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Conceptualization of a Patient Safety Management Model as Practical Approach toward Benchmarking and Improving Healthcare Outcomes

Bahjat Al-Awa¹, Isabelle Devreux², Agnes Jacquerye³, Abeer Alhazmi¹, Hussam AlBaz¹, Hamed Habib⁴ and Osama Rayes⁴

¹King Abdulaziz University Hospital, Jeddah

²Faculty of Applied Medical Sciences, King Abdul Aziz University

³Ecole de Santé Publique, Université Libre de Bruxelles

⁴Faculty of Medicine, King Abdul Aziz University

alawabahjat@gmail.com

Abstract: Introduction: Patient safety is a major concern in the Kingdom of Saudi Arabia. Organizations and investigators are alike in searching for ways to improve delivery and safety of patient care. Many have reported that the existence of a patient safety and risk management system will have an effective impact on the overall patients' outcomes. **Aims:** To study the effectiveness of a patient safety model on patient safety indicators when implemented in a university hospital. **Methods:** A task force constituted by various patient safety experts was established to design a practical concept of patient safety management based on a nine steps model and applied by all hospital departments. Patient safety indicators (780) were monitored over a four years period and the model's effectiveness was analyzed on 40 selected indicators. **Results:** A statistical significant improvement by 67.5% (27/40) of initially measured patient safety indicators was evidenced mainly in the domains of peri-operative mortalities, neonatal mortality, return to surgeries, healthcare associated infections, safety and medication use, blood transfusion reactions, cardio pulmonary resuscitations, patient adverse events, and occurrence variance reporting. However, 12.5% (5/40) of the indicators of hospital standardized mortality and specific mortality were not improved by the model's implementation while others, 20% (8/40) of the patients safety indicators were maintained as their initial baselines were satisfactory. **Conclusions:** The implementation of a patient safety management model was found to be effective in improving patient safety practices (PSP) as well as patient safety indicators (PSIs) and finally patient outcomes.

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Key words: patient safety indicators, patient safety model, risk management, health care associated infection, patient's adverse events.

1. Introduction

Patients' safety continues to concern consumers, health professionals, policymakers, insurers, and researchers, as well as the general public in the Kingdom of Saudi Arabia. These common interests have been fuelled, in a part by news which was related to individuals who were victims of serious medical errors and which were originated from both private and public hospitals. To err is human^{1,2}, especially in hospitals where patients could consult various health care providers in multiple settings and no one might have complete information about their condition. Many types of "process errors" have been attributed to this "non-system," including medication errors, administrative mistakes, treatment delivery problems, and miscommunication.^{3,4} In this situation, both patient safety and quality of healthcare might be affected.

Organizations and investigators are alike in searching for ways to improve delivery and safety of patient care. Many are intent on embedding patient safety practices into healthcare. A patient safety

practice is defined as "a type of process whose application reduces the probability of an adverse event", however, evidence for the incorporation of various safety practices, including incident reporting, root cause analysis, and the promise of promoting a "culture of safety," are still in their theoretical approach.⁵⁻⁷

Monitoring the impact of its own patient safety initiatives and available outcomes using selected patient safety indicators (PSIs) require close attention.^{8,9} Additionally, the complexity of patient care is still a major concern and the challenge for the patient safety practices.^{3,10} The prevention of accidental harm through analysis and redesign of vulnerable patient systems such as ordering, preparation and dispensing of medications, infection control, falls, patient identification, accidents or incidents involving injury, sentinel events etc., should also be closely monitored.

Determining the factors that are associated with the provision of safe patient care is crucial for today's healthcare environment.¹¹ Researchers and

experts in patient safety and its evaluation methods have recommended the use of a theoretical and logic model and have found that the measurement, intervention and reporting contexts will improve the science of patient safety.¹²

Patient safety and risk management constitutes the main core of quality improvement, hospital performance and patient outcomes with a special focus on effectively managing and mitigating clinical and safety-related risks. Similarly, a patient safety management approach supports the organizations' understanding of an acceptable level of quality and continuum of care in health care practices. It also aims at continuously raising the bar with regards to quality improvement initiatives and serves the purpose of enhancing the end-users awareness and perception of quality care.^{13,14}

A common understanding is that providing a framework which assists in the creation and implementation of systems and processes can improve operational effectiveness and enhance positive health outcomes.^{13,14} Such systems can be started by the concept of understanding patient safety from theory to practice, and by improving working conditions as well as promoting the capacity building and organizational learning. It is essential to consider that patient safety practices can be only improved through the engagement of all staffs from various levels through. Therefore, the present patient safety management model is empowered by staff opinion and input, team work and expanded training that could lead to better patient outcomes.^{15,16}

2. Methodology

The present research was conducted in the King Abdulaziz University Hospital (KAUH) which operates 750 beds and 200 ambulatory care beds. It has an average of 4.5 FTE employees per bed with 740 physicians and 1,250 nurses.

A task force of patient safety experts specialized and experienced the field of patient safety has been created in 2006. Team members originated from various hospital departments and included doctors, nurses, respiratory therapists, physical therapists, infection control nurses, laboratory physicians and technicians, pharmacists, as well as department chairmen. The terms of reference of the present task force were to determine the theoretical components of a patient safety model that would be followed and implemented at King Abdulaziz University Hospital (KAUH).

To that effect, the following steps were initiated:

Step 1- SWOT Analysis (strengths, weaknesses, opportunities and threats)

Meetings and brainstorming sessions have been conducted at higher level of the hospital management and quality department, and a primary SWOT

analysis was made with the objective to improve healthcare and patient safety outcomes. Additionally, all departments were invited to perform their own specific SWOT analysis in terms of patient safety. Findings have been submitted to the hospital's quality department.

Step 2- Patient Risk Identification

Nursing and quality departments, in collaboration with the various task force members, identified more than 400 patient safety concerns and risks as outcomes of the SWOT analysis and information from all available sources. Preliminary patient safety concerns were identified and supported by obtaining additional information, data and input from various resources. Pertinent and interesting findings were regularly discussed in the medical board meetings to highlight and identify patients' risks in the organization.

Step 3 - Patient Risk Analysis

The analysis and review of the patient safety concerns were retained according to their impact on patients and healthcare outcomes (such as high volume, high risk, high cost, problem prone and quick win). The evaluations of patients' risks with the trending between the past and the present situations completed by the possible degree of evolution have been possible by additional ongoing assessments and the review of the updated available information.

Step 4 - Patient Safety Action Plan

The task force team created a patient safety action plan in response to issues that have been identified and assessed, and that could be prevented through a vertical and horizontal interventional plan. The plan combined strategic goals, action and tactics, performance measures, baseline, targets, responsibilities in addition to the required resources for implementation. The present patient safety action plan has therefore been limited to 40 patient safety indicators.

Step 5 - Patient Risk Financing

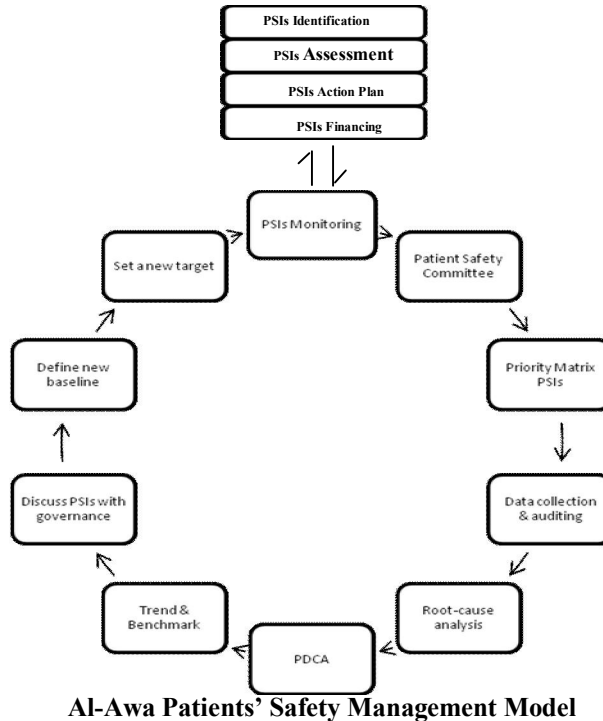
Based on the possible funds that the hospital management is willing to allocate in the risk management process, human and financial resources and benefits have been specified in the patient safety action plan.

Step 6 - Concept of Patient Safety Management Model

The patient safety task force team has thereafter designed a practical concept of patient safety management to be followed by all departments which started by monitoring and assessing patient safety indicators as per specifically defined criteria. The following steps were implemented and were summarized as follows: It is at first required to create a patient safety committee composed of experts in the field. This task force will prioritize

patient safety indicators as per a feasibility matrix, create tools for data collection and auditing, and conduct effective root cause analyses. These tasks are completed by the actions of trending and benchmarking aiming at improving benchmarks through a process of PDCA (Plan-Do-Check-Act). Whenever necessary, it appears essential to discuss

benchmarks with the concerned departments, and discuss the results of patient safety indicators with governance. In addition, it is advised over time to define new baselines and new targets to reach, and finally to continue monitoring or defining new patient safety indicators for improvement



Al-Awa Patients' Safety Management Model

Step 7 - Patient Safety Indicators (PSIs) Classifications and Benchmarks

The monitored patient safety indicators have been grouped in various categories such as standardized mortality, surgery/invasive procedures, healthcare associated infections, medication safety, blood product, codes, patient adverse events, and occurrence variance reporting as quality indicators.

Step 8- Statistical Analysis

Patient safety indicators were collected on a monthly basis over the period of four years and comparative analysis was made using a One-way Anova test and a global *P*-value was calculated for each indicator using the Statistical Package for Social Sciences.

Step 9 - Study of the Model's Impact

Results sensitive to the model's implementation and non-sensitive results have been classified into two groups with average benchmarks for the four years of data collection.

3. Results

The results are based on data collected during the period 2006 to 2009. Patients' safety indicators and identified risks were collected through various methods and monitored on a monthly basis manually or through the hospital information system (HIS). The following results are classified as patient safety indicators (PSIs) sensitive to model implementation and resumed in the tables (1) and (2).

Table 1: Patient Safety & Quality Indicators Sensitive to Model Implementation

No.	Patient Safety Indicators	2006	2007	2008	2009	Average & Benchmark	<i>P</i> -Value
Hospital Standardized Mortality Rates							
1	Perioperative deaths/month	1.08	0.42	0.25	0.17	0.48	.002
2	Perioperative mortality/1000 surgeries	1.81	0.61	0.4	0.22	0.76	.003
3	Perioperative mortality/1000 total deaths	22.28	7.29	4.10	2.38	9.01	.001
4	Neonatal mortality/100 NICU admissions	46.14	20.24	23.83	18.32	27.13	.001
Surgery/Invasive Procedures							

1	Average unplanned returns to surgery \leq 48 hours	2.33	0.83	0.58	1.67	1.35	.009
2	Unplanned returns to surgery / 100 operations	0.37	0.13	0.10	0.19	0.20	.013
Health Care Associated Infections (HAIs) & Hand Hygiene							
1	HAIs targeted infections / 1000 hospital day	4.18	3.00	2.15	2.27	2.9	.001
2	Clean surgical site infections per 1000 operations	7.12	4.52	1.81	4.30	4.44	.001
3	Neonatal HAIs/1000 patient days	11.40	9.22	7.87	4.79	8.32	.008
4	Blood stream infections per 1000 patient days	1.17	0.78	0.17	0.52	0.66	.001
5	Central line BSIs / 1000 device days in Adult ICU	15.18	6.98	6.29	4.21	8.17	.000
6	Central line BSIs / 1000 device days in NICU	17.34	30.04	40.17	12.81	25.09	.008
7	Urinary tract HAIs / 1000 patient days.	1.34	0.90	0.28	0.88	0.85	.001
8	UTIs / 1000 device days in Adult ICU	12.68	6.88	2.45	2.79	6.20	.001
9	Respiratory tract HAIs / 1000 patient days	0.27	0.26	0.04	0.31	0.22	.022
10	VAP infections / 1000 device days in Adult ICU	23.78	8.53	7.35	8.12	11.95	.001
11	Skin and soft tissue HAIs / 1000 patient days.	0.62	0.62	0.49	0.16	0.47	.003
12	Average use of alcohol hand disinfectant in liter / bed	0.45	0.63	1.23	1.72	1.00	.000

CPR: Cardio Pulmonary Resuscitation HAIs: Healthcare Associated Infections VAP: Ventilated Associated Pneumonia BSIs: Blood Stream Infections UTI: Urinary Tract Infection

Table 2: Patient Safety & Quality Indicators Sensitive to Model Implementation

No.	Patient Safety Indicators	2006	2007	2008	2009	Average & Benchmark	P-Value
Safety of using medications							
1	Average numbers of medication errors reported	0.58	1.42	1.00	2.17	1.29	.039
2	Reported medication errors / 100 admissions	0.02	0.05	0.03	0.07	0.04	.050
3	Reported medication errors / 100 hospital beds	0.10	0.21	0.15	0.33	0.20	.050
Blood Products Use							
1	Numbers of blood transfusion reactions	3.25	1.33	1.25	3.17	2.25	.002
2	Rates of blood transfusion reactions / 100 transfusions.	0.37	0.32	0.15	0.29	0.27	.018
Codes: Cardiopulmonary Resuscitations							
1	Average patients survival post first CPR.	21.08	23.08	33.08	38.08	28.83	.001
Patient Adverse Events							
1	Pressure ulcers developed / 1000 admissions.	2.83	1.74	1.95	1.56	2.02	.020
2	Average Length of Stay	4.10	3.47	3.31	3.28	3.54	.000
OVRs Reporting as Quality Indicators							
1	Numbers of occurrence variance reports	186.08	257.25	320.08	247.50	252.73	.000

CPR: Cardio Pulmonary Resuscitation HAIs: Healthcare Associated Infections VAP: Ventilated Associated Pneumonia BSIs: Blood Stream Infections UTI: Urinary Tract Infection

4. Discussion

The implementation of the designed management model of patient safety was resumed in monthly reports to the management. The reported indicators were signed on a format of data collection by the concerned department heads which emphasized increased staff awareness and allowed the management to review the departments' performance, bench marking and patient safety practices. In addition, patient safety practices and benchmarks were discussed in the medical board meetings and in the quality and nursing departments' management which largely contributed to the creation of a system process. This process encouraged each department to improve their data to meet acceptable international standards.

