrane of lysosomes (De Duve et al., 1955) as a result of the disruption of the membrane by copper, or due to the proliferation of lysosomes in attempt to sequester copper. On the contrary, ACP activity decreased significantly at 0.5, 1.0 and 2.0 mg Cu^{2+}/L exposures in kidney and gill, at 2.0 mg Cu^{2+}/L exposure in liver and at all copper exposures in spleen. This reduction might be attributed to the escape of the enzymes from the lysosomes out of the cell due to the damage of lysosomal membrane (Malbica and Hart, 1971) by copper. The decreased lysosomal membrane stability was observed by Regoli et al. (1998) on exposure to copper.

ALP, a ubiquitous plasma membrane-bound enzyme, is often employed to assess the integrity of the plasma membrane (Akanji et al., 1993), and any perturbation in the membrane property caused by interaction with xenobiotics could lead to alteration in ALP activity (Molina et al., 2005). In the present study, ALP activity increased significantly at 0.2 mg Cu^{2+}/L exposure in kidney, at 0.1 and 0.2 mg Cu^{2+}/L exposures in liver, and at 0.2 and 0.5 mg Cu^{2+}/L exposures in gill. Such increase might be attributed to the increase in functional activity of these organs/tissues leading to the synthesis of the enzyme molecule (Umezawa and Hooper, 1982; Yakubu et al., 2001), or regard it as an adaptive response in mitigating copper toxicity at low concentration. On the contrary, ALP activity decreased significantly at 1.0 and 2.0 mg Cu^{2+}/L exposures in kidney and brain, at 2.0 mg Cu^{2+}/L exposure in liver and gill, at all copper exposures in spleen. Such reduction might be attributed to the loss of ALP from plasma membrane into the extracellular fluid (Malbica and Hart, 1971) and the reduction in concentration or total absence of specific phospholipids required by this membrane-bound enzyme to express its full activity (Yakubu et al., 2002) under the interaction of copper with plasma membrane, or due to inhibition of the enzyme activity at the cellular/molecular level (Akanji et al., 1993) and inactivation of the enzyme molecule *in situ* (Umezawa and Hooper, 1982) by the binding of copper to ALP directly. In addition, the little effect of copper on phosphatase activity in muscle revealed that the membranes of lysosomes and plasma in muscle have been little disrupted by copper.

In this study, ACP activity in liver and ALP activity in kidney, liver and gill increased at lower copper concentration and then went down with the increased copper concentration. The relation between copper concentration and enzymatic activity showed the inverted U-shaped curve, which is usually called as hormetic effect (Rodricks, 2003). Hormetic effect, a phenomenon characterized by low-dose stimulation and high-dose inhibition, has been frequently observed in organisms exposed to heavy metals (Oller and Bates, 2004) and considered to be a general rule in the study of environment toxicology, but the mechanisms underlying it induced by environmental agents still remain an enigma. Nevertheless, the mechanism of hormetic effect in this study might be interpreted as the result of instantaneous balance between the synthesis and degradation of enzyme protein under the interaction of copper with membranes of lysosomes and plasma.

In the present study, copper-induced alterations in phosphatase activities in different organs/tissues were variable, this may be relate to the different physiological functions of organs/tissues. Moreover, Atli et al. (2006) reported that the responses of enzyme activities in different organs/tissues to heavy metals also depend on metal bioaccumulation ability of these organs/tissues. Kidney is the excretory and immune organ of fish, in which the higher metal bioaccumulations were observed (Dautremepuits et al., 2004; Palaniappan and Karthikeyan, 2009). In the present study, compared with the control, ACP and ALP activity in kidney both appeared the significant change at three different copper concentrations, this suggests the sensitivity of the both enzymes to copper stress in the kidney. This sensitivity may be related to the higher copper bioaccumulation in kidney. In previous studies, the kidney was found to be a organ with the highest ALP activity by Cvancara et al. (1978), but in this study, compared the control values of the both enzymes in kidney with that in the other organs/tissues, we found the kidney of *Carassius auratus gibelio var* has not only the highest ALP activity but also the highest ACP activity.

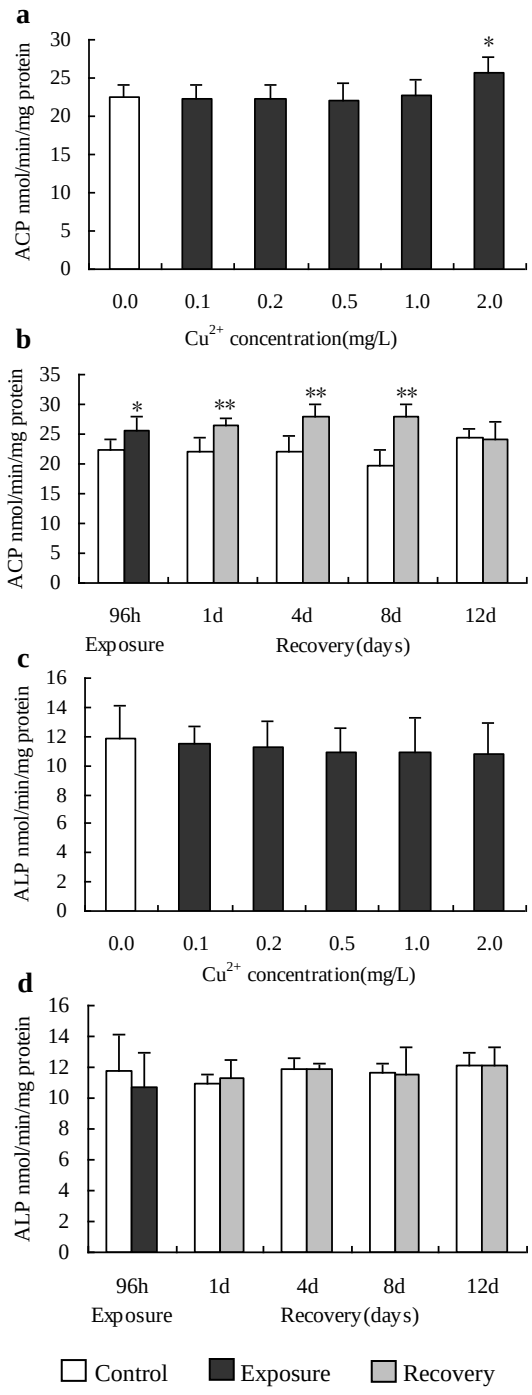

Figure 5. Changes of ACP and ALP activities in muscle of *Carassius auratus gibelio var* after a 96-h copper exposure and recovery of enzyme activity after removed 2.0 mg/L exposure. The values are expressed as mean ±SD (n=5). Compared with the control, "*" respresents the significant difference (p <0.05), "**" respresents the highly significant difference (p <0.01); Compared with the 96-h copper exposure, "♦" respresents the significant difference (p <0.05), "♦♦" respresents the highly significant difference (p <0.01).

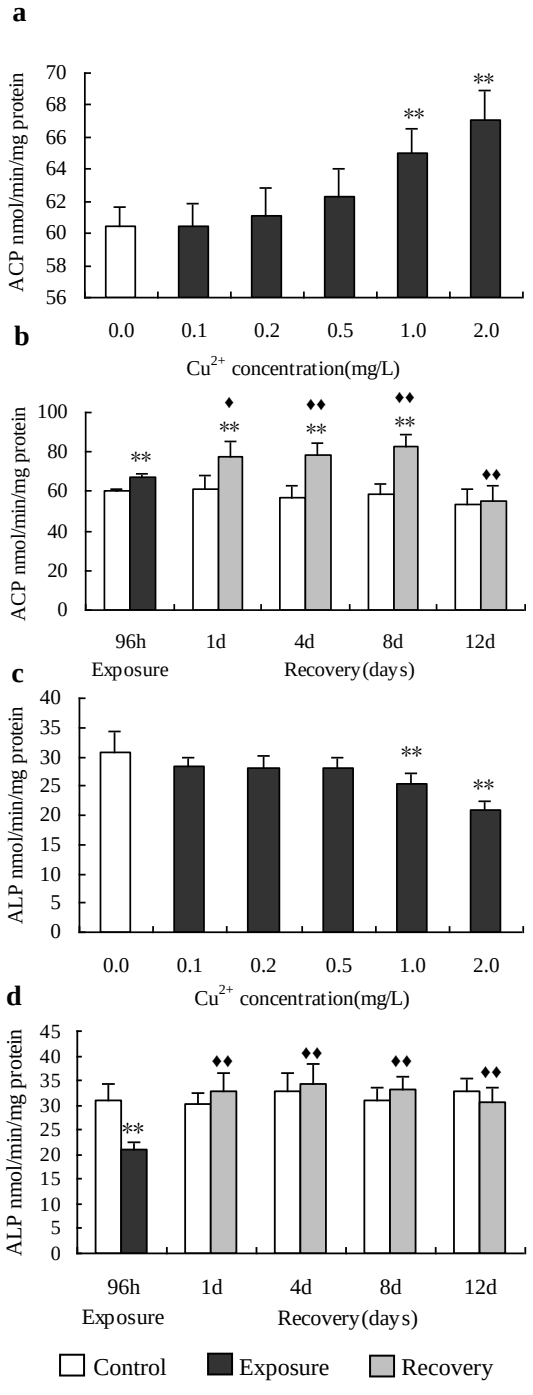

Figure 6. Changes of ACP and ALP activities in brain of *Carassius auratus gibelio var* after a 96-h copper exposure and recovery of enzyme activity after removed 2.0 mg/L exposure. The values are expressed as mean ±SD (n=5). Compared with the control, "*" respresents the significant difference (p <0.05), "**" respresents the highly significant difference (p <0.01); Compared with the 96-h copper exposure, "♦" respresents the significant difference (p <0.05), "♦♦" respresents the highly significant difference (p <0.01).

Fish liver is not only one of the vital detoxifying organs, but also the important compartment of heavy metal accumulation (Jarić et al., 2011; Fallah et al., 2011). In the present study, the effect of copper on ACP and ALP activities in liver all showed the typical hormetic dose-response, which may be associated with the detoxification function of fish liver. Marr et al. (1995) pointed out that a metal-binding protein, the metallothionein (MT), could be induced by heavy metals in liver, and there existed a positive correlation between MT and heavy metals. MT in liver can attenuate cytotoxicity induced by heavy metals by sequestering these metals and reducing their intracellular concentration. In the present study, increased ACP and ALP activities at low copper concentrations suggested the hydrolysis of phosphate esters to release energy in view of the synthesis of MT and the enhancement of the detoxification function of liver. With the increasing copper concentration, more copper ion entered into liver cells and was sequested by MT firstly, but when the higher copper concentrations in liver was beyond the regulation capacity of MT by Cu-binding, the surplus copper could direct bind to –SH groups on enzyme molecule and cause the decrease of ACP and ALP activities. At the same time, the detoxification capacity of liver decreased and this would lead to the liver damage eventually. Sharkoori et al. (1992) have suggested the decrease (or) inhibition of ACP and ALP activities might be taken as indexs of necrosis in hepatocytes.

Gill is the vital respiration organ, which was targeted by lots of xenobiotics due to their extensive surface area directly contacted with water environment and the reduced distance between the internal and external medium. In this study, in gill, ACP activity and ALP activity both changed significantly at three copper concentrations compared with the control. It was suggested that ACP and ALP in gill, like the kidney, were more sensitive to copper. In addition, ACP activity was inhibited by all copper exposures, but ALP activity was induced at low copper exposures and inhibited at high copper exposures. This result was at odds with the previous literatures which showed that ACP and ALP activities in gill were all inhibited after exposure to some contaminants (Karuppasamy, 2000; Bhavan and Geraldine, 2004). This discrepancy could be due to the difference of contaminant type and fish species.

Spleen plays an important role in immune protection in fish. In this study, ACP and ALP activities in spleen were significantly inhibited at all copper concentrations, and the significant negative correlation was found between copper exposure concentration and enzymatic activity. Nevertheless, there are few literatures to our knowledge about the ACP and ALP activities in fish spleen. The data in this study suggested that ACP and ALP in spleen were the most sensitive to copper exposure among the organs/tissues. This sensitivity may be related to the immune function of spleen, because the immune system is vital for the fish to prevent from infectious agents and immune impairment by environmental pollutants (Spromberg and Meador, 2005).

Fish muscle is an important tissue to conduct movement. Contractile proteins are rich in muscle and have a high affinity for calcium and a low affinity for heavy metals (Palaniappan and Karthikeyan, 2009). Thus, fish muscle usually has the lower accumulation ability to heavy metals. In addition, fish muscle is consumed by the general public as food. Owing to consumers healthy demand, metal bioaccumulation of fish muscle has been paid more attentions and studied extensively by many investigators (Storelli et al., 2006; Ploetz et al., 2007; Agah et al., 2009; Palaniappan and Karthikeyan, 2009). The results from these studies further confirmed that muscle had the lowest accumulation level for the most of the heavy metals among the organs/tissues in fish. In the present study, copper has little effect on ACP activity and no effect on ALP activity in muscle of *Carassius auratus gibelio var*, it was suggested to be associated with the lower copper bioaccumulation of this fish muscle.

Fish brain is the major component of the central nervous system and the main target of the pollutants (Mieiro et al., 2011). Water contaminants can effect the activities of various enzymes in brain (Bagnyukova et al., 2005; Modesto and Martinez, 2010) and even cause the neurodegenerative damage (Berntssen et al., 2003) by passing through the fish blood-brain barrier into the brain tissue. In this study, the increased ACP activity and the decreased ALP activity after an 96-h copper exposure were observed in the brain of *Carassius auratus gibelio var*. This result is in agreement with the elevation of ACP activity and the inhibition of ALP activity in brain of *Channa punctatus* after 96-h $HgCl_2$ exposure as reported earlier by Sastry and Sharma (1980). The functional significance of ACP and ALP in brain is involved in various secretory and transport processes, and the ALP also involved in blood-brain barrier mechanisms (Shaffi, 1979). The alterations in the both phosphatases activities in the present study indicate the copper-induced disturbances in the normal functioning of the brain of *Carassius auratus gibelio var*.

Studies of contaminant-induced alterations in phosphatase activities reported that the changes in ACP and ALP activities could damage the cells and organs/tissues and adversely affect their physiological

functions (Butterworth and Moss, 1966; Ramalingam and Vimaladevi, 2002; Akanji et al., 2008). In the present study, in the group of 2.0 mg/L exposure, after 96-h copper exposure, ACP and ALP activities in six organs/tissues all significantly changed except ALP activity in muscle. This showed the membranes of lysosomes and plasma have been heavy disrupted by copper at this high copper concentration. ACP activity significantly decreased in kidney, liver, gill and spleen, this decrease reflected the damage of lysosomes by copper, and the injured lysosomes would release hydrolytic enzymes into cytoplasm leading to auto degradation of cellular proteins and subsequent cell necrosis (Kågedal et al., 2001). ACP activity significantly increased in muscle and brain, this could result in indiscriminate hydrolysis of phosphate esters (Butterworth and Moss, 1966) and consequently autolysis and cell death which constitute a possible threat to the well being of the organs (Akanji et al., 2008). ALP activity significantly decreased in kidney, liver, gill, spleen and brain, such reduction would hinder adequate transportation of required ions or molecules across their cell membrane (Akanji et al., 1993) and also adversely affect other metabolic processes where the enzyme is involved such as the synthesis of nuclear proteins, nucleic acids, phospholipids and cleavage of phosphate esters (Ramalingam and Vimaladevi, 2002). However, the both enzymatic activities in the six organs/tissues all could normalize within 12 days after copper was removed, it was demonstrated that the copper toxicity on the lysosomal and the plasma membrane was transient and the enzymatic activities were reversible.

In recovery process, the recovery speed of ACP and ALP activities in different organs/tissues was different. ACP activity in gill and ALP activity in brain normalized on the first day in the fastest speed. ACP activities in liver, muscle and brain, and ALP activity in kidney normalized on the twelfth day in the slowest speed. Such discrepancy is probably related to the different regulation mechanisms of the organs/tissues. Moreover, the enzymes in differernt organs/tissues exhibited different recovery pattern. After 96-h copper exposure, ACP activities in kidney and gill, and ALP activities in kidney, liver, spleen and brain significantly decreased compared with the control, but after copper exposure was removed, enzyme activities increased continuously compared with the 96-h exposure group, which showed the consistency between the change of enzymes activities and the elimination of copper. ACP activities in muscle and brain increased significantly compared with the control after 96-h copper exposure. After copper exposure was free, they did not decrease immediately compared with the 96-h exposure group, but increased continuously from Day 1 to Day 8. The Reason might be that the higher level of acid phosphatase mRNA made the enzyme synthesis continue, although environmental factor to induce ACP activity had been eliminated. After 96-h copper exposure, ACP activity in liver and spleen, and ALP activity in gill significantly decreased compared with the control, but after copper exposure was removed, enzyme activities decreased firstly and then increased compared with 96-h copper exposure. It is difficult to explain these changes, so the further studies still need to be carried out to provide the more evidences.

In summary, our study unambiguously demonstrates for the first time that ACP and ALP in spleen of *Carassius auratus gibelio var* are very sensitive to copper stress and can be accepted as sensitive biomarkers to assess copper contamination in aquatic ecology. This study also suggests the changes of ACP and ALP activities in different organs/tissues of *Carassius auratus gibelio var* exposed to copper are different, but the changes of the both enzyme activities in the highest copper concentration group (2.0mg/L) are recoverable, and the different recovery pattern in differernt organs /tissues may be due to the physiological regulation of these organs/tissues and the enzyme complement mechanism at the cellular level. However, the more experiments are still required to carried out to provide the more evidences to better understand the response mechanism of ACP and ALP activities in fish to copper exposures and recovery process.

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Remote Sensing as a Tool in Assessing Water Quality

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Abstract: Remote sensing techniques can be used to detect the water quality against different dates. The aim of this study is to determine the relation between the water quality of AL-Abshiet drain and its reflectance using satellite images. Four different dates of SPOT satellite images and an image of World View satellite are used to measure the reflectance of the water through the selected 6 points along Al-Abshiet drain. The results of monitoring the water reflectance of 6 points along Al-Abshiet drain (as polluted water) and one point taken from Nile River (Damietta Branch as clear water) show that there is a high effect of the growth of aquatic plants and suspense-materials in the year 2011 in the all year and there is high difference with the clear water sample (Damietta Branch). There are high differences in the water reflectance which is mainly due to the growth of aquatic plants as well as the suspended water in Al-Abshiet drain. The results of the chemical analyses of Al-Abshiet drian water show that the Nitriate-Nitrogen values in sites 5 and 6 ranged from 18.03 mg/l to 18.68 mg/l were higher than the maxiumum limit value (15 mg/l). The ammonia-N value in sites 5 and 6 ranged from 5.10 mg/l to 5.52 mg/l) was less than the maxiumum limit value (5 mg/l). The ECw of Al-Abshiat drain in all sites (ranged from 2.6 dS/m to 3.2 dS/m) were higher than the maximum limit value (2 dS/m). The Boron element was very high increase in Al-Abshiet drain in all 6 sites (ranged from 5.16 mg/l up to 32.99 mg/l) than the maxiumum limit (0.75 mg/l). Therefore, the water quality of Al-Abshiet drian is not recomended to be used it for irrigation (Accoding to Ayers and Westcst, 1994).
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Keyword: water and aquatic plant reflectance; water quality and pollution; remote sensing; Al-Abshiet drain.

1. Introduction

Suspended sediments and aquatic plants are the most common pollutant both in terms of weight and volume in surface waters of freshwater systems. Suspended sediments may serve as a surrogate contaminant in agricultural watersheds since phosphorus, insecticides, and metals adhere to fine sediment particles. Suspended sediments increase the radiance emergent from surface waters in the visible and near infrared proportion of the electromagnetic spectrum (Ritchie and Schiebe 2000). Significant relationships have been shown between suspended sediments and radiance or reflectance from spectral wave bands or combinations of wave bands on satellite and aircraft sensors (Brando, V.E., & Dekker, A.G. 2003). Aquatic plants are the main sources of loosing water in the open water channels as a result of their high evapotranspiration due to its viperous vegetative growth.

Phosphorous and nitrogen are both key parameters for aquatic plant life. The ammonium ion is the preferred nitrogen source for plant growth (Smolders et al. 2002; James et al. 2004; Jampeetong and Brix 2009). Several studies have shown that phosphate and ammonium concentrations in water affect the composition of aquatic plant communities (Kohler 1975; Carbiener et al. 1990).

Remote sensing techniques can be used to assess several water quality parameters (e.g., suspended sediments (turbidity), chlorophyll, temperature). Monitoring the concentration of the chlorophyll (algal/phytoplankton) is needed to manage eutrophication in lakes, water bodies, irrigation and drainage canals. Remote sensing can be used to measure chlorophyll concentrations and patterns in water bodies. As with suspended sediment measurements, remote sensing of chlorophyll in water is based on developing relationships between radiance/reflectance in narrow bands or band ratios and chlorophyll (Greulich S, Bornette G 1999).

Remote sensing techniques, ground truth, and laboratory analysis are used in this study to determine the relation between the water of Al-Abshiet drain and its reflectance using satellite images.

2. Materials and methods

2.1. Materials used:

2.1.1. Satellite images:

Four different dates of SPOT satellite images and an image of World View satellite are used to measure the reflectance of the water through the selected 6 points along Al-Abshiet drain as polluted water and one clear water sample from Nile River (Damietta Branch). The following satellite images were used.

No.	Satellite Type	Date	Resolution
1	World Veiw2	Summer 2011	2 meters
2	SPOT5	Summer 2005	2.5 meters
3	SPOT4	Summer 2000	20 meters
4	SPOT4	Summer 1995	20 meters
5	SPOT2	Summer 1989	20 meters

2.1.2. GIS Software

Dynamic SWERI data collector system (SDC) using Global Position System (GPS) is developed to define the location of the 6 sites. Water samples of water of Al-Abshiet drain (as polluted water), and one sample from Nile River (Damietta Branch as clear water) were collected. The ERDAS Imagine 9.1 is used for image enhancement, correction and cut purposes, while Arc.GIS 9.2 and ILWIS 3.7 software's are used as a system provides its users with state of the art data gathering, data input, data storage, data manipulation, analysis, and data output capability by integrating conventional GIS.

2.1.3. Location of the studied area

The area located between Tanta city and El Mahalah city. Al-Abshiat drain is passing from the southern to northern part. Figure (1) and Table (1) show the Location and the coordinates of the 6 Sites along Al-Abshiet drain.

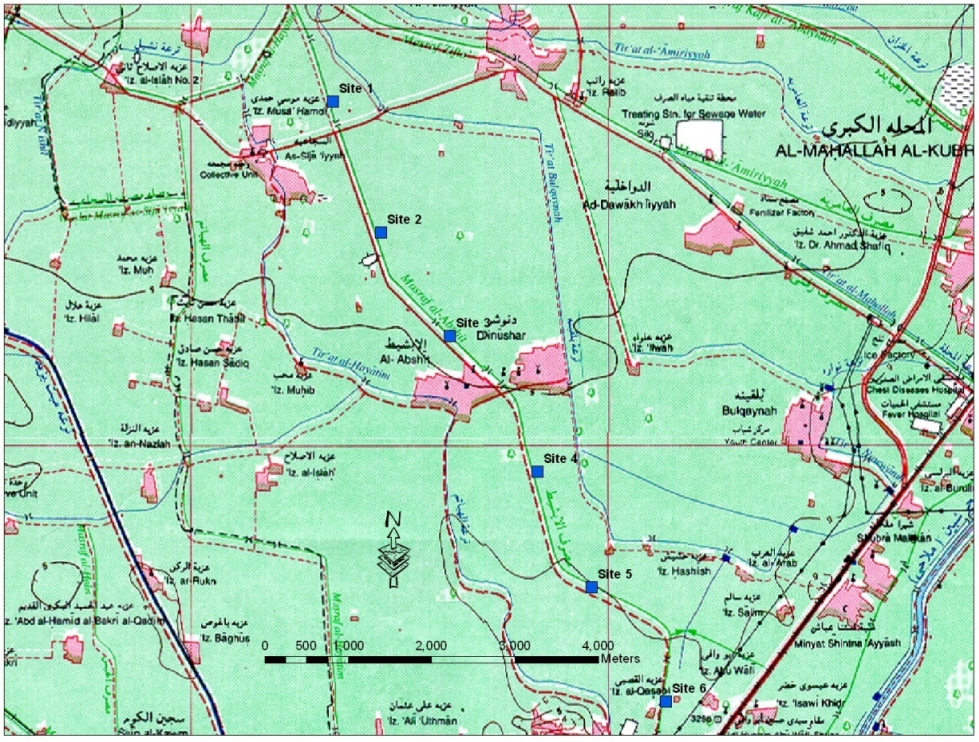


Figure (1) The Location of the 6 Sites along Al-Abshiet drain

Table (1) the Longitudes and Latitudes of the 6 Sites along Al-Abshiet drain and Nile River (Damietta Branch)

Sites	Long	Lat
Site 1	31°0 4' 12.872" E	30° 59' 02.997" N
Site 2	31°0 4' 34.675" E	30° 58' 11.972" N
Site 3	31° 05' 05.659" E	30° 57' 31.613" N
Site 4	31° 05' 45.433" E	30° 56' 38.650" N
Site 5	31° 06' 09.725" E	30° 55' 53.484" N
Site 6	31° 06' 43.353" E	30° 55' 09.029" N
Nile River (Damietta Branch)	31° 14' 06.207" E	30° 54' 17.180" N

2.1.4. Image Processing

Image processing techniques were followed to produce the best possible, enhanced image. Colour enhancement was done to create new images from original in order to increase the amount of information that can be visually interpreted from the data. In this procedure three bands were selected for red, green and blue to create false colour composite (3, 2, 1) for all SPOT images and false colour composite (7, 5, 3) for World View image. For each site the reflectance of the three bands used to plot in graphs to see the effects of growth the aquatic plants and suspense-materials on Al-Abshiet drain.

2.1.5. Water samples

Six sites water samples were collected. Its cover the distance from the connection with drain No.8 (sites 1-3), to the north of Al-Abshiet Village and three sites from the south of Al-Abshiet village to the nearest point to textile factor on the Al-Abshiet drain (sites 4-6), and the distance between the sites is 2 Km. One water sample was taken from Nile River (Damietta Branch) as clear water to test the difference of the spectral reflection and water analysis.

The pH value was determined in water samples using pH meter according the procedure described by Jackson (1976). The electrical conductivity (EC) was determined using the conductivity meter as described by Jackson (1976) and the values were corrected at 25º C.

Harvey and micro elements (B, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, P, Pb, and Zn) in the water samples were extracted by AB-DTPA according to Soltanpour, (1991). The Harvey and micro elements in extracts were determined using Inductively Coupled Spectrometry Plasma (ICP) Model Ultima 2-Jobin Yvon.

Nitrogen forms (NH_4^+ and NO_3^-) were determined using Technicon Auto Analyzer II.

2.2.5. Water Quality for irrigation used

The guidelines are general in nature, and should not be with any specific type of irrigation method. Ayers and Westcst, 1994 have been based mainly on guidelines for the Food and Agricultural Organisation (FOA). Table (2) shows the maximum limit of water quality parameters were used for irrigation.

2.1.6. Laboratory activities

Table (2) The guidelines of maximum limit of water quality parameters were used for irrigation (source: Ayers and Westcst, 1994)

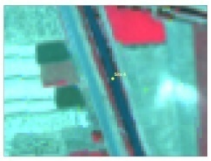
Parameters	Unit	Maximum limit
Chloride (Cl)	mg/l	250
Sulphate (S)	mg/l	500
Nitrate-Nitrogen (NO3-N)	mg/l	15
Ammonia-N (NH4-N)	mg/l	5
Electrical Conductivity	dS/m	2.0
Boron (B)	mg/l	0.75
Cadmium (Cd)	mg/l	0.01
Chromium (Cr)	mg/l	0.10
Cobalt (Co)	mg/l	0.05
Copper (Cu)	mg/l	0.20
Iron (Fe)	mg/l	5.0
Lead (pb)	mg/l	5.0
Manganese (Mn)	mg/l	0.2
Molybdenum (Mo)	mg/l	0.01
Nickel (NI)	mg/l	0.2
Zinc (Zn)	mg/l	2.0

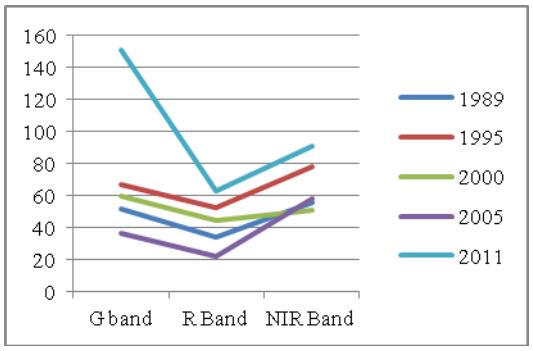
3. Results and Discussion

3.1. Al-Abshiet drain reflectance

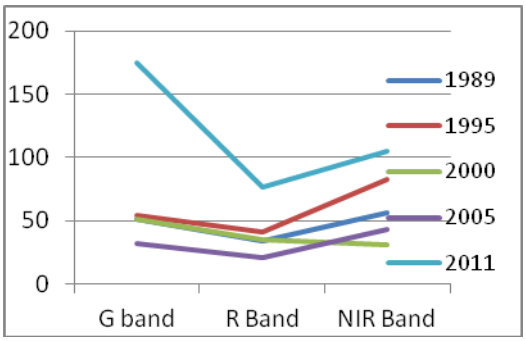
The selected six sites out of 6 sites were used to determine the reflectance of the three bands which are used to create false color composite (3, 2, 1) for all SPOT images (SPOT5, SPOT4, and SPOT2) and false color composite (7, 5, 3) for World View image. The DN values of the false color composite satellite images were collected and create in Excel sheet (Table 3). Figure (2) show the results of the reflectance graphs of polluted of Al-Abshiet drain.

Table (3) The Digital Number (DN) values of the three bands Green (G), Red (R), and Near Infra Red (NIR) for five years

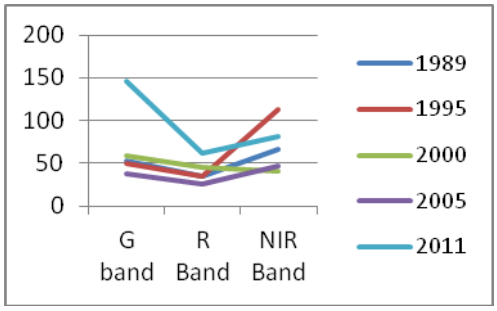
World veiw2 2011	Sites	Bands		Years				
				1989	1995	2000	2005	2011
	Site 1	DN value	G	62	67	60	37	151
			R	34	63	45	22	63
			NIR	56	78	41	58	91
	Site 2	DN value	G	51	54	51	32	175
			R	34	41	35	21	76
			NIR	56	83	31	43	105
	Site 3	DN value	G	52	50	59	38	146
			R	35	34	45	26	61
			NIR	59	113	41	46	82
	Site 4	DN value	G	55	61	61	37	120
			R	38	52	44	23	65
			NIR	59	98	41	78	89
	Site 5	DN value	G	55	62	58	37	121
			R	39	42	46	24	60
			NIR	61	86	42	61	83
	Site 6	DN value	G	55	52	60	48	160
			R	39	44	59	30	85
			NIR	62	80	46	63	119
	Nile River (Damietta Branch)	DN value	G	40	42	46	30	10
			R	23	29	25	19	6
			NIR	20	24	19	14	4



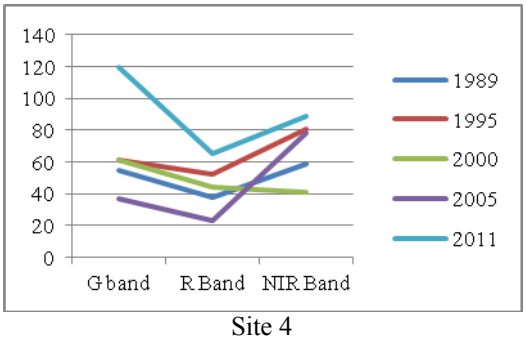
Site 1



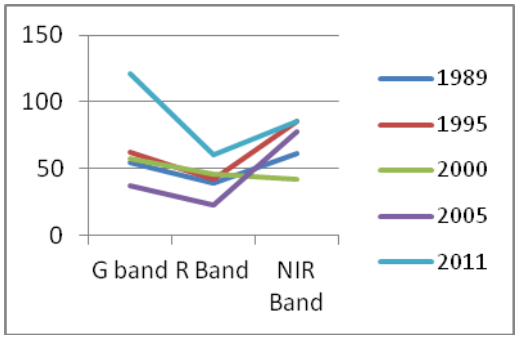
Site 2



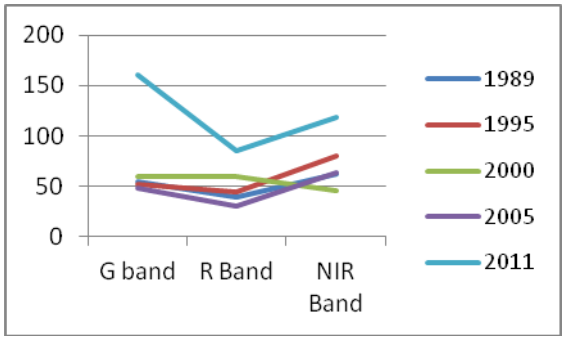
Site 3



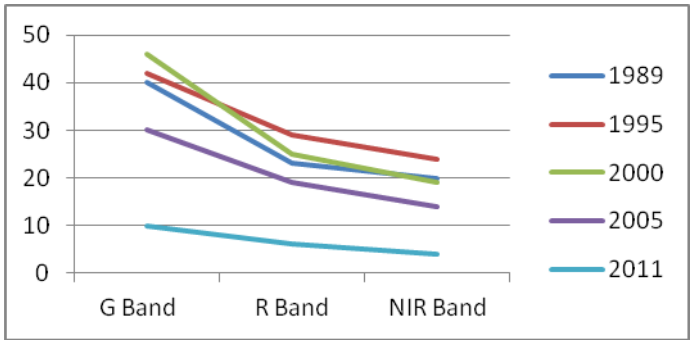
Site 4



Site 5



Site 6



Nile River Damietta Branch

Figure (2) the reflectance graphs of the DN value of three bands in 6 sites on Al-Abshiet drain and clear water sample

The results illustrated the following remarks:

(1) the graph of year 2000 give the normal distribution of the three bands of water reflectance curve which is higher in Green band and goes down in the Red band and Near Inferred band. This can be explained as the cleaning of Al-Abshiet drain from the grow plants.

(2) The graph of years 1989, 1995, and 2005 give the normal distribution of the three bands of plant reflectance curve which is high in Green band and goes down in the Red band and higher in Near Inferred band, but stile within the ranges of water reflectance. This can be explained as the grow plants along Al-Abshiet drain.

(3) The graph of year 2011 gives the normal distribution of the three bands of plant reflectance curve which is high in Green band and goes down in the Red band and higher in Near Inferred band, and higher from the ranges of water reflectance curve and lower than the plant reflectance curve. This can be explained due to two main reasons which are the grow plants along Al-Abshiet drain and the effects of polluted water from the textile factory.

(4) The graph of Nile River (Dumyat Branch years) of years 1989, 1995, 2000 and 2005 give the normal distribution of the three bands of water reflectance curve which is high in Green band and goes down in the Red band and downer in Near Inferred band, Also year 2011 give the same ranges of water reflectance but they are very low. This can be explained as the percentages of the sediments in the water are low in year 2011 and the water much deeper in this year.

3.2. Water analysis

To correlate the data of water reflectance with the quality of the water the pH and EC of the water of Al-Abshiet drin, in the twelve sites were represented in Table (4). The results illestrated that the ECw of Al-Abshiet drain were higher in the 6 sites and the domint salt is soudum chloride. The pH is normal and ranged from 7.1 up to 7.7. There is siginfican differece between the EC of the Nile River (Damietta branch) as clear water and the EC of the water of Al-Abshiet drain.

Table (4) The chemical analysis of the water samples

Site No.	Water type	pH	EC dS/m
1	Al-Abshiet drain	7.6	2.5
2		7.6	2.9
3		7.6	2.9
4		7.5	2.8
5		7.5	3
6		7.7	3.2
Nile River Damytta Branch		7.7	0.3

3.3. The Micro, Heavy, NH_4, NO_3, and K Elements of the water samples

Table (5) shows the results of the Micro Elements (Fe, Zn, Mn, and Cu), heavy elements (Pb, Cd, Ni, Co, B, Mo, and Cr), NH_4, NO_3, and K Elements of the water sample and clear water sample from Nile River (Damietta Branch). The results show that there is high differece between the micro elements, heavy elements, and elements of NH_4, NO_3, and K of the water of the Nile River (Damietta branch) as clear water than the same elemants of the water of Al-Abshiet drain.

Table (5) The Micro Elements, Heavy Elements, and Elements of NH_4, NO_3, and K of the water samples

Water type	Point location	Micro Elements in mg/l				Heavy Elements in mg/l							Elements		
		Fe	Zn	Mn	Cu	Pb	Cd	Ni	Co	B	Mo	Cr	NO_3	NH_4	K
Al-Abshiet drain	Site No.1	0.071	0.009	0.186	0.007	0.005	0.001	0.075	0.003	5.664	0.017	0.005	9.83	0.39	13.57
	Site No.2	0.077	0.018	0.183	0.011	0.005	0.001	0.079	0.003	7.399	0.021	0.003	11.42	0.26	14.3
	Site No.3	0.091	0.046	0.156	0.01	0.006	0.002	0.083	0.002	13.21	0.022	0.003	6.99	0.01	13.57
	Site No.4	0.117	0.075	0.116	0.006	0.009	0.002	0.081	0.003	23.87	0.022	0.009	12.62	0.13	15.6
	Site No.5	0.119	0.079	0.118	0.009	0.009	0.002	0.094	0.004	32.87	0.025	0.01	18.15	5.15	38.6
	Site No.6	0.139	0.089	0.138	0.009	0.009	0.003	0.098	0.006	32.99	0.027	0.011	18.68	5.52	38.82
Nile River Damietta Branch		0.022	0.012	0.005	0.004	0	0	0.001	0.0001	0.002	0.001	0	0.014	Nil	0.021

3.4. Water Quality for irrigation used

Accoding Ayers and Westcst (1985), the water quality of Al-Abshiet drian is not recomended to use it for irrigation for the following indecators:

1- The Nitriate-Nitrogen values in sites 5 and 6 ranged from 18.03 mg/l up to 18.68 mg/l were higher than the maxiumum limit value (15 mg/l).
2- The ammonia-N value in sites 5 and 6 ranged from 5.1 mg/l up to 5.52 mg/l) were small increase than the maxiumum limit value (5 mg/l).
3- The ECw of Al-Abshiat drain in all sites (ranged from 2.6 dS/m to 3.2 dS/m) were higher than the maximum limit value (2 dS/m).
4- The Boron element was very high increase in Al-Abshiet drain in all 6 sites (ranged from 5.16 mg/l up to 32.99 mg/l) than the maxiumum limit (0.75 mg/l). And the Boron element was increased from site 1 up to site 12 which is near the textile factory.
5- The 8 elements (Cd, Cr, Co, Fe, Pb, Mn, Ni, and Zn) were very low than the maxiumum limit value of these elements.
6- Molybdenum element was lettel higher than the maxiumum limit value (0.01 mg/l).

4. Conclusion and Recommendations

The integrated methodology of this study could be considered as a ready module for applying at different locations and represents a significant participatory management tool for detect water pollution in Egypt. As the results of this paper show that the important of using satellite images for monitoring the polluted water in lakes, water bodies, irrigation and drainage canals.

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Anatomical, histological and histochemical adaptations of the avian alimentary canal to their food habits: *I-Coturnix coturnix*

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Abstract: The present work is the first in a series of studies aiming at establishing a connection between the food habits of aves and the anatomical, histological and histochemical structures of their alimentary canal. In this study the gross anatomy, histology and histochemistry of the alimentary canal of common quail*, Coturnix coturnix,* a granivorous bird, have been investigated. This study revealed that, the oesophagus is not ably long with a well developed crop; thus stomach is differentiated into a glandular proventriculus and a muscular ventritculus or gizzard. The gizzard is much more developed having a thick hard cuticle, its wall consists of two strong smooth muscles, the small intestine is divided into duodenum, jejunum and ileum and the transition from the jejunum to ileum is indicated by the vitelline (Meckel's) diverticulum, and the ileum was the longest part of the small intestine. The large intestine consists of paired well developed caeca and a short rectum. The present histological studies revealed that the alimentary tract showed the usual four laminae: serosa, musculosa, submucosa and mucosa. The oesophageal mucosa of the quail was thrown into numerous longitudinal folds. The mucosa of oesophagus is lined with stratified squamous epithelium. The proventricular glands are simple tubular to simple branched tubular glands. The mucosal surface of the ventriculus is indented by deep, broad crypts into which simple to branched tubular gastric glands open. A thick gastric keratinoid material covers the mucosa of the ventriculus. The intestinal mucosa is thrown into intestinal villi which show a marked variation in density, shape and size in the different regions of the intestine. The goblet cells gradually increase in frequency from the duodenum to the rectum. Also, the histochemical study revealed the existence of a high amount of mucopolysaccharides in the oesophageal glands, PAS and Alcian blue positive mucin granules as well (neutral and acid mucin, respectively). The ventriculus mucosa is covered by a thick keratinized laminated layer of koilin membrane which is formed of proteinous material similar to keratin and stained positive for PAS and Alcian blue indicating the presence of neutral and acid mucin within its contents. The proventriculus mucosa shows folds lined by simple columnar cells containing PAS and Alcian blue positive mucin granules. The goblet cells and crypts of Lieberkühn have acid and neutral mucopolysaccharide secretions and the luminal surface of the columnar cells and the lamina propria of the intestine contains proteins.
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Key Words: Anatomical, histological, histochemical, alimentary canal, birds.

1. Introduction

Several studies have been conducted to examine how dietary habits have shaped the physiological and the morphological features of the digestive system (Penry and Jumars, 1968; Martı´nez del Rio and Karasov, 1990; Martı´nez del Rio *et al*., 1992; Lopez-Calleja *et al*. 1997; Hume, 1998; Caviedes-Vidal and Karasov, 2001; Sabat and Veloso, 2003; Sassi *et al.*, 2007; Naya *et al.*, 2008).

El-Banhawy *et al*. (1993 a) studied the comparative histochemistry of the proventriculus and ileum of the piscivorous bird, the black headed gull *Larusridi bundus*, as well as those of granivorous bird, the palm dove *Strepto peliasene galensis.* The authors found that the proventriculus of both birds have a moderate polysaccharides content. The brush borders as well as the goblet cells of the ileum have a high amount of that material.

Stevens & Hume (1995) added that the proventriculus function is the secretion of gastric juice during the passage of food into the gizzard.

Klasing (1999) studied the avian gastrointestinal anatomy and found that the avian gastrointestinal tract is a double-ended open tube (as seen in mammals) that begins at the beak and finishes at the vent. In sequential order it is composed of a mouth, oesophagus, crop, proventriculus, ventriculus (gizzard), intestine, caeca, rectum and cloaca. Some of these structures may be vestigial or even lost during the evolution of some species. The author found that the oesophagus extends down the neck into the thoracic cavity and terminates in the proventriculus. The peristaltic contraction of the inner circular and the

outer longitudinal muscles in the musculosa propels the food posteriorly through the oesophagus. Also, the author mentioned that the proventriculus of granivorous bird is elongated and relatively small in diameter in relation to the gizzard. The author found that the duodenal loop of the intestine encircles the pancreas and receives the pancreatic and hepatic ducts. The epithelium of the intestine contains villi and intestinal crypts. The author added that the liver has two lobes and the pancreas has three pancreatic lobes.The progress of food through the tract follows a specific digestive sequence including premoistening and softening, acidifying, grinding, hydrolyzing, emulsifying and propulsion of the end products.

Denbow (2000) and Taylor (2000) stated that, in birds that eat hard food items, the proventriculus is relatively thin-walled and glandular. The ventriculus is muscular, thick-walled and powerful. The intermediate zone connects the two.

Taylor (2000) stated that, in general, the jejunum is thought to begin just after the ascending duodenal loop begins to turn back on itself, where the jejunal branches of the cranial mesenteric artery begin. The ileum is thought to begin at the vitelline (Meckel's) diverticulum and end at therecto-caecal junction.

Vukicevic *et al*. (2004) mentioned that the small intestine of Ratites is differentiated into duodenum, jejunum and ileum.

Kadhim *et al*. (2011) studied the histomorphology of the stomach, proventriculus and ventriculus of the Red jungle fowl. They found that both chambers presented folds of the mucosa lined by a simple prismatic epithelium that was positive for neutral mucin. Simple tubular glands occupied the lamina propria of both chambers; in the ventriculus of older birds, they showed a coiled base. These ventricular glands were lined by simple cuboidal cells represented by the chief cells and a few large basal cells. The luminal and tubular koilin rodlets and folds of the ventriculus were positive to periodic acid Schiff (PAS) stain. The proventricular glands were situated between the inner and outer layers of the muscularis mucosae. Cells lining the tubulo-alveolar units of the proventricular glands showed a dentate appearance. Vacuoles were not observed, and the cells were negative for Alcian–PAS stain. The submucosa was very thin in the proventricular wall. In the ventriculus, it was not separated from the lamina propria owing to the absence of any muscularis mucosae. The musculosa of the proventriculus was formed by a thick inner layer of circular smooth muscle fibres and a thin outer layer of longitudinal fibres. In addition to these layers, oblique muscle fibres formed the most internal layer of the musculosa in the ventriculus.

With the purpose of correlating the molophology of the alimentary tract and the feeding habits of birds in general, we carried out histological and histochemical studies of the alimentary tract of the common quail, *Coturnix coturnix* .

2. Material and Methods

Coturnix coturnix (Order: Galliformes, Family: Phasianidae), was used in the present investigation. Healthy ten specimens of common quail were trapped alive from quail farms; Egypt. It was used as a model of granivorous birds (Fig.1). The specimens were anaesthetized by chloroform, and then they were carefully dissected for studying the gross anatomy of the alimentary canal (Fig.2). In addition, in two specimens, the alimentary canal was cut longitudinally to describe the structure of the internal surface as the folds, the villi and valves. For the general histological studies, the contents of the alimentary canal were drained by saline solution; small pieces of the various segments; the oesophagus, the stomach (proventriculus, ventriculus), small intestine (duodenum, ileum), and large intestine (caecum, rectum). Were fixed rapidly in alcoholic Bouin's solution or 10% neutral formalin for 24 hours and then kept in a mixture of 70% ethyl alcohol.

After fixation, the different parts of the alimentary tract were dehydrated through ascending grades of alcohol, cleared in cedar wood oil and finally embedded in parablast. Transverse sections of the different studied samples were cut at thickness of 7 microns. Sections were stained with differential double stained haematoxylin and eosin (Castro and Camargo, 1951) for general histological structures. For the histochemical studies, the following techniques were implied:

1- General carbohydrates were demonstrated by using the periodic acid-schiff (PAS) technique (Pearse, 1968). In this procedure, sections were placed in 0.5% periodic acid for the liberation of aldehydes, and treated with Schiff's regent for 2 minutes. A positive reaction is indicated by the appearance of magenta colouration resulting from the reaction between aldehydes and the decolourized solution (leucofuchsin) of Schiff's regent.
2- Acid and neutral mucopolysaccharides were demonstrated by using the Alcian blue- PAS method according to Mowry(1956). By this method, acid mucins exhibit blue stainabilities where as neutral mucins take a reddish colouration, and the mixture of both mucins acquire a purple stainability.
3- For the detection of total proteins, the mercury bromophenol blue method (Mazia *et al*., 1953) was employed. The existence of a dark blue

stainability denotes the occurrence of total proteins.

4- Nucleic acids (DNA and RNA) were demonstrated by using the methyl green-pyronin method (Kurnick, 1955).While the application of Feulgen reaction was used for demonstration of DNA only (Stowel, 1945).

Photomicrographs were taken to illustrate the histological, histochemical structure of the different studied samples; oesophagus, proventriculus, ventriculus, small intestine and large intestine.

3. Results

The Gross Anatomy

The alimentary tract consists of a buccal cavity, pharynx, oesophagus, proventriculus, ventriculus, small intestine (consisting of duodenum, jejunum and ileum), large intestine (consisting of paired caeca and a rectum) and cloaca, which opens to the outside by the cloacal opening (Fig.3)**.**

The oesophagus:

The oesophagus is a thin walled distensible tube. It lies dorsal to the trachea in the anterior regions of the neck and then runs along the right side. The oesophagus transports the food from the pharynx to the gastric region allowing birds to swallow their food whole. Thus, it contains a number of longitudinal folds which provide distensibility. It is distinguished into three distinct anatomical portions: the cervical part, the crop and the thoracic part. The cervical part of the esophagus is considerably larger than the thoracic part.

The oesophagus has a single outpouching to form the crop**;** it is located just outside the body cavity in the neck region. It is a storage place for food. From here food passes through the lower oesophagus into the proventriculusand it contains longitudinal folds on the inner surface thus making it distensible. Beyond the crop, the oesophagus continues as the thoracic part connects with the proventriculus.

The stomach

The stomach is formed of two distinct parts; the glandular portion, gastric proventriculus or true stomach (pars glandularis) which is caudal to the oesophagus and the muscular portion, gastric ventriculus or gizzard (pars muscularis) which is located caudal to the proventriculus. The two parts of the stomach are connected together by an intermediate zone. The proventriculus secretes HCL and pepsin which is needed for protein digestion. The proventriculus being buff while the gizzard bluish-red.

The proventriculus

The proventriculus is a spindle shaped organ located between the oesophagus and the ventriculus. It arises from the oesophagus without adistinct demarcation, the wall was thicker than that of the oeosphagus, and the caudal extent of the proventriculus was marked by a constriction, the isthmus gastris. The mucosal surface of the proventriculus revealed the presence of raised papillae, papillae proventriculus, over its entire surface.

The ventriculus

The gizzard is a small spheroid organ; the ventriculus lies in the left dorsal and ventral regions of the thoracoabdominal cavity, placed partly between the lobes and partly behind the left lobe of the liver. It is much larger and more muscular than the proventriculus. It is consists of two pairs of opposing muscles. The caudoventral and craniodorsalthin muscles line the caudal and cranial sac of the ventriculus, respectively. The cranioventral and caudodorsal thick muscles are responsible for the powerful grinding contractions seen in the ventriculus. The asymmetrical arrangement of these four muscles provides mixing and grinding actions during contraction. The ventriculus is lined by the koilin, a cuticle layer, which acts as a grinding surface and protects the underlying mucosa from the acid and pepsin produced by the proventriculus, which frequently appeared green or yellow in colour due to the regurgitation of bile.

The pyloric region of the stomach opens distally into the duodenum by pyloric orifice which is guarded by a small pyloric valve. This lies at the right angle of the pyloric orifice.

The small intestine

The small intestine extends from the pyloric end of the stomach to the junction of the small intestine, caeca and colon. It is long and consists of a coiled mass forming a series of loops and lies within the abdominal cavity. It is distinguished into three main parts, the duodenum, jejunum and ileum.

The duodenum begins at the end of ventriculus and forms an elongated loop about 20 cm long. The pancreas lies between the arms of the loop and being attached to each arm of the duodenum actually holds the two arms together. The jejunum and the ileum are very long and coiled, commence at the caudal end of the duodenum where the bile and the pancreatic ducts are located and terminate at the ileo-caecal-colic junction. This junction is where the small intestine, the two caeca and the colon all meet. The external diameter of which is roughly uniform, so the transition between them is anatomically unrecognizable, but, we have found that Meckel‘s diverticulum marks the end of the jejunum and the start of the ileum. Meckel‘s diverticulum appears as a small projection on the outer surface of jejunum, the projection is where the yolk sac was attached during the development of the embryo.

The large intestine

The rectum of the *Coturnix coturnix* is extending from the end of the small intestine until it opens distally in the cloaca in the form of a short and straight tube. Also, the large intestine contains a pair of outpocketing caeca that project from the proximal part of the rectum at its junction with the small intestine. Internally, the mucous membrane of the rectum is thrown into numerous distinct longitudinal folds.

These caeca, the right and left caeca arise from the lateral walls of the rectum, close to the junction with ileum, they are well developed. Each caecum could be divided externally into a short proximal neck, a long middle thin-walled body, and a short apex.

The cloaca

The cloaca extends from the end of the rectum to the cloacal opening.It is divided, as it is generally the case in birds, into three chambers namely, coprodaeum, uerodaeum and proctodeum.

The liver:

The liver was dark red–brown in colour. Placed in its natural position. It consists of two lobes, namely; the right and the left lobes, the former is much larger than the left one and the latter is sub divided into two lobes. The right and the left lobes are united together antero-dorsally by a thin isthmus. It is connected to both the diaphragm and the ventral body wall by means of the falciform ligament.

The gall bladder is partially embedded in the right lobe of the liver at the posterior surface. It is somewhat elongated and oval-shaped thin –walled sac, dark green in colour. Two bile ducts emerge from the right lobe. One of these originates from the gall bladder and the second provides a direct connection from the liver to the small intestine.

The pancreas:

The quail pancreas, which is a pale-yellow organ with a finely lobulated surface located between the ascending and descending loops of the duodenum, was determined to be composed of the dorsal, ventral, third and splenic lobes united together by a broad median bridge (Fig .1).