It is to note that the improvement which was observed in the peri-operative mortality was certainly due to the opening of pre-anesthesia clinic and the increased team effort that took place to reduce the operating room cancellation rate. This last was based on the improvement of operating room booking

process using the Hospital Information System (HIS) in addition to the credentialing and privileging of surgeons, better hospital bed bookings for surgical cases, and the support of the hospital leadership. This also was certainly impacted positively on the peri-operative deaths which were reduced by 238%. In the contrary, there was no improvement identified in the standardized hospital mortality rate of 1.98% of admissions. However, it was noticed that the average unplanned returned to surgery were significantly reduced by ($P \leq .009$) which was positively correlated with the peri-operative mortality. Also, the mortality of neonates has been decreased by 170% as compared to 2006, and as confirmed by other surveys, this is mainly explained by enhanced teamwork to control and reduce the cross infections and the implementation of strict environmental and hand hygiene.^{5,17,18}

Results analysis identified that the healthcare associated infections (HAIs) in general have significantly improved. The implemented policies and the dissemination of information regarding the

importance of controlling nosocomial infections through education, posters, news letter, and the increased availability of hand disinfectant dispensers have in our opinion contributed to this excellent result. For instance, the average utilization of hand disinfectant was multiplied by 3.82 as compared to 2006. Additionally, it is worth to mention that the significant ($P \leq 000$) reduction of length of stay (LOS) which was our focus by 0.82 days per admission have contributed positively to less acquired infections.^{19,20,21} Similarly, the implementation of evidence based policies that central line insertions are to be inserted in the operating room rather than in the acute care wards, has significantly improved the central line blood stream infections ($P,000$).^{5,22,23} Additionally, the implementation of the bundle for the prevention of ventilator associated pneumonia has significantly reduced the VAP ($P \leq 001$). The present findings were also supported by the international literature.²⁴⁻²⁶

As in the present study population, 90% of our nursing staffs were foreigners and it might appear that they were less familiar with the process of reporting medication errors or were fearful of an established culture of blame.²⁷ It appeared thus essential in a risk management approach related to the medication use to encourage clinical workers to report with blame free.^{28,29} The average medication errors reported over four years has significantly improved ($P \leq 0.039$) and have been multiplied by 3.74 as well as, the reported medication errors per 100 beds has significantly improved with ($P \leq 0.050$). The increase in reporting medication errors was certainly one of the steps of acquiring patient safety culture.^{5,30,31} In the same idea, the sentinel events reporting have decreased from 12 events per year in 2006 to 8 events in 2009. The question remains however to identify whether this progress was due to less sentinel events effectively occurring or due to a lack of reporting by the healthcare providers. The survey culture conducted in the present hospital, as compared to the United States, showed that the studied hospital was still presenting a lower percentage of feedback and communication about error with ($P \leq 001$). The communication openness sum was in average 36% as compared to 60% in the International Hospital in United States with ($P \leq 001$). The average teamwork in the unit was 68% as compared to 74% with ($P \leq 005$). Finally, regarding the organization learning and continuous improvement, the studied hospital's score was in average of 74% as compared to 71% while no significant difference was evidenced. Additional benchmark studies identified that the hospital management and the patient safety support was in average of 61% as compared to 60% with no

significant difference. Additionally, the frequency of events reported in average represented 57% as compared to 52% ($P \leq 0.050$) and for the overall patient safety, our survey results evidenced a score of 45% as compared to 57% ($P \leq 001$) in international benchmarks.

The benchmarking of blood transfusion reactions in 2006 was initially of 0.37% and showed a decrease to 0.27% over the period of four years with a marginal gain of 0.10%. This was explained by increased evidence-based practices in the whole management of blood transfusion which included cross matching, blood storage, staff education, and the implementation of newly developed policies in the early 2006.

Many indicators of nursing care have also improved over the studied period, as well as many satisfactory indicators of patient safety and quality of care which were maintained at their initial baseline level and were judged of acceptable benchmark as compared to other institutions.³⁶ The implementation of patient assessment risk for falls (Waterloo Scale) and the compulsory implementation of a patient risk plan for all nursing areas have contributed to reduce the average number of developed pressure ulcers per 1000 admission from 2.83 to an average of 2.02 with ($P \leq 0.020$). There is no doubt that a significant reduction in the length of stay ($P \leq .000$) as in table 2 have contributed positively to the reduction of patients' adverse events.

Such practical implementation of a patient safety management model and the policy of blame free in addition to the clinical guidelines and a performance improvement program contributed to very positive changes over the years. It also appeared that the creation of patient safety committee identifying patients' safety indicators, the utilization of a matrix PSI's, as well as organized data collection, monitoring and auditing were contributing factors to the improvements. The management model was also based on the implementation of root-cause analysis, PDCA, trends analysis and benchmarking in addition to the discussion of patient safety indicators with the hospital's governance to define new baselines of action. It also appeared that the value of establishing new targets in patient safety monitoring and risk management made people proud instead of fearful of reporting incidents and occurrences as this reporting was significantly increased over the four years of research to reach an average 252.73 occurrence variance reports per month ($P \leq 000$).

Conclusion

The conceptualization and implementation of a communicated patient safety management was

effective in improving patients' safety practices and patients' safety indicators.

As demonstrated in this research, patient's safety indicators sensitive to improvement were mainly in the domains of peri-operative and neonatal mortalities, return to surgeries, healthcare associated infections and medication use. In the same line, blood transfusion reactions, cardio-pulmonary resuscitations, patient adverse events, and occurrence variance reporting also showed significant improvements. However, it was observed that the hospital standardized and specific mortalities were not improved by the model's implementation while some patient safety indicators were maintained at their initial acceptable baseline.

The present study highlights the importance of clinical governance by developing a patient safety culture based on positive reporting and discussion with the higher authorities to create a dynamic and continuous process of patient safety improvement.

Corresponding author

Dr. Bahjat Al-Awa

King Abdulaziz University Hospital, Jeddah-KSA.

alawabahjat@gmail.com

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Relation of Work-related Musculoskeletal Disorders and Over-commitment of Rehabilitation Staff in Saudi Arabia

Isabelle C.N. Devreux¹, Bahjat Al-Awa², Khaled Mamdouh¹, Enas Elsayed¹

¹ College of Applied Medical Sciences, King Abdul Aziz University-Jeddah.

² King Abdul Aziz University Hospital- Jeddah.

devreuxic@hotmail.com

Abstract: Background: Physical and occupational therapy professions are perceived as physically demanding. Occupational positions, patients handling techniques and therapeutic applications may result in job-related and postural stresses. **Objectives:** The research aimed at identifying the musculoskeletal disorders reported by physical and occupational therapists in the region of Jeddah and the relation to the level of Over-commitment and work factors. Demographic variables, productivity and job stress were studied in correlation with the musculoskeletal problems expressed by the therapists. **Methods:** A cross sectional survey approach by questionnaire identified the musculoskeletal disorders and related Effort-Reward Imbalance and over-commitment amongst therapists in various physical rehabilitation departments of public, profit making and teaching hospitals. **Results:** Results indicated that 50.6 % of the surveyed staff (N=166) reported to experience physical stress related to their work and whilst 39 % of the staff claimed to suffer from physical symptoms regularly after their working day. It was found that 56.6 % of the staff felt their symptoms were proportional with the workload intensity and severe enough to force them to take time off work. The main work-related complaints were back, neck and shoulder pain and for 25 % of the staff myalgia in different areas of the body. Complaints were significantly correlated to the Over-commitment score and to the number of patients treated when associated to absences from work. The incidence of the musculoskeletal problems appeared higher amongst males and in teaching and private rehabilitation departments. **Conclusion:** Musculoskeletal disorders expressed by rehabilitation staff in the area of Jeddah appeared to be strongly related to the level of Over-commitment in work. The work-related complaints varied according to gender, age and hospital type. The main symptoms were back pain and generalized myalgia which were severe enough to lead to sick leave and medical consultations. The prevention of exaggerated efforts at work and recognition of the hardship related to the professional occupation could be emphasized in management strategies of physical rehabilitation services.

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Keywords: Musculoskeletal disorders, work stress, over-commitment, Effort-Reward Imbalance measure, physiotherapist, occupational therapist, occupational injuries.

1. Introduction

Rehabilitation services' professions, particularly physical therapists, have been considered at risk of developing work-related musculoskeletal disorders in various countries [1-6]. During patients' treatments, physiotherapists frequently need to use manual handling and awkward postures increasing the risk of developing musculoskeletal injury associated with the physical handling of the patients [7, 8]. Physical stress could also increase with additional workload and the work situation of the staff. Previous studies have shown that job stress, measured by the Effort and Reward Imbalance model, is strongly correlated with the types and number of treated patients per day and the number of worked hours per week [9]. An intrinsic element of Effort- Reward Imbalance is considered as "over-commitment". This has been defined as an enduring cognitive-motivational pattern of maladaptive coping with demands characterized by excessive striving and an inability to withdraw from obligations

[10]. The present study aimed at identifying work factors and related physical work stress factors amongst rehabilitation services staff in the region of Jeddah in Saudi Arabia.

2. Methods

Study design:

A cross sectional survey by a written questionnaire followed approval by relevant ethics committees of the university hospital and other concerned hospitals.

Subjects:

222 therapists working full time in the physical rehabilitation services were included in the survey of ten randomly selected healthcare facilities. They included the Ministry of Health, private sector and teaching hospitals located in the region of Jeddah in Saudi Arabia. The inpatient hospitals ranged from capacity of 250 to 700 beds and several outpatient

healthcare facilities that were also included in the survey.

Methods:

Two written survey questionnaires were specifically adapted for this research and simultaneously distributed to each subject. The first questionnaire aimed at identifying the job stress based on the Effort- Reward Imbalance measure [11] and its intrinsic component by the over-commitment score. Over-commitment was assessed by a uni-dimensional scale composed of six Likert-scaled items. In these the respondents indicated to what extent they personally agreed or disagreed with the given statements on a four-point rating scale. The items were as follows: (1) I get easily overwhelmed by time pressures at work; (2) As soon as I get up in the morning I start thinking about work problems; (3) When I get home, I can easily relax and “switch off” work; (4) People close to me say I sacrifice too much for my job; (5) Work rarely lets me go, it is still on my mind when I go to bed; (6) If I postpone something that I was supposed to do today I’ll have trouble sleeping at night. The score ranges from 6 to 24 with higher scores reflecting higher Over-commitment [10]. The questionnaire included twenty three questions without modifications from the original standardized questionnaire. The sum of the values of the efforts was related to the values of rewards and a ratio of “1” is considered as the optimal reference Effort-Reward Imbalance value at work [11].

The second survey questionnaire included questions to precise demographic data, working

situation, productivity of the staff. Questions graded on a five point Likert scale also investigated the physical complaints and symptoms related to work and perceived as increasing with workload (Appendix). Both questionnaires sections were personally distributed to each subject and collected within a period of two weeks.

Statistical Analysis:

The results of the questionnaires and collected data were encoded in an SPSS (Statistical Package for Social Sciences) program for analysis. Data were computed by descriptive statistics *P*-values less than 0.05 were considered as significant. Variables of hospital category, position, specialty, nationality, age and gender of the therapists were analyzed and compared in a cross tabulation analysis. Normality test of the distribution by one-sample Kolmogorov-Smirnov test was made. Pearson test for significance and Kruskal Wallis nonparametric tests for ranks were made and Spearman-rho test was applied with numerical variables correlations for the number of patients treated per day and Effort-Reward Imbalance ratio.

3. Results

The survey was completed by 166 rehabilitation therapists with a response rate of 74.9 %. Results indicated that 50.6 % of the surveyed staff reported to regularly experience physical stress related to their work and 39 % of the staff reported also to suffer physical symptoms after their working day.

Table (1): Percentage of staff with work related complaints

Statements related to work related complaints	Percentage of Staff
Experience of physical stress related to work	50.6
Complains of symptoms after work day	39.0
Symptoms are increasing with workload	56.6
Have consulted a medical expert for the symptoms	31.0
Complaints have resulted in sick leave or work absence	25.9
N =166	

A primary symptom was expressed by 45% of physical rehabilitation staff and 22.3 % of the subjects have mentioned a second symptom in addition to the first one. Table (2) resumes the various symptoms per percentage of staff.

Table (2): Work related complaints per percentage of rehabilitation staff

Work related complaints	Staff with primary symptom (%)	Staff with a secondary symptom (%)
Back pain	23.5	5.4
Myalgia (not specified)	6.6	4.2
Neck/ Shoulder pain	4.2	3.0
Lower limbs pain	3.0	2.4
Wrist pain	1.8	1.2
Others (fatigue, stress, headache)	6.0	6.0
Total staff with complaints (N =166)	45.2 %	22.2 %

Back pain was the most commonly expressed symptom in all categories of positions and more specifically low back pain (13.9 %) and this for 50 % of the occupational therapists and 57.7 % of the physiotherapists. Respiratory therapists complained more of lower limbs pain for 33.3 % of them and 23.3 % expressed other complaints such as stress, fatigue or headaches. Back pain appeared the most amongst therapists assigned to treat both inpatients and outpatients. On average, a higher number of therapists treating mainly adult patients mentioned suffering from work related musculoskeletal problems compared to the therapists with a pediatric caseload. It was also found that the symptoms of low back pain were reported by most of the staff working in the teaching or private hospital categories.

Results indicate that 57% of the staff aged between 21 and 30 years were in general expressing

back pain and generalized myalgia while 50% of the therapists aged between 31 to 40 years old complained mainly of backache and 18.8% of them of generalized myalgia. Staff aged of 41 years and above reported stress, headaches and general fatigue.

While 58% of the male therapists were suffering from back pain symptoms, analysis showed 28 % of the male therapist expressed work-related physical complaints in general and reported an increase in their symptoms with additional work (32.2 %, $P < 0.005$) and obliging them to take sick leave ($P < 0.002$). Results indicated a significantly ($P < 0.023$) higher value for the mean number of patients per day for the staff expressing physical stress after work (mean of 10.99 ± 3.98 compared to 9.59 ± 3.82) and for the staff having related sick leave or absence days ($P < 0.010$) with a mean number of 11.45 ± 3.57 compared to 9.7 ± 4.05).

Table (3): Mean Over-commitment score per expressed work symptoms

Statements related to work symptoms	Over-commitment Score			
	N	Mean \pm SD	Chi ²	P-value
Experience physical stress related to work	81	15.55 \pm 2.94	.233	.003**
Complains of symptoms after work day	63	15.66 \pm 2.93	.213	.007**
Symptoms are increasing with workload	63	15.61 \pm 2.76	.270	.001**
Have consulted a medical expert for the symptoms	39	16.31 \pm 3.01	.207	.001**
Complaints have resulted in sick leave or work absence	49	15.98 \pm 2.97	.313	.001**

** . Correlation is significant at the 0.01 level (2-tailed).

Analysis outlined a highly significant correlation ($P < 0.001$) between the over-commitment score and all the statements indicating the work related symptoms. While there was no significant correlation evidenced, higher mean Effort-Reward Imbalance ratio was also seen for the staff reporting experiencing work related physical complaints and suffering from symptoms after a working day. A higher mean Effort-Reward Imbalance ratio was seen for the persons that felt their symptoms increased with additional workload though correlation analysis failed to reach significance. Finally, results identified a significantly ($P < 0.001$) higher mean Effort-Reward Imbalance for the staff who mentioned taking sick leave or time off work and having consulted a medical expert regarding their occupational related symptoms.

4. Discussion

Confirming other studies, rehabilitation services staffs in the present survey expressed to suffer from muscular strains and musculoskeletal pain related to work and appearing to increase with their professional activity [12,13]. The severity of the symptoms lead one

fourth of the staff to consult medical experts or to take sick leave from work. In addition, one fourth of the surveyed staff expressed to suffer from cumulated symptoms with mainly lower back, neck and shoulder pain.

The present research outlined a clear evidence of the relation between over-commitment and work-related complaints expressed by the therapists. Overcommitted persons are driven by their high need for control and approval, thereby repeatedly overtaxing their own resources and, thus, precipitating exhaustion and breakdown in the long run [14]. Several studies have suggested that over-commitment increases musculoskeletal pain [15] and coronary heart disease risk [11,14,16]. In this context, "over-commitment is thought to magnify stressful experience resulting from high cost/low gain conditions at work because it induces exaggerated efforts which are not met by extrinsic rewards" [11].

Job stress measure based on the Effort-Reward Imbalance and the workload intensity measured by the daily average number of patients treated per therapists were not significantly correlated to the complaints of

musculoskeletal pain. An increased number of patients and higher workload were however associated to the severity of musculoskeletal disorders and resulting in work absences caused by sick leave. It could then be supposed that in the present study population, repeated exaggerated efforts in work, more than the factors of rewards, are identified as strongly related factors to musculoskeletal discomfort.