Three pancreatic ducts opening into the duodenum proceed directly from the dorsal, ventral and third lobes, respectively.

Histological studies of alimentary canal of the common quail (*Coturnix coturnix*).

The oesophagus:

The oesophageal wall consists of the usual layers of a tubular digestive organs, i.e. mucosa, submucosa, musculosa and adventitia or serosa (Fig.4a).

The mucosa is formed of stratified squamous epithelium of about seven to eight longitudinal folds of different shapes. These folds are shallow and narrow 7-10 cells thick. The lamina propria is formed of loose connective tissue. Fine connective tissue fibers, fibroblasts and areas of lymphocyte infiltration can be seen in this layer. This layer is considered as an extension of the submucosa. The muscularis mucosa, which is a very well developed continuous layer, is formed of smooth muscle fibers. The submucosais composed of loose connective tissue with larger blood vessels, lymphatics and nerve fibers. The simple branched mucus glands called oesophageal glands, scattered throughout the submucosa, are present in both the cervical and the thoracic parts of oesophagus. The glands are less developed in the cervical part in comparison to the thoracic part of the organ (Figs.4a& b). An important characteristic feature of the oesophagus in common quail is the presence of mucous glands in the crop (Fig.4c). The musculosa of the oesophagus is composed only of smooth muscle and is composed of two distinct layers: an inner circular and an outer longitudinal layer. The circular layer is thicker than the longitudinal layer. The two layers are separated by connective tissue fibers in which runs a nerve plexus (Fig.4d). Peristaltic contractions of the inner circular and the outer longitudinal muscles propel food posteriorly through the oesophagus. The adventitia is composed of loosely arranged connective tissue which binds the organ to the surrounding tissues. Nerve fibers and blood vessels can be found in this layer. The thoracic part of the oesophagus that extends below the diaphragm is covered by a serosa.

Stomach

Microscopically, the wall of the stomach (proventriculus and gizzard) consists of four distinct functional tunicae namely, mucosa, submucosa, musculosa and outermost serosa.

The proventriculus

The proventriculus mucosa, unlike that of the oesophagus, is thrown into folds with varying heights; the folds were lined by a simple columnar epithelium. Lamina propria is occupying the center of the mucosal folds. This layer is dense irregular connective tissue with collagen fibers, fibroblasts and lymphoid infiltration. Delicate smooth muscle fibers are scattered in the deepest part of this layer and between the proventricular glands. That extends to hold two types of gastric glands, the deep and the superficial gastric glands (Fig. 5a).

The superficial gastric glands are of simple tubular type appearing in the form of numerous folds of the mucosal epithelium. The walls of these glands are composed of columnar mucus-secreting epithelial cells with centrally located nuclei and a cytoplasm filled with translucent mucous secretion (Fig.5b).The submucosais a narrow connective tissue layer sandwiched between the circular layer of the

musculosa and the main mass of the muscularis mucosae.

Compound tubulo-alveolar proventricular glands formed the greatest thickness of the proventricular wall. The proventricular glands are distributed throughout the entire organ. The glands are composed of rounded, oval, hexagonal or polymorphic lobules separated from each other by thin perilobular connective tissue sheath containing fibroblasts and few smooth muscle fibers. The wall of each lobule is formed of numerous secretory alveoli or tubules opening together into a wide central cavity, from which a wide duct originates. Ducts from several lobules joined together to form a short main duct which is connected to the apex of the raised mucosal papillae and open into the narrow lumen of the proventriculus. The duct system of the proventricular glands is lined with tall columnar epithelium with oval or vesicular nuclei which are located sometimes at different levels giving the epithelium pseudostratified appearance. The proventricular gland alveoli were formed from one cell type (oxynticopeptic cell), the proventriculus cells secrete hydrochloric acid & proteolytic enzymes. The proventricular tubules are composed of cuboidal cells. Their nuclei were nearly rounded and located near to the basement membrane. The secretory cells are oriented obliquely to the long axis of the glandular tubules of the proventriculus and are separated from each other by relatively narrow spaces giving the epithelial cells a serrated appearance (Figs.5c).

The muscularis mucosa of the proventriculusis formed of two small muscle fibers layers; inner isolated muscular bands arranged in a longitudinal manner and outer band arranged in a circular manner. The deep proventricular glands are located between the inner and the outer layers of the muscularis mucosa. The musculosa is moderately thick and consists of an inner circular and an outer longitudinal muscle layer. The inner layer is two times thick as the outer layer. Serosa was constituted by connective tissue, containing many blood vessels and nerves, lined by mesothelium.

The ventriculus

The mucous membrane of the gizzard presents low folds which are lined by columnar epithelial cells possessing generally rounded nuclei. Over the mucous membrane, a thick cuticle is disposed. The lamina propria is constituted by a dense connective tissue and it is occupied by numerous deep simple tubular glands which expand in the base of the fold, partially located between them. Those glands are lined by a simple columnar epithelium, which is lower in the base of the glands and higher in their upper portion in the interior of the glands. There are crypts in the base of the folds (Fig.6a). We observed eosinophilic secretion fillets continuous with the cuticle. Glandular tubes are narrower, while others are wider (Fig.6b). No muscularis mucosa is present making no partitions between the lamina propria and the submucosa. The musculosa is well developed forming the main bulk of the gizzard wall and represented by smooth muscle fibers arranged mostly in a circular manner. The muscular bundles are interspersed with bands of connective tissue. The musculosa is very thick to support the mechanical force of grinding.

The serosa, which is constituted by connective tissue lined by methelium is rich in blood vessels and nerve endings. It is followed by a subserosal layer (Fig.6b).

The small intestine

The small intestine is conveniently divided into three main regions namely, the duodenum, the jejunum and the ileum. All the three divisions show the usual tunicae namely; mucosa, submucosa, musculosa and serosa. The mucosa of the intestine is thrown into villi which show a marked variation in density, shape and size in the different regions of the intestine. Intestinal villi gradually decrease in length and size moving from the duodenum to the ileum.

The mucosa is built up of a lamina propria of loose connective tissue supporting the mucosal membrane which is thrown into deep, narrow finger-like villi in the duodenum while the villi are relatively short, somewhat broad and numerous in the ileum (Figs. 7a.8a).

The mucosa consists of a simple columnar epithelium and a tunica propria. The muscularis mucosa is represented by a narrow part of longitudinally arranged smooth muscle fibers towards the side of the submucosa, but on the side of the lamina propria, it is represented by vertically arranged smooth muscle fiber strands. The columnar cells possess elongated nuclei and a clear cytoplasm.

Goblet cells frequently occur amongst the columnar or absorptive cells. Each cell is rounded or oval in shape. The goblet cells are more numerous in the ileum than in the duodenum (Figs. 7b. 8b). The goblet cells increase from the duodenum towards the rectum. The goblet cells are positive to the stains specific for mucus. Lymphocytes are scattered amongst the bases of the columnar epithelial cells. They are small more or less spherical and their nuclei are rounded and darkly stained. Crypts of Lieberkühn, in the form of simple tubular stands, occur at the bases of the villi, being more numerous and too crowded in the duodenum. They are built of cells similar in structure to those of the mucosal epithelium.

The cores of the villi are formed of the areolar connective tissue of the tunica propria. They

contain blood vessels and capillaries, lymph vessels and numerous darkly stained lymphocytes.

The submucosa is thin, narrow and hardly distinguished in some regions. The submucosa connective tissue holds few blood vessels. The muscularis mucosais composed of thin layer of longitudinal muscle fibers which merges gradually into the submucosa and extends into the core of the villi. The musculosa consists of two smooth muscle layers; outer longitudinal layer and a thick circular muscle layer. All muscle fibres are of the unstriated type. Two muscle layers surround the intestine, the inner circular and outer longitudinal layers that allow mixing and propulsion of the digesta through the intestinal tract. The serosa is made up of flattened simple squamous epithelium

The rectal caeca

Each rectal caecum is distinguished into three main regions: proximal, middle and distal. Its mucous membrane is raised into simple villi. These show a gradual change in their depth and width from end to end. Thus, the height of the villi increased as the villi neared the proximal caecum. Conversely, the villi height decreased as the position moved far from the proximal caecum. The villi are lined by a simple columnar epithelium whose cells resemble those of the small intestine. villi were found and had globet cells and crypts, and the muscle layer was thicker (Fig.9). No villi in the middle zone caecum was found. The muscularis mucosa is composed of thin layer of longitudinal muscle fibers. Accordingly, the narrow and thin submucosa connective tissue layer merges into that at the lamina propria. The musculosa consists of two layers of unstriated muscle fibres: an outer longitudinal layer and inner circular one. These muscle layers are in the proximal part of the caecum, continuous with those of the ileum and of almost the same thickness. In the middle and distal parts, the outer longitudinal muscle layers are quite thin. In the middle zone caecum the muscle layer was thin with saw-shaped or parallel ridged tract lumen. The distal caecal muscle layer was thinner than that in the proximal caecum and its surface was saw-shaped and no parallel ridges or villi were found. The caecum is covered externally with a thin serosal layer; formed of simple squamous epithelial cells which possess flattened nuclei.

The rectum

Apart from minor differences such as the thickness of the various coats and the shape of the villi, the musculosa and the serosa are similar to those described above for the small intestine. The wall of the rectum is made up of serosa, musculosa, submucosa, muscularis mucosa and mucosa. The serosa is a thin layer composed of simple squamous epithelium with flattened nuclei. The musculosa is made up of two muscle layers; an outer thin longitudinal and thick circular one. The submucosa consists of loose connective tissue holding blood vessels. The muscularis mucosa is composed of longitudinal muscle fibers. This layer extends inside the mucosal folds as vertical muscle fiber strands (Fig.10a). The mucosa is thrown up into numerous leaf-like villi, all covered by simple columnar epithelium containing goblet cells. The goblet cells are numerous in number and open into the lumen. At the base of the mucosal folds, rectal glands (simple tubular) are noticed. These glands are crypts as in the small intestine, lined with simple columnar epithelium and goblet cells (Fig. 10b).

Histochemical studies of the alimentary canal of the common quail (*Coturnix coturnix)*

Carbohydrates (PAS-positive material):

The oesophagus:

The esophageal glands are composed of typical alveoli .These glands react positively with the periodic acid Schiff (PAS) stain. These glands were loaded with positively stained material (Fig. 11).

The stomach

In the proventriculus, The cells of the surface lining epithelium of the mucosal folds, showed PAS- positive mucin granules occupying the supra nuclear area of the cells . The ductular cells that lined the ducts of the proventricular glands showed PAS positive reaction in their apical ends. The secretory cells were negatively stained with PAS stain (Fig. 12). The luminal and tubular koilin rodlets and folds of the ventriculus were positive to PAS stain (Fig.13).

The small intestine (the duodenum and the ileum)

Application of PAS method indicated that, the mucosa of the small intestine revealed a strong magenta colouration in the goblet cells of both the villi and the crypts of Leiberkühn as well as the apical plasma membranes of the columnar epithelial (absorptive) cells. However, the ground cytoplasm of the columnar epithelial cells exhibits moderate PAS-reactivity (Figs.14&15).

The rectum

General carbohydrates are localized in the rectal glands, goblet cells and in the surface mucous epithelium with PAS method in the form of magenta colouration (Fig.16).

Mucopolysaccharides:

The oesophagus

The Alcian blue - PAS method, showed that the nature of the oesophageal glands of quail in the form of acid mucopolysaccharides (the blue colour, Fig. 17).

The stomach

Neutral mucopolysaccharides are abundantly found in the gastric glands. On the other hand, the superficial glands secrete acid and neutral

mucopolysaccharides since they give blue and red colour with Alcian- PAS stain. Cells lining the tubulo-alveolar units of the proventricular glands showed a negative reaction for Alcian-PAS stain (Fig.18).

In the ventriculus, the mucosal epithelium was PAS and Alcian blue positive especially at the apical portion of the cells indicating the presence of both neutral and acid mucin, similar to the proventriculus. The ventriculus mucosa covered by a thick keratinized laminated layer of koilin membrane which is formed of proteinous material similar to keratin and stained positive for PAS and Alcian blue indicating the presence of neutral and acidic mucin within its contents (Fig.19).

The small intestine

The goblet cells and crypts of Leiberkühn have acid and neutral mucopolysaccharide secretions (Figs.20&21)

The rectum

Application of Alcian blue PAS method revealed that the rectal glands contain acid and neutral mucopolysaccharides. Also, the columnar cells contain neutral mucopolysaccharides while the goblet cells contain acid mucopolysaccharides (Fig. 22)

Total proteins:

The oesophagus

Application of mercuric bromophenol blue method on the oesophagus of the quail proved an exaggerated amount of proteinic elements in the cytoplasm of its stratified squamous epithelium. On the other hand, the oesophageal glands showed a negative response to the above mentioned method (Fig.23)

The stomach

In the proventriculus, the cytoplasm of cells of the surface lining epithelium of the mucosal folds and the ductular cells that lined the ducts of the proventricular glands show weak reaction with bromophenol blue method (Fig. 24).The luminal and tubular koilin rodlets of the ventriculus showed a strong response to the bromophenol blue method, while a weak reaction was noticed in the cell lining folds.(Fig. 25).

The small intestine and the rectum

In the small intestine and the rectum of the *Coturnix coturnix,* the bromophenol blue stain reacts positively with the absorptive columnar cells. A similar feature is also noted in the lamina propria.While a weak reaction was noticed in the cytoplasm of the goblet cells (Figs. 26&27).

Nucleic acids:

Histochemical demonstration of DNA revealed the appearance of adense product in the nuclei of the oesophageal, gastric and intestinal mucosal cells. Such A positive staining product is present in the place of the chromatin substances containing DNA (Figs.28-30). Application of methyl green pyronin methods proved the existence of a considerable amount of RNA inside the cytoplasm of the columnar epithelial cells in the different gut regions of quail (Figs.31-35).

4. Discussion

The anatomical observation of the alimentary canal of *Coturnix coturnix* detects that the oesophagus of *Coturnix coturnix*is a long tube since, it is characterized by three main parts; the upper part starts at the end of the pharynx, the next part is the crop which represent a musculo membranous pouch and the lower part which is connected to the proventriculus. These results are in agreement with those obtained by Klasing (1999).In *Coturnix coturnix*, the presence of the crop serves as a storage receptacle for the swallowed grains since this bird has to take a large quantity of is food in a fast and quick manner similar finding were observed by Wallace (1961).The stomach of *Coturnix coturnix* is a smooth muscular organ, located between the esophagus and the intestine, and it is constituted of two different portions: glandular and muscular stomach and its gastric ventriculus is constituted by four semi-autonomous muscles, two thick and dark colored, the caudodorsalis and the cranioventralis, and two with fine thickness and clear colored, the craniodorsalis and the caudoventralis, that are responsible to crush the victuals ingested. This observation is similar to that of Suganuma *et al*., 1981; Dyce *et al*., 1996; Bailey *et al*., 1997; Bacha & Bacha, 2000. Anatomically, the present study revealed that, the ventriculus of *Coturnix coturnix*, was thick-walled and powerful. The intermediate zone connects the two .This observation is similar to that of Denbow (2000) and Taylor (2000). The differences in proventriculus structure may also be related to diet. The large walled quail proventriculus allows it to accommodate dry bulky food, since both the proventriculus and the gizzard contain rocks.

In the present results the small intestine of *Coturnix coturnix*, is differentiated into the duodenum, jejunum and ileum. These results agree with Taylor (2000) and Vukicevic *et al*. (2004). The present study revealed that, the rectum of the *Coturnix coturnix* extends from the end of the small intestine until it opens distally in the cloaca in the form of a short and straight tube. Similar observation had been pointed out in *Bubo bubo* (Abo-Shaeir, 2001). The rectal villi are shorter than that of the small intestine. These results agree with Klasing (1999). Quail are granivorous and have a pair of well developed caeca. This agrees with Chen *et al*.(2002) in geese.

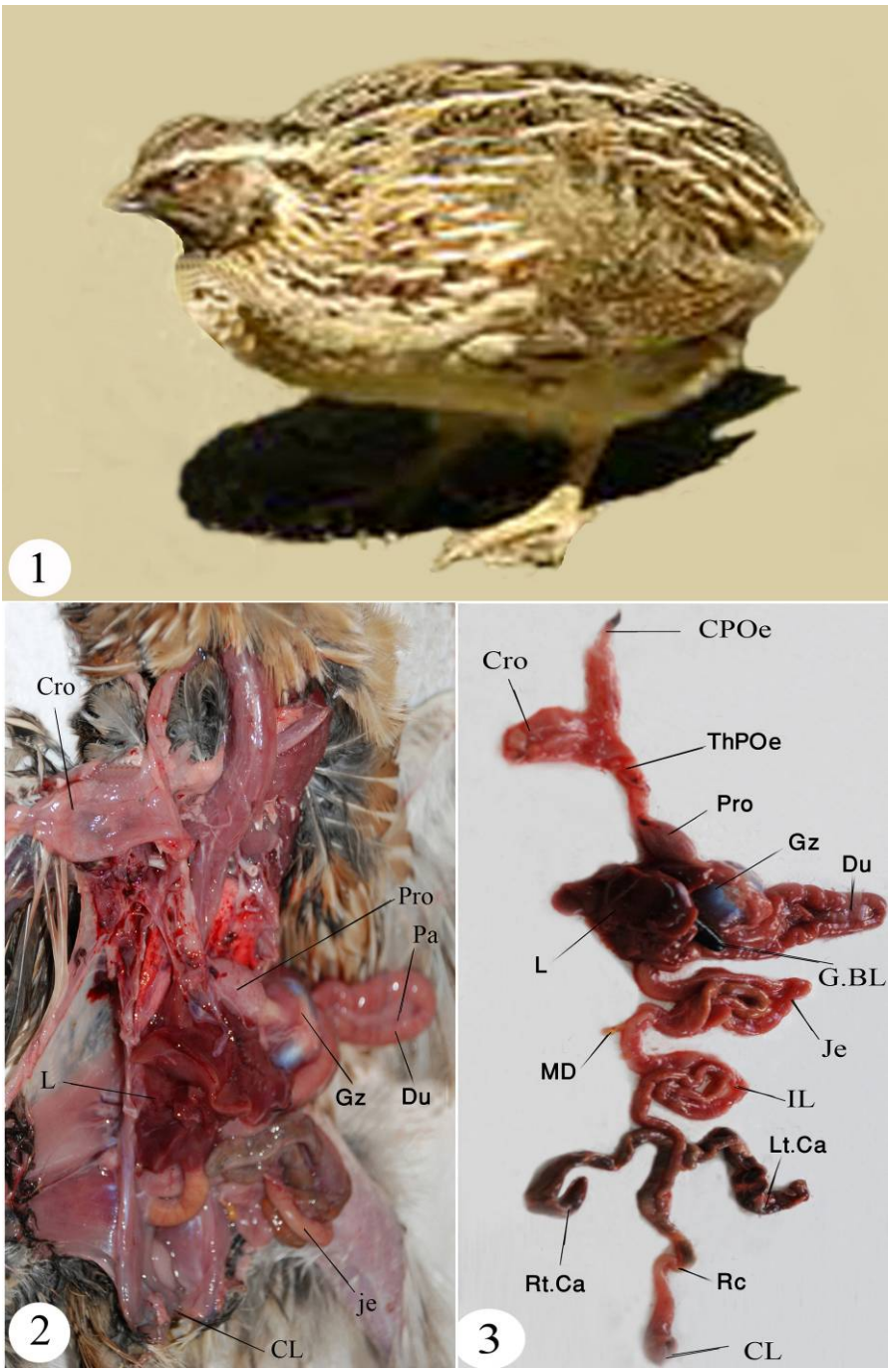

Fig. (1): Photograph of the common quail, *Coturnix coturnix*.

Fig. (2): Photograph of the dissection of the alimentary tract of *Coturnix coturnix* showing crop (Cro), proventriculus (Pro), Gizzard (Gz), duodenum (Du), jejunum (je), cloaca (CL), liver (L), and pancreas (pa)

Fig. (3): Photograph of a fresh isolated alimentary tract of *Coturnix coturnix* showing the cervical part of oeosphagus (CPOe), thoracic part of oesophagus (ThPOe), crop (Cro), proventrculus (Pro), Gizzard (Gz), duodenum (Du), jejunum (je), ileum (il), Caecum (Ca), liver (L), Mechel's deverticulum (MD), rectum (Rc) , and cloaca (CL)**.**

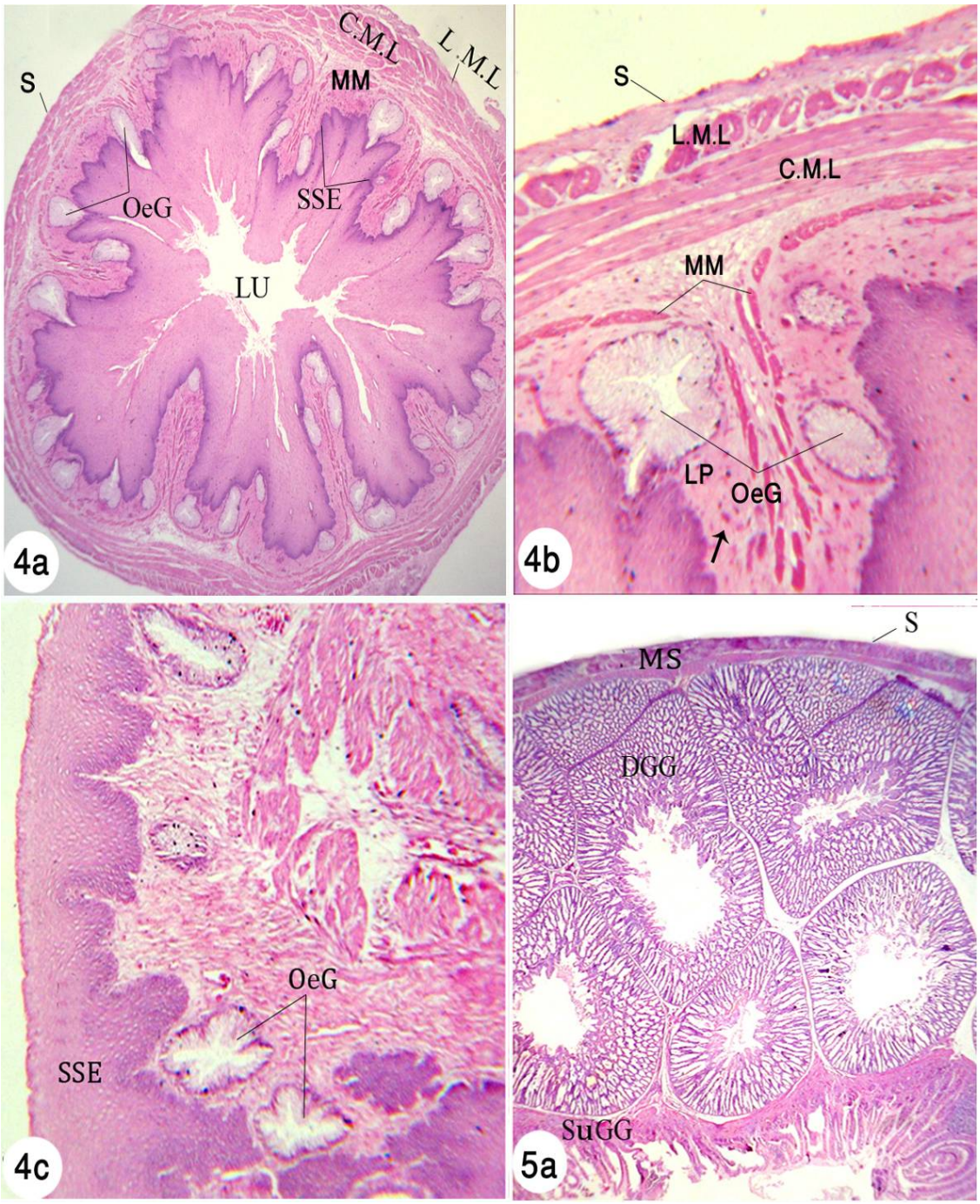

Fig. (4a): Photomicrograph of a transverse section of the thoracic part of oesophagus in the *Coturnix coturnix* showing the lumen(LU), oesophageal gland(OeG),stratified squamous epithelium(SSE), mucosa(Mu), muscularis mucosa (M.M) musculosa (circular and longitudinal layers(CML&LML) and serosa(S). H&E stain, X56.

Fig. (4b): Photomicrograph of the enlarged portion of the cervical part of the oesophagus in the Coturnix coturnix showing the fibro blasts and areas of lymphocyte in filtration in the lamina propria (arrow), H&X 182.

Fig. (4c): Photomicrograph of a transverse section of the crop of *Coturnix coturnix*. Showing the oesophageal gland (OeG), stratified squamous epithelium (SSE), H&E stain, X100.

Fig. (5a): Photomicrograph of a transverse section of the proventriculus of *Coturnix coturnix*. Showing the serosa(S), musculorosa (Ms), deep gastric gland (DGG), and superficial gastric gland (SuGG). H&E *stain*, X 40.

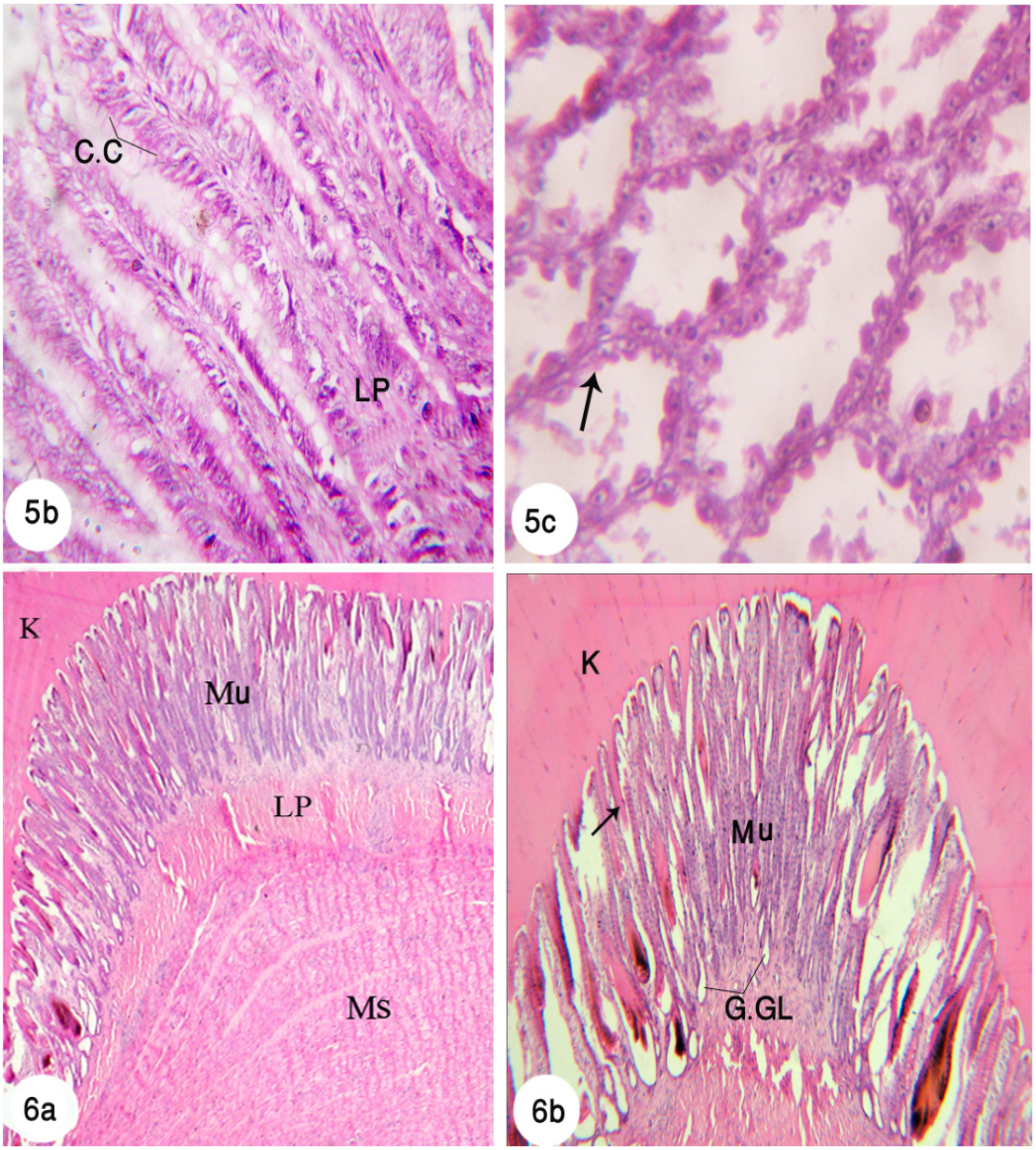

Fig. (5b): Photomicrograph of enlarged portion of the proventriculus of *Coturnix coturnix*. Showing lamina propria (Lp) and columnar cells (C.C) lining the superficial glands H&E *stain*, X 400.

Fig(5c): Photomicrograph of enlarged portion of the proventriculus of *Coturnix coturnix*. Showing serrated appearance of the glandular tubules and cuboidal cells lining the tubules(arrow). H&E *stain*, X 600.

Fig. (6a): Photomicrograph of a transverse section of the ventriculus of *Coturnix coturnix*. Showing the mucosa (Mu), muscularosa(Ms), lamina propira(LP)and koilin(K). H&E stain, X100.

Fig. (6b): Photomicrograph of enlarged portion of the ventriculus of *Coturnix coturnix*. Showing the secretion fillets continuous with the cuticle (arrow). and glandular tubes, gastric gland (G.Gl) are narrower, while others are wider. H&E stain, X150.

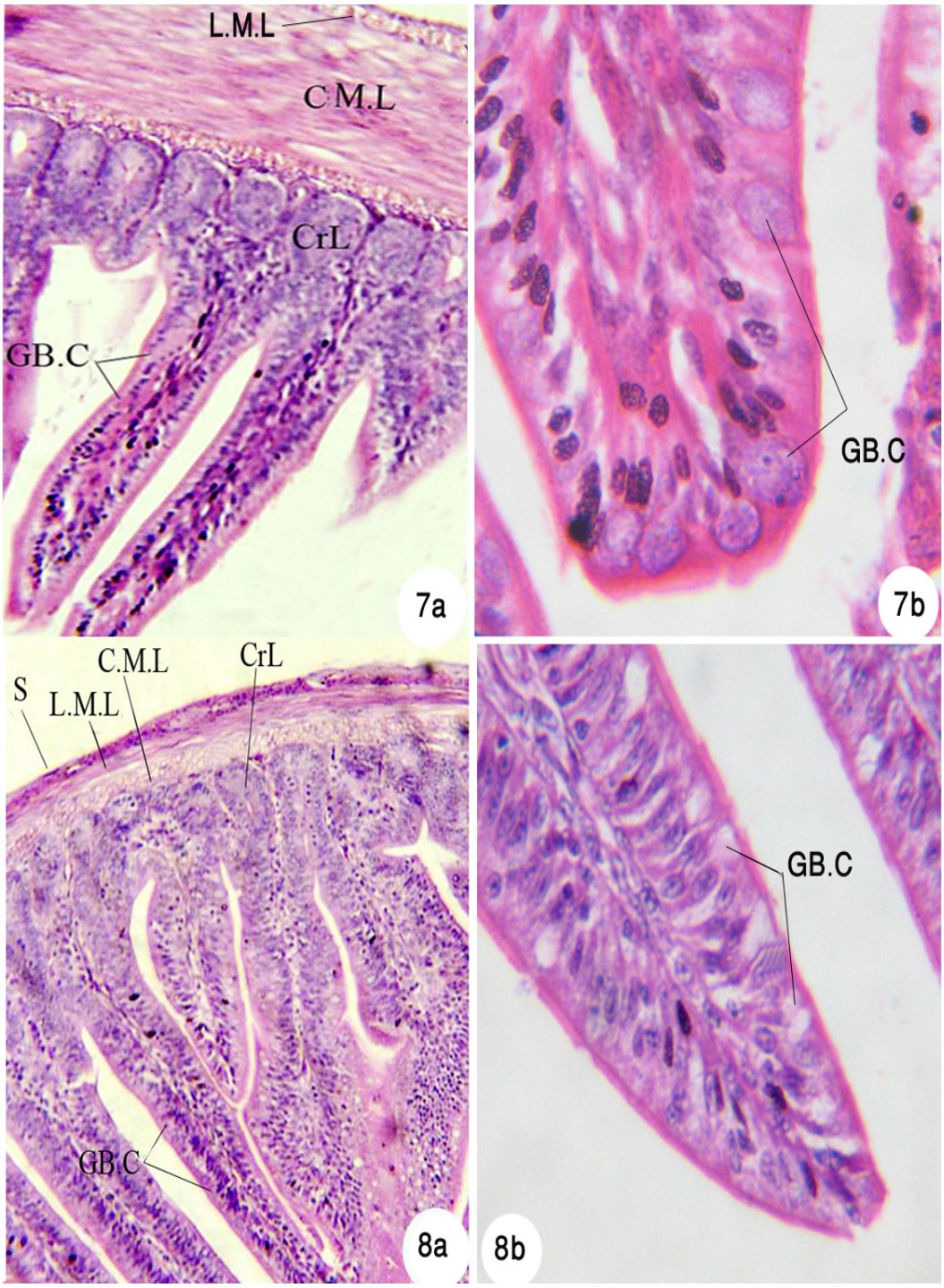

Fig. (7a): Photomicrograph of a transverse section of the duodenum of *Coturnix coturnix* showing the musculosa (circular and longitudinal layers (CML&LML), goblet cell (GB.C) and crypts of leiberkühn(CrL) H&E stain, X60.

Fig. (7b): Photomicrograph of enlarged portion of duodenum of *Coturnix coturnix* in the mucosal layer showing goblet cells (GB.C). H&E stain, X400.

Fig. (8a): Photomicrograph of a transverse section of the ileum of *Coturnix coturnix* showing the musculosa, circular and longitudinal layers (CML&LML), serosa(S), goblet cell (GB.C). H&E stain, X100.

Fig. (8b): Photomicrograph of enlarged portion of ileum of *Coturnix coturnix*, showing goblet cells (GB.C), in the mucosal layer. H&E stain, X400.

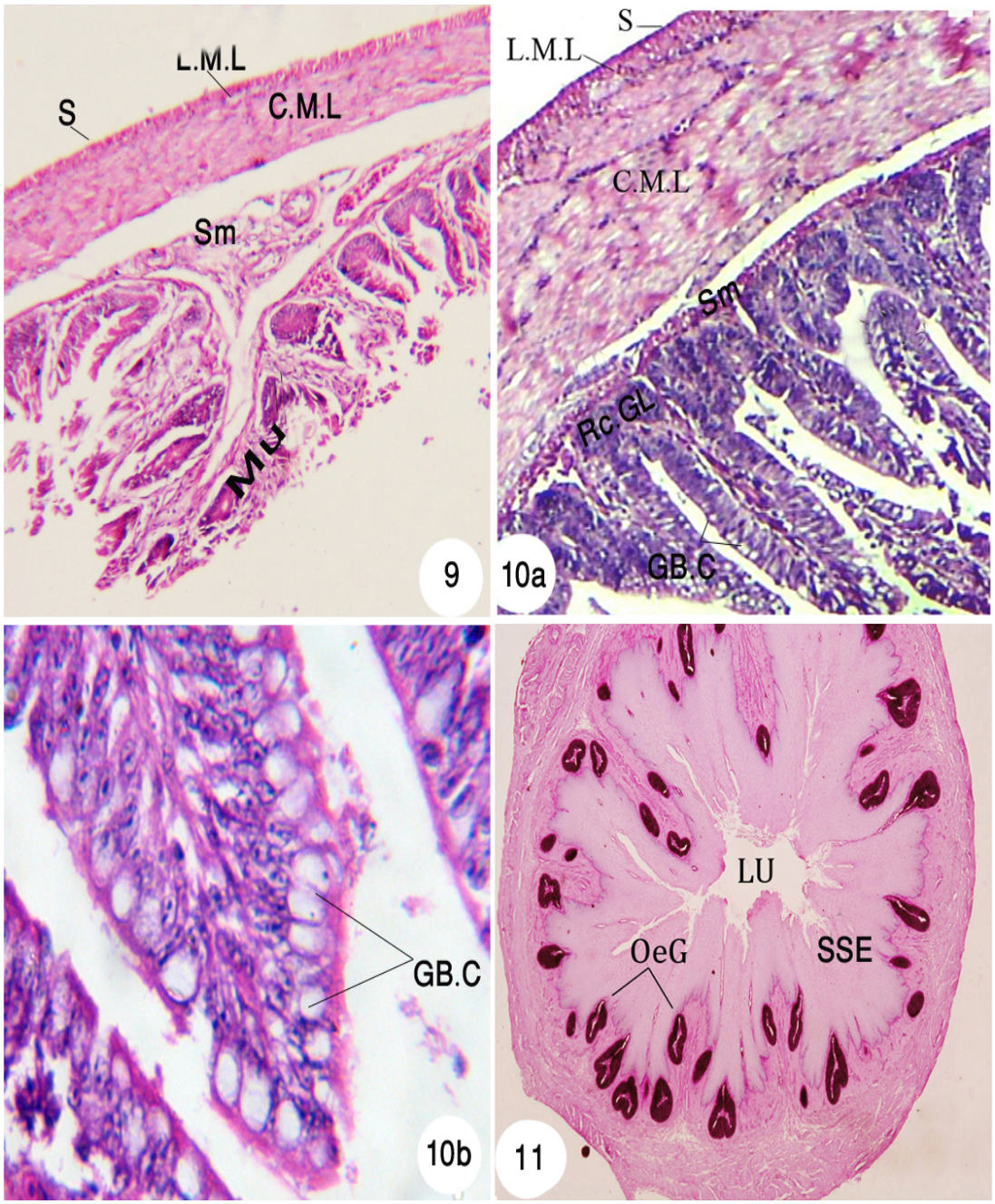

Fig. (9): Photomicrograph of a transverse section of the proximal caecum of *Coturnix coturnix.* Showing the serosa (S) muscularosa (circular and longitudinal layers CML&LML), (H&E stain, X100.

Fig. (10a): Photomicrograph of a transverse section of the proximal caeca of *Coturnix coturnix.* Showing the serosa (S) muscularosa (circular and longitudinal layers (CML&LML), and mucosa (Mu) H&E stain, X100.

Fig. (10b): Photomicrograph of enlarged portion of rectum of *Coturnix coturnix* in the mucosal layer showing goblet cells (GB.C) H&E stain, X400.

Fig. (11): Photomicrograph of a transverse section of the oesophagus of *Coturnix coturnix*. Showing the carbohydrate content (PAS-positive stain) X40.

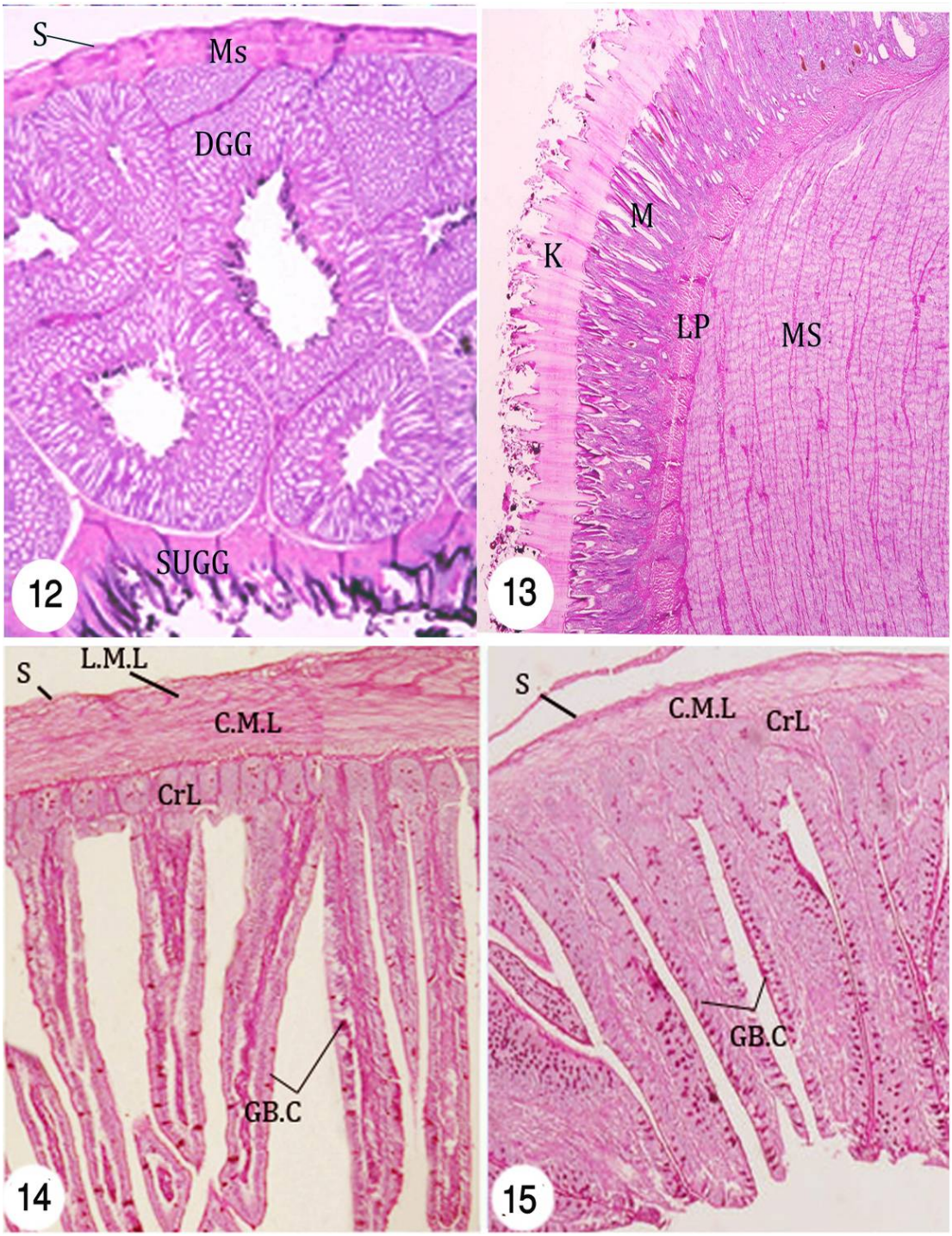

Fig. (12): Photomicrograph of a transverse section of the proventriculus of *Coturnix coturnix*. Showing the carbohydrate content: (PAS-positive stain) X40.

Fig. (13): Photomicrograph of a transverse section of the ventriculus of *Coturnix coturnix*. Showing the carbohy drate content. (PAS-positive stain) X656.

Fig. (14): Photomicrograph of a transverse section of the duodenum of *Coturnix coturnix*. Showing the carbohydrate content. (PAS- positive stain) X164.

Fig. (15): Photomicrograph of a transverse section of the ileum of *Coturnix coturnix*. Showing the carbohydrate content. (PAS- positive stain) X 656.

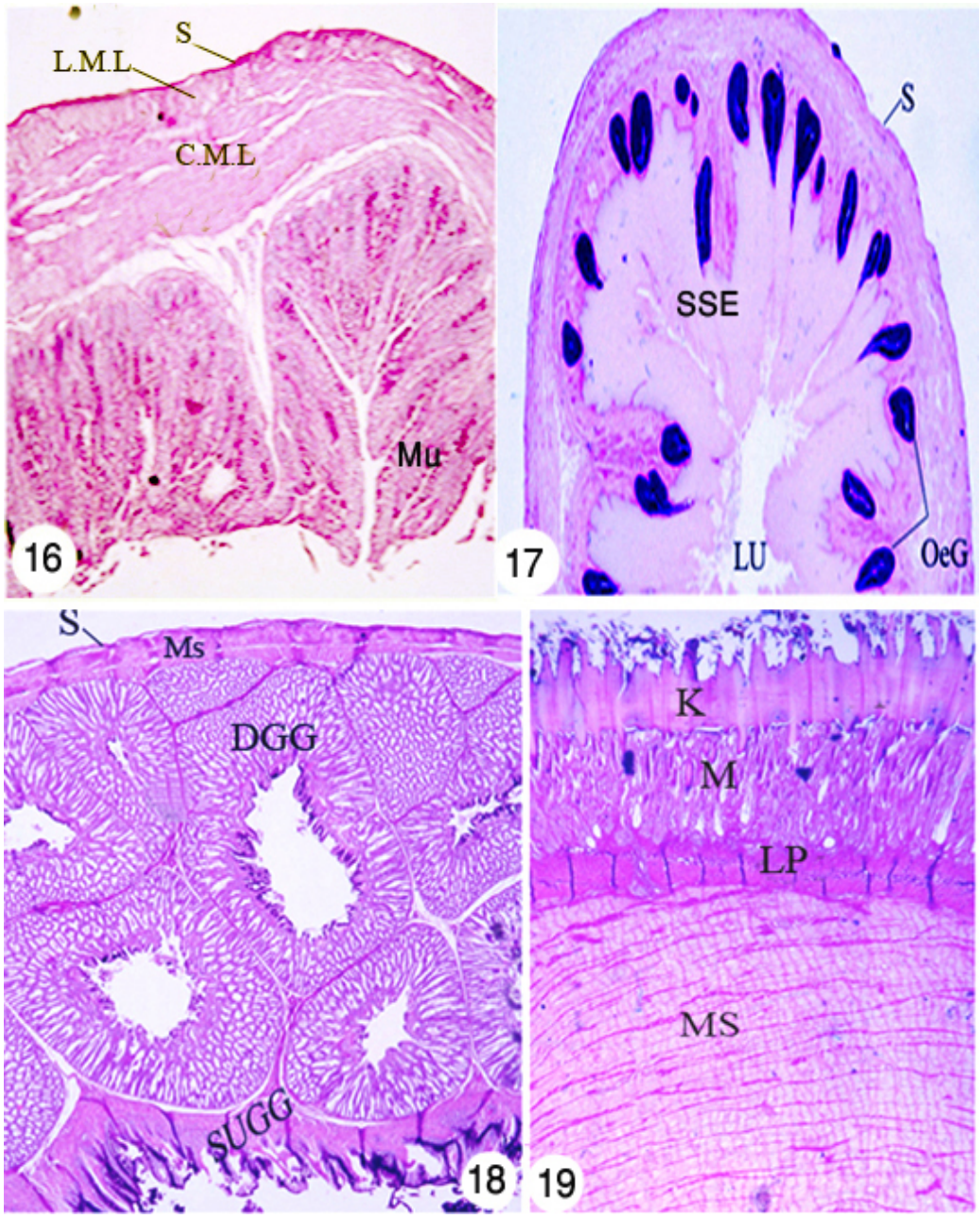

Fig. (16): Photomicrograph of a transverse section of the rectum of *Coturnix coturnix*. Showing the carbohydrate content (PAS- positive stain) X600.

Fig. (17): Photomicrograph of a transverse section of the oesophagus of *Coturnix coturnix*. Showing the mucopolysaccharide content (PAS- Alcian blue stain) X 600.

Fig. (18): Photomicrograph of a transverse section of the proventriculus of *Coturnix coturnix* showing the mucopolysaccharide content (PAS-Alcian blue stain) X 164.

Fig. (19): Photomicrograph of a transverse section of the ventriculus of *Coturnix coturnix* showing the mucopolysaccharide content (PAS-Alcian blue stain) X 600.

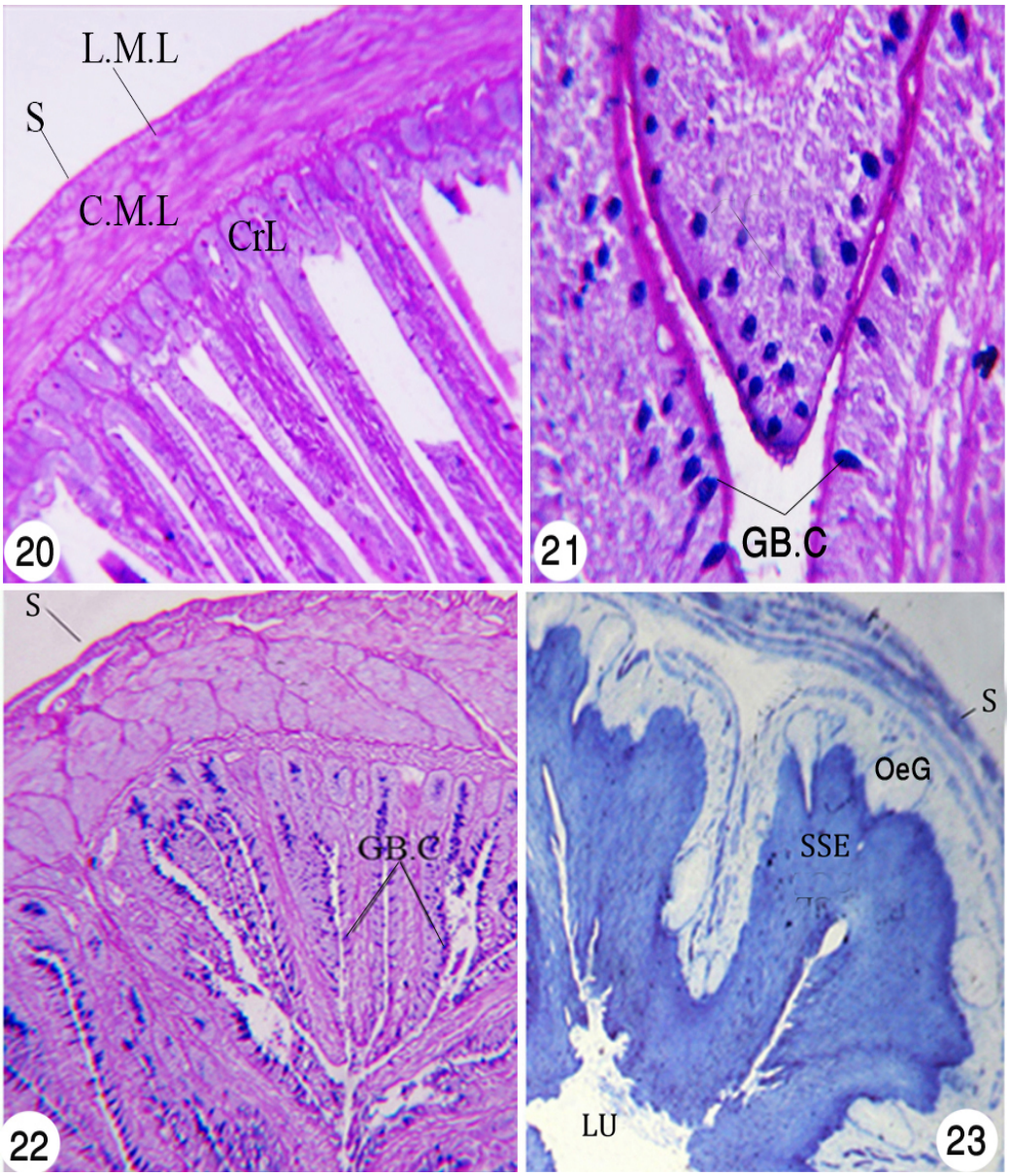

Fig. (20): Photomicrograph of a transverse section of the duodenum of *Coturnix coturnix* showing the mucopolysaccharide content. (PAS-Alcian blue stain) X 140.

Fig. (21): Photomicrograph of a transverse section of the ileum of *Coturnix coturnix* showing the mucopolysaccharide content. (PAS-Alcian blue stain) X 560.

Fig. (22): Photomicrograph of a transverse section of the rectum of *Coturnix coturnix* showing the mucopolysaccharide content (PAS-Alcian blue stain) X560.

Fig. (23): Photomicrograph of a transverse section of the oesophagus of *Coturnix coturnix* showing the protein content (Bromophenol blue stain) X 600.