Previous researchers have also identified a link between job strain and job turn-over and musculoskeletal disorders in physical therapists [2,17].

While the main musculoskeletal complaints appeared to be back pain principally in the lumbar region, other complaints included generalized muscle pain, shoulder and neck pain as well as lower limbs pain. These results are in line with other research [12] and some older therapists had expressed to suffer from headaches and generalized fatigue related to their work which may be reflections of stress at work. Several researches have confirmed the relation between occupational stress and subjective health complaints in healthcare professionals and physiotherapists [18-20].

In accordance with other studies, back pain appeared more prevalent amongst the young therapists which could possibly be related to higher workload [3] with repetitive transfers or mobilization of patients performed by that category of staff. Lifting and transferring dependent patients have been outlined as the main factors likely to contribute to musculoskeletal disorders [2,13,21]. In accordance to a heavier professional workload in their professional activity, male therapists appeared more prone to develop musculoskeletal complaints compared to their female colleagues.

From their educational background, physical and occupational therapists are very well aware of work ergonomics though the physical workload appears mainly related to their specific work tasks and proportional to the level of commitment. In accordance to other studies [17], a higher incidence of musculoskeletal disorders was seen in male staff and confirmed by a higher proportion of males that have sought medical advice for their problem.

The educational degree of the therapists was related to the expression of work-related musculoskeletal symptoms. Master and doctoral degree holders were complaining of back pain in general, while bachelor and diploma holders were mainly complaining of low back pain possibly due to a higher proportion of patient treatments in their assignments. This could be attributed to non-clinical duties such as education, administrative duties or documentation as well as complex medical cases which are more often assigned to senior staff. Increased pressure and work demands on qualified staff is often associated with high professional standards to the detriment of related physical stress. It was also noted that the severity of the

symptoms were more evident in the profit making and teaching hospitals. This was supported by a higher number of patients-therapist ratio in these hospital categories associated with a higher Effort- Reward Imbalance [9].

In comparison between specialties, occupational therapists appeared to have a similar risk of developing back pain or upper extremity strains in the wrist or the shoulder and no significant differences have been found between the rehabilitation specialties. Similar risks in other categories of health professions such as nurses were identified in other researches [21,22].

The recommendations for practice guidelines and load management have been outlined by Cromie *et al.* [2] and the need of specific strategies for work load management by cases distribution could be also emphasized. The importance of a positive work environment and decreased over-commitment or exaggerated efforts was recognized to affect the musculoskeletal strains at work among rehabilitation staff.

CONCLUSION

The musculoskeletal complaints expressed by rehabilitation staffs in the area of Jeddah were related to their professional activity and severe enough to lead to sick leave and medical consultation.

The main symptoms were back pain and generalized myalgia which were related to the work stress caused by the over-commitment at work and a high number of treated patients. These symptoms were also high amongst young therapists and of higher incidence in the teaching and profit making hospitals.

Preventive strategies to adopt compensatory mechanisms by modifying body mechanics, monitoring the workload but also analyzing the factors contributing to the over-commitment could be encouraged. An emphasis should be considered on the decrease of exaggerated efforts at work and on the recognition of the hardship related to the professional occupation in physical rehabilitation services.

Competing Interests

The authors declare that they have no competing interests.

Corresponding author

Isabelle C.N. Devreux

College of Applied Medical Sciences, King Abdul Aziz University-Jeddah, KSA.

devreuxic@hotmail.com

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Appendix: Questions relevant to the musculoskeletal disorders and strains related to work from the questionnaire.

Relating to the statement on the left, please select the number that best describes your viewpoint.

Thank you for answering all the questions.

On the present job, this is how I feel about the following statements:

1. I strongly disagree
2. I disagree
3. I sometimes agree
4. I agree
5. I strongly agree

Question1: I experience physical stress complaints related to the work on regular basis.

Question 2: I am regularly suffering of physical symptoms experienced after a working day

Question3: Mention the main symptom related to work:.....

Question 4: These symptoms increase as the work intensity increase.

Question 5: These symptoms/complaints are sometimes forcing me to take sick leave or time off from work.

Question 6: I have consulted a medical expert for relieving these symptoms.

Some Studies in Baraki Sheep Intoxicated with Cadmium.

Mona. S. Zaki* and Mohamed M.I.**

*. Department of Hydrobiology National Research Center, Egypt

** Department. of Animal Poultry Nutrition and Production National Research Center, Egypt

dr_mona_zaki@yahoo.co.uk

Abstract: Eighteen rams (27 - 28 kg. B.wt and 6 - 10 months old) were divided into 3 equal groups (gps. 1-3) and kept on a basal ration to evaluate the cadmium toxicity on the digestibility, blood picture and reproductive status, beside the hepatic and renal functions. Gp. (1) was the control. Gps. (2 and 3) were orally given 50 and 100 mg. Cadmium chloride/kg. Bwt respectively for 4 weeks. Heparinized and non-heparinized blood samples were collected for blood picture and serum separation, respectively. The serum was used for the determination of some biochemical blood parameters. Atrophy and necrosis of the testes, liver and kidneys were associated with clinicopathological changes. A significant decrease was detected in the values of RBCs, PCV, Hb, LH, FSH, Testosterone, total proteins, zinc concentration and digestion coefficient. On the other hand, there was significant increase in levels of ESR, WBCs, ALT, AST, Urea, Creatinine, Sodium, Potassium and Cadmium.

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Keywords: Baraki Sheep, Cadmium toxicity, liver function, kidney function.

1. Introduction

The heavy metals are toxic due to the low rate of its elimination from the body. The environmental contamination with heavy metals such as lead, cadmium, zinc, mercury and copper are widely distributed in the agricultural land and water, **Adriana (1986), Hires et al., (1999), Bryant and Rose (1995)**. The heavy metals may be absorbed from digestive tract of the animal, some by grazing, some of these metals are toxic virtually for every system of human body, **Kabata and Peido (1999)** and may cause serious health problems in man, depending on their levels of contamination, **Fayed and Abdallal (1997)**. Industrial agriculture like coal and oil combustion byproducts chemical and chloride plant emissions, fertilizers and sludge used in agricultural lands **Kajikawa et al. (1991)**. Sewage effluents, some types of plastics and pesticides are considered the primary source of lead and cadmium pollutions for animals and fish, **Abe and Itakawa (1993)**. Heavy metals are cumulative poisons for man and animals, therefore the current study was planned to estimate the effect of cadmium on the nutritional status of sheep together with its effect on the liver, kidney and reproductive organs from the clinicopathologic aspect increased.

Anoxia, depression, emaciation, tucked up abdomen pluse and respiration rates with laboured breathing, exophthalmia and diarrhea beside frequently odema were encountered after 30 days on treatment.

The objectives of the present work were to study the effects of cadmium toxicosis on digestibility, hematology and reproductive status

beside the liver and kidney functions of sheep.

2. Material and Methods

Eighteen rams (6-10 month old and 27-28 kg Bwt) were equally divided into 3 groups (gps. 1-3) and kept on a balanced ration. (Table 1). Gp. (1) was the control. Gp. (2) was orally given 50 mg cadmium chloride/kg B wt/day. Gp. (3) was orally given 100 mg cadmium chloride/kg Bwt/day. The experiment extended for 4 successive weeks.

Blood was collected from the jugular vein after 30 days of the start of the experiment in heparinized test-tubes for determination of blood picture according to **Jain (1986)** and non heparinized test-tubes for serum collection where test tubes were centrifuged at 3000 r.p.m. for 15 minutes and then the sera were kept in deep freeze at -20°C. Determination of total testosterone was done by **Radiimmunoassay method according Ismail (1986)**. Evaluation of follicular stimulating hormone (F.S.H) and leutinizing hormone (L.H.) were determined by **Kulin and Santer (1977) and Fuquay (1983)**. Serum zinc, sodium, potassium and cadmium were estimated by atomic absorption according to **Joseph and Roger (1979)**.

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), beside the total protein, urea and creatinine were determined by using commercial kits (diagnostic kits-Bio Merieux France).

Three digestion trials were conducted to evaluate the digestion coefficients of nutrients of the three groups. The animals of each treatment were

penned individually in the digestion cages. The digestion trials lasted for 21 days. The preliminary period lasted for the first 14 days and the collection period lasted for the other 7 days. The fecal samples were collected daily and dried at 60°C for 72 hrs and

men stored in screw-top glass jars for determining the different chemical constituents according to **A.O.A.C. (1984)**. Statistical analysis of the obtained data were determined by using student (t) test according to **Gad and Weil (1983)**.

Table (1): Composition of the basal diet for sheep, according to the national research. Council (1976): Nutrient requirements of domestic animals, National Academy of science, Washington D.C, 5th edition (4): 10 -26.

Ingredient	Percentage
Cotton seed cake.	30
Corn yellow.	25
Roughages.	25
Wheat bran.	15.5
Calcium chloride.	2
Sodium chloride.	1
Vitamin and mineral mixture	2

Calculated Nutrient Composition:

Crude protein.	18.525
Energy (ME/ kg).	1772.5
Crude fiber.	13.51
Ether extract.	2.85
Calcium.	2.111
Phosphorus.	0.649

Table (2): Some hematological parameters after 30 days of experiments (Means ± SE)

Groups Parameters	Control 1	Cadmium chloride 50mg 2	Cadmium, chloride 100mg 3
RBCS 10 ⁶ /mm ³	9.08 ± 0.03	8.75 ± 0.01*	8.00 ± 0.43**
PCV%	34 ± 0.25	32 ± 0.14*	30 ± 0.27**
HB g/dl.	12.7 ± 0.72	11.8 ± 0.08*	10.9 ± 0.012**
ESR mm/2hrs	1.04 ± 0.32	2.00 ± 0.79*	1.07 ± 0.98**
WBCS 10 ³ /mm ³	7.94 ± 0.32	8.01 ± 0.73	8.09 ± 0.88**

**P<0.01

*P<0.05

PCV = Packed cell volume. ESR = Sedimentation rate HB = Hemoglobin W.B.C.S = White blood cells.

RBCs = Red blood corpuscles.

Table (3): Effect of cadmium chloride on some biochemical parameters after 30 days of experiments (Means \pm SE)

Parameters \ Groups	Control 1	Cad cl 50mg 2	Cad cl 100mg 3
AST U/L	22.7 \pm 0.22	23 \pm 0.49*	33.5 \pm 0.29**
ALT U/L	19.0 \pm 0.18	20.5 \pm 0.37*	23 \pm 0.28**
Total protein gm/dl	6.97 \pm 0.85	6.72 \pm 0.12*	6.3 \pm 0.27**
Urea mg/dl	14.00 \pm 0.09	14.50 \pm 0.73*	15.5 \pm 0.70**
Creatinine mg/dl	1.81 \pm 0.03	1.50 \pm 0.92*	1.62 \pm 0.64**
Sodium MEq/L	11.00 \pm 0.24	11.22 \pm 0.12*	124.3 \pm 0.17**
Potassium MEq/L	9.3 \pm 0.72	10.7 \pm 0.98*	11.9 \pm 0.62**
Serum zinc, ppm	2.00 \pm 0.72	1.85 \pm 0.74*	1.25 \pm 0.07**
Cadmium ppm.	0.6 \pm 0.45	0.84 \pm 0.20*	1.49 \pm 0.67**

**P<0.01 *P<0.05

Table (4): Effect of cadmium chloride on some hormonal parameters after 30 days of experiments (Means \pm SE)

Parameters \ Group	Control 1	Cad cl 50 mg 2	Cad. Cl. 100 mg 3
LH IU/L	1.67 \pm 0.01	0.89 \pm 0.04*	0.70 \pm 0.06**
Testosterone ng/ml	2.74 \pm 0.07	2.52 \pm 0.31*	2.15 \pm 0.23**
F.S.H U/L	1.87 \pm 0.24	1.72 \pm 0.06*	1.50 \pm 0.04**

**P<0.01 *P<0.05

Table (5): Digestion coefficient of the different experimental rations (Means \pm SE).

Groups	Nutrients digestibility %					
	DM	OM	CP	EE	CF	NFE
1	62.3 \pm	63.5 \pm	60.8 \pm	58.7	55.3*	63.1 \pm *
2	61.5 \pm	62.8 \pm	60.3 \pm	59.4	54.7	62.5 \pm **
3	43.25 \pm **	43.49 \pm **	39.2 \pm **	35.4**	30.1**	42.7 \pm **
SE	14.3	12.7	15.3	11.8	12.7	13.5

**P<0.01 OM = Organic matter DM = Dry matter. CP = Crude protein.
EE = Ether extract. CF = Crude fiber. NFE = Nitrogen Free extract

3. Results

There was a decrease in the body weight. Necrosis and calcification were detected in the kidney and liver of the dead animals. There was a significant decrease in the RBC, PCV and Hemoglobin ($P < 0.01$) while there was a significant increase in ESR and WBC count (Table 3).

There was a significant increase in AST, ALT, urea, creatinine, sodium, potassium and serum cadmium, while a significant decrease in TP and zinc concentration were recorded as shown in table (4). There was a significant decrease in mean LH, FSH and testosterone (Table 5). Table (6) shows that the digestion coefficients were significantly decreased

in gp (2) and highly significantly decreased in gp. (3).

4. Discussion

Cadmium is apparently non-essential element that is virtually absent from the body of man and animal at birth. Air pollution with cadmium from industrial sources may be transmitted to man and animals through contaminated food stuffs **Catalaba and Yarland (1986)**, **Bryant and Rose (1995)**, **Fiberg et al. (1996)**, **Sharl et al. (1999)**.

Significant decreases were observed in RBCS, PCV and hemoglobin. On the contrary, there were significant increases in the ESR and WBC. Similar results were obtained by **Fiberg et al. (1996)**. It is well known that the toxicity of cadmium inhibits reproduction in animals. [**Kumimata and Miruo (1986)**, **Hew et al. (1993)**, **Mircda (1996)**, **Fayed and Abdallal (1997)**]. There was a reduction in the level of LH, FSH and testosterone in comparison with the control. The available literature concerning the effect of cadmium on the levels of LH, FSH and testosterone are very scarce. These results are in accordance with those obtained by **Kuo et al. (1995)**, **Watanabe et al. (1998)**, and **Santner et al., (1981)** who reported that the LH, FSH, testosterone were significantly decreased in mice. The fertility and libido were lost after treatment with cadmium chloride 5mg/kg Bwt and decreased after treatment with 2mg/kg Bwt, in mice. Such effects could be the result of vascular damage of the testes and the leydig cells, **Nishiyama and Nakamura (1984)**. The pollution with cadmium adversely affected the fertility and libido of the exposed animals.

The ALT, AST, urea, creatinine, Na⁺, K⁺ and cad⁺ concentrations were significantly increased. This may be attributed to the necrosis of both kidney and liver. These results are coincident with **Gabiani et al., (1974)**, **Mamkiewicz et al., (1975)**, **Ferguson (1980)**, **Berraw and Deaves (1984)**, **Adriana (1986)**, **Abu Salem (1991)**, **Mansi et al., (1993)**, **Bryant and Rose (1995)** and **Fiberg et al (1996)**. Moreover total protein decreased probably due to necrosis of liver cells.

It could be concluded that cadmium toxicity markedly suppressed the LH, FSH and Testosterone and caused degeneration of testes. On the other hand, it caused atrophy of the liver, kidney, which showed renal calcification due to deposition of calcium.

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In vitro Effect of Pomegranate Peel Extract on *Trichomonas tenax*

Gehad T. El-Sherbini¹ and Nahla M. Shoukry²

¹ Department of Parasitology, Faculty of Pharmacy, October 6 University Cairo, Egypt.