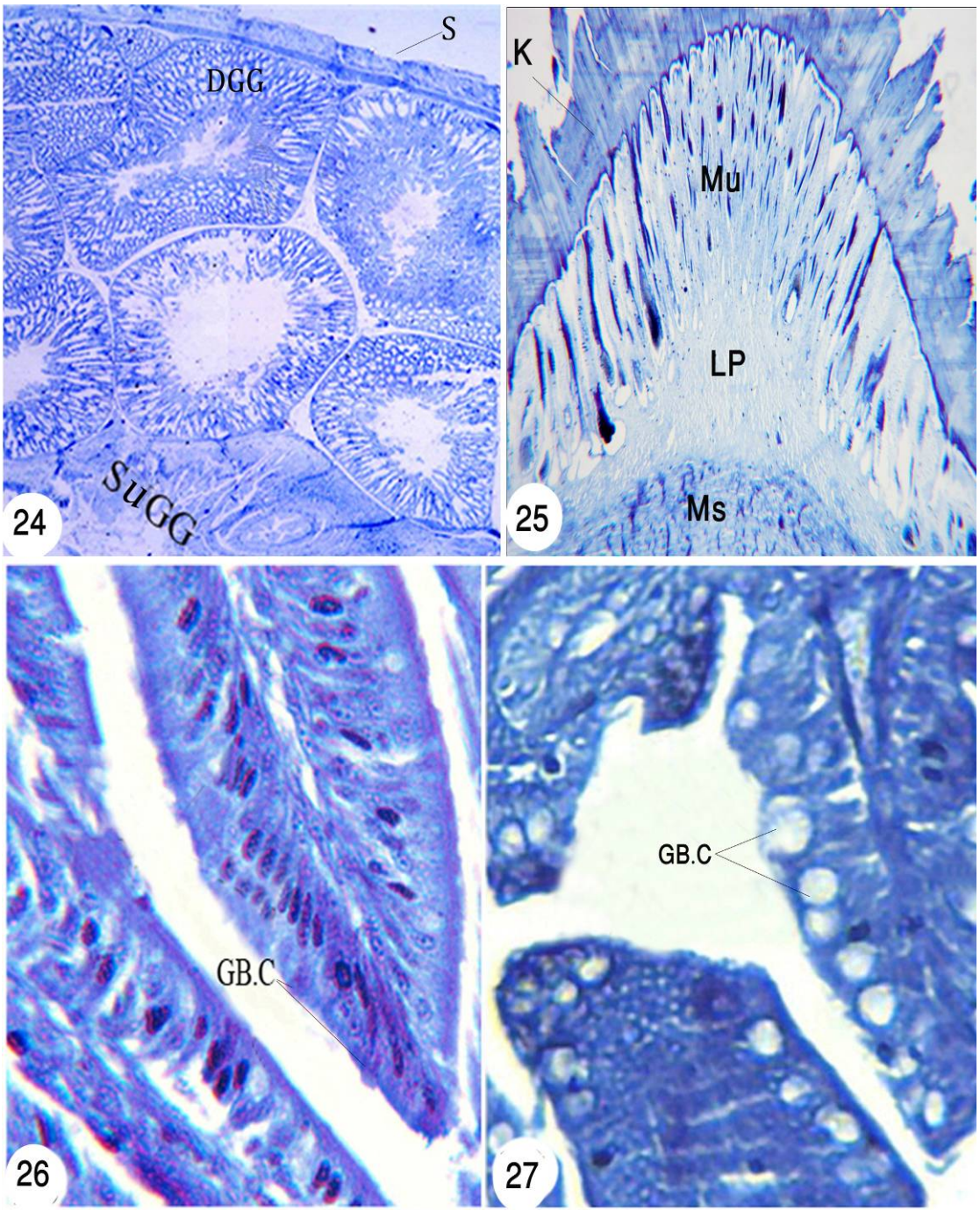

Fig. (24): Photomicrograph of a transverse section of the proventriculus of *Coturnix coturnix*, showing the protein content (Bromophenol blue stain) X 600.

Fig. (25): Photomicrograph of a transverse section of the ventriculus of *Coturnix coturnix* showing the protein content (Bromophenol blue stain) X 600.

Fig. (26): Photomicrograph of a transverse section of the ileum of *Coturnix coturnix* showing the protein content (Bromophenol blue stain) X 600.

Fig. (27): Photomicrograph of a transverse section of the rectum of *Coturnix coturnix* showing the protein content (Bromophenol blue stain) X 600.

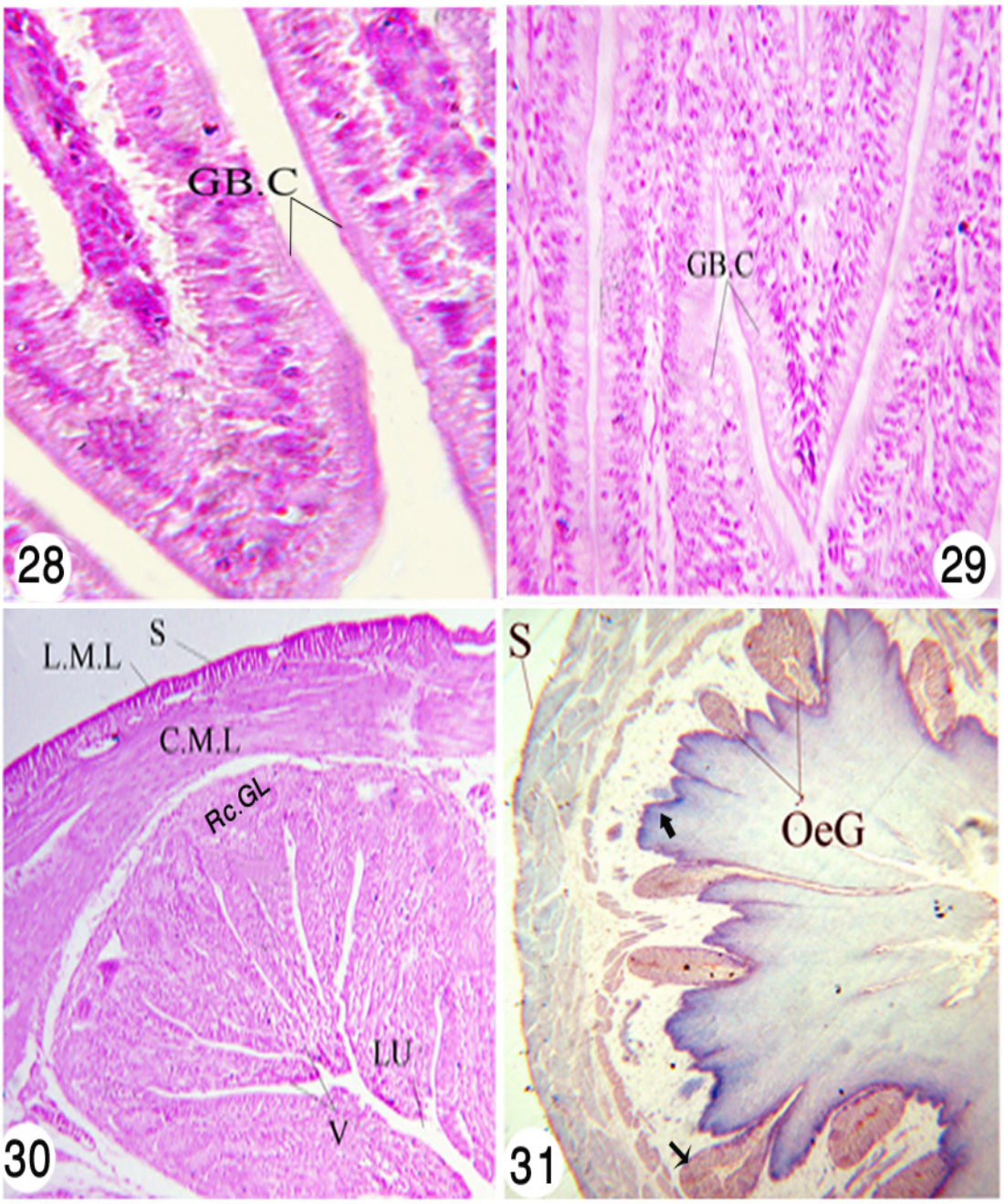

Fig. (28): Photomicrograph of a transverse section of the duodenum of *Coturnix coturnix* showing the DNA content. (Feulgen technique) X 560

Fig. (29): Photomicrograph of a transverse section of the ileum of *Coturnix coturnix* showing the DNA content. (Feulgen technique) X 560

Fig. (30): Photomicrograph of a transverse section of the rectum of *Coturnix coturnix* showing the DNA content. (Feulgen technique) X 560

Fig. (31): Photomicrograph of a transverse section of the oesophagus of *Coturnix coturnix* indicating the DNA (small arrow) and RNA content (large arrow) (Methyl green-pyronin stain) X560.

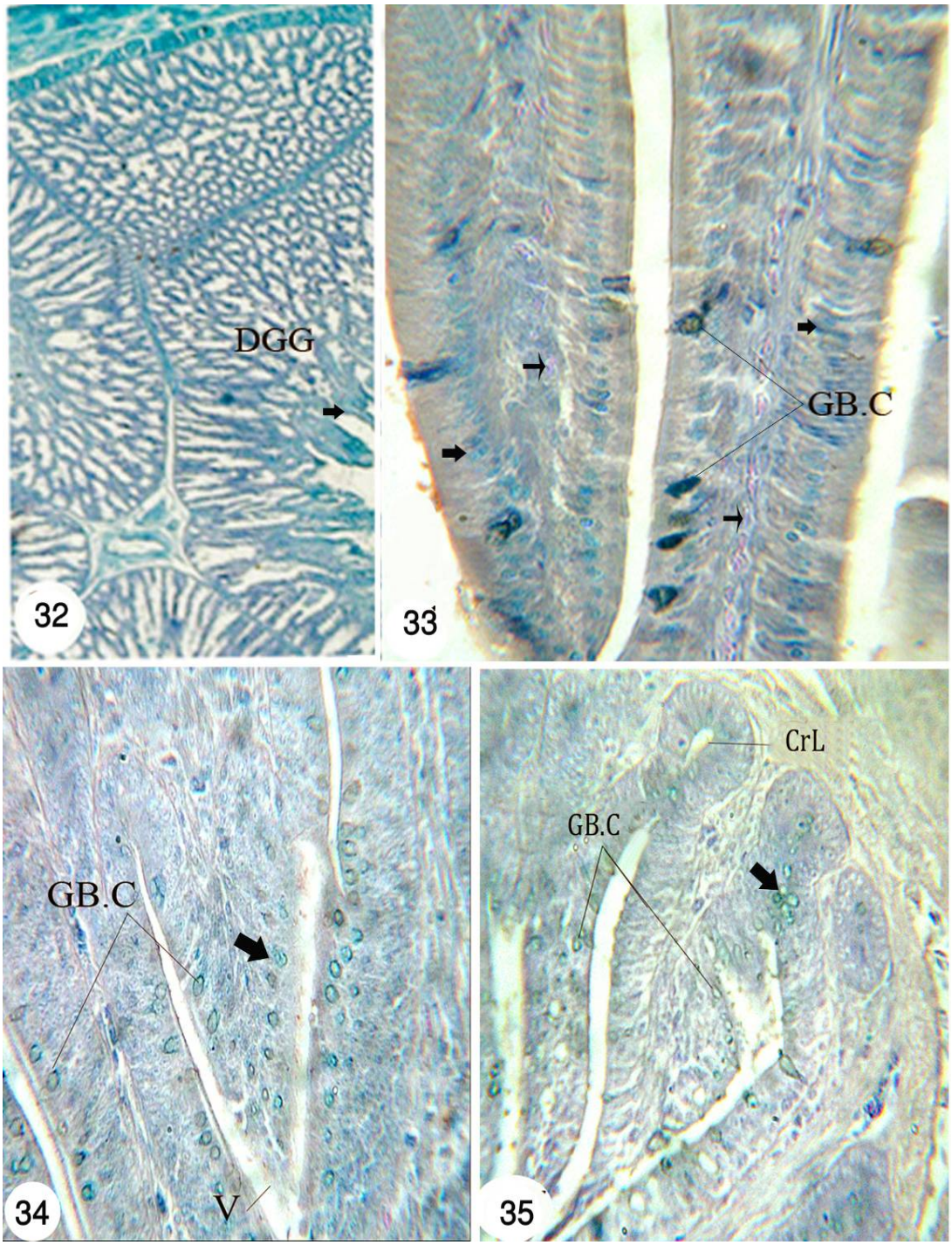

Fig. (32): Photomicrograph of a transverse section of the proventriculus of *Coturnix coturnix* indicating the DNA content (small arrow) (Methyl green-pyronin stain) X560.

Fig. (33): Photomicrograph of a transverse section of the duodenum of *Coturnix coturnix* indicating the DNA (small arrow) and RNA content (large arrow) (Methyl green-pyronin stain) X560.

Fig. (34): Photomicrograph of a transverse section of the ileum of *Coturnix coturnix* indicating the DNA (small arrow) and RNA content (large arrow) (Methyl green-pyronin stain) X560.

Fig. (35): Photomicrograph of a transverse section of the rectum of *Coturnix coturnix* indicating the DNA (small arrow) and RNA content (large arrow) (Methyl green-pyronin stain) X560.

Our results indicate that well-developed caeca also occur in omnivorous and some granivorous species. However, this is due to the inclusion in both categories of Galliform species from Leopold's data (1953) in which he designates quails, partridges, and pheasants as "seed"-eating species (granivorous) that also consume greens, fruits, and insects (omnivorous). Never the less, a common component of both diets is the insoluble carbohydrate, cellulose. Several studies have demonstrated that as the amount of cellulose in the diet increases, whether in natural or commercial diets, so do the lengths of the caeca (Lewin 1963; Moss 1972). If caecal length is an indicator of ingested cellulose, then species consuming the cell walls of higher plants would be expected to have well-developed caeca, and those species consuming nectar, fruits, and animal proteins would be expected to have less caecal development because these foods are easily digested by endogenous lipases, proteases, and carbohydrases Duke (1986).

The caeca and rectum may provide area for use symbiotic bacteria to aid in the digestion of fibrous components of food. This agrees with Klasing (1999).

The liver of *Coturnix coturnix* is bilobed organ. It is composed of two lobes right and left. It contains the gall bladder which is a spherical greenish sac. These results are in agreement with those observed by Leake (1975), Klasing (1999) and Abo-Shaeir (2001).In the present work, the quail pancreas is composed of 4 lobes (dorsal, ventral and splenic lobes and a third lobe) as described in chicken Rawdon (1998), in geese Gulmez (2003) and in quail (Baumel *et al*., 1993), the pancreas fills the gap between the duodenal limbs, while in the duck and goose, the pancreas is too short to reach the end of these limbs (Getty, 1975; Nickel *et al*., 1977). The shortest pancreas is found in bustards, which consist of two lobes (Bailey *et al*., 1997). According to suggestions by Gussekloo (2006) on chicken and other birds that feed on grains and seed, they need more enzymatic activity to compensate for their lack of teeth and hydrolytic enzymes in their saliva. Our data agreed with Nickel *et al*. (1977) who reported that there are three efferent pancreatic ducts in the fowl and pigeon, two of which arise from the ventral and one from the dorsal lobe. These ducts enter the proximal loop of the ascending duodenum, there are only two ducts in goose (Gulmez, 2003) and duck, in addition to the first pancreatic duct from the dorsal pancreatic lobe, which enters the duodenum loop between its descending and ascending limb (Liu *et al*. 1998).

The histological studies of the present investigation revealed that, the alimentary canal wall of *Coturnix coturnix*is differentiated into the same basic four layers of other birds. These layers are; serosa, musculosa, submucosa, and mucosa. Similar findings were achieved by Chikilian & De Speroni (1996) and Abou-Dief & El-Akkad (1999). Histologically, the wall of the oesophagus in *Coturnix coturnix* is formed from the same layers; serosa, musculosa, submucosa, and mucosa. This result agrees with that mentioned by Mclelland (1979).The serosa is a thin layer formed of simple squamous epithelium. It is present only in the lower part of the oesophagus while a fibrous layer surrounds the upper part. The musculosa consists of two distinct muscle layers; circular and longitudinal. This result agrees with that mentioned by Salem (1984). Also, Klasing (1999) added that the peristaltic contraction of inner circular and outer longitudinal muscles propels food posteriorly through the oesophagus. The mucosal folds are lined by stratified squamous epithelium interrupted by the ducts of the mucosal glands. This observation is similar to that of the fowl Bradley (1915) and Calhoun(1933) and in *Tyto alba* (Ismail,2000). In *Coturnix coturnix*, mucosa contains oesophageal glands which are tubulo-alveolar type. Similar observations were reported by Abd El- Aziz (1984), Salem (1984), El-Bahrawy *et al.,* (1989), El-Banhawy *et al.,* (1993b) and El-Sayyad (1995) and the presence of these mucous glands could be considered as another kind of oesophageal adaptation with the nature of food items.

The secretion of the crop mucous glands may serve to moisten seeds for a certain degree which allows the bird to avoid the unpleasant feeling of swallowing rough and dry seeds (Leznicka,1971). Similarly, the mucosal glands of the lower oesophagus may serve to lubricate the moisten food reducing its tackiness ability which may cause difficulty in the process of swallowing. In agreement with Selvan *et al*.(2008), the current study showed that the wall organization of the proventriculus is according to the general pattern that specified most of the digestive organs; tunica mucosa, tunica submucosa, tunica muscularosa and tunica serosa. In agreement with Okamoto & Yamada(1981),Prasad & Kakade (1990),Imai *et al*. (1991) and Liman *et al*. (2010) the current study showed that the proventricular folded mucosal surface is not smooth but it is covered by several projections or papillae. The proventricular glands which form the most thickness of the proventricular wall open at the apex of this papilla by ducts elaborating pepsinogen, hydrochloric acid and mucus discharge into the stomach lumen. In agreement with Langlois (2003) and Rahman *et al*.(2003), the current study showed that the surface lining epithelium of the proventriculus is of a simple columnar type however the glandular epithelium is

formed by only one principle exocrine cells of a simple cuboidal type; oxyntico-peptic cells.

The occurrence of two types of gastric glands in proventriculus of the bird under investigation was previously reported by Calhoun (1933) in *Gallus domesticus* and Langlois (2003). The glands located between the inner and outer layers of the muscularis mucosa. The location of the proventricular glands is a matter of controversy between the authors. Some authors reported that the proventricular glands present in the lamina propria of duck proventriculus Calhoun (1954) in similar to our results, however many studies suggested the glands in other species to be submucosal Farner (1960) contradicting our results. These differences in the location of the glands may due to species variation but may be also due to developmental stages differences. In agreement with Prasad and Kakade (1990) in duck and Rahman *et al*. (2003) in chickens, the current study showed that lymphocytes and lymphatic nodules were seen in the lamina propria of the quail proventricular surface mucosa. That is likely, indicates a sort of participation of the proventriculus in the quail immune response. In our results, the musculosa was represented by a thick inner circular layer and a thin external longitudinal layer of smooth muscle fibres. These data are in agreement with those of Hodges (1974) but differ from the results of Catroxo *et al*. (1997) and Ogunkoya & Cook (2009), who described the musculosa of the proventriculus in three layers, viz. an inner longitudinal, a middle circular and an external longitudinal layer of smooth muscles. In contrast to our findings, however, Rossi *et al*., (2005) mentioned only two layers, i.e. the inner longitudinal and outer circular layers. In agreement to other reports, the current study showed that the ventriculus wall in *Coturnix coturnix* made by mucosa, submucosa, muscularosa and serosa (Gabella ,1985 and Bailey *et al*., 1997).The cuticagastrica in *Coturnix coturnix* is injunction with stones taken into the ventriculus with food forms an effective surface for hard food. These findings agree with Leake (1975) who added that the cuticagastrica produced by the gastric tubular glands. The absence of lamina muscularis mucosae of ventriculus in this study is in agreement with the report of Catroxo *et al*. (1997) but does not support the findings of Rocha and De Lima (1998). The observation of the submucosa beneath the ventricular glands in the current study does not agree with the report of Chikilian and De Speroni (1996), Catroxo *et al*. (1997), and Rocha and De Lima (1998). The observation that the tunica musculosa is made of two layers coincides with the report of Gabella (1985), Catroxo *et al*. (1997) and Rocha and De Lima (1998). And contrasts with the report of Hodges (1974) who observed three layers with different directions of muscle fibres.

The histological studies showed that, the ileal wall is built up of the same layers; serosa, musculosa, submucosa, muscularis mucosa, and mucosa. The serosa is the outermost layer made up of flattened simple squamous epithelium. The musculosa consists of two layers of muscle fibres; outer longitudinal and inner circular muscle layers. This finding is accordance with that of Abd El-Aziz (1984) in *ardeola ibis ibis*. The submucosa is a thin loose connective tissue containing a number of blood capillaries. The muscularis mucosa is represented by a narrow part of longitudinally arranged smooth muscle fibers. The mucosa is in vaginated at the bases of the villi into straight tubular glands (crypts of Leiberkühn) which are continuous with the columnar epithelium linning the villi. The same condition has been found in *Strothio* Bezuidenhout& Van Aswegen (1990) in *Larusridi bundus*&*Strepto peliasen egalensis* El-Banhawy *et al.* (1993a) and in *Cattle egret* Abou-Dief & El-Akkad (1999).

Also, goblet cells in the ileum which have slender bases containing elongated nuclei secrete mucous that protect the intestinal epithelium**.** Leznicka (1971) mentioned that these cells (goblet cells) are greatly correlated with the consistency of the bird's food items.

The mucosa of the rectum is thrown up into numerous leaf-like villi, all covered by simple columnar epithelium containing goblet cells. The goblet cells are numerous in number and open into the lumen. This agrees with Abd El-Aziz (1984). Rectal glands are noticed at the base of the mucosal folds. This observation was recorded by Abo-Shaeir (2001) in *Tyto alba*.

The histochemical study revealed the existence of a high amount of mucopolysaccharides in the oesophageal glands. These results agree with El-Bahrawy *et al.* (1989) and El- Banhawy*et al.* (1993a). Moreover, Leznicka (1971) reported that food composed of green plants or food of high content of starch had a stimulating effect on increasing the number of the oesophageal mucous glands and consequently the amount of secreted substances. On the other hand, Leznicka (1971) added that the size of the oesophageal mucous glands also, changed depending on the kind of food and the amount of water in it. The proventriculus mucosa shows folds lined by simple columnar cells containing PAS and Alcian blue positive mucin granules (neutral and acid mucin, respectively) as reported by Hodges (1974) in domestic fowl. The presence of neutral and acid mucin acts as a barrier to protect the proventricular mucosal surface Mogil'naia *et al*. (1978). The secretory cells, oxyntico-peptic cells are separated from each other by

narrow spaces giving the cells a serrated appearance, due to the presence of cell junctions at the lower lateral portions of the cells but not at the upper ones. The cells stained negatively for PAS and Alcian blue indicating that these cells may not be of a mucous secretory function, but certainly secrete HCL and pepsin analogues to that of mammalian stomach secretion Hodges (1974). The gizzard mucosa is covered by a thick layer of koilin membrane which is formed of proteinous material similar to keratin and stained positive for PAS and Alcian blue indicating the presence of neutral and acidic mucin within its contents; in agreement with reports of other authors (Selvan *et al*., 2008). The goblet cells and the crypts of Leiberkühn have acid and neutral mucopolysaccharide secretions and the lamina propria of the intestine contains proteins, these findings are in agreement with El-Banhawy *et al*.(1993 a) and El-Sayyad (1995).

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Study of Beta 2 Glycoprotein 1 Antibodies in HCV Positive Patients on HD and Its Relation to Vascular Access Thrombosis

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Abstract: Background: Although the precise physiological role of B2-GPI is not known. B2-GPI has been shown to inhibit intrinsic pathway activation and prothrombinase activities. Hemodialysis access failure is a leading cause of morbidity and hospitalization for patients with end-stage renal disease. **Patients and methods:** our study was conducted on forty patients under regular hemodialysis randomly selected from hemodialysis units of Ain Shams University Hospitals. Patients were divided into Group A: 20 patients with positive hepatitis C virus antibodies, and group B: 20 patients with negative hepatitis C virus antibodies. All patients were subjected to: full clinical examination, routine CBC, ESR and quantitative CRP, routine chemistry including (BUN, serum creatinine), serum Na, serum K, serum Ca, serum PO4, serum albumin, and total proteins. Liver enzymes (AST, ALT), routine coagulation profile (PT, INR, PTT), HBsAG and HCVab, B2 glycoprotein I antibodies IgM, IgG titers by ELISA and assessment of fistula flow by Doppler ultrasound were also done. **Results:** We found that the frequency of B2IgM and B2IgG positive or borderline in group A was 20 % (4 patients), 25% (5 patients) respectively while the frequency of B2 IgM and B2IgG in group B was 30% (6/20 patients), 35% (7/20 patients) respectively. No relation was found between B2-glycoprotein I antibodies and HCV in prevalent hemodialysis patients. Our study revealed the frequency of B2 IgM was 40% and B2 IgG was 40% positive or borderline in patients with AVF with positive history of vascular access occlusion. Elevated B2GPI IgM titre is associated with decreased fistula volume of flow by Doppler.
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Key words: Beta2glycoprotein1 antibodies – HCV- HD – Vascular access thrombosis.

1. Introduction:

Beta 2 glycoprotein 1 (beta 2 GP1, also called apolipoprotein H) is a 326 amino acid synthesized by hepatocytes, endothelial cells and trophoblast cells (1).

B_2GP_1, *in vitro* studies suggest that it likely functions as a natural anticoagulant. B_2GP_1 has been shown to inhibit intrinsic pathway activation and prothrombinase activities on the surface of activated platelets and synthetic phospholipids vesicles. B_2GP_1 also inhibits the activity of activated protein C on procoagulant surfaces. B_2GP_1 inhibits ADP-induced platelet aggregation (2).

B_2GP_1 has become well-known as a co-factor for anticardiolipin auto-antibodies. Autoantibodies against B_2GP_1 are described for various autoimmune disease. The presence of anti-B_2GP_1 antibodies can be related to the development of arterial and venous thromboses, venous thromboembolism, thrombocytopenia and fetal loss. Anti-B_2GP_1 antibodies are found in the immunoglobulin classes IgG, IgM and IgA (3).

Anti-B_2GP_1 IgG antibody titers correlate well with the clinical status of the patients in thrombosis, thromboembolism and repeated fetal loss, while anti-B_2GP_1 IgM antibodies show significant association with thrombosis and thrombocytopenia(3).

Sands *et al.* (2001)(4) showed that haemodialysis patients had elevated anti-B_2GP_1 antibodies and patients with PTFE grafts had elevated antibodies most frequently versus fistulas and tunneled catheters and this study concluded that haemodialysis patients with PTFE grafts frequently have elevated antibodies to beta 2 GP_1 and the presence of elevated antibody levels is associated with an increased thrombotic risk.

Previously, there were attempts to detect anticardiolipin antibodies IgM, IgG in HCV positive hemodialysis patients. However, the relation between its presence and thrombotic events including fistula thrombosis was not proved. Elevated IgM aCL titer was present in 17.4% of chronic HD patients. Results suggest recurrent vascular access thrombosis of synthetic or native fistula may not be caused by elevated IgM-aCL (IgM-anticardiolipin antibody) titer in these patients (5).

Ozmen *et al.* (2009)(6), showed that prevalence of IgG-aCL (IgG-anticardiolipin antibody) in chronic HD patients was 14.6% and no patient had a positive value of the IgM-aCL test.

2. Patients and Methods

This study was conducted on forty patients with ESRD on regular hemodialysis (3 sessions per week) randomly selected from hemodialysis units of Ain Shams University Hospitals between September 2010 and September 2011. The patients were divided into 2 groups:

Group A: 20 patients with positive hepatitis C virus antibodies.

Group B: 20 patients with negative hepatitis C virus antibodies.

We excluded from the study ESRD patients on regular hemodialysis having diabetes mellitus, collagen disorders, coagulation abnormality other than that associated with chronic liver disease including drug induced liver disease. Smokers were excluded from the study.

All patients were subjected to full clinical examination including vascular access examination (synthetic or native fistula or catheter).

Routine complete blood count (CBC), ESR and quantitative CRP, BUN, serum creatinine, serum Na, serum K, serum Ca, serum PO_4, serum albumin, total proteins, liver enzymes (serum ALT, serum AST), routine coagulation profile (PT, INR, PTT), HBsAg and HCVab, B_2 glycoprotein I antibodies IgM, IgG titer by ELISA.

Methods:

All routine investigations were done by conventional methods at Ain Shams University Hospital laboratories.

Beta-2 glycoprotein I estimation principle of the test

Highly purified beta-2 glycoprotein I is bound to microwells. Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgA immunologically detects the bound patient's antibodies forming a conjugate/antibody/antigen complex washing of the microwells remove unbound conjugate.

An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometricaly at 450 nm.

The amount of colour is directly proportional to the concentration of IgA antibodies present in the original sample.

Interpretation of results

Quality control:

This test is only valid if the optical density at 450 nm for positive control (1) and negative control (2) as well as for the calibrators A and F complies with the respective range indicated on the quality control certificate enclosed to each test kit. If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

Interpretation of results

In a normal range study with serum samples from healthy blood donors, the following ranges have been established with the anti-beta-2- glycoprotein I test.

Anti-beta-2 glycoprotein I Ab IgM, IgG (U/ml)

Normal: < 5

Borderline: 5-8

Positive: > 8

Statistical analysis of data

T-test and paired t-test were used to compare means of parametric data group while Mann Whitney test was used for the non parametric data.

Pearson chi-square correlation test were used to correlate parametric values and spearman correlations was used for the non parametric data.

3. Results

Participants in group A were 16 females (80%) and 4 males (20%). The available access was natural arteriovenous fistula (AVF) in 17 patients, polytetrafluoroethylene (PTFE) grafts in 2 patients and cuffed catheter in 1 patient.

Participants in group B were 13 females (65%) and 7 males (35%), two patients had a history of cerebrovascular stroke and B_2-GPI was negative, one patient with DVT with borderline B_2 IgG-1. Two patients died after termination of our study (one was due to pulmonary edema and the other was due to hematemesis).

HCV PCR done for patients with negative HCV antibody with positive B_2-GPI antibody, was found to be negative. Patients were further classified into 2 equal groups according to vascular access occlusion:

First group: Patients having positive history of vascular access occlusions (group C).

Second group: Patients having negative history of access (occlusion (group D).

4. Discussion

Antiphospholipid (aPL) antibodies (Abs) were discovered in 1952 in patients suffering from systemic lupus erythematosus (SLE), it has the ability to prolong coagulation time in vivo from which the name lupus anticoagulant (LA) arose (7).

Later on, it was discovered that aPL do not act as anticoagulants in vivo, and can be found not only in SLE patients but also in apparently healthy individuals (8).

At the beginning of the 1990s, beta-2 glycoprotein 1 (B2-GPI) was shown to be a major antigen for anti-phospholipid antibodies. Although anticardiolipin antibodies (aCL) Abs and Abs against B_2-GPI (anti-B_2-GPI Abs) often coexist, they are not identical.

Interestingly, the highest risk of thrombosis was associated with the presence of both LA with anti-B_2GPI.

Table (1): Comparison of quantitative variables in group (A) and group (B)

	HCV state	N	Mean	SD	t*	*P*-value	Sig.
Age in years	HCV+ve (group A)	20	47.65	±12.571	-0.147	>0.05	NS
	HCV-ve (group B)	20	47.05	±13.153			
Systolic B.P. in mmHg	HCV+ve (group A)	20	124.500	±19.86136	0.793	>0.05	NS
	HCV-ve (group B)	20	130.000	±23.84158			
Diastolic b.P. in mmHg	HCV+ve (group A)	20	77.00	±10.31095	0.000	>0.05	NS
	HCV-ve (group B)	20	77.000	±11.74286			
WBcs 10^6/cmm	HCV+ve (group A)	20	6.220	±2.3761	0.376	>0.05	NS
	HCV-ve (group B)	20	6.500	±2.3317			
HGB in mmHg	HCV+ve (group A)	20	10.450	±1.9503	-0.986	>0.05	NS
	HCV-ve (group B)	20	9.895	±1.5906			
PLT 10^6/cmm	HCV+ve (group A)	20	218.45	±68.241	0.899	>0.05	NS
	HCV-ve (group B)	20	239.10	±76.730			
BUN mg/dl	HCV+ve (group A)	20	61.35	±27.601	0.841	>0.05	NS
	HCV-ve (group B)	20	67.85	±20.790			
Creat mg/dl	HCV+ve (group A)	20	7.74	±2.223	2.233	0.032	**S**
	HCV-ve (group B)	20	9.45	±2.606			
Alb gm/dl	HCV+ve (group A)	20	3.40	±0.592	1.854	0.071	NS
	HCV-ve (group B)	20	3.68	±0.299			

*** t-test was used**

Table (2): Comparison of quantitative variables in group (A) and group (B) (Cont..)

	HCV state	N	Mean	SD	t*	*P*-value	Sig.
Na meq/l	HCV+ve (group A)	20	136.75	5.025	1.007	>0.05	NS
	HCV-ve (group B)	20	138.10	3.275			
K meq/l	HCV+ve (group A)	20	5.205	0.7287	0.414	>0.05	NS
	HCV-ve (group B)	20	5.315	0.9382			
Ca mg/dl	HCV+ve (group A)	20	8.575	0.8058	0.728	>0.05	NS
	HCV-ve (group B)	20	8.790	1.0467			
Po4 mg/dl	HCV+ve (group A)	20	4.295	1.5463	2.505	0.017	**S**
	HCV-ve (group B)	20	5.415	1.2671			
Ast	HCV+ve (group A)	20	19.40	6.44	0.259	> 0.05	NS
IU	HCV-ve (group B)	20	21.05	27.757			
Alt	HCV+ve (group A)	20	17.30	5.222	-.221	>0.05	NS
IU	HCV-ve (group B)	20	16.35	18.488			
Total bilirubin	HCV+ve (group A)	20	0.69	0.268	1.493	>0.05	NS
mg/dl	HCV-ve (group B)	20	0.83	0.323			
INR	HCV+ve (group A)	20	1.0035	0.05284	-1.144	>0.05	NS
	HCV-ve (group B)	20	0.9850	0.04936			
PTT sec	HCV+ve (group A)	20	35.805	7.6490	0.137	>0.05	NS
	HCV-ve (group B)	20	36.200	10.3417			
Doppler flow	HCV+ve (group A)	20	740.865	134.3721	-.624	>0.05	NS
ml/min n=17	HCV-ve (group B)	20	706.824	180.2599			
Intradialytic	HCV+ve (group A)	20	2.7250	1.19731	-1.167	>0.05	NS
weight gain	HCV-ve (group B)	20	2.300	1.10501			

*** t-test was used**

Table (3): Comparison of group (A) and group (B) as regard ESR and CRP

	HCV state	N	Median	Std. Error of Mean	Mann Whitney*	*P*-value	Sig.
Duration of	HCV+ve (group A)	2	14	10	0.827	>0.05	NS
hemodialysis n =20	HCV-ve (group B)	3	13	7	0.694		
ESR 1st hour	HCV+ve (group A)	20	47.75	46.693	199.000	>0.05	NS
	HCV-ve (group B)	20	38.60	31.149			
CRP mg/dl	HCV+ve (group A)	20	11.70	20.275	165.000	>0.05	NS
	HCV-ve (group B)	20	9.30	5.667			

***Mann Whitney u test was used was used**

Table (4): Comparison of group (A) and group (B) as regard B2 IgM and B2 IgG:

		HCV state				Value of chi-square test*	*P* value > 0.05 NS < 0.05 S	Sig.
		Positive A		Negative B				
		No.	Percent	No.	Percent			
B2 IgM u/ml	Negative	16	80%	14	70%	0.533	0.465	NS
	Positive	4	20	6	30			
B2-IgG u/ml	Negative	15	75	13	65	0.476	0.490	NS
	Positive	5	25	7	35			

*** Chi square test was used**

No statistical significance as regard correlation between b2 glycoprotein antibodies IgM, IgG and HCV state (positive or negative) (*p* value > 0.05).

Table (5): Comparison of group (A) and group (B) as regard B2 IgM and B2 IgG tire.

	HCV State				Value of chi-square test*	*P* value > 0.05 NS < 0.05 S	Sig.
	Positive A		Negative B				
	Median	Std. error	Median	Std. error			
B2 IgM titre u/ml	3.000	0.6729	4.000	1.1056	143.000	.120	NS
B2-IgG titre u/ml	3.75	0.268	4.00	0.447	161.500	.293	NS

*** Mann Whitney u test was used**

No statistical significance as regard correlation between b2 glycoprotein antibodies IgM, IgG and HCV state (positive or negative) (*p* value > 0.05).

Table (6): Risk estimation via odds ratio for: B2 IgM & B2 IgG positive or borderline

	Odds ratio	95% confidence interval	
		Lower	Upper
B2 IgM u/ml (negative/positive)	0.583	0.136	2.498
B2 IgG u/ml (negative/positive)	0.619	0.158	2.429

This table 3.10 shows that:

B2 IgM negative is more likely to be among group A "HCV +ve patients" than B "HCV –ve patients" by a ratio of 0.583. B2 IgG negative is more likely to be among group group A "HCV +ve patients " than B "HCV –ve patients" by a ratio of 0.619.

Table (7): Correlation between B2-IgM, B2-IgG and other variables in HCV +ve (group A)

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
Age (years) (n=20)	-0.027	0.911	NS	0.103	0.667	NS
Duration of hemodialysis "n=30" in years	-0.081	0.735	NS	0.195	0.41	NS
Systolic BP "n=20" in mmHg	-0.329	0.157	NS	0.197	0.406	NS
Diastolic BP "n = 20" in mmHg	-0.241	0.306	NS	0.276	0.239	NS
Wbc "n=20" 10^6/cmm	-0.342	0.14	NS	-0.112	0.639	NS
Hb "n=20" gm/dl	0.229	0.332	NS	0.251	0.286	NS
Plt "n=20" 10^6/cmm	-0.067	0.779	NS	-0.062	0.794	NS
BUN "n=20" mg/dl	0.168	0.48	NS	-0.507(*)	0.027	**S**
Cr 'n=20" mg/dl	-0.167	0.481	NS	-0.494(*)	0.027	**S**
Albumin "n=20" gm/dl	-0.186	0.432	NS	0.22	0.352	NS
Na "n=20" meq/L	0.142	0.551	NS	-0.219	0.354	NS
K 'n = 20" meq/L	0.142	0.551	NS	-0.219	0.354	NS
Ca "n=20" mg/dl	0.14	0.557	NS	0.383	0.096	NS
Po4 "n=20" mg/dl	0.248	0.291	NS	-0.046	0.849	NS
Ast IU	0.391	0.089	NS	-0.108	0.651	NS
Alt IU	0.281	0.23	NS	0.039	0.869	NS

* Spearman test was used

Table (8): Correlation between B2-IgM, B2-IgG and other variables in HCV +ve (group A) (Cont…)

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
Bil total mg/dl	0.463	0.04	S	-0.018	0.941	NS
INR	0.348	0.133	NS	0.012	0.959	NS
PTT sec	0.121	0.61	NS	0.176	0.459	NS
ESR 1st hour	0.225	0.341	NS	0.057	0.81	NS
CRP mg/dl	-0.258	0.272	NS	0.07	0.77	NS
Doppler flow ml/min n=17	-0.209	0.420	NS	-0.271	0.292	NS
B2-IgG u/ml	0.142	0.55	NS			

* Spearman test was used

Table (9): Correlation between B2-IgM, B2-IgG and other variables in HCV -ve (group B)

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
Age (years) (n=20)	-0.187	0.431	NS	-0.31	0.183	NS
Duration of hemodialysis "n=30" in years	0.396	0.084	NS	-0.198	0.402	NS
Systolic BP "n=20" in mmHg	0.142	0.55	NS	0.437	0.054	NS
Diastolic BP "n = 20" in mmHg	-0.045	0.849	NS	0.312	0.181	NS
Wbc "n=20" 10^6/cmm	0.21	0.35	NS	0.373	0.106	NS
Hb "n=20" gm/dl	-0.119	0.617	NS	0.092	0.699	NS
Plt "n=20" 10^6/cmm	0.252	0.283	NS	0.122	0.61	NS
BUN "n=20" mg/dl	0.407	0.075	NS	0.246	0.296	NS
Cr 'n=20" mg/dl	0.127	0.594	NS	-0.221	0.348	NS
Albumin "n=20" gm/dl	-0.301	0.197	NS	-0.127	0.593	NS
Na "n=20" meq/L	-0.136	0.567	NS	-0.016	0.945	NS
K 'n = 20" meq/L	0.151	0.526	NS	-0.243	0.302	NS
Ca "n=20" mg/dl	0.278	0.235	NS	0.169	0.477	NS
Po4 "n=20" mg/dl	-0.108	0.651	NS	0.017	0.942	NS
Ast IU	0.198	0.403	NS	0.224	0.342	NS
Alt IU	0.325	0.162	NS	0.169	0.476	NS
Bil total mg/dl	0.161	0.497	NS	0.221	0.35	NS
INR	-0.174	0.464	NS	-0.417	0.068	NS

* Spearman test was used

Table (10): Correlation between B2-IgM, B2-IgG and other variables in HCV -ve (group B) (Cont…)

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
PTT sec	0.046	0.848	Ns	-0.148	0.533	NS
ESR 1st hour	0.318	0.172	NS	-0.181	0.444	NS
CRP mg/dl	0.062	0.796	NS	0.03	0.901	NS
Doppler flow ml/min n=17	-0.254	0.326	NS	-0.028	0.914	NS
B2-IgG u/ml	0.26	0.268	NS	0.26	0.268	NS

* Spearman test was used

Table (11): Comparison of quantitative variables in patients with positive and negative history of vascular access occlusion

	HCV state	N	Mean	SD	t*	*P*-value	Sig.
Age in years	Positive	20	45.45	12.672	0.945	>0.05	NS
	Negative	20	49.25	12.769			
Systolic B.P. in mmHg	Positive	20	124.000	21.373	0.940	>0.05	NS
	Negative	20	130.500	22.354			
Diastolic b.P. in mmHg	Positive	20	76.00	10.9544	0.575	>0.05	NS
	Negative	20	78.00	11.050			
WBcs 10^6/cmm	Positive	20	6.550	2.5264	-0.511	>0.05	NS
	Negative	20	6.170	2.1599			
HGB in mmHg	Positive	20	9.985	1.4339	0.662	>0.05	NS
	Negative	20	10.360	2.0894			
PLT 10^6/cmm	Positive	20	217.70	71.506	-0.966	>0.05	NS
	Negative	20	239.85	73.467			
BUN mg/dl	Positive	20	64.70	24.883	-0.026	>0.05	NS
	Negative	20	64.50	24.436			
Creat mg/dl	Positive	20	8.47	2.747	0.295	>0.05	NS
	Negative	20	8.71	2.386			
Alb gm/dl	Positive	20	3.50	0.358	0.486	>0.05	NS
	Negative	20	3.58	0.590			
Intradialytic weight gain	Positive	20	2.600	1.11921	-0.473	>0.05	NS
	Negative	20	2.425	1.21693			

*** t-test was used**

Table (12): Comparison of quantitative variables in patients with positive and negative history of vascular access occlusion (cont…)

	HCV state	N	Mean	SD	t*	*P*-value	Sig.
Na meq/L	Positive	20	136.90	4.941	0.779	>0.05	NS
	Negative	20	137.95	3.456			
K meq/L	Positive	20	5.325	0.8608	-0.490	>0.05	NS
	Negative	20	5.195	0.8172			
Ca mg/dl	Positive	20	8.755	1.1532	-0.489	>0.05	NS
	Negative	20	8.610	0.6545			
Po4 mg/dl	Positive	20	4.655	1.2458	0.837	>0.05	NS
	Negative	20	5.055	1.7380			
Ast IU	Positive	20	23.55	27.657	-1.056	>0.05	NS
	Negative	20	16.90	5.281			
Alt IU	Positive	20	20.05	18.193	-1.547	>0.05	NS
	Negative	20	13.60	4.083			
Total bilirubin mg/dl	Positive	20	0.82	0.341	-1.270	>0.05	NS
	Negative	20	0.70	0.250			
INR	Positive	20	0.9890	0.05848	0.642	>0.05	NS
	Negative	20	0.9995	0.04395			
PTT sec	Positive	20	38.665	11.6042	-1.940	>0.05	NS
	Negative	20	33.340	3.9948			
Doppler flow ml/min n=17	Positive	20	383.514	177.9665	1.260	>0.05	NS
	Negative	20	752.075	139.2072			

*** t-test was used**

Table (13): Comparison of patients with positive and negative history of vascular access occlusion as regard ESR and CRP:

	History of vascular access occlusion	N	Median	Std. error of mean	t*	*P* value	Sig.
ESR 1st hour	Positive	20	46.85	41.808	-0.584	>0.05	NS
	Negative	20	39.50	37.658			
CRP mg/dl	Positive	20	9.60	6.573	0.382	>0.05	NS
	Negative	20	11.40	20.033			

*t-test was used

Table (14): Comparison of patients with positive and negative history of vascular access occlusion as regards B2-IgM and B2-IgG

		Vascular access state				*Value of chi-square test*	*P* value	Sig.
		Positive history vascular access occlusion "n=20"		Negative history of vascular access occlusion "n=20"				
		No.	Percent	No.	Percent			
B2 IgM u/ml	Negative < 5	12	60	18	90	4.800	0.028	**S**
	Positive ≥5	8	40	2	10			
B2-IgG u/ml	Negative < 5	12	60	16	80	1.905	0.168	NS
	Positive ≥5	8	40	4	20			

* Chi-square test was used

Table (15): Comparison of group (A) and group (B) as regard B2 IgM and B2 IgG tire.

	Vascular access state				*Value of Mann-Whitney U	*P* value	Sig.
	Positive history vascular access occlusion "n=20"		Negative history vascular access occlusion "n=20"				
	Median	Std. error	Median	Std. error			
B2 IgM u/ml	4.000	1.1349	3.000	0.5781	121.000	0.031	**S**
B2-IgG u/ml	4.00	0.335	4.00	0.414	176.00	0.512	NS

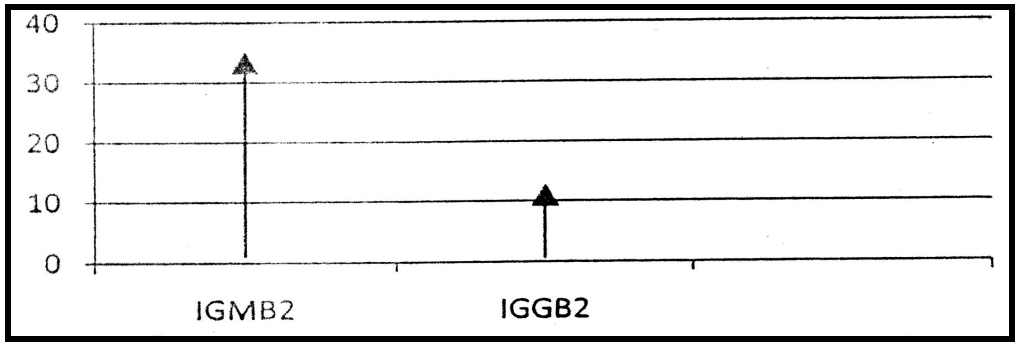
* Mann Whitney u test was used

Table (16): Risk estimation via odds ratio for B2 IgM & B2 IgG

	Odds ratio	95% confidence interval	
		Lower	Upper
B2 IgM u/ml (negative/positive)	6.00	1.082	33.274
B2 IgG u/ml (negative/positive)	2.667	0.648	10.972

B2 IgM positive is more likely to be among patients with positive history of vascular access occlusion than patients with negative history of vascular access occlusion by a ratio of 6.

So patients with positive B2 IgM have the risk of thrombosis 6 times than individuals with negative B2-IgM. B2 IgG positive more likely to be among patients with positive history of vascular access occlusion than patients with negative history of vascular access occlusion "by a ratio of 2.3.



Odds ratio for: B2 IgM and B2 IgG in patients with positive and negative history of vascular access occlusion.

Table (17): Correlation between B2-IgM and other variables in patients with positive history of vascular access occlusion (group C)

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
Age (years) (n=20)	0.016	0.945	NS	0.137	0.565	NS
Duration of hemodialysis "n=30" in years	0.112	0.637	NS	-0.357	0.122	NS
Systolic BP "n=20" in mmHg	-0.062	0.795	NS	0.336	0.148	NS
Diastolic BP "n = 20" in mmHg	-0.05	0.834	NS	0.423	0.063	NS
Wbc "n=20" 10^6/cmm	-0.004	0.986	NS	0.176	0.459	NS
Hb "n=20" gm/dl	0.011	0.963	NS	0.225	0.341	NS
Plt "n=20" 10^6/cmm	0.152	0.523	NS	0.026	0.914	NS
BUN "n=20" mg/dl	0.29	0.216	NS	-0.408	0.074	NS
Cr 'n=20" mg/dl	0.12	0.614	NS	-0.347	0.133	NS
Albumin "n=20" gm/dl	-0.37	0.108	NS	-0.052	0.827	NS
Na "n=20" meq/L	-0.1	0.675	NS	0.188	0.428	NS
K 'n = 20" meq/L	0.508	0.022	**S**	-0.062	0.794	NS
Ca "n=20" mg/dl	0.162	0.495	NS	0.246	0.297	NS
Po4 "n=20" mg/dl	0.34	0.143	NS	0.152	0.523	NS
Ast IU	0.422	0.064	NS	-0.103	0.667	NS
Alt IU	0.169	0.477	NS	-0.307	0.189	NS
Bil total mg/dl	0.503	0.024	**S**	-0.214	0.366	NS
INR	0.19	0.422	NBS	-0.164	0.489	NS
PTT sec	-0.065	0.786	NS	0.287	0.22	NS

* Spearman test was used

Table (18): Correlation between B2-IgM and other variables in patients with positive history of vascular access occlusion (group C) cont)

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
ESR 1st hour	0.222	0.347	NS	0.226	0.339	NS
CRP mg/dl	-0.039	0.87	NS	0.308	0.187	NS
Doppler flow ml/min n=17	0.577	0.031	**S**	-0.116	0.692	NS
B2-IgG u/ml	0.048	0.842	NS			

*Spearman test was used

Table (19): Correlation between B2IgM, B2IgG and other variables in patient with negative history of vascular access occlusion (group D)

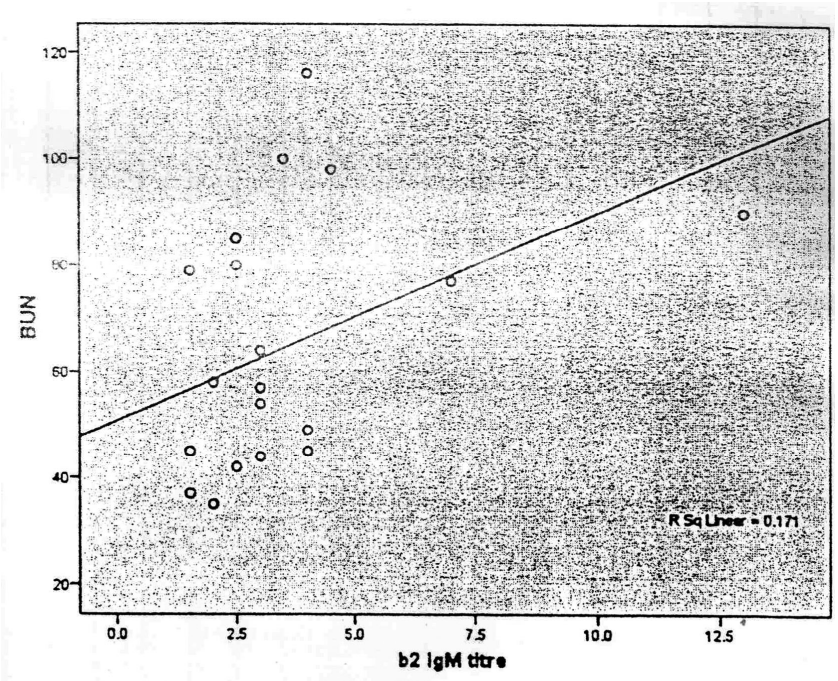
	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
Age (years) (n=20)	-0.07	0.77	NS	-0.288	0.218	NS
Duration of hemodialysis "n=30" in years	-0.304	0.193	NS	0.143	0.547	NS
Systolic BP "n=20" in mmHg	0.165	0.487	NS	0.39	0.089	NS
Diastolic BP "n = 20" in mmHg	-0.024	0.919	NS	0.204	0.388	NS
Wbc "n=20" 10^6/cmm	-0.125	0.6	NS	0.174	0.462	NS
Hb "n=20" gm/dl	0.027	0.909	NS	0.065	0.784	NS
Plt "n=20" 10^6/cmm	0.12	0.613	NS	0.104	0.662	NS
BUN "n=20" mg/dl	0.509	0.022	**S**	0.176	0.458	NS
Cr 'n=20" mg/dl	0.157	0.508	NS	-0.099	0.679	NS
Albumin "n=20" gm/dl	-0.068	0.775	NS	0.251	0.258	NS
Na "n=20" meq/L	0.28	0.232	NS	-0.35	0.131	NS
K 'n = 20" meq/L	-0.172	0.469	NS	-0.069	0.771	NS
Ca "n=20" mg/dl	0.344	0.138	NS	0.311	0.183	NS
Po4 "n=20" mg/dl	0.149	0.531	NS	0.024	0.919	NS
Ast IU	-0.025	0.917	NS	0.003	0.999	NS
Alt IU	-0.061	0.779	NS	0.297	0.203	NS
Bil total mg/dl	0.131	0.582	NS	0.406	0.076	NS
INR	0.06	0.801	NS	-0.242	0.304	NS
PTT sec	-0.088	0.712	NS	-0.341	0.141	NS

* Spearman test was used

Table (20): Correlation between B2 IgM and other variables in patient with negative history of vascular access occlusion (group D) (cont).

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
ESR 1st hour	0.248	0.292	NS	-0.363	0.115	NS
CRP mg/dl	-0.104	0.662	NS	-0.207	0.381	NS
Doppler flow ml/min n=17	0.027	0.909	NS	-0.050	0.836	NS
B2-IgG u/ml	0.19	0.423	NS			

* Spearman test was used



Correlation between B2-IgM and BUN in patients with negative history of vascular access occlusion

IgG Abs, and LA with aCL IgG Abs, when considered separately, venous thrombosis was more strongly related to LA Abs, while arterial thrombosis was more closely associated with aCL (mainly IgG class) and anti-B_2-GPI IgG Abs.

Recent studies have shown a high prevalence of antiphospholipid antibodies (lupus anticoagulant and anticardiolipin antibodies) among patients on hemodialytic treatment (9).