² Department of Zoology, Faculty of Science, Suez Canal University, Suez, Egypt.
nahla_shoukery@yahoo.com and gody_55@yahoo.com

Abstract: The incidence of *Trichomonas tenax* (*T. tenax*) in patients with acute ulcerative gingivitis has been demonstrated in several published reports. Metronidazole was known as the most effective drug for human trichomoniasis, however, drug resistance and toxicity appeared. This study was designed in vitro to investigate the inhibitory activity of *Punica granatum* (*P. granatum*) ethanol extract on the growth and motility of *T. tenax* in comparison to metronidazole. Pomegranate ethanol extract group was treated with concentrations of 12.5, 25, 50, and 100 µg/ml. Metronidazole group and blank control were included. At 12 h, 24 h, 48 h and 72 hr after drug treatment, the anti-*T. tenax* effect of pomegranate ethanol extract was tested by microscope counting method. The results showed 60% motility of *T. tenax* trophozoite after treatment with 12.5 µg/100 ml of pomegranate ethanol extract group and 25 µg/ml showed higher anti-*T. tenax* ($P < 0.01$). The ethanol extract of pomegranate peel has a remarkable effect on *T. tenax*, and among the groups, 60% ethanol extract shows the best anti-*T. tenax* activity.

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Keywords: *Trichomonas tenax*, *Punica granatum*, Metronidazole, Herbal medication

1. Introduction

Serum The human oral cavity is home to numerous microorganisms. *Trichomonas tenax* (*Trichomonas buccalis*) is a regular guest of human oral cavity microorganism (1). It is an anaerobic species that lives as a commensal in the mouth of humans' oral cavity. It is frequently associated with pyogenic organisms in pus pockets or at the base of teeth. There are studies relate to its prevalence in patients with Marginal Chronic Periodontitis (2). Transmission is through saliva, droplet spray, and kissing or use of contaminated dishes and drinking water (3). World widely, its prevalence in the mouth ranges from 4 to 53% (4,5).

The detection of *T. tenax* in the human oral cavity is an indication of poor oral hygiene, so that its incidence increases significantly in patients with periodontal problems, this being three to four times higher than in periodontal healthy subjects. (6)

Since the organism is believed to enter the respiratory tract by aspiration from the oropharynx and then cause bronchopulmonary trichomoniasis, the importance of oral infections has been increased (7).

The development of drug resistance in human pathogens against commonly used treatment has necessitated a search for new therapeutic agents from other sources. Recently, there has been considerable interest in the use of plant materials as an alternative method to control pathogenic microorganisms (8, 9). Many compounds of plant products have been shown to be specifically targeted against resistant pathogens (10).

Punica granatum, which belongs to the family of Punicaceae, is commonly known as pomegranate, grenade, granats and punica apple (11). *P. granatum* has been used extensively as a traditional medicine in many countries (12) for the treatment of dysentery, diarrhea, helminthiasis, acidosis, hemorrhage and respiratory pathologies (13,14) In addition, *P. granatum* is reported to have antioxidant (15,16) anti-atherosclerotic (17,18), antibacterial (19,20), and antiviral (21) properties. The constituents of *P. granatum* include gallo catechins, delphinidin, cyanidin, gallic acid, ellagic acid, pelargonidin and sitosterol, which are very well known for their therapeutic properties (22). *P. granatum* peel is used to treat infections found

in human sexual organs as well as mastitis, acne, folliculitis, pile, allergic dermatitis, tympanitis, scalds, diarrhea, dysentery and as an antioxidant. In addition, it is reported that the extracts of *P. granatum* have antimicrobial activity against *Salmonella* (23).

Pomegranate components have properties that could promote oral health, including reducing the risk of gingivitis. However, to date, no studies regarding the anti-*T. tenax* activity of *P. granatum* extract have been conducted. Therefore, the goal of this study is to evaluate the anti-*T. tenax* activity of the extracts of *P. granatum* peel *in vitro*.

2. Materials and Methods

Patients

The periodontiums of 51 patients were clinically examined, and diagnosis and classification of the periodontium was done according to the Periodontal Screening and Recording (PSR) I, in agreement with the Military Academy of Periodontology and Dental Association criteria. Twenty patients were diagnosed with gingivitis (EG1), 22 with periodontitis (EG2) and 9 presented a healthy periodontium (CG). The patients were also asked about the use of medications and systemic conditions which might predispose them to the development of periodontal disease.

Sample collection

Samples of saliva and dental biofilm/calculi were collected from all patients in the morning, before any oral hygiene. After determining the frontal mandibular area most affected by periodontal disease (by means of PSR), dental biofilm/calculi samples were collected by scraping the area with sterile periodontal curettes. Unstimulated saliva samples were collected as recommended by Navazesh (24). All samples were placed in sterile Petri dishes and diluted with saline at room temperature (25 to 28°C). Immediately after dilution, the samples were examined under a light microscope.

The removal of dental calculus, as well as debris and plaque was performed according to (24). Dental calculus removed from the experimental group was ground prior to planting and microscopic observation using previously sterilized glass rods.

Transport of samples:

The samples were placed in vials containing transport medium (sterile Ringer solution) and taken to the laboratory for further planting and microscopic observation.

Inoculation of the samples:

Once the samples were taken to the laboratory, 0.1 ml of Ringer's solution containing the inoculum was inoculating in the broth selective Kupferberg, used for the growth

of *T. tenax* ("Kupferberg Trichomonas Broth. "Difco Laboratories, Detroit, Michigan, USA), to which 0.1 g of chloramphenicol was added to prevent the growth of bacteria and other microorganisms. Two plantings were made for each patient, an aerobically and anaerobically, using the jug designed for this purpose (Gas Pak). Seeded culture media were taken to the oven at 37 ° C for 72 hours.

Microscopic Observation:

For the identification of *T. tenax* microscopic observations were made three times for each patient in order to determine what opportunities exist in the ability to view the scourge. These observations were made in the first instance on the same day of sampling, taking a drop of inoculum containing transport medium with the previously sterile platinum loop and placed onto a glass slide. The two remaining microscopic observations were performed at 72 hours of selective media incubated in an oven, taking a drop in the previously sterile platinum loop, both the stock that was planted under aerobic conditions as was shown in the anaerobic placing in each case on the surface of its respective blade slide.

In each of the above described cases, samples were examined microscopically using the light microscope (Leitz), focusing first with a low magnification lens (10 x) and then with higher magnification lenses (20 x 40 x).

Extraction of plant material

Preparation of the Plant Extract

Fresh pomegranates (500 gram) were obtained (in order to prepare fresh extraction) from a public market. The peels of pomegranate were separated and oven dried at 33°C for 7 days. The dried peels were powdered in an electric grinder and stored in plastic bags for the next step. A sample of 100 gm powder was extracted using 200ml ethanol (99.9%) in an electric blender for 30 min. This suspension was filtered three times per day for 30 days. New methanol was used each time. Then methanol was removed in a rotary evaporator to produce a dry powder. The final material was dissolved in ethanol for obtaining concentrations of 12.5µg/ml, 25µg/ml, 50µg/ml and 100 µg/ml of dry plant powder (23, 24, 25).

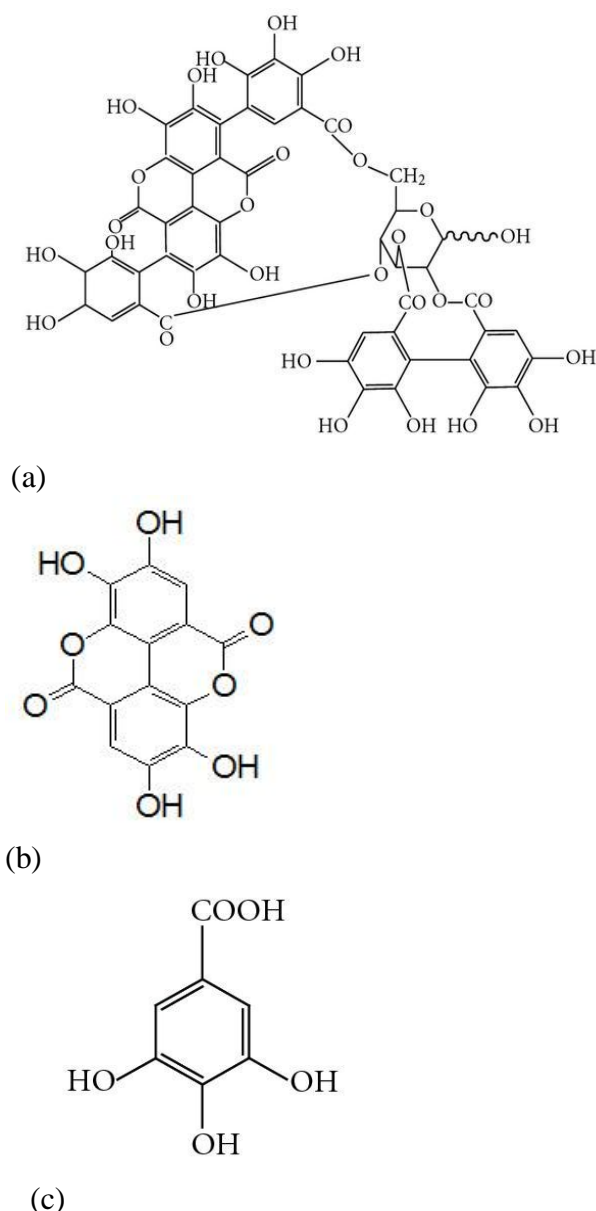


Fig. 1: The chemical structure of *P. granatum* peel: punicalagin (a), ellagic acid (b) and gallic acid (c)

Metronidazole

It was supplied as 500 mg tablets (Rhone Poulenc Rorer, France).

Tablets were dissolved in distilled water, and then diluted in incubation medium to yield 12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml (26).

Growth inhibition Assay

The effect of *P. granatum* on the growth of the *T. tenax* trophozoites was studied as follows: 2x10⁵ trophozoites were incubated in Selective Kupferberg (KTB) medium with different concentrations of *P. granatum* and metronidazole (12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml), for 12, 24, 48, and 72 h at 37°C. In addition, controls were included (cultures containing only

the parasites) and submitted to the same procedures used for the experimental cultures.

Evaluation of the drug efficacy was done by:

1. Counting the number of trophozoites using the haemocytometer (Neubauer cell-counter chamber).
2. Calculation of the percent of inhibition of multiplication according to the equation:
Percent inhibition of growth = $\frac{a-b}{a} \times 100$

Where;

a=Mean number of trophozoites in control tubes and

b= Mean number of trophozoites in test tubes [27].

3. Calculation of the percent of motility of trophozoites which is the ratio of motile to total number of parasites counted per 10 high power field (HPF).
4. The minimal lethal concentration (MLC) of *P. granatum* extract and metronidazole was determined.

3.Results

The flagellate in some cases move in a circular motion and other times not moving and isolated. Usually, the flagellar morphotypes of *T. tenax* can be seen with four flagella, but sometimes can be seen only with one or two flagella (Fig.2). Growth of *T. tenax* in culture medium is demonstrated in fig 3&4.

The present study was carried out to investigate in vitro the activity of Ethanol Extract of *P. granatum* Peel (EEP GP) on the growth and motility of *T. tenax*, compared to the standard drug metronidazole. The results showed that the degree of growth inhibition was dependent upon the concentration of *P. granatum* and metronidazole.

Effect of *P. granatum* extract in specified times and concentrations on *T. tenax* was assessed. The inhibitory effect of extract on *Trichomonas* was assessed by counting the alive parasites 12h, 24 h, 48h and 72 hours after exposure with extracts.

Findings of this study showed that ethanol extract *P. granatum* at concentration of 12.5, and 25 µg/ml resulted in motility percentage of the parasites 60% & 40% after 12h respectively and 50% & 10% after 24h of exposure respectively. Lower dose of ethanol extract *P. granatum* (12.5 µg/ml) showed complete inhibition of growth after 48 hours (Table 2).

Both of *P. granatum* and metronidazole were able to inhibit the motility of the parasite with increasing percent of immotile trophozoites after 72 hr.

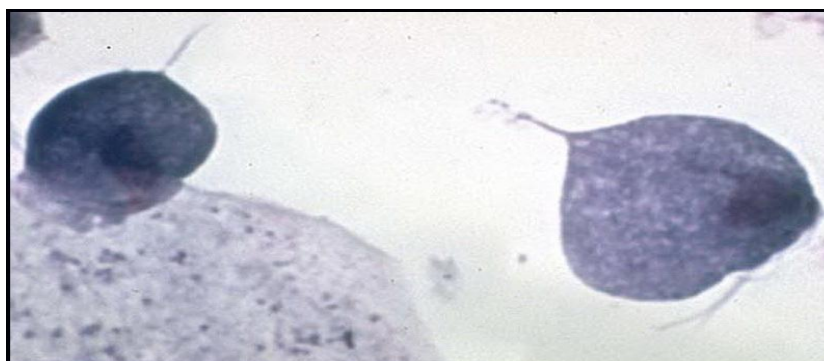
Table 1: Motility percentage of *T. tenax* after exposure to various concentrations of metronidazole in comparison to normal control

Concentration ($\mu\text{g/ml}$)	% of motility			
	12 hr	24 hr	48 hr	72 hr
12.5	98%	80 %	60 %	non motile
25	80%	70 %	20 %	non motile
50	30%	10 %	no motility	non motile
100	No organism	No organism	No organism	No organism
NTC	98%	85 %	50%	non motile

NTC = Non Treated Culture Control

Table 2: Motility percentage of *T. tenax* after exposure to various concentrations of *P. granatum* ethanol extract in comparison to normal control

Concentration ($\mu\text{g/ml}$)	% of motility			
	12 hr	24 hr	48 hr	72 hr
12.5	60 %	50 %	Non motile	No organism
25	40 %	10 %	No organism	No organism
50	20%	No organism	No organism	No organism
100	No organism	No organism	No organism	No organism
NTC	100%	98 %	85%	50 %

**Fig. 2:** *Trichomonas tenax***Fig.3:** *T. tenax* by light microscope, evidencing a flagellum (40x).

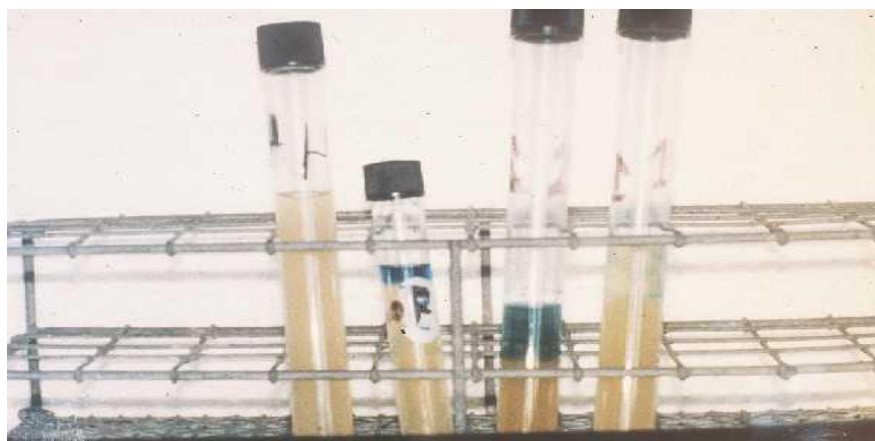


Fig.4: Growth of *T. tenax* in culture medium



Fig 5: Selective Kupferberg (KTB).

4. Discussion

In the recent years, the use of plants with preventive and therapeutic effects contributes to health care needs (28).