Anticardiolipin antibodies represent a group of antibodies from the family of antiphospholipid antibodies. Antiphospholipid antibodies have been implicated in a variety of diseases bu the exact meaning in uremia is not understood clearly. For some scientists, the presence of anticardiolipin antibodies represent a risk factor for vascular access thrombosis(10).

The B_2-GPI induced by infections may bind to "self" aPL thus forming an immunogenic complex against which a PL are then produced (11).

aPL have also been found to be raised in a large number of infections diseases, such as syphilis, HIV infection, malaria, leprosy, and viral infections, including hepatitis C (HCV), where they are not usually associated with the clinical complications attributed to them(12).

The prevalence of aPL in infections has mainly been reported for the IgG and IgM isotypes, although a few recent studies have also investigated the IgA isotype in some infections(13).

Vascular access

Thrombosis is a major problem in hemodialysis units, resulting in increased patient morbidity, hospitalization and overall dialysis cost (14).

Despite the great prevalence of HCV among hemodialysis patients, no available study have discussed the relation of HCV and presence of Beta-2-glycoprotein I antibodies and its thrombotic complications including vascular access in hemodialysis patients.

In our study, we assessed the frequency of anti-B_2 glycoprotein I antibodies and its possible relation to thrombotic complications including vascular access dysfunction in hepatitis C seropositive hemodialysis patients in a sample of Egyptian patients on regular hemodialysis.

At present the arteriovenous fistula (AVF) is still considered to be the vascular access of choice (15).

Unfortunately, creation of an AVF is not always possible. Nowadays, polytetrafluoroethylene (PTFE) is the most used graft-material. Unfortunately arteriovenous graft (AVG) appear to be associated with a significantly higher risk of thrombosis and infection as compared with AVF (16).

Both group A (HCV+ve) and group B (HCV-ve) were similar as regard age, sex, duration of hemodialysis and type of vascular access.

Our study revealed the frequency of B_2-IgM positive or borderline was 25% (10/40 patients) [4/20 HCV +ve patients, 6/20 HCV-ve patients] while the frequency of B_2 IgG was 30% (12/40 patients) [5/20 HCV +ve patients, 7/20 HCV-ve patients].

This result was different from **Sands *et al.* (2001)**(4), who studied B_2-GP I antibodies and vascular access thrombosis in hemodialysis patients. The frequency of B_2-GPI antibodies was 10.2% (9/88 of patients).

In our study, we didn't find a significant difference in B_2-glycoprotein IgM & IgG between HCV +ve patients and HCV-ve patients. This was confirmed by the results of **Leroy *et al.*, (1998)** (17), who performed his study on chronic hepatitis C patients without renal involvement following alpha-IFN and he found similar result as ours and he found no significant difference between treated and control groups as regard B_2-glycoprotein I titre.

In HCV infection, anti B_2-GPI independent aCL are reported to be raised in 17-44% of patients, whereas raised anti-B_2-GPI and aPL are seldom found. In small cohort of HCV patients, studying all three aPL isotypes, **Gugliemone *et al.* (2001)**(18) found that 30% were positive for anti-B_2 GPI. Elevated levels of aCL were found by **Ordi-Ros *et al.* (2000)** (19), in 3.3% of patients with HCV infection and these were all beta-2 glycoprotein I (B2-GPI)-independent.

Sthoeger *et al.* (2000)(20), found elevated levels of aCL in 44% of HCV patients but once again no relationship to any APS related clinical manifestations were evident.

Dalekos *et al.* (2000)(21) found that 37.3% of their HCV patients in Northern Greece had aCL positivity.

Cacoub *et al.* (2000)(22), studying 321 patients, found aCL positivity in 27% of their patients with chronic HCV infection.

In our study, on comparing HCV positive patients and HCV negative patients as regard inflammatory markers (ESR,CRP), we didn't find a significant difference between the two groups.

This result agrees with that of **Zumrutdal *et al.* (2007)** (23), who had investigated the influence of anti-HCV positivity on markers of malnutrition and inflammation in hemodialysis (HD) patients. Serum levels of CRP and ESR, showed no statistical significant difference between HCV positive and HCV negative patients.

To our knowledge, we are the first to study the level of B_2 glycoprotein I in HCV positive prevalent hemodialysis patients and we found no relation between HCV and B_2 glycoprotein I nor fistula flow.

In our study, we assessed the possible relation of anti-B_2 glycoprotein I antibodies to thrombotic complications including vascular access.

We classified the patients according to history of vascular access occlusion into two groups each of them was 20 patients. The first group had a positive history of vascular access occlusion (group C) and the available vascular access was 14 AVF, 4 PTFE grafts and 2

catheters while the second group had negative history of vascular access occlusion (group D) and the available vascular access was 20 AVF.

In our study, elevated B_2-glycoprotein IgM titre was present in 40% (8/20 in patients with previous vascular occlusion, group C) and 10% (2/20 in patients with no previous vascular access occlusion, group D). On comparing both groups, we found significant association between recurrent vascular access thrombosis and B_2-glycoprotein IgM antibodies ($p = 0.028$).

This was in contrast to the study done by **Sands *et al.* (2001)**(4), who found elevated, B_2 – glycoprotein antibodies was present in 10.2% (9/88 patients), increased thrombotic rates were not associated with elevated anti-human B_2-GPI antibody level in PTFE.

On the other hand, elevated B_2-glycoprotein IgG tire was present in 40% (8/20) in patients with previous access occlusion and 20% (4/20) in patients with no previous access occlusion. There was no significant association between recurrent vascular access thrombosis (VAT) and elevated B_2- glycoprotein IgG antibodies.

This result was similar to that of **Sands *et al.* (2001)**(4), who studied human B_2-glycoprotein 1 (B_2 – GPI) and found no association between elevated anti-human B_2 – GPI antibody levels and increased thrombotic rates.

In our study, patients having positive B_2-glycprotein IgM antibodies had thrombotic risk more than 6 folds than negative one and most of them had AVF (6/8) patients, while (2/8) patients had graft.

This result was similar to **Sands *et al.* (2001)**(4), who found that patiens with PTEE grafts and antibodies to one or more of these proteins (prothormbin, factor V, and B_2-glycoprotein I) plasma proteins had over six fold higher thrombosis rates despite the different type of vascular access.

This difference between our results and **Sands *et al.* (2001)**(4), may be due to the predominance of AVF presence in our patients, and about 50% of our patients are HCV positive, and lastly that our patients are a different population. In addition, **Sands *et al.* (2001)**(4), studied multiple antibodies, his study included diabetic patients, smokers and collagen diseases that are excluded in our study while we studied glycoprotein I IgM, IgG antibodies as separate factor.

In our study on comparing the two groups we found no statistical difference as regards variables especially serum albumin, hemoglobin and intradialytic weight gain despite they are inerinminated in causing recurrent vascular access occlusion.

On the other hand, our results was in contrast to that of **Cheng *et al.* (2003)**(24), who found significant difference as regards serum albumin between patients with recurrent vascular access failure versus patients with longer vascular access survival, being higher in longer vascular access survival patients.

In our study, we compared ESR, CRP in patients with positive history of vascular access occlusion and patients with negative history of vascular access occlusion and we found no significant difference between the two groups.

Our results were different from that of **Che *et al.* (2009)** (25), who used Cox regression with adjustments for age, systolic blood pressure and vascular access types, serum C-reactive protein (CRP). These parameters were found to be independently associated with an increased risk for vascular access thrombosis, with a hazard ratio of 1.4 (95% confidence interval: 1.01-1.27, $p = 0.017$). High serum CRP levels was associated with the development of vascular access thrombosis in chronic HD patients.

Univariate analysis of B_2 glycoprotein I IgM titre with other variables was also assessed in our study, and we found significant negative correlation between volume of flow and B_2-IgM titre ($p = 0.03$), we concluded that elevated B_2 – GPI IgM titre is associated with decreased fistula volume of flow by Doppler .No related studies had assessed similar relations.

Conclusion:

Our study had showed significant association between recurrent vascular access thrombosis and elevated. B_2-glycoprotein IgM antibodies and negative correlation between volume of flow by Doppler and B_2-IgM titre.

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Obesity Degree and Cardiometabolic Risk among School Students

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Abstract: Rationale: Childhood obesity is a risk factor for developing cardio metabolic diseases in adulthood. **Objective:** Studying the association of cardio metabolic risk factors in students (7 - 16 years) with different degrees of obesity. **Methods:** Cross-sectional study including 169 student: 72 obese [body mass index (BMI) > 95th percentile] and 97 extremely obese (BMI > 97th percentile) for age and gender based on Egyptian Growth Reference Charts. Interrelationship between risk factors prevalence: hypertension, high waist circumference (WC), impaired fasting glucose, hyperinsulinieamia, insulin resistance, and dyslipideamia (abnormal TC, LDL-C, HDL-C, and triglyceride), according to age groups and degree of obesity were assessed. A set of cardio metabolic risk factors were defined for each individual, ranging from 0 (no risk factors) to 9 (all risk factors). **Results:** In younger age group (7 - 11 years), extremely obese students were proven to have higher frequencies of cardio metabolic risk factors in comparison to obese group, with highly significant differences regarding fasting glucose level and WC. Older students aged 12- 16 years recorded insignificant differences in the frequency of cardio metabolic risk factors between obese and extremely obese ones. For both age groups, elevated total and LDL-Cholesterol were significantly linked to disturbances of carbohydrate metabolism; indicated by fasting glucose level. Highly significant positive interrelationships between WC and triglycerides for children, and diastolic blood pressure for adolescents were detected. Among extremely obese students, 81% of younger and 60% of older had a cluster of at least three risk factors or more in comparison to only 56.7% and 48.7% of obese. **Conclusion:** Cardio metabolic risk factors are associated with degree of obesity in young age (7-11 years), but not in those aged 12-16 years. Elevated triglycerides are the most common risk factors in both age groups.

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Key words: Obesity; risk factors; children, adolescents.

1. Introduction:

Overweight and obesity are an important risk factor for cardiovascular diseases. Although the clinical manifestations of these diseases occur in adulthood, studies have demonstrated that comorbidities such as dyslipidemias, hypertension and insulin resistance may be present in childhood and adolescence **(Botton et al, 2007)** , and are responsible for the increased risk of morbidity and mortality in adulthood **(Baker et al, 2007; Berkowitz, 2010).**

In Egypt, as in other parts of the world, the obesity epidemic affects a growing number of children and adolescents **(El-Masry, 2007, Shaaban et al, 2008).** The study of **Jackson et al (2003)** among female adolescents showed that 35 % of the girls were overweight and 13 % were obese. In the final report of Diet, Nutrition and Prevention of Chronic non communicable diseases in Egyptian Adolescents **(DNPCNCD, 2008)**, it was found that about 20.5% of the adolescents were either overweight or obese with higher prevalence among urban than rural and females compared to males.

Few studies correlating obesity with cardiovascular risk factors have been conducted among adolescents in developing countries, particularly in Egypt **(Hassan et al, 2011a, b)**. However, according to the World Health Organization's report **(WHO 2006)**, children and adolescents from low socioeconomic levels are as exposed to obesity and cardiovascular risk factors as their peers from high socioeconomic levels. For the development of more efficient clinical prevention and intervention programs, studies targeted at this population are necessary.

In fact, obesity-related diseases may depend on the ethnic or genetic background. Therefore, studies including Egyptian children and adolescents with obesity of all degrees are necessary to examine the effects of adiposity on health in this age group and its association with cardio metabolic risk factors.

The aims of this study are: 1). to determine whether the prevalence of obesity-related risk factors in Egypt is as high as reported for other parts of the world; 2) to examine whether a clustering of several risk factors occurs; and 3) to explore the prediction of single and multiple comorbidities by the degree of obesity as defined by BMI adjusting for potential confounders as age.

2. Subjects and methods:

Between October 2007 and April 2009, 5798 students (2655 boys and 3143 girls), with age range 7-16 years, were studied in a cross-sectional survey for evaluation of overweight and obesity. These students were recruited from 6 public schools (two Primary Schools, two preparatory and two secondary schools) situated in Giza governorate. Permission to perform the study was granted by the Ministry of Education, and the directors of the school included in the research. Parents were informed about the purpose of the study and their permission in the form of written consent was obtained. Another assent was taken from the students to be involved in this research. The protocol was approved by the "Ethical Committee" of the "National Research Centre". The agreement reference number is 07/091.

Of the total sample, four hundred and sixty-two students only (8.0%) complaint of obesity; 174 boys and 288 girls; their mean age was 13.43± 2.65 years**,** 26.4 % aged 7 – 11 years and 73.6% aged 12 – 16 years. Obese students were excluded if they had a prior major illness, including type 1 or 2 diabetes, took medications or had a condition known to influence body composition, insulin action or insulin secretion (e.g. glucocorticoid therapy, hypothyroidism and Cushing's disease). All overweight students refused to participate. Of the obese students eligible to the study, informed consent and assent were only obtained from the parents of 169 students for the laboratory data; 72(45 boys and 27 girls), were defined obese (with body mass index (BMI) equal to or greater than the 95th - 97th percentile for age and gender based on the Egyptian Growth Reference Charts **(Ghali et al, 2008)** and 97 (28 boys and 69 girls) as extremely obese (with BMI higher than the 97th percentile). Data of BMI percentiles, waist circumference (WC), blood pressure (BP), fasting lipids and glucose were collected.

Each student subjected to a simple questioner as past history of chronic diseases, assessment of socioeconomic status; and a complete physical examination, including anthropometric measures. The height was measured to the nearest 0.5 cm using a Holtain portable anthropometer, and the weight was determined to the nearest 0.1 kg using a Seca scale Balance with the subject dressed minimum clothes and no shoes. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Waist circumference was measured at the level of the umbilicus with subject standing and breathing normally, using non-stretchable plastic tape to the nearest 0.1 cm. Each measurement was taken as the mean of three consecutive measurements, using standardized equipments and following the recommendations of International Biological programmes (**Hiernaux and Tanner, 1969**). Blood pressure was measured with a standard mercury sphygmomanometer after the subjects had rested at least 10 min. Systolic blood pressure was recorded at the appearance of sounds, and the diastolic blood pressure was recorded at the disappearance of sounds. The blood pressure measurement was repeated for 3 consecutive days, if it was high to be sure from the diagnosis of hypertension.

Venous blood samples were obtained to measure plasma glucose, plasma insulin levels and lipid profile in the morning by venipuncture after overnight fasting. Plasma glucose was determined by the glucose oxidase method. Plasma insulin was measured using ELIZA immunoassay (DRG Diagnostic Products Corporation, Los Angeles, CA). Plasma concentrations of total cholesterol and triglycerides were estimated in serum by using calorimetric assay kit produced by P.Z. cormay, Lublin, Poland. High-density lipoprotein-cholesterol (HDL-C) was determined in serum by using calorimetric assay kits produced by Stanbio laboratory, Boerne, Texas. Low-density lipoprotein-cholesterol (LDL-cholesterol) was calculated as follows:

$$\text{LDL} = \text{Total cholesterol} - \text{HDL} = \frac{\text{TG}}{5}.$$

WC was considered high if > 90th percentile for age and sex. BP related to age and sex was considered elevated if systolic or diastolic blood pressure above the 90th percentile of Egyptian reference ranges **(Zaki et al, 2006).** *Dyslipideamia* (abnormal lipid levels) were defined according to the American Heart Association **(Grundy et al, 2004)** as follows: total cholesterol (TC) >210 mg/dL, high-density lipoprotein (HDL-C)-cholesterol <40 mg/dL, low-density lipoprotein (LDL-C)-cholesterol > 130 mg/dL, or triglycerides (TG) >110 mg/dL. Any one elevated level out of the following was classified for "Impaired carbohydrate metabolism" according to modified WHO criteria adapted for children **(1999);** impaired fasting glucose tolerance>6.1 mmol/L, hyperinsulinieamia was defined from norms for age: 7- 11 years> 15 mU/L; and 12 – 18 years>30 mU/L; insulin resistance is defined as the levels of the HOMA-IR greater than 3.16, according to **Keskin et al (2005**). HOMA-IR (The homeostatic model assessment for insulin resistance) was calculated as follow:

HOMA-IR= fasting insulin (μU/mL) x fasting glucose (mmol/L)/22.5.

Statistical analysis

Statistical analyses were carried out with calculations of the proportions of students exposed to different risk factors: hypertension, high WC, impaired fasting glucose, hyperinsulinieamia, insulin resistance, and dyslipideamia (abnormal TC, LDL-C, HDL-C, triglyceride), in the obese and in the extremely obese groups, with the respective odds ratios (OR) and 95% confidence intervals (CI).Interrelationship between risk factors prevalence according to the age groups were assessed by chi-square test. Data was examined by one

sample Kolmogorov-Smirnov Z test for normal distribution, which revealed that the data was not normally distributed. So, bivariate Spearman's correlation tests were used to examine the association between contributing risk factors. Variables with significant associations only ($p < 0.05$) in the bivariate analysis were included in the correlation tests. Finally, a set of cardiovascular risk factors was defined as the number of conditions present in each individual, ranging from 0 (absence of all conditions) to 9 (presence of all the conditions mentioned).The prevalence of combination of multiple risk factors was examined also. Statistical significance was taken as $p < 0.05$. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS/Windows Version 16.0, SPSS Inc., Chicago, IL, USA).

3. Results:

According to BMI, 42.6% were obese, their BMI >95th up to 97th percentile, and 57.4 % were extremely obese, their BMI > 97th percentile. Girls presented somewhat more frequently (56.8%) than boys (43.2%). In spite of insignificant differences in the age of boys and girls (mean age of boys 12.32 $\pm$2.51, while that for girls 12.78 $\pm$2.77), girls had higher degree of obesity (71.9% of girls were extremely obese) than boys (38.4%), while obesity; BMI between 95th -97th percentile; was more prominent in boys (61.6%) than girls (28.1%), namely that girls tended to be extremely obese than boys (table 1).

Insignificant sex differences were observed in both age groups; 7-11 and from 12 to 16 years; regarding WC, BP and all the laboratory data. So, the analysis was completed without sex differentiation.

The frequencies and odds ratios(OR) with respective 95% confidence intervals for the variables of cardiovascular risk factors in relation to BMI degree in the two age groups are presented in tables 2 and 3. In the younger age group (7 to 11 years), extremely obese students were proven to have higher frequencies of abnormal triglycerides and fasting glucose levels, hypertension and high WC in comparison to the obese group, with highly significant differences regarding fasting glucose level and WC. Waist circumference > 90th percentile was recorded in 91.9% (crude OR = 7.16).The frequency of abnormal glucose levels was 35.1% (crude OR = 7.85). Hypertension was present in 27.0% of extremely obese (crude OR=7.0). Approximately 78% of the extremely obese students had high triglyceride levels (crude OR = 1.99). However, in the older students aged 12- 16 years (table 3), insignificant differences in the frequency of different variables of cardiovascular risk factors between the obese and extremely obese students were recorded.

Table 1: Frequency distribution of the sample

	BMI>95		BMI >97		
	Boys	Girls	Boys	Girls	Total
	N (%)	N (%)	N (%)	N (%)	N (%)
7 – 11 years	20 (29.4)	11(16.2)	12(17.6)	25(36.8)	68(40.2)
12 –18 years	25(24.8)	16 (15.8)	16(15.8)	44(43.6)	101(59.8)
Total	45(26.6)	27 (16.0)	28 (16.6)	69 (40.8)	169 (100.0)

Table 2: Frequencies (%) and odds ratio (OR) with their respective 95% confidence intervals (CI) for the variables related to cardiovascular risk factors in children aged 7 -11 years using Chi- square test

Variables	BMI>95			BMI >97			Crude OR (95% CI)	P
	N	yes		N	yes			
		N	%		N	%		
WC > 90th percentile	31	19	61.3	37	34	91.9	7.16 (1.79-28.57)	0.003
Glucose >6.1 mmol/L	31	2	6.5	37	13	35.1	7.85 (1.61-38.28)	0.007
Insulin > 15 mU/L	31	6	19.4	37	3	8.1	0.37 (0.08-1.61)	0.282
HOMA > 3.16	31	6	19.4	37	3	8.1	0.37 (0.08-1.61)	0.282
Triglycride>110 mg/dL	31	20	64.5	37	29	78.4	1.99 (0.68 – 5.84)	0.319
TC > 210 mg/dL	31	13	41.9	37	9	24.3	0.45 (0.16-1.25)	0.198
HDL-C<40 mg/dL	30	17	56.7	37	20	54.1	0.90 (0.34-2.37)	1.000
LDL-C > 130 mg/dL	30	15	50.0	37	15	40.5	0.68 (0.26-1.8)	0.600
Hypertension	31	1	3.2	37	6	27.0		
SBP> 90th percentile	31	1	3.2	37	7	18.9	7.00(0.81-60.43)	0.063
DBP> 90th percentile	31	1	3.2	37	4	10.8	3.64 (0.39-34.38)	0.366
Cardiovascular risk factors								
1 CV risk	31	3	9.7	37	1	2.7		0.202
2 CV risk	31	7	22.6	37	6	16.2		
3 CV risk	31	17	56.7	37	30	81.0		

Table 3: Frequencies (%) and odds ratio (OR) with their respective 95% confidence intervals (CI) for the variables related to cardiovascular risk factors in children aged 12 -18 years using Chi- square test

Variables	N	BMI>95 yes		N	BMI >97 yes		Crude OR (95% CI)	P
		N	%		N	%		
WC > 90th percentile	37	14	37.8	60	15	25.0	0.55 (0.23-1.33)	0.266
Glucose >6.1 mmol/L	41	11	26.8	60	14	23.3	0.83 (0.33-2.07)	0.869
Insulin > 15 mU/L	41	2	4.9	58	1	1.7	0.61 (0.26-1.41)	0.568
HOMA > 3.16	33	9	27.3	48	15	31.3	1.21 (0.46-3.23)	0.891
Triglycride>110	40	29	72.5	60	41	68.3	0.82 (0.34-1.98)	0.824
TC > 210 mg/dL	38	11	28.9	60	18	30.0	1.05 (0.43-2.57)	1.000
HDL-C<40 mg/dL	39	19	48.7	56	39	69.6	2.42 (1.03-5.64)	0.065
LDL-C > 130 mg/dL	40	13	32.5	58	18	31.0	0.94 (0.39-2.22)	1.000
Hypertension	37	6	16.2	60	17	28.3		
SBP> 90th percentile	37	3	8.1	60	8	13.3	1.74 (0.43-7.04)	0.524
DBP> 90th percentile	37	4	10.8	60	15	25.0	2.75 (0.84-9.05)	0.116
Cardiovascular risk factors								
1 CV risk	41	7	17.1	60	8	13.3		0.696
2 CV risk	41	12	29.3	60	15	25.0		
3 CV risk	41	19	48.7	60	36	60.0		

Table 4: Interrelationships between adverse cardiovascular risk factors by Spearman correlation test.

Risk factors		Waist Circumference		Fasting glucose level	
		7- 11 years	12 – 16 years	7- 11 years	12 – 16 years
SBP	*r*	.178	.198	-.168	-.147
	p	.147	.051	.171	.150
DBP	*r*	-.110	.306**	-.028	-.142
	p	.371	.002	.823	.167
Triglycerides	*r*	.483**	-.120	-.228	.223*
	p	.000	.244	.061	.026
Total cholesterol	*r*	.106	-.121	.268*	.214*
	p	.388	.238	.027	.033
HDL	*r*	.041	-.119	.083	-.246*
	p	.743	.254	.502	.015
LDL	*r*	.038	-.033	.272*	.353**
	p	.761	.753	.026	.000
Insulin level	*r*	.235	.119	-.204	-.368**
	p	.053	.252	.095	.000
HOMA	*r*	.226	.114	-.063	-.117
	p	.064	.273	.610	.249

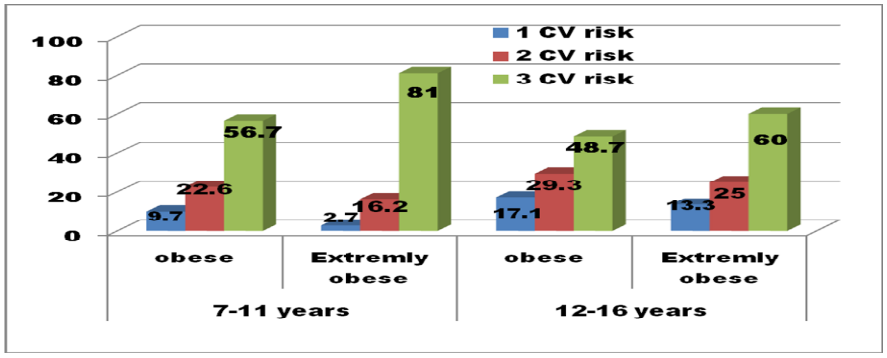

Figure 1: Prevalence of cardiovascular risk factors

Frequency distribution of cardiovascular risk factors is shown in **Tables 2, 3** and Figure 1. Six students only had no risk factors: 4 obese students aged 7 -11 years, and 2 students aged 12- 16 years (1 obese and 1 extremely obese). Of the extremely obese students, 81% of younger students aged 7- 11years and 60% of older ones aged 12- 16 years had a cluster of at least three risk factors or more in comparison to only 56.7% and 48.7% of the obese students. The most common association of 2 risk factors in both age groups was elevated triglycerides and decreased HDL-C. In case of existence of 3 risk factors or more, high WC, elevated triglycerides and LDL-C were the common associations in the young age (7-11 years), while elevated triglycerides, total cholesterol and decreased HDL- were the common association in the older students (12 – 16 years).

Interrelations between CV risk factors using Spearman's correlation test revealed highly significant positive interrelationships between waist circumference and triglycerides in young age group (7 -11 yrs), and diastolic BP for older age group (12 – 16 yrs). Elevated total cholesterol and LDL-cholesterol were each significantly linked to disturbances of carbohydrate metabolism; indicated by fasting glucose level; in both age groups. Moreover, increased triglycerides decreased HDL-C and elevated insulin level were also significantly associated with increased fasting glucose level in older students aged 12- 16 years (Table 4).

4. Discussion:

Although the clinical symptoms of cardiovascular diseases occur in adulthood, the atherosclerotic process starts in childhood, and obesity is one of its main determinants **(McMahan et al, 2006).** The presence of cardiovascular risk factors including dyslipidemia, hypertension and abnormal baseline insulin levels characterizes the metabolic syndrome **(Agirbasil et al, 2006; Lottenberg et al, 2007; Sun et al, 2008).**

This study included 169 school students suffering from various degree of obesity. The association between the degree of obesity and cardio-metabolic risk factors was investigated. Despite the higher prevalence of obesity among girls (56.8%) than boys (43.2%), yet the WC, BP and all the laboratory data were similar for both sexes as there were insignificant sex differences. This coincided with previous studies **(Jackson et al, 2003, El-Masry, 2007; Shaaban et al, 2008 Hassan et al, 2011)** which recorded higher prevalence of obesity among girls compared to boys.

Results revealed that obese children and adolescents had adverse CV risk factors, which related to degree of obesity in children only and not in adolescents. Extremely obese children were proven to have higher frequencies of high waist circumference (91.9%), abnormal triglycerides (78.4%), high fasting glucose levels (35.1%) and hypertension (27.0%) compared with obese ones (61.3%, 64.5%, 6.5% and 3.2% respectively). Extremely obese children were found to have nearly 7 fold (times) risk for having high WC and fasting glucose levels than obese ones, and the differences were highly significant. However, in adolescents, differences in the frequency of cardiovascular risk factors between the obese and extremely obese students were minimal and insignificant. These results coincide with the previous studies **(Steinberger and Daniels, 2003; Wannamethee et al,. 2005; Kohli and Greenland 2006; Saland, 2007)** which concluded that high levels of body mass index among children and adolescents has been reported to be associated with abnormal levels of lipids, insulin, blood pressure and all components of the metabolic syndrome**.** Other large epidemiologic studies **(Suk et al, 2003; Wang et al, 2005; Yusuf et al, 2005)** stressed on **t**he importance of WC in predicting cardio metabolic risk factors and their adverse outcomes (e.g., diabetes, CHD, and death rate). They attributed that to the finding that the body fat distribution; mainly excess abdominal fat (also known as central or upper-body fat) is an important risk factor for obesity-related diseases. However, precise measurement of abdominal fat content requires the use of expensive radiological imaging techniques. Therefore, waist circumference (WC) is often used as a surrogate marker of abdominal fat mass, because WC correlates with abdominal subcutaneous and intra-abdominal fat mass, and is associated with cardio metabolic disease risk **(Pouliot et al, 1994)).**

Freedman et al (1999) in the Taipei Children Heart Study, reported a significant relation between obesity and high fasting glucose level**.** While **Geiss et al (2001)** in Korean children and adolescents recorded that fasting glucose concentrations did not increase according to the increase in weight status.

Literature reviews proved that adiposity leads to increased triglycerides in obese children **(Baranowski et al, 2006; Weiss et al, 2004). Chu et al (1998)** have shown a strong relationship between triglycerides and obesity (OR 7.1 (5.8–8.6)) among US children. The same was recorded by **Weiss et al (2004) and Kim et al (2006)** among the obese children of Central European studies.

Reinehr et al (2005a, b) demonstrated in a longitudinal study that hyperinsulineamia may be the central abnormality in obese children and adolescents, and this contributes to dyslipideamia. The physiological mechanism suggested for this process is that the intra-abdominal fat has a high and intense metabolic activity, permitting the triglyceride depots concentrated in this region to be more easily mobilized to the blood stream, thus leading to an increased production of free-fatty acids and LDL-c in the liver **(Björntorp 1997).**

The present study showed that ***extremely obese*** adolescents *aged 12- 16* years (69.6%) had marked

lowering of the HDL-cholesterol concentration compared to *obese* ones (48.7%). They were found to have a 2 times risk for having low concentrations of HDL-C than obese ones. On the other hand, the frequency of having low HDL-C was nearly similar concerning the younger age group aged 7 to 11 years (56.7% of obese children and 54% of extremely obese ones). However, the differences were insignificant for both age groups. Previous studies of **Weiss et al (2004)** and **Baranowski et al (2006)** proved that adiposity leads to markedly lowered HDL-cholesterol concentrations. As observed in the general population, HDL-cholesterol concentrations are lowered by puberty or age >12 years, most probably caused by increasing androgens **(Bao et al, 1996; Williams et al, 2002)** or reduced physical inactivity **(Akerblom et al, 1999)**

Results of this study recorded that obese (50%) and extremely obese children (40.5%) had high frequency of elevated LDL- C than obese (32.5%) and extremely obese (31%) adolescents. This coincided with **Morrison et al (2003)** who concluded that pubertal obese adolescents had a lower probability of elevated LDL-cholesterol concentrations, which was explained by the increase of estradiol concentrations both with puberty and adiposity, even in male subjects**.** Inspite of that, the present study detected that LDL-cholesterol level was very weakly related with the degree of obesity for both studied children (OR 0.68(0.26- 1.8)) and adolescents (OR 0.94(0.39- 2.22)). This finding supports previous observations that LDL-cholesterol is not related to BMI or to the metabolic syndrome **(Baranowski et al, 2006; Kim et al, 2006; Weiss et al, 2004).** However, **Freedman et al (1999)** in population based studies; and particularly in younger children; recorded the effect of BMI on LDL-cholesterol.

Over the past decade, BP in children increased substantially **(Ford et al, 2004; Muntner et al, 2004)** and was associated with the concurrent increase of body weight **(Couch and Daniels 2005)**, even as early as in 2- to 5-year-old children in a study of **Falkner et al (2006)** in a primary care setting**.** Irrespective of the effects of obesity, elevated BP in childhood is predictive of sustained hypertension and target-organ damage in young adulthood **(Sorof et al, 2003; Vos et al, 2003; Lande et al, 2006).** The National Health and Nutrition Examination Survey (NHANES) on children and adolescents, increased obesity; especially abdominal obesity indicated by WC; was observed to explain part of the tendency to elevated blood pressure levels, because there is an association between hypertension and hyperinsulinism **(WHO 2006).** The prevalence of elevated BP in the present study was roughly similar in both extremely obese children (27%) and adolescents (28%), but higher compared with obese children (3%) and adolescents (16%). **Sharpe et al (2006)** found that diastolic hypertension was more often elevated than systolic.

Another important observation in this study was the clustering of 2 or more CV risk factors, being closely associated with the degree of excess weight. The most common association of 2 risk factors in both age groups was elevated triglycerides and decreased HDL-C. In case of clustering of 3 risk factors or more, the most prevalent was elevated triglycerides level for both obese and extremely obese students which were associated with high WC and LDL-C in young children, and impaired total cholesterol and HDL-C, in adolescents (12- 16 years). Similar results were reported in previous studies **(Weiss et al, 2004; Baranowski et al, 2006).**

A clustering of three or more adverse CV risk factors has been shown to be the most severe obesity-related health hazard. This is supported by the observation that there is a close interaction between increased triglycerides, BP, and impaired carbohydrate metabolism. Triglyceride-induced insulin resistance and hyperinsulinemia may be the common pathophysiological link between these parameters, but the exact nature of the relation between increased fat mass, insulin resistance, and arterial hypertension in children remains unclear **(Sinaiko et al . 2002).**

The present study demonstrated that disturbances of carbohydrate metabolism; indicated by fasting glucose level; was significantly associated with the decreased HDL-cholesterol and insulin level in adolescents aged 12- 16 years, and with elevated total cholesterol and LDL-cholesterol in both children and adolescents. These results are consistent with many other studies as dyslipidemia is well documented in older children and adults who are obese **(Boyd et al, 2005; Reinehr et al, 2005)**, and associations have been found between serum fasting insulin concentrations and serum lipids in 2–3-y-old Hispanic children **(Shea et al, 2003).**

A significant positive correlation between waist circumference and diastolic BP for adolescents only was detected also in the present study. This comes in agreement with the German study of **Reinehr et al (2005)**, who found that the degree of body mass index correlates positively with diastolic hypertension**.**

Obesity tracks from childhood to adulthood, and childhood adiposity is a strong predictor of obesity, insulin resistance **(Steinberger et al, 2001),** and abnormal lipids in adulthood **(Srinivasan et al, 1996).** Moreover, the rate of increase in adiposity during childhood was significantly related to the development of cardiovascular risk in young adults **(Sinaiko et al, 1999).** However, BMI only accounts for 60% of the variance of insulin resistance in adults **(Abbasi et al, 2002),** which suggests that other factors are important. It has been reported that a transient insulin-resistant state occurs in children during normal pubertal

development **(Caprio et al, 1989; Moran et al, 1999).** Studies with euglycemic insulin clamps have shown that insulin resistance increases at the beginning of puberty, peaks at mid puberty, and returns to near-prepubertal levels by the end of puberty **(Moran et al , 1999).** The increase in growth hormone, sex hormone, and insulin-like growth factor-1 levels that occurs during puberty is thought to be the cause of this form of insulin resistance **(Moran et al, 2002).** Thus the insulin level in children and adolescents is influenced not only by the obesity degree but also by the normal increase in growth hormone, sex hormone, and insulin-like growth factor-1 levels that occurs during normal pubertal development.

In summary: This study revealed that obese children and adolescents did show adverse cardio metabolic risk factors. The most prevalent risk factor was elevated triglycerides level which were associated with impaired carbohydrate metabolism, as well as with insulin resistance, hypertension and high waist circumference.

Conclusion:

Cardio metabolic risk factors tend to cluster, being closely associated with the degree of excess weight,; particularly in young children. Thus a comprehensive screening is crucial for all obese children and adolescents to identify cardio metabolic risk factors as early as possible aimed at reducing morbidity and mortality in adulthood.

For the development of more efficient clinical prevention and intervention programs, studies targeted at this population are necessary. Evaluation of the nutritional status is, therefore, essential in the clinical practice, aiming at detecting and preventing obesity and the associated cardio metabolic risk factors. Changes in lifestyle by encouraging physical activities and an adequate balanced diet are key strategies for maintaining healthy weight.

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Occult Hepatitis B Infection in Patients with Chronic Hepatitis C

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Abstract: Occult hepatitis B virus infection (OBI) is characterized by the presence of HBV DNA in the liver tissue or in the serum of HBsAg negative individuals. Although OBI was detected frequently in patients with chronic hepatitis C, the clinical implication of this co-infection is still not fully clarified. The aim of the present study was to assess the prevalence and the possible clinical impact of occult HBV infection in patients with chronic hepatitis C. A total of 60 chronic HCV patients who were HBsAg negative, were enrolled into the study. Serum samples from the studied patients were tested for the presence of anti-HBs and total anti-HBc antibodies by ELISA technique and HBV DNA by real time PCR assay. The results showed that 8 (13.3%) patients were HBV DNA positive; 6 (75%) patients were anti-HBc positive while 3 patients (37.5%) were anti-HBs positive. There was no significant difference between chronic HCV patients with or without HBV DNA in duration of infection, ALT level, histological score or HCV viral load. In conclusion, a considerable proportion of patients with chronic hepatitis C had occult HBV infection. Occult HBV infection was significantly higher among anti-HBc positive patients. Occult HBV infection did not seem to modify the progression of chronic HCV-related liver disease.
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Keywords: Occult HBV infection; Chronic hepatitis C; HBsAg-negative; HBV-DNA; Real time PCR

1. Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are major public health problems worldwide. Egypt has the highest prevalence of hepatitis C virus (HCV) worldwide, ranging from 6-28% with an average of approximately 15% in the general population (Nafeh *et al.,* 2000; Mohamed, 2004 & El-Zanaty and Way, 2009) and intermediate prevalence of HBV (2-8%) (WHO, 2004).

Hepatitis B and hepatitis C viruses' co-infection may occur because of the common modes of viral transmission, particularly in areas where the two viruses are endemic (Liu and Hou, 2006). Patients with chronic HCV infection are at high risk of acquiring HBV infection in the absence of serological markers for HBV which called occult hepatitis B infection (OBI) (Cacciola *et al.,*1999 & Fukuda *et al.,*1999). In such patients, OBI may be associated with flare of the liver enzymes (Kannangai *et al.,*2007), increased incidence of cirrhosis (Cacciola *et al.,*1999 & De Maria *et al.,*2000), and hepatocellular carcinoma (HCC) (Branco *et al.,*2007 & Miura *et al.,* 2008). Occult HBV infection may also play a role in the poor response of HCV viremia to alpha interferon and rebavirin therapy irrespective of HCV genotype (Fukuda *et al.,*2001 & Mrani *et al.,* 2007).

Occult hepatitis B infection is simply defined by the presence of HBV-DNA in the liver tissue or in the serum of HBsAg negative individuals (Raimondo *et al.,*2008). Although, the exact pathogenesis of OBI is not fully understood, it is probably due to both host and viral factors which are important in suppressing viral replication and keeping the infection under control (Hollinger and Sood, 2010).

The gold standard for diagnosis of OBI is detection of HBV-DNA from liver tissue or serum (Urbani *et al.,*2010). It is strongly recommended to utilize a highly sensitive and specific approach based on ‘‘nested’’ or ‘‘real time’’ polymerase chain reaction (PCR) techniques and the use of oligonucleotide primers specific for different HBV genomic regions (Raimondo *et al.,*2010).

Occult HBV infection is world-wide spread however, its prevalence is closely related to the endemicity of HBV infection (Bréchot *et al.,*2001) and the characteristics of the studied population, being more common in patients with chronic liver disease and less common among healthy blood or organ donors (Conjeevaram and Lok, 2001). Herein, the frequency and the impact of occult hepatitis B infection in patients with chronic hepatitis C are still under discussion. The aim of the present study was twofold; to assess the prevalence of OBI in patients with chronic hepatitis C and to evaluate its possible impact on liver disease progression regarding the liver enzymes level and fibrosis activity.

2. Patients and methods:

Study design and patients

This is a cross sectional study conducted at Departments of Medical Microbiology & Immunology, Clinical Pathology and Internal Medicine of Soahg University hospital, Egypt, during the period from June to December 2011. The study included 60 chronic

HCV patients who were recruited from outpatient clinics of Internal Medicine Department, Sohag university hospital. All studied patients were HBsAg negative, HCV positive and without manifestations of hepatic decompensation. The diagnosis of chronic HCV infection was based on clinical, laboratory and histologic evaluation. Laboratory diagnosis of chronic HCV patients was based on detection of anti-HCV antibodies by ELISA at least twice within 6 months before enrollment of the patients, and HCV RNA by real time PCR. Patients with autoimmune or metabolic liver disease, schistosomiasis, hepatocellular carcinoma, serological evidence of HIV infection, history of hepatotoxic drugs or current antiviral therapy for HCV and those on hemodialysis were excluded. All eligible patients were subjected to full clinical assessment, routine laboratory investigations and abdominal ultrasonography. Signed written informed consents were obtained from the participants.

Liver biopsy

Liver biopsy was performed for all studied patients as a part of pre-treatment evaluation for HCV infection. The degree of hepatic fibrosis and portal inflammation was evaluated according to the METAVIR scoring system. The stage of fibrosis varied from 0 to 4 (F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis with few septa; F3 = septal fibrosis, without cirrhosis; F4 = cirrhosis). The grade of inflammatory activity classified into; none, mild, moderate and severe (METAVIR, 1994).

Serodiagnosis of HBV

Anti-HBs and total anti-HBc antibodies were detected by ELISA technique according to the manufacturers' instructions (DiaSorin diagnostic kits, Italy).

HBV DNA amplification and detection by real time PCR assay

HBV DNA was extracted from patients' serum samples by QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA was extracted from 200 µl serum samples, eluted in 200 µl of buffer and stored at –20°C. HBV-DNA was determined quantitatively by real-time PCR assay using Rotor-Gene Q instrument (Qiagen, Germany). Oligonucleotide primers were selected from highly conserved regions of the HBV S gene; FOR 4 (5′-CCTATGGGAGTGGGCCTCA- 3′, nucleotides 639–657) and HBV REV 7 (5′-CCCCAATACCACATCATCCATATA-3′, nucleotides 761–738) (Invitrogen, USA) yielding a 123-bp. The probe sequence was selected within a conserved region of the 123 bp amplicon (5′ - CACTGAACAAATGGCACTAGTAAACTGAGCCA - 3′) (Zanella et al., 2002). The PCR reaction mix for real-time quantification contained 10 µl of the extracted DNA, 1×TaqMan Universal PCR Master Mix (Applied Biosystems, USA), 45 pmol of each primer and 10 pmol of probe, in a final volume of 50 µl. The TaqMan Universal PCR Master Mix contains AmpErase® uracil-N-glycosylase (UNG) to prevent the reamplification of carryover PCR products by removing any uracil incorporated into single- or double-stranded DNA, AmpliTaq Gold, PCR buffer, dNTPs and the rhodamine derivative ROX as a passive reference dye. Thermal cycling conditions were as follows: initial activation of AmpErase UNG at 50°C for 2 min followed by activation of Taq Gold at 95°C for 10 min. Subsequently, 45 cycles of amplification were performed at 95°C for 30 sec and 59°C for 1 min (Zanella *et al.*,2002). Standard curves were done using the Rotor-Gene Q Series Software version 2.0.2.4 (Qiagen, Germany). A sample result is accepted only when the internal control is amplified.

Statistical analysis

Statistical analysis was done using Statistical package for social sciences version 10 (SPSS Inc., Chicago, IL, USA). Data were presented as median and range or as an absolute number and percentage. Chi-square test was used for categorical data. Quantitative data with uneven distribution were analyzed with the Mann-Whitney U test. $P < 0.05$ was considered statistically significant.

3.Results

The study group comprised of 60 patients with chronic HCV, 45 males (75 %) and 15 females (25 %). The median age of the participants was 45 years and a range from 22-59 years. According to METAVIR score, 70% of the studied patients have moderate inflammatory activity while about 47% showed portal, without septal, fibrosis (F1). Patients' characteristics were shown in Table 1.

Normal values of AST (up to 40 IU/L), ALT (up to 41 IU/L), Total biliruben (up to 1 mg/dL), Albumen (3-5 g/dL), Alkaline phosphatase (up to 256 IU/L)

Out of 60 chronic HCV patients included in the study, 27 (45 %) patients were positive to total anti-HBc antibodies and 6 (10 %) patients were positive to anti-HBs antibodies (Table 2).

Among the studied patients, the serum samples of 8 patients (8/60; 13.3%) were positive for HBV DNA by the real time PCR assay, documenting an occult HBV infection. Out of 8 OBI/HCV dually infected patients, 6 (75%) patients were anti-HBc positive while 3 patients (37.5%) were anti-HBs positive. This result was statistically highly significant (P=0.001). There was no significant difference between chronic HCV patients with or without occult HBV infection in terms of clinical characteristics including gender distribution, age, histological score, liver function tests and HCV viral load (Table 3).

Table 1: Patients' characteristics

Characteristics	Patients (N= 60)
Age; (years)	45 (22 - 59)
Gender; n (%)	
Male	45 (75 %)
Female	15 (25 %)
BMI (kg/m^2)	27.6 (21.5 – 30.4)
Duration of HCV infection; (years)	6 (2 – 18)
Liver function tests:	
Serum albumin (g/dL)	4.2 (3.3 – 6)
Liver enzymes (IU/L)	
ALT	50.5 (8 – 134)
AST	41.5 (18 -102)
Total bilirubin (mg/dL)	0.9 (0.5 – 1.4)
Alkaline phosphates (IU/L)	111.5 (57 -281)
Basal HCV RNA load (IU/ml)	272,669 (1747 - 5,230,677)
METAVIR Score	
inflammatory activity	
Mild	16 (26.7 %)
Moderate	42 (70 %)
Severe	2 (3.3 %)
Fibrosis	
F1	28 (46.7 %)
F2	22 (36.7 %)
F3	10 (16.6 %)
F4	0 (0 %)

Data were presented as median (range) or number (%)
Abbreviations: BMI; Body mass index, ALT; Alanine transaminase, AST; Aspartate transaminase.

Table 2: HBV Serological patterns of the studied patients

Serological markers	Anti-HBc (-) Anti-HBs (-)	Anti-HBc (+) Anti-HBs (-)	Anti-HBc (-) Anti-HBs (+)	Anti-HBc (+) Anti-HBs (+)
N (%)	29 (48.3 %)	25 (41.7 %)	4 (6.7 %)	2 (3.3 %)

4. Discussion

As HBV and HCV share similar transmission routes, co-infection with the two viruses is not a rare event in areas where the two viruses are endemic and among subjects with high risks of parenteral infections (Saravanan *et al.,* 2009). Occult HBV infection has been frequently reported in patients with chronic HCV with a prevalence ranging from 0-52% (Fukuda *et al.,* 1999 and Goral *et al.,* 2006). Considerable data suggested that occult infection may contribute to chronic liver damage, poor response to antiviral therapy, and the development of HCC (Miura *et al.,* 2008 and Mrani *et al.,* 2007).

The present study reported that 13.3% of patients with chronic hepatitis C had detectable HBV DNA in the serum, despite the absence of circulating HBsAg. This result was consistent with other reports which revealed prevalence of OBI ranging from 11%-14.8% among chronic HCV patients (Zignego *et al.,* 1997; Kao *et al.,* 2002; Silva *et al.,* 2004 and Selim *et al.,* 2011). However, a wide variation of the prevalence of occult HBV infection in patients with chronic hepatitis C has been reported. Some authors failed to detect HBV-DNA in both serum and liver samples of HCV patients (Pontisso *et al.,* 1993 and Goral *et al.,* 2006) while others reported an exceedingly high prevalence reaching up to 90% among HCV-infected patients (Uchida *et al.,* 1997 and Koike *et al.,* 1998). This dissimilarity among studies might be due to the geographic variations regarding the HBV prevalence, the number of patients' samples investigated in each study or the different sensitivities of the assays used to detect HBV-DNA and the different types of specimens used to detect the presence of HBV (serum or liver).

Table 3: **Characteristics of OBI/HCV dually infected and HCV mono-infected patients**

Characteristics	OBI/HCV dual infection(N= 8)	HCV mono-infection (N= 52)	*P* value
Age; (years**)**	45 (24 – 56)	44.5 (22 -59)	0.90
Gender; n (%)			
Male	5 (62.5%)	40 (76.9%)	0.38
Female	3 (37.5%)	12 (23.1%)	
BMI (kg/m^2)	25.6 (21.9 – 30.2)	27.7 (21.5 – 30.4)	0.50
Duration of HCV infection; (years)	5 (2 -12)	6 (2 -18)	0.32
Liver function tests:			
Serum albumin (g/dL)	4.6 (3.3 – 4.8)	4.1 (3.4 – 6)	0.60
Liver enzymes (IU/ml)	48.5 (30-134)	50.5 (8 – 132)	0.90
ALT			
AST			
Total Bilirubin (mg/dl)	48.5 (28 – 68)	37 (18 – 102)	0.47
Alkaline phosphatase (IU/ml)	1 (0.6 – 1.1) 87 (80 – 198)	0.9 (0.5 -1.4) 126 (57 -281)	0.35 0.47
Basal HCV RNA load (IU/ml)	276,939 (250,334 – 4,956,255)	260,459 (1747 – 5,230,677)	0.24
METAVIR Score inflammatory activity			
Mild	3 (37.5%)	13 (25.0%)	0.67
Moderate	5 (62.5%)	37 (71.2%)	
Severe	0 (0.0%)	2 (3.9%)	
Fibrosis			
F1	3 (37.5%)	25 (48.1%)	0.18
F2	5 (62.5%)	17 (32.7%)	
F3	0 (0.0%)	10 (19.2%)	
Serological markers for HBV			**0.001***
Anti-HBc(-)/Anti-HBs (-)	1 (12.5%)	28 (53.9%)	
Anti-HBc(+)/Anti-HBs (-)	4 (50.0%)	21 (40.4%)	
Anti-HBc(-)/Anti-HBs (+)	1 (12.5%)	3 (5.8%)	
Anti-HBc(+)/Anti-HBs (+)	2 (25.0%)	(0.0%)	

Data expressed as median (range) or number (%)
*Significant when HBV DNA positivity in anti-HBc(+) was compared with anti-HBc(-) patients

In agreement with some reports, the present study suggested that occult HBV could be predicted by serological markers of HBV infection (Cacciola *et al.,* 1999; Kao *et al.,* 2002 and El-sherif *et al.,* 2009). Seventy five percent of the patients with detectable HBV DNA had anti-HBc antibodies. This proportion constituted 22.2% (6/27) of the total anti-HBc positive patients indicating that about a quarter of the patients with positive anti-HBc definitely had OBI. Meanwhile, 3 (37.5%) patients had anti-HBs antibodies. This could be explained by the notion that occult HBV infection is frequently a late phase of overt chronic HBV infection or serologically recovered acute HBV infection while titres of anti-HBs decreases over years to undetectable levels, anti-HBc antibodies only persists. Another possible hypothesis is that HCV infection may block the circulating viral expression of HBV but anti-HBc in the serum and HBV DNA in the hepatocytes may persist (Zollner *et al.,* 2006). On the other hand, other studies found no association between prevalence of HBV DNA and HBV serological markers (Khattab *et al.,* 2005; Emara *et al.,* 2010 and Selim *et al.,* 2011).

Most cross sectional studies that addressed the issue of OBI did not report a strong correlation between ALT/AST levels and occult hepatitis B (Silva *et al.,* 2004; Torbenson *et al.,* 2004). Many studies, including our failed to demonstrate a relationship between occult HBV infection and high aminotransferases levels in chronic HCV patients (Georgiadou *et al.,* 2004; Branco *et al.,* 2007; Chen *et al.,* 2010 and Emara *et al.,* 2010). From the other point of view, a relationship between OBI/HCV co-infection and high aminotransferases levels has been suggested by few studies (Fukuda *et al.,* 1999; Kannangai *et al.,* 2007; Saravanan *et al.,* 2009 and Selim *et al.,* 2011). Because of these inconsistent data, it was evidenced that aminotransferases levels in patients with chronic HCV could not predict the presence of OBI.

Several studies showed an association of occult HBV infection with progressive HCV-related liver disease evidenced by the presence of biochemical activity, increased HCV viral load or increased histological activity and fibrosis (Cacciola *et al.,* 1999; Fukuda *et al.,* 2001 and Branco *et al.,* 2007). The authors of these studies explained this finding by accelerated inflammation of the hepatic cells induced by OBI due to increased HBV DNA replication, immune activation, and subsequent liver injury. On the other hand, the present study and some other studies did not find any association between occult HBV infection and virologic or histologic data (Kazemi-Shirazi *et al.,* 2000; Kao *et al.,* 2002; Silva *et al.,* 2004 and Georgiadou *et al.,* 2004). Collectively, the present study showed no correlation between clinical outcome and severity of HCV- related liver disease and silent HBV infection. This finding could be attributed to the small number of co-infected patients or that ALT levels correlate well with the inflammatory activity on biopsy, consequently, the ALT level was comparable to the reported mild to moderate degree of inflammation. Another possible explanation is that most of these studies were cross sectional which have the drawback of evaluating the disease at certain point of time and therefore, longitudinal studies should be designed to evaluate the real impact of OBI on liver disease progression.

Conclusion

This study reported that a considerable proportion of patients with hepatitis C had occult HBV infection. The study also supported the use of anti-HBc (either alone or with other markers of previous HBV infection) as a surrogate marker for occult HBV infection in patients with chronic hepatitis C and HBsAg should not be used alone as the golden marker for the diagnosis of HBV infection. Patients who may be at risk for acquiring and/or transferring occult HBV should be examined for the presence of HBV DNA by PCR. There was no correlation between occult HBV infection and the severity of the liver disease. However, further studies including larger number of patients, are needed to clarify the clinical significance of occult HBV for accelerating the natural course of chronic HCV infection.

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5/12/2012

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