There are three main reasons for interest in the treating and healing power of plant extracts. First, pharmacological studies have demonstrated that many of plants are known to possess antimicrobial agents; second, people are becoming aware of the side effects associated with the over prescription of traditional antibiotics; third, time to time resistant microorganisms against antibiotics are increasing. Among these plants, *P. granatum* has an important role in folk medicine. omegranate is known as a rich source of pharmacological properties which have been evaluated due to antiparasitic, antibacterial, antifungal, antiproliferative, apoptotic and anti-cancer effects as well as protection against herpes virus, inhibition of LDL oxidation and decrease in atheromatous plaque formation and reduction of systolic blood pressure (29, 30, 31).

While the vast majority of studies of oral Microbiology relate to various aspects of bacteriology, buccal Protozoology has virtually

forgotten, despite the high prevalence of infections in adults where *T. tenax* etiologic agent. (32)

In this regard there are many publications that reported the complexity of the microbiota residing in dental plaque, not only in quantitative terms but also qualitative including bacteria, fungi, mycoplasma and protozoa, among them *T. tenax* was observed in the oral cavity (33, 34).

Microscopic observation in the cool of the samples from dental calculus and subgingival plaque by using light microscopy is essential if you want to achieve faster discovery *T. tenax*, being able to visualize usually with one or two flagella. However, provided samples should be inoculated in the most appropriate culture media and allow accurate identification of this species. (35, 36)

Conclusions

The incidence of *T. tenax* was higher in patients and this study suggests that EEPGP might be used as an antiparasitic agent in controlling oral *T. tenax* infections.

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Conflict of interest: None declared.

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First Isolation and Identification of Ovine Herpesvirus 2 Causing Malignant Catarrhal Fever Outbreak in Egypt

Iman M. Bastawecy¹ and Abd El-Samee, A.A.²

¹Dept. of Virology, Animal Health Research Institute, Dokki, Giza

²Dept. of Int. Medicine and Infectious Diseases, Faculty of Vet. Med. Cairo University

imanbaz@hotmail.com

Abstract: Ovine herpesvirus 2 (Ov HV-2) was isolated for the first time from cattle and water buffalos during an outbreak of malignant catarrhal fever (MCF) in Egypt, 2012. The isolated virus was characterized as herpesvirus with negative staining electron microscopy (EM). Further identification using polymerase chain reaction (PCR) and nucleotide sequencing of the PCR product. GenBank confirmed it as ovine herpesvirus 2, complete genome with query coverage 100% and maximum identity 100% and ovine herpesvirus 2 strain BJ 1035, complete genome with query coverage 100% and maximum identity 99%. Separation of susceptible animals from sheep and goats specially during lambing is recommended and euthanasia of animals which were clinically infected with MCF is advised.

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Keywords: Ovine herpesvirus 2, malignant catarrhal fever, isolation, electron microscopy, sequencing.

1. Introduction

Malignant catarrhal fever (MCF) is an acute, highly fatal, infectious disease of domestic cattle, buffaloes, many wild ruminant species (Anthony and Werner, 1992) and pigs (Bremer, 2010).

Alcelaphine herpesvirus 1 (Al HV-1) and ovine herpesvirus 2 (OvHV-2) belongs to subfamily Gammaherpes virinae. They are the causative agents of MCF. These viruses were previously included in the genus Rhadinovirus, but have been reassigned recently to the genus Macavirus (Maclachlan and Dubovi, 2011) Latent infection of Gammaherpes viruses occurs in lymphoid tissue (Brooks *et al.*, 1998)

In South Africa, approximately 90% of MCF polymerase chain reaction (PCR)-positive cases are associated with Al HV-1 and only 10% with OvHV-2 (Bremer, 2010). In USA, the prevalence of OvHV-2 in adult sheep was 99% as determined by nested PCR (Li *et al.*, 1995). OvHV-2 – associated disease occurs throughout the world (OIE, 2008).

The disease is characterized by the development of an erosive stomatitis, erosions in the upper respiratory tract, gastroenteritis, keratoconjunctivitis, encephalitis, cutaneous exanthema and lymph node enlargement. It may occur sporadically or in explosive outbreaks (Blood *et al.*, 1983) where epizootics of acute high-morbidity syndrome may occur (Katz *et al.*, 1991).

The epidemiology of the two major types of MCF viruses within their natural well adapted hosts differs significantly. Whereas intense virus shedding from the wildebeest occurs predominantly during the first 90 days of life, lambs do not shed virus until

after 5 months of age. Wildebeest – associated MCF of cattle occurs most frequently in Africa during the wildebeest calving season whereas the sheep-associated form of MCF occurs year – round in cattle with a modestly increased incidence during the lambing season (Maclachlan and Dubovi, 2011).

The excreted infectious virus can be transmitted from carriers to clinically susceptible hosts through nasal and ocular secretions by direct contact, or by poorly defined air borne routes. Mechanical vector or contaminated feed or water plays a role in transmission (Brown and Torres, 2008).

Clinically, a presumptive diagnosis of MCF can be made when nasal and ocular lesions are observed with a persistent high temperature, enlargement of the peripheral lymph nodes and terminal encephalitis particularly with a history of exposure to sheep, goats, antelope or wildebeest during parturition (Radostits *et al.*, 2000).

Identification of MCF virus can be made by immuno-fluorescent (IF) staining of infected cells. Virus neutralization test (VNT) provides the most reliable method of specific virus identification. Electron microscopy may be used to morphologically identify herpesviral particles. (Anthony and Werner, 1992).

Alcelaphine herpesvirus-2 could be isolated in monolayer cultures of ruminant origin but OvHV-2 has never been identified formally (OIE, 2008) although it could be isolated by Schuler *et al* (1990). Also, OvHV-2 has been transmitted experimentally to rabbits and hamsters which develop lesions characteristic of MCF (OIE, 2008).

Viral DNA has been detected in clinical material from cases of MCF caused by both A1 HV-1 and OvHV-2 using the polymerase chain reaction (PCR), and this is becoming the method of choice for diagnosing the OvHV-2 form of the disease (OIE, 2008).

Serological tests as VNT, IF, complement fixation test, agar gel precipitation test (Anthony and Werner, 1992) and competitive inhibition enzyme linked immunosorbent assay (OIE, 2008) could be used to identify animals infected with MCF virus but some of which may be asymptomatic virus carriers such as sheep and goats. Those animals clinically affected with MCF that die actually may lack detectable serum antibodies. The aim of the present study is the accurate diagnosis of suspected cases to be infected with MCF and with history of exposure to sheep during lambing. They are assumed to be infected with OvHV-2. Trials for isolation of the causative virus were attempted with identification using negative staining EM, PCR and nucleotide sequencing of the PCR product.

2. Material and Methods:

Animals:

Foreign breed cows and water buffalos of all ages and both sex were subjected for this study. They belonged to Gharbia, El-kalyobia and Alexandria governorates, Egypt. These animals were suffering from fever (41-41.5 C), rapid pulse rate (100-120/min), and clinical signs as nasal and ocular discharges, erosions in nasal and oral mucosae, excessive salivation of ropey and bubbly saliva, hyperemia and edema of conjunctiva, injection of scleral vessels, bilateral corneal opacity, arthritis severe inflammation of the hoof, lameness, trembling, in-coordinated gait and sometimes nystagmus.

Samples:

Tongue epithelia were collected from the affected animals for isolation and identification. Samples were submitted to the laboratory on ice without delay.

Cell Culture:

Madden Derby Bovine Kidney (MDBK) cell culture was provided by Virology Department, Animal Health Research Institute, Dokki, Giza, Egypt and subjected for isolation.

Diagnostic Methods:

Isolation:

Samples were subjected for inoculation onto MDBK cell culture according to Anthony and Werner (1992). The cells were maintained in Eagle's essential medium containing 2% fetal calf serum, 100 mg of streptomycin per ml and 100 IU of penicillin per ml. The inoculated cultures were incubated at 37

C. Cell cultures should be examined for cytopathic effect (CPE) for 5 to 10 days. If no CPE is detected, cultures should be frozen and thawed 3 times and used for inoculation up to 3 blind passages.

Negative staining electron microscopy:

Negative staining EM was conducted according to Payment and Trudel (1993). Supernatants of the tongue epithelia and suspensions of the inoculated MDBK cell cultures showing CPE were mixed with a droplet of 3% phosphotungstic acid (PTA). A copper grid coated with carbon formvar was dipped into the mixture. After drying, the grid was examined by EM.

Polymerase Chain Reaction and Sequencing of PCR Product:

The PCR products were constructed and sequenced by Macrogen, Southern Korea and PCR study was performed according to Dunowska *et al.*, (2001). Viral DNA was extracted from the tongue epithelia of affected animals and infected MDBK cells. A set of primers for OvHV-2 (glycoprotein B) were

	ORF8	F	5'-
			GGGCCTTTATCTAACGTATGAGA-3'
	ORF8	R	5'-
			TCACAATGCAAACACTTATGAGTAA-3'

Reaction conditions for PCR were 94 C/2min. (1X). 10 cycles were performed (94 C for 10 seconds, 60 C for 30 seconds, 72 C for 2 minutes), followed by 20 cycles with the same denaturation and annealing conditions, but with 5 sec. added to each successive elongation cycle and a final elongation step (72 C for 7 min). The PCR products were electrophoresed and subjected for nucleotide sequencing.

3. Results:

Animals:

The tested animals showed erosions on the tongue (which were local or diffuse and the epithelium was fragile leaving eroded and hyperemic surface), pressed head, corneal opacity (Fig. 1), teats with dry tenacious scabs (Fig. 2) and ulcers on the gum below the incisors (Fig. 3).

Isolation:

The inoculated MDBK cell cultures revealed CPE characterized by formation of multinucleated syncytial giant cells which degenerate rapidly by contraction and rounding followed by detachment from the surface (Fig. 4).

Negative Staining Electron Microscopy:

Herpesviral particles were detected in supernatants of tongue epithelia and suspensions of inoculated MDBK cell cultures revealing CPE and subjected for EM (Fig. 5).

PCR and Sequence Analysis of PCR Products:

Supernatants of tongue epithelia and suspensions of inoculated MDBK cell cultures which showing CPE and revealed herpesvirus particles in negative staining EM, revealed PCR positive for OvHV-2 which when sequenced, they revealed OvHV-2, complete genome with query coverage

100% and maximum identity 100% and OvHV-2 strain BJ1035, complete genome with query coverage 100% and maximum identity 99% (Fig. 6) using NCBI software when they were submitted to GenBank.



Fig. (1): Cow showing pressed head, excessive salivation of ropey and bubbly saliva, corneal opacity, and frayed tongue epithelium leaving hyperemic and eroded surface.



Fig. (2): Teats with dry tenacious scabs.



Fig. (3): Cow showing ulcers on the gum below the incisors.

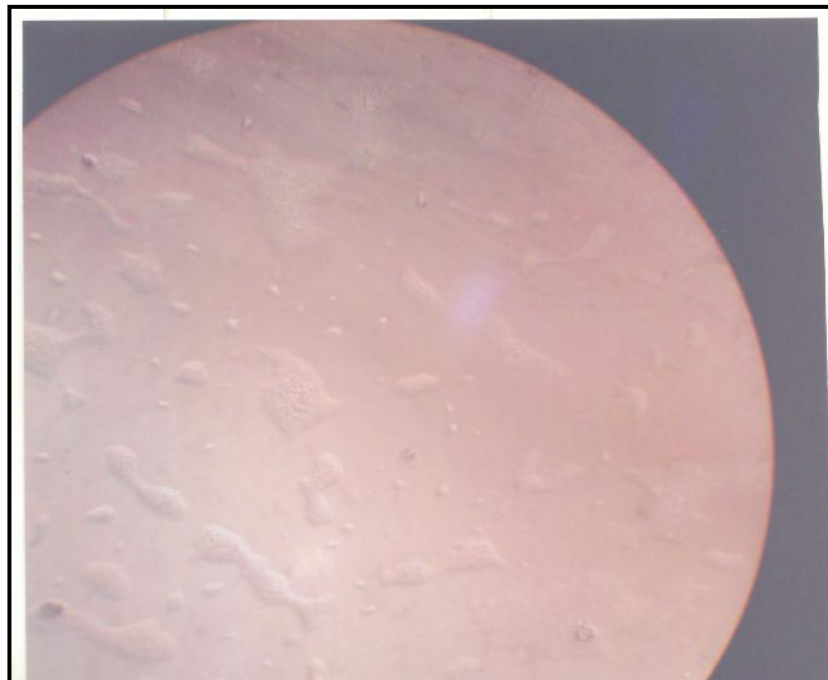


Fig. (4): Inoculated MDBK cell culture revealed CPE characterized by formation of multinucleated syncytial giant cells.

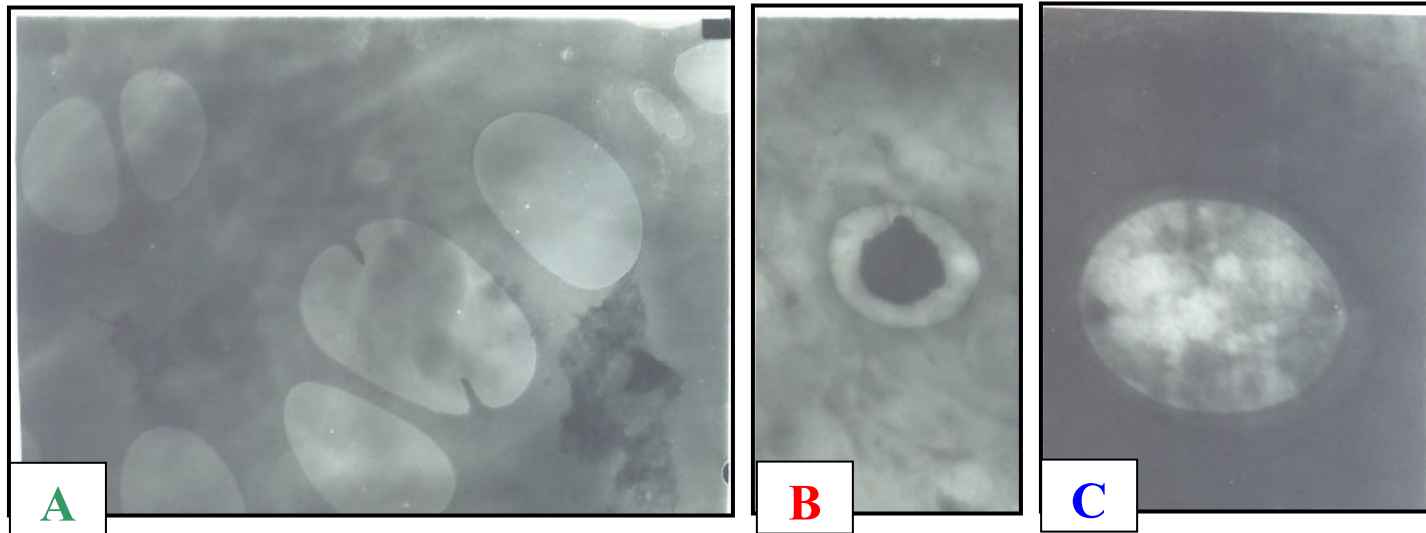


Fig. (5): Electron micrographs showing:
 (A): An intact, negatively stained herpesviral particles, the intact envelop is not permeable to negative stain. (35.000 X)
 (B): Herpesviral particle showing envelop surrounding cubic capsid. (21.000 X)
 (C): Capsid containing DNA permeated with negative stain and appears as thread-like structures on the surface of the core. (56.000 X)

Descriptions

Legend for links to other resources: **U** UniGene **E** GEO **G** Gene **S** Structure **M** Map Viewer **PubChem** BioAssay

Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
DQ198083.1	Ovine herpesvirus 2, complete genome	3.285e+04	3.285e+04	100%	0.0	100%	
AY839756.1	Ovine herpesvirus 2 strain BJ1035, complete genome	3.212e+04	3.220e+04	100%	0.0	99%	
JN133502.1	Bovine herpesvirus 4 strain V.test long unique region, complete sequence	58.4	58.4	0%	0.002	100%	
AF318573.1	Bovine herpesvirus 4 long unique region, complete sequence	58.4	58.4	0%	0.002	100%	

Fig. (6): Result of neocleotide sequencing submitted to Gen Bank illustrated OvHV-2, complete genome with query coverage 100% and maximum identity 100% and OvHV-2 strain BJ1035, complete genome with query coverage 100% and maximum identity 99%.

4. Discussion:

A tentative diagnosis of MCF can be made based on clinical signs and a history of contact with sheep, goats or alcelaphine especially during the period of parturitions in these species (Anthony and Werner, 1992).

Ovine herpesvirus 2 was suspected to be the etiology of MCF outbreak in Egypt since there is no evidence of contact for cattle and buffalos with wildebeest but all the infected animals were in contact with sheep and goats during lambing. Cattle and buffalos developed clinical signs characteristic for MCF, ranged from mild to sever even sudden death especially in calves due to the disease occurs in a number of forms, the peracute, the alimentary tract form, the common "head and eye" form and the mild form, but these are all gradations. Cases being classified on the prominent clinical signs where serial transmission with one strain of the virus all of these forms may be produced as mentioned by Blood *et al* (1983).

Previous investigations among dairy cattle supports our observation in the disease severity of the susceptible animals showed that although OvHV-2 infection was positively associated with the development of MCF, not all OvHV-2 positive cattle developed disease (Dunowska *et al.*, 2001). Another observation was the continuous of cases to occur for months because of the long incubation period (OIE, 2008) and the greatest incidence of the disease is

during late winter, spring and summer months (Blood *et al.*, 1983).

We found that buffalos are more susceptible and these results agreed with OIE (2008) because OHV-2 appears to fit an emerging pattern in which a mild or subclinical infection with herpesvirus that is lethal for other animal species provides an advantage not to the virus, but also to its host (Brown, 1997) where morbidity and mortality are according to the species (Bratanich *et al.*, 2012).

Our trial for isolation of the etiology of MCF was succeeded although most references mentioned its difficulty for isolation but some stated that lymphoblastoid cell lines propagated from affected animals contain OvHV-2 specific DNA and virus particles have been observed in these cells (OIE, 2008). Due to most monolayer cultures of ruminant origin are probably susceptible and develop CPE (OIE, 2008), we use MDBK cell cultures for isolation. The causative agent was identified as herpesvirus using negative staining electron microscopy as mentioned by Anthony and Werner (1992). EM has the advantages of ease for sample preparation and rapid analysis (same day result) and the undirected "open view" of electron microscopy allows rapid morphologic identification and differential diagnosis of different agent present in the specimen (Hazelton and Gelderblom, 2003; Bastawecy *et al.*, 2007). Because of this capability, EM must be a frontline method (Green *et al.*, 2002).

This step of identification excludes infection with foot and mouth disease (FMD) which is suspected due to presence of oral and hoof lesions.

Further identification was performed with PCR and sequencing. Primers used in ORF 75 (coded for tegument protein) PCR do not react with A1 HV-1 (Li *et al.*, 1994). However, they may not react with all isolates of OvHV-2 and furthermore, they may be able to cross react with viruses similar to OvHV-2 (Dunowska *et al.*, 2001).

Glycoprotein B (encoded by ORF 8) is one of the most conserved herpesvirus glycoproteins (Pereira, 1994). It plays a role in virus entry and spread between cells. The gB sequence has been used for estimating phylogeny between herpesviruses and it is predictive of the more accurate phylogenetic relationships based on the analysis of several conserved genes (Mc Geoch *et al.*, 1995). These primers were designed on the partial OvHV-2 sequence from ORF 6 to ORF 9 determined (Dunowska *et al.*, 2001).

Neocleotide sequence obtained in the present study when it was submitted to the GenBank, it confirmed it as ovine herpesvirus 2, complete genome with query coverage 100% and maximum identity 100% and ovine herpesvirus 2 strain BJ 1035, complete genome with query coverage 100% and maximum identity 99%. Our results are in agreement with Mc Geoch *et al.* (2000) who mentioned that herpesviruses appear to co-evolve with their host and thus diverge over time for alphaherpesviruses and betaherpesviruses but less clear for members of Gamma herpesvirinae.

On conclusion, the current study illustrated that OvHV-2 was responsible for MCF outbreak in Egypt, 2012 as sheep associated form. When we have less experience with disease as MCF which may be misdiagnosed with other diseases, negative staining electron microscopy is recommended as frontline to give "open view" along with trials for isolation and confirmation with PCR and sequencing. Because of the field observation that sheep and goats are important (potential reservoir species) in the spread of the disease especially during lambing, separation of susceptible animals from them is recommended. Since animals clinically infected with MCF may remain virus carriers, euthanasia of animals which were clinically infected with MCF is advised.

Corresponding author

Iman M. Bastawecy

Dept. of Virology, Animal Health Research Institute, Dokki, Giza

imanbaz@hotmail.com

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Feed and Water Consumptions, Digestion Coefficients, Nitrogen Balance and Some Rumen Fluid Parameters of Ossimi Sheep Fed Diets Containing Different Sources of Roughages

Hamed A.A. Omer, Mohamed A. Tawila and Sawsan M. Gad

Animal Production Department, National Research Center, Dokki, Giza, Egypt
hamedomer2000@yahoo.com

Abstract: Five digestibility trials were carried out to investigate the effect of different roughage sources on feed and water intakes, water metabolism, nutrient digestibility coefficients, nitrogen utilization and some rumen fluid parameters. Twenty mature male Ossimi sheep were randomly assigned to five experimental groups (four animals in each treatment). Animals were fed on 3% DM of live body weight and received one of the following diets. All the experimental diets contained 50% concentrate feed mixture (CFM) plus 50% roughage. Control diet contained berseem hay (BH), while the other four experimental diets were replaced BH in control diet by peanut vein hay (PVH); beans straw (BS); kidney beans straw (KBS) or linseed straw (LS). Results showed that, source of roughage were affected on the chemical composition of the experimental diets. Diet contained BH showed the highest value of CP (15.69%), followed by PVH and LS diets (14.52 and 14.23%, respectively). On the other hand CP content of BS and KBS diets were in the same range (12.65 and 12.42%). Beans straw recorded the highest value of neutral detergent fiber (53.16%), acid detergent fiber (37.84%) and cellulose contents (28.30%), however, PVH diet showed the lowest value of ADL (7.30%). Inclusion PVH, BS, KBS and LS in sheep diet significantly increased ($P<0.05$) feed consumption as DM, TDN, CP and DCP intakes compared to the BH containing diet. Sheep received BS diet significantly increased ($P<0.05$) drinking water (4650 ml/h/day), total water intake (4829 ml/h/day) compared to the other diets. Inclusion different sources of roughage in sheep diet had no significant effect on DM digestibility. While, sheep received KBS diet significantly ($P<0.05$) increased OM, CP and CF digestibilities compared to the other different diets. Introduce PVH, BS, KBS and LS in sheep diets significantly decreased ($P<0.05$) total digestible nutrient and digestible crude protein values compared to the control diet. All groups were positive nitrogen balance and sheep received LS diet recorded the highest values of nitrogen retention (21.7 g) and nitrogen retention % of digested nitrogen (81.61%) compared to the other diets. Dietary treatments significant affected ($P<0.05$) on pH value, ammonia nitrogen ($\text{NH}_3\text{-N}$) and total volatile fatty acids (TVFA's) concentrations. Three hrs post feeding significantly ($P<0.05$) decreased ruminal pH value, while, it significantly ($P<0.05$) increased both ruminal $\text{NH}_3\text{-N}$ and TVFA's concentrations. It could be concluded that we can using alternative sources of roughage successfully in sheep diets as a good sources of roughages instead of berseem hay with better feed intake, digestion coefficient, nitrogen utilization and ruminal fermentation. Also we can use the tested materials to formulate cheap diets for sheep.

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Keywords: Sheep, roughage sources, feed and water intakes, water metabolism, digestibility coefficients, nitrogen balance, ruminal fermentation.

1. Introduction

Ruminant species occupy an important niche in modern agriculture because of their unique ability to digest certain feedstuffs, especially roughages, efficiently. In future, the direct demands for grain by human beings will make efficient utilization of roughages increasingly important. A basic understanding of ruminant digestion is essential for good management and sound nutrition of beef cattle, sheep and goats (Visser, 2005).

One of the important limiting factors for animal production in Egypt is the availability of feedstuffs. Locally produced feeds are not sufficient to cover the nutritional requirements of livestock (Abou-Akkada, 1988).

In Egypt, the total area planted by clover hay reached about 2 million feddans (EMA, 2003). Recently, according to the national policy, the berseem area was decreased to increase the wheat area. Using non-traditional feed in animal feeding led to some advantages such as participates in solving the problem of feed shortage, decrease the cost of feeding and alleviate the pollution problems (Abdel-Magid et al., 2008).

Recently, the agricultural policy in Egypt aimed to increase the area cultivated by strategically crops on behalf of that cultivated by berseem. At the same time, several crops such as chick pea, pea, peanut, beans, kidney beans, linseed, lentil and others are cultivated in the newly reclaimed lands. So, significant amounts of

the straws of these crops are produced annually as residues, about 25 thousand tons from chick pea straw (EMA, 2003) and 13 thousand tons from pea straw (AIEG, 2005).

Groundnut vines hay is one of the important feed resources grows in new reclaimed sandy soil. A vast amount 35 thousand tons from groundnut vines hay is produced annually as by-products (AIEG, 2005). Groundnut vines hay had been demonstrated as good animal feed for calves (Ahmed and Pallot, 1979), goat (Gelaye et al., 1990) and sheep (Awadalla et al., 1997; Etman and Soliman, 1999; Talha, 2001; Talha et al., 2001; Mahmoud et al., 2003 and Talha et al., 2005). All explored good response in animals performance when including it in their rations to replace almost other leguminous hay. Mahmoud et al. (2003) found no significant difference in feed intake, nutrients digestibility, daily gain and feed conversion with groundnut vines hay in contrast with alfalfa hay. Moreover, feeding groundnut vines hay was more economic.

On the other hand, several researches have shown that these straws such as chick pea straw, pea straw, peanut vein hay, beans straw, kidney beans straw, linseed straw, lentil straw and others had considerable amounts of nutrients that of suitable digestibilities (Etman and Soliman, 1999; Tawila 1999; Talha 2001; Talha et al., 2001; Mahmoud et al., 2003; Talha *et al* 2005 and Abdel-Magid 2005).

Several studies have investigated the effects of roughage source and/or level on DMI and performance by feedlot cattle fed high-concentrate diets, literature data make it clear that roughage source and level can have substantial effects on DMI by cattle fed concentrate diets (Defoor et al., 2002 and Galyean and Defoor, 2003) and El-Bedawy et al. (2004a) & (2004b); Abdel-Magid et al. (2008) and Khorshed (2008) with sheep.

The effect on dry matter intake of adding a concentrate supplement to forage depends on the digestibility of the forage. Concentrate added to forage of low digestibility tends to be consumed in addition to the forage, but when added to forage of high digestibility it tends to replace the forage (McDonald et al., 1995).

Roughage play a major role as feed for ruminants, also, seasonal patterns affect the availability and quality of the roughages, particularly during the dry season (Wanapat, 1999).

It is evident from the literature that forage or roughage alone can not supply sufficient energy especially for high producing animals, therefore concentrate supplementation is always needed for maximizing intake and consequently improving overall performance of ruminant animal (Morita et al., 1996).

Ruminal ammonia nitrogen ($\text{NH}_3\text{-N}$) has been reported to be an important compound in supporting efficient rumen fermentation and it is the major nitrogen source for microbial protein synthesis and growth (Wanapat and Pimpa, 1999). Perdok and Leng (1990) found that a higher level of ruminal $\text{NH}_3\text{-N}$ (15-20 mg %) increased dry matter intake and digestibility in cattle fed with low quality roughage. Erdman et al. (1986) found that a higher level of $\text{NH}_3\text{-N}$ can increase the rate of fermentation *in vivo*, depending on the potential fermentation of feed. Paengkoum et al. (2010) noticed that TVFA's was affected by type of roughage.

The objective of this study was carried out to evaluate the effect of using some agriculture by-products (peanut vein hay, beans straw, kidney beans straw and linseed straw instead of berseem hay in the diets as sources of roughage on feed and water consumption, digestion coefficients, nitrogen balance and ruminal fermentation.

2. Materials and Methods

Twenty mature male Ossimi sheep of about 52 kg live body weight approximately on average were used to investigate the effect of different roughage sources on feed and water intakes, water metabolism, nutrient digestibility coefficients, nitrogen utilization and some rumen fluid parameters. The animals were randomly assigned to five experimental groups (four animals in each treatment).

Experimental diets

Sheep fed diets contained 50% of concentrate feed mixture (CFM) that composed of 45% yellow corn, 35% undecorticated cotton seed meal, 17% wheat bran, 1.5% lime stone, 1% sodium chloride and 0.5% mineral and vitamins mixture. The five experimental groups were considered as the following:

G₁: 50% berseem hay+50% concentrate feed mixture. (BH).

G₂: 50% peanut vein hay+50% concentrate feed mixture. (PVH).

G₃: 50% beans straw+50% concentrate feed mixture (BS).

G₄: 50% kidney beans straw+50% concentrate feed mixture. (KBS).

G₅: 50% linseed straw+50% concentrate feed mixture. (LS).

Apparent digestibility, nutritive values and nitrogen balance

Animals were housed in individual metabolic cages. Cages allowed catching feces separately from the urine which was collected in attached glass containers containing 50 ml sulphoric acid 10%. The digestibility trial consisted of 14 days as a preliminary period followed by 7 days for feces and urine collection.

The animals were fed on 3% of live body weight. Rations were offered in two portions, CFM at 8.00 a.m. followed by different roughage sources (BH, PVH, BS, KBS or LS) at 9.00 a.m. Water was offered twice daily at 11.00 a.m. and 2.00 p.m. During the collection period, feces and urine were quantitatively collected

from each animal once a day at 7.00 a.m. before feeding. Actual quantity of feed intake and water consumption were recorded. A sample of 10% of the collected feces from each animal was sprayed with 10 % sulphuric acid and 10% formaldehyde solutions and dried at 60 °C for 24 hrs. Samples were mixed and stored for chemical analysis. Composite samples of feeds and feces were finely ground prior to analysis. Also 10% of the daily collected urine from each animal was preserved for nitrogen determination. The nutritive values expressed as the total digestible nutrient (TDN) and digestible crude protein (DCP) of the experimental rations was calculated by classical method.

Chemical composition and cell wall constituents (%) of ingredients and experimental rations are presented in Tables (1 and 2). Feeds and water intakes were also recorded during the digestibility trials.

Rumen fluid parameters

Rumen fluid samples were collected from all animals at the end of the digestibility trial before feeding and 3 hrs post feeding via stomach tube and strained through four layers of cheesecloth. Samples were separated into two portions, the first portion was used for immediate determination of ruminal pH and ammonia nitrogen concentration, while the second portion was stored at -20 °C after adding a few drops of toluene and a thin layer of paraffin oil till analyzed for TVFA's.

Analytical procedures

Chemical analysis of ingredients, experimental rations and feces samples were analyzed according to A.O.A.C. (2000) methods.

Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined in ingredients and experimental rations according to Goering and Van Soest (1970). Hemicellulose was calculated as the difference between NDF and ADF, while cellulose was calculated as the difference between ADF and ADL. Ruminal pH was immediately determined using digital pH meter. Ruminal ammonia nitrogen (NH₃-N) concentrations were determined applying NH₃ diffusion technique using Kjeldahle distillation method according to A.O.A.C (2000). Ruminal total volatile fatty acids (TVFA's) concentrations were determined by steam distillation according to Warner (1964).

Statistical analysis

Collected data of feed and water consumption, digestibility, nutritive values and nitrogen balance were subjected to statistical analysis as one way analysis of variance using the general linear model procedure of SPSS (1998). On the other hand, collected data of ruminal fluid parameters (pH, NH₃-N and TVFA's concentrations) were subjected to statistical analysis as two factors-factorial analysis of variance using the

general linear model procedure of SPSS (1998). Duncan's Multiple Range Test (1955) was used to separate means when the dietary treatment effect was significant.

3. Results and Discussion

Chemical analysis and cell wall constituents of feed ingredients and experimental rations

Chemical analysis and cell wall constituents of feed ingredients are presented in Table (1) showed that berseem hay recorded the highest value of dry matter (92.63%), organic matter (90.83%), crude protein (15.19%) and nitrogen free extract (48.58%); however, it recorded the lowest value of ash (9.17). On the other hand peanut vein hay showed the highest value of ash content (14.11%). Beans straw showed the highest value of crude fiber (43.52%) and the lowest value of ether extract (0.61%). Meanwhile, kidney beans straw contained the lowest value of crude protein (8.65%). However, linseed straw showed the highest value of ether extract (3.14%).

Beans straw contained the highest values of NDF (66.39%), ADF (47.43% and cellulose contents (37.18%). But peanut vein hay showed the lowest value of ADL content (5.77%), however, linseed straw contained the lowest value of hemicellulose (5.10%).

These ingredients reasonably comparable in chemical composition to that reported by Ibrahim (2000); Talha et al. (2001 & 2002); El-Bedawy et al. (2004a & 2004b); Abdel-Magid (2005); Talha et al. (2005); Abdel-Magid et al. (2008).

Chemical analysis and cell wall constituents of the experimental rations are presented in Table (2). Results obtained showed that source of roughage was affected on the chemical composition of the experimental diets. Diet contained BH showed the highest value of CP (15.69%), followed by PVH and LS diets (14.52 and 14.23%), respectively. On the other hand CP content of BS and KBS diets were in the same range (12.65 and 12.42%). PVH diet contained the highest value of ash content (10.18%). However, BS diet contained the highest value of crude fiber (26.37%) followed by KBS diet contained 20.78% CF. Linseed straw (LS) diet showed the highest value of ether extract (4.62%) followed by KBS diet contained 4.09% EE.

Beans straw diet (BS) recorded the highest value of NDF (53.16%), ADF (37.84%) and cellulose content (28.30%). Also, our data cleared that PVH diet showed the lowest value of ADL (7.30%). Whoever, LS diet showed the lowest value of hemicellulose (8.39%). These variations in chemical composition of ration used in this study related to differ in chemical composition of ingredients that used in ration formulations.

Table 1. Chemical analysis and cell wall constituents of feed ingredients

Item	CFM*	Berseem hay	Peanut vein hay	Beans straw	Kidney beans straw	Linseed straw
Dry matter	90.36	92.63	91.56	90.69	90.52	90.74
<i>Chemical analysis on DM basis</i>						
Organic matter	93.74	90.83	85.89	90.31	88.48	87.86
Crude protein	16.17	15.19	12.85	9.12	8.65	12.27
Crude fiber	9.22	25.23	24.78	43.52	32.34	28.28
Ether extract	6.09	1.83	2.04	0.61	2.08	3.14
Nitrogen free extract	62.26	48.58	46.22	37.06	45.41	44.17
Ash	6.26	9.17	14.11	9.69	11.52	12.14
<i>Cell wall constituents</i>						
NDF	39.92	38.47	47.30	66.39	53.40	44.28
ADF	28.23	20.50	32.50	47.43	43.96	39.18
ADL	8.81	11.25	5.77	10.25	17.06	9.72
Hemicellulose**	11.69	17.97	14.80	18.96	9.44	5.10
Cellulose***	19.42	9.25	26.73	37.18	26.90	29.46

*CFM: Concentrate feed mixture composed of 45% yellow corn, 35% undecorticated cotton seed meal, 17% wheat bran, 1.5% lime stone, 1% sodium chloride and 0.5% mineral and vitamins mixture.

NDF: Neural detergent fiber. ADF: Acid detergent fiber. ADL: Acid detergent lignin.

** Hemicellulose= NDF – ADF. *** Cellulose= ADF – ADL.

Table 2 Chemical analysis and cell wall constituents of the experimental diets

Item	Experimental diet				
	BH	PVH	BS	KBS	LS
Dry matter	91.50	90.96	90.53	90.44	90.55
<i>Chemical analysis on DM basis</i>					
Organic matter	92.29	89.82	92.03	91.11	90.80
Crude protein	15.69	14.52	12.65	12.42	14.23
Crude fiber	17.23	17.00	26.37	20.78	18.75
Ether extract	3.47	4.07	3.36	4.09	4.62
Nitrogen free extract	55.90	54.23	49.65	53.82	53.20
Ash	7.71	10.18	7.97	8.89	9.20
<i>Cell wall constituents</i>					
NDF	39.20	43.61	53.16	46.66	42.10
ADF	24.37	30.37	37.84	36.10	33.71
ADL	10.04	7.30	9.54	12.94	9.27
Hemicellulose	14.83	13.24	15.32	10.56	8.39
Cellulose	14.33	23.07	28.30	23.16	24.44

BH: 50% berseem hay + 50% CFM. PVH: 50% peanut vein hay + 50% CFM. BS: 50% beans straw+ 50% CFM. KBS: 50% kidney beans straw + 50% CFM. LS: 50% linseed straw + 50% CFM.

Feed intake

Dry matter, TDN, CP, DCP intakes by the experimental groups fed the experimental diets are presented in Table (3). The results showed that inclusion PVH, BS, KBS and LS in sheep diet significantly increased ($P<0.05$) feed consumption as DM, TDN, CP and DCP intakes in comparison with the BH containing diet. Feeding sheep on PVH containing diet recorded the highest values of DM, TDN, CP and DCP intakes this may be related to high palatability of PVH by sheep compared to the other sources of roughage.

These results were agreement with those obtained by El-Basiony (1992) who found that calves fed

berseem hay consumed less ($P<0.05$) DM and also, with those found by Abdel-Magid et al. (2008) who observed that Rahmani lambs fed berseem hay consumed less ($P<0.05$) DM compared to pea straw. Pathirana and Ørskov (1995) reported increased nutrient intake as a result of increases of forage legumes as supplements to low quality basal diets.

Bartle et al. (1994) fed alfalfa and cottonseed hulls at 10, 20, or 30% of the dietary DM to finishing beef cattle; they found that within each roughage level, DMI was decreased compared to control diet.

Guthrie et al. (1996) fed heifers diets with alfalfa, cottonseed hulls, and sorghum Sudan grass hay at either 7.5 or 15% of DM in whole shelled corn-based

diets. The DMI was greater by heifers fed the cottonseed hull and sorghum Sudan grass hay diets than by those fed alfalfa.

Galyean and Defoor (2003) noted that formulating diets to a specific NDF concentration with different roughage sources probably accounts for most

of the effect of roughage source and level on DMI, but it does not fully account for the aggregate of small differences in fiber sources that might affect chewing time and kinetics of digestion and passage of roughage and grain.

Table 3. Dry matter, TDN, CP, DCP intakes by the experimental groups fed the experimental diets

Item	Experimental diets					SEM
	BH	PVH	BS	KBS	LS	
Animal No.	4	4	4	4	4	-
Live body weight, kg	51.50	50.80	52.00	51.60	51.30	1.12
<i>Feed consumption (g/h/day) as:</i>						
Dry matter	1289 ^d	1819 ^a	1709 ^b	1571 ^c	1675 ^b	43.31
TDN	943 ^d	1276 ^a	1210 ^{ab}	1140 ^c	1182 ^{bc}	25.83
CP	202 ^d	264 ^a	216 ^c	195 ^d	238 ^b	6.06
DCP	145 ^d	196 ^a	155 ^c	154 ^{cd}	170 ^b	4.28

a, b, c and d: Means in the same row having different superscripts differ significantly (P<0.05).

BH: 50% berseem hay + 50% CFM. PVH: 50% peanut vein hay + 50% CFM.

BS: 50% beans straw+50% CFM. KBS: 50% kidney beans straw + 50% CFM. LS: 50% linseed straw+50% CFM.

Water intake and water metabolism

Water consumptions and water metabolism by the experimental groups fed the experimental rations are presented in Table (4). Our data cleared that feeding sheep on BS containing diet significantly increased (P<0.05) drinking water (4650 ml/h/day), total water intake (4829 ml/h/day) compared to the other diets.

Also the same group showed the highest value of and insensible losses (2785 ml/h/day), while group sheep that fed PVH containing diet recorded the lowest value of insensible losses (1109 ml/h/day). On the other hand, there were no significant effect (P>0.05) among groups that fed on BH, PVH, KBS and LS containing diets on drinking water and total water intake.

Table 4 Water intake and water metabolism by the experimental groups fed the experimental diets

Item	Experimental diets					SEM
	BH	PVH	BS	KBS	LS	
Animal No.	4	4	4	4	4	-
Live body weight, kg	51.50	50.80	52.00	51.60	51.30	1.12
<i>Water consumption, ml:</i>						
Drinking water	3088 ^b	3742 ^b	4650 ^a	3660 ^b	3038 ^b	167.2
Feeds water	119 ^c	181 ^a	179 ^a	167 ^b	175 ^{ab}	5.43
Total water intake	3207 ^b	3923 ^b	4829 ^a	3827 ^b	3213 ^b	169.6
<i>Water metabolism:</i>						
Urinary losses, ml	867 ^{bc}	1685 ^a	1168 ^b	525 ^c	500 ^c	120.8
Feces water, ml	920 ^b	1129 ^a	876 ^b	734 ^c	899 ^b	33.16
Total water losses, ml	1787 ^{bc}	2814 ^a	2044 ^b	1259 ^d	1399 ^{cd}	143.8
Insensible losses, ml	1420 ^{bc}	1109 ^c	2785 ^a	2568 ^a	1814 ^b	160.2

a, b, c and d: Means in the same row having different superscripts differ significantly (P<0.05).

BH: 50% berseem hay + 50% CFM. PVH: 50% peanut vein hay + 50% CFM. BS: 50% beans straw+ 50% CFM. KBS: 50% kidney beans straw + 50% CFM. LS: 50% linseed straw + 50% CFM.

Because DMI and water intake are positively associated (NRC, 1996), the increased DMI noted with higher dietary concentrations of NDF from roughage could be linked to a positive effect on acid load simply by an associated increase in water intake and dilution of acid. Incomplete mixing of water with ruminal contents (Allen, 1997) would tend to lessen the effects of greater water intake. In addition, increased water intake might merely shift site of acid absorption (i.e.,

rumen vs. intestines) and thereby not greatly alter total metabolic acid load; however, the temporal pattern of acid absorption would perhaps be altered so as to spread the metabolic acid load more evenly over time (Galyean and Defoor, 2003).

Digestion coefficients and nutritive values

Digestion coefficients and nutritive values of the experimental diets are illustrated in Table (5). Data showed that inclusion different sources of roughage in

sheep diet had no significant effect on DM digestibility. Sheep received KBS containing diet significantly ($P<0.05$) increased OM, CP and CF digestibilities compared to the other different diets, and it was recorded the highest values of the same parameters of nutrient digestibilities. Sheep fed on PVH, KBS and LS containing diets significantly

($P<0.05$) decreased NFE digestibility, while it significantly ($P<0.05$) increased CF digestibility in comparison with the BH diet (control). Both PVH and KBS diets significantly ($P<0.05$) increased CP digestibility compared to the other diet (BH, BS and LS).

Table 5 Digestion coefficients and nutritive values of sheep fed the experimental diets

Item	Experimental diets					SEM
	BH	PVH	BS	KBS	LS	
<i>1- Nutrient digestion coefficient</i>						
DM	69.83	67.84	68.40	69.21	68.38	0.32
OM	73.85 ^{ab}	73.30 ^{ab}	73.32 ^{ab}	74.20 ^a	72.23 ^b	0.27
CP	70.79 ^b	74.19 ^a	70.63 ^b	75.59 ^a	70.48 ^b	0.60
CF	46.59 ^d	58.36 ^c	62.31 ^b	66.82 ^a	56.64 ^c	1.62
EE	87.13 ^a	84.21 ^b	81.64 ^c	84.59 ^b	80.30 ^c	1.32
NFE	79.28 ^a	76.92 ^{bc}	78.19 ^{ab}	75.60 ^c	77.09 ^{bc}	0.34
<i>2- Nutritive values on (DM basis) %</i>						
TDN	73.12 ^a	70.13 ^c	70.18 ^c	72.54 ^b	70.54 ^c	0.35
DCP	11.25 ^a	10.77 ^b	9.09 ^c	9.81 ^d	10.15 ^c	0.18

a, b, c, d and e: Means in the same row having different superscripts differ significantly ($P<0.05$).

BH: 50% berseem hay +50% CFM. PVH: 50% peanut vein hay + 50% CFM. BS: 50% beans straw+50% CFM.

KBS: 50% kidney beans straw + 50% CFM. LS: 50% linseed straw + 50% CFM.

Abdel-Magid et al. (2008) fed sheep on chick pea straw (CPS) or pea straw (PS) instead of berseem hay (BH) as control. They found that pea straw (PS) containing diet and control containing berseem hay (BH) had similar values of digestibilities of OM, CP, CF and NEF of experimental diets, being higher than those of PS containing diets. Differences reached significances ($P<0.05$) with OM digestibility and NFE digestibilities. Foster et al. (1988) observed that lambs given 30% ground maize and 70% chopped forage of 0, 25, 50, 75 or 100% pea hay with Lucerne showed linear decrease in DM digestibility of the diet from 59.7 to 53.3% with increasing proportion of pea hay.

Santini et al. (1992) found that an increase in ADF intake led to a decrease DM digestibility. The lower digestibility of CF of animals fed berseem hay diet might be a result of their high intake of concentrate which might depress cellulolytic activity of rumen microbes (Stewart, 1977). Rapid starch fermentation probably reduced cellulolytic activity due to decrease pH values associated with accumulation of fermentation products. The higher digestion coefficients of other nutrients with clover hay diet might be due to the low CF and high NFE contents resulted in increase of apparent digestibility of CP and NFE (Taie et al., 2005).

Valdes et al. (2000) noted that, the digestibility of fiber in the total tract depends on the intrinsic characteristics of the ration, its passage rate and the microbial activity.

The higher CF digestibility might results from the slower passage rate in the rumen (Gado, 1992 and Taie et al., 1996).

Nutritive values of sheep fed the experimental rations are presented in Table (5). Data obtained showed that introduce PVH, BS, KBS and LS in sheep diets significantly decreased ($P<0.05$) total digestible nutrient (TDN) and digestible crude protein (DCP) values compared to the control diet that contained BH. These results were in agreement with those found by Taie (1993 and 1998) and Taie et al. (2005) that feeding sheep on clover hay containing diet lead to improvement in nutritive value could be due to one or more of the following; higher fermentation rate, better $\text{NH}_3\text{-N}$ concentration or longer retention time and slower flow rate in the rumen. Abdel-Magid et al. (2008) noted that chick pea straw (CPS) diets showed lower TDN value than those obtained for PS diet with significant difference but without significance with control diet. Also, no significant difference in DCP content was found among experimental diets. However, the CPS diet tended to be lower in DCP content than the control or PS diets.

Nitrogen utilization

Data of nitrogen utilization by sheep fed the experimental diets are illustrated in Table (6) was affected by the dietary treatments and these due to the differences in nitrogen intake, fecal nitrogen (FN), urinary nitrogen (UN), total nitrogen excretion and digested nitrogen (DN) significantly differed among experimental animal groups.

On the other hand, sheep received PVH containing diet significantly increased ($P<0.05$) nitrogen intake (NI), fecal nitrogen, urinary nitrogen, total nitrogen excretion and digested nitrogen (DN). While, it significantly ($P<0.05$) decreased NR% of NI and NR% of DN in comparison with the other dietary treatments. These may be related to increase the total

nitrogen excretion and N of PVH diet was utilized less efficient as compared with those of the other diets. All groups were positive nitrogen balance and sheep received LS containing diet recorded the highest values of NR (21.7 g) and NR % of DN (81.61%) compared to the other diets used.

Table 6 Nitrogen utilization by sheep fed the experimental diets

Item	Experimental diets					SEM
	BH	PVH	BS	KBS	LS	
<i>3- Nitrogen balance</i>						
Nitrogen intake (NI), g	32.80 ^{de}	42.20 ^a	35.09 ^c	32.59 ^c	38.65 ^b	0.89
Fecal nitrogen (FN), g	9.47 ^b	12.28 ^a	10.31 ^b	7.95 ^c	12.06 ^a	0.40
Urinary nitrogen (UN), g	7.93 ^b	14.86 ^a	9.98 ^b	4.71 ^c	4.89 ^c	0.91
Total nitrogen excretion, g	17.40 ^{bc}	27.14 ^a	20.29 ^b	12.66 ^d	16.95 ^c	1.17
Digested nitrogen (DN), g	23.33 ^c	29.92 ^a	24.78 ^c	24.64 ^c	26.59 ^b	0.57
Nitrogen retention (NR),g	15.40 ^b	15.06 ^b	14.80 ^b	19.93 ^a	21.70 ^a	0.78
NR % of NI	46.95 ^b	35.69 ^c	42.18 ^{bc}	61.15 ^a	56.14 ^a	2.33
NR % of DN	66.01 ^b	50.33 ^c	59.73 ^b	80.88 ^a	81.61 ^a	2.99

a, b, c, d and e: Means in the same row having different superscripts differ significantly ($P<0.05$).

BH: 50% berseem hay + 50% CFM. PVH: 50% peanut vein hay + 50% CFM. BS: 50% beans straw+ 50% CFM. KBS: 50% kidney beans straw + 50% CFM. LS: 50% linseed straw + 50% CFM.

Forster et al. (1988) mentioned that N retention was not affected by diet when lambs were fed 30% ground maize and 70% chopped forage of 0, 25, 50, 75 or 100% pea hay with Lucerne.

Abdel-Magid et al. (2008) showed that NB of lambs fed chick pea straw (CPS) was significantly lower compared among the other two diets berseem hay (BH) and pea straw (PS) which recorded no significant values. Also NB values expressed as % from N intake indicating that N of CPS diet was utilized less efficient as compared with those of the control or PS diets.

Taie et al. (2005) noted that the highest value of both N digested (ND) as well as NB followed the same pattern of NI, being the greatest for low clover hay level, the lowest for high clover hay level and intermediate for medium clover hay level (MH-groups). Differences were significant ($P<0.05$ & $P<0.01$). El-Sayed et al., (2002) indicated that NB values were 3.01; 5.91 and 5.71 for adult goats fed four different biological treated roughages.

Rumen fluid parameters

Ruminal pH value

Effects of dietary treatments on rumen fluid parameters of the experimental rations are shown in Tables (7). Data obtained showed that dietary treatments significant affected ($P<0.05$) on pH value, ammonia nitrogen ($\text{NH}_3\text{-N}$) and total volatile fatty acids (TVFA's) concentrations. Sheep received BS containing diet significantly ($P<0.05$) increased pH value compared to the other experimental diets.

On the other hand sheep fed PVH diet recorded the lowest pH value (5.65). There were no significant effects on ruminal pH value among sheep fed BH, KBS or LS diets.

Ruminal pH was maintained within the values of 6.6 to 6.9, the optimal value for microbial growth and digestion of fiber pH 6.0-7.0 (Weimer, 1996). Mould and Orskov (1984) demonstrated that cellulose digestion was limited when ruminal pH was below 6.0.

Staples et al. (1984) noted that the optimum pH value for rumen cellulolytic bacteria was ranged "between" 5.8-6.3. This range was almost in the same range to that obtained in our study.

Allen (1997) noted that the balance between production of fermentation acids and secretion of salivary buffers was the primary determinant of ruminal pH. Hence, with a higher NDF intake per unit of grain, one might expect a higher, or at least more stable, ruminal pH. The resulting lower metabolic acid load also could be lower simply because the proportion of fermentable substrate per bite would be less, and the greater proportion of NDF in each bite might stimulate more chewing and saliva secretion. If the total number of bites increases until acid load becomes limiting, total energy intake might exceed what would be expected from compensation for energy dilution alone. The level of NDF from roughage required to elicit overcompensation in DMI likely differs among roughage sources and within a roughage source as NDF concentration of the source changes with maturity (Galyean and Defoor, 2003).

Galyean and Defoor (2003) and Moor et al. (1987) found that ruminal pH was numerically greater for the diet containing wheat straw than for those containing alfalfa or cottonseed hulls (6.2 vs. 5.9 and 5.8, respectively), but did not differ among the three roughage sources. Thus, wheat straw, but not cottonseed hulls, seemed to alter chewing time and ruminal pH, even though both of these high-NDF roughages tended to increase DMI relative to alfalfa. Similarly, Shain et al. (1999) reported that steers fed a dry-rolled corn-based diet containing wheat straw spent more total time ruminating than steers fed a dry-rolled corn-based diet containing alfalfa; however, ruminal pH did not differ between cattle fed diets containing alfalfa or wheat straw ground to pass through a 2.54-cm screen. Pitt et al. (1996) reported a fairly strong relationship between ruminal pH and the NDF concentration of dairy, beef, and sheep diets. In contrast, Nocek (1997) reported that NDF accounted for approximately 5% of the variation in ruminal pH in lactating dairy cows.

Allen (1997) noted that forage NDF as a percentage of the DM was significantly related to ruminal pH, which supports the concept that NDF from roughage might be related to ruminal pH, thereby accounting, at least in part, for the relationship that we observed between NDF from roughage and DMI by beef cattle fed high-concentrate diets. The statistical analyses conducted by Pitt et al. (1996), Allen (1997) and Nocek (1997) did not seem to use mixed-model methodology that would have allowed random study effects to be accounted for, which might explain some of the variation in results among these studies. In addition, animal-to-animal variation in ruminal pH and the ability to handle an acid load seems fairly substantial, even in model systems where a relatively constant acid load is applied (Brown et al., 2000). Such variation, as well as potentially large diurnal fluctuations in ruminal pH, would decrease the ability of dietary NDF to account for a substantial proportion of the variation in mean ruminal pH.

Table 7 Effect of dietary treatments and sampling time on rumen fluid parameters of the experimental rations

Item	Sampling time										SEM	P<0.05
	Before feeding					3 hrs post feeding						
	BH	PVH	BS	KBS	LS	BH	PVH	BS	KBS	LS		
pH	6.60 ^a	5.83 ^c	6.60 ^a	6.45 ^a	6.50 ^a	5.70 ^{cd}	5.47 ^d	6.20 ^b	5.55 ^d	5.65 ^{cd}	0.07	*
NH ₃ -N (mg/dl)	18.11 ^{bcd}	16.93 ^{cd}	13.03 ^e	11.03 ^e	16.44 ^d	25.53 ^a	18.93 ^{bcd}	20.05 ^b	20.22 ^b	24.10 ^a	0.73	*
TVFA's (meq/dl)	7.35 ^e	4.40 ^f	8.25 ^{de}	10.10 ^c	7.90 ^{de}	9.00 ^{cd}	10.23 ^{bc}	10.25 ^{bc}	14.60 ^a	11.65 ^b	0.44	*

a, b, c, d, e and f: Means in the same row having different superscripts differ significantly (P<0.05).

BH: 50% berseem hay + 50% CFM. PVH: 50% peanut vein ha y + 50% CFM. BS: 50% beans straw+50% CFM
KBS: 50% kidney beans straw + 50% CFM. LS: 50% linseed straw + 50% CFM.

Wanapat et al. (2000) found that the rumen samples of microbial populations, obtained from animals kept under traditional village conditions in the Northeast of Thailand, had similar pH value for both species, but had significantly difference in the numbers of microorganism.

Ruminal ammonia nitrogen (NH₃-N) concentration

Data obtained of ruminal NH₃-N concentration (Table 7) cleared that, dietary treatment significantly (P<0.05) affected ruminal NH₃-N concentration among different experimental groups. Sheep received LS diet had no significant effect on NH₃-N concentration compared to BH diet, however, it significantly (P<0.05) increased in comparison with PVH, BS and KBS diets. KBS diet showed the lowest content of ruminal NH₃-N concentration (15.62).

Briggs et al. (1957) noted that an increasing in ruminal TVFA's concentration caused a reduction in ruminal pH value. Ruminal pH is one of the most important factors affecting the fermentation and influences its functions. It varies in a regular manner depending on the nature of the diet and on the time it is measured after feeding and reflects changes of organic acids quantities in the ingesta. The level of NH₃-N and

TVFA's as end products of fermentation and breakdown of dietary protein, have been used as parameters of ruminal activity by Abou-Akkada and Osman (1967).

It should be noted that, TVFA's concentration in the rumen is governed by several factors such as dry matter digestibility, rate of absorption, rumen pH, transportation of the digesta from the rumen to the other parts of the digestive tract and the microbial population in the rumen and their activities (Allam et al., 1984).

It is unknown whether roughage source and level affects absorption of acids from the rumen or acidity in the small and large intestines. It seems unlikely that increasing NDF supplied by roughage in a high-concentrate diet would directly affect absorption of VFA from the rumen. Similarly, direct effects of roughage on absorption of acids from the intestines seem unlikely (Galyean and Defoor, 2003). Allen (1997) suggested that changes in ruminal papillae surface area among diets might affect the susceptibility of cattle to acidosis, which could be related to differences resulting from dietary NDF supplied by roughage. Whether roughage source or level in feedlot

finishing diets affects ruminal surface area for absorption is unknown. The NDF supplied by roughage might exert effects on digesta kinetics and associated water flux that affect digesta flow through the intestines and absorption of acid post ruminally.

Ruminal total volatile fatty acids (TVFA's) concentration

Data of total volatile fatty acids (TVFA's) concentration of Table (7) established that KBS diet significantly ($P < 0.05$) increased TVFA's concentration compared to the other dietary treatments. Sheep received PVH diet recorded the lowest value of TVFA's (7.32), while KBS diet showed the highest value of TVFA's (12.35). Slyter et al. (1979) and Pan et al. (2003) demonstrated that increased ruminal $\text{NH}_3\text{-N}$ (22.5 mg %) might increase ruminal pH, TVF's production and stimulated cellulolytic bacteria activity in the rumen. Wanapat and Pimpa (1999) also found that higher levels of ruminal $\text{NH}_3\text{-N}$ at 17.6 mg% resulted in the highest total purine derivatives, indicating highest rumen microbial protein synthesis. Kajanaputhipong and Leng (1998) showed that protozoan, fungal and bacterial populations in the rumen were influenced by the levels of ruminal $\text{NH}_3\text{-N}$.

Effect of sampling time on rumen fluid parameters

Effect of sampling time on rumen fluid parameters is presented in Table (7). Results obtained showed that 3hrs post feeding significantly ($P < 0.05$) decreased ruminal pH value, while, it significantly ($P < 0.05$) increased both ruminal $\text{NH}_3\text{-N}$ and TVFA's concentrations. These results were agreement with those recorded by Taie et al. (2005) who noticed that values of pH were higher before feeding in all groups then declined after feeding to reach their lowest values at 4 hrs in low clove hay diet group and 6 hrs for both medium and high levels of clover hay diet groups. Taie (1993 and 1998) found that the values of pH declined at 3-4 hr post- feeding and then increased in sheep fed corn-cobs or clover hay containing diets.

Khorshed (2008) found that pH value was significantly ($P < 0.05$) decreased after 3hrs post feeding compared to before feeding (zero hrs), the reduction in pH values with advancing sampling time post feeding was mainly due to increase fermentation after feeding

Taie et al. (2005) reported that within the experimental groups, the highest TVFA's concentration was observed at 4 hrs post-feeding for low clover hay level diet, whereas it was at 6 hrs post-feeding for medium and high clover hay levels diets. Also, similar trends of TVFA's values and relationship with the time were observed by Taie (1993 and 1998); however, TVF's levels were lower than theirs which may be due to differences in dietary CF levels. The overall averages of TVFA's concentration proved that the LH diet had higher fermentable materials which usually are degraded to TVFA's by the rumen

microorganisms during digestion (Sultan and Loerch 1992). The increases in TVFA's post- feeding were associated with a decrease in rumen pH. This in agreement with the findings of Bonsembinate et al. (1988) and Taie (1993) and Taie et al. (2005) that the increase in TVFA's concentrations is paralleled the reduction in ruminal pH. The TVFA's concentrations were generally increase as the proportion of concentrate mixture of the experimental diets increase (Punia and Sharm, 1990 and Taie et al., 2005).

The peak concentration of ammonia-nitrogen at 3 hrs after feeding may be because of degradation of protein and hydrolysis of NPN substances (Reddy et al., 1989). On the other hand Chandra et al. (1991) noted that the peak of ruminal $\text{NH}_3\text{-N}$ at 3 hours after feeding may be due to deamination of amino acids in the rumen. Gado et al. (2007) noted that values of sampling time on ammonia-N concentrations were at the minimum at 0 hrs before feeding and increased to its maximum levels at 4hrs after feeding then values tended to decrease gradually as the time passed up to 6 hrs after feeding.

It is worthy to notice that the balance between $\text{NH}_3\text{-N}$ and TVFA's concentrations reflect the pH values in the rumen liquor and effect of fungi might be related to the more utilization of the dietary energy and positive fermentation in the rumen (El-Shafie et al., 2007).

Obtained data also, showed that there were significant interaction ($P < 0.05$) between roughage sources and sampling time on ruminal pH, $\text{NH}_3\text{-N}$ and TVFA's concentrations (Table 7).

4. Implications

Changes in roughage source affect dry matter intake, water intake and metabolism, nutrient digestibility coefficients, nitrogen utilization and ruminal fermentation by sheep. Although neutral detergent fiber supplied by roughage might provide a useful basis for exchanging roughages in sheep diets, the biological reasons for changes in dry matter intake associated with changes in roughage source need further study.

From this study, it could be concluded that we can using alternative sources of roughage successfully in sheep diets as a good sources of roughages instead of berseem hay with better feed intake, digestion coefficient, nitrogen utilization and ruminal fermentation. Also we can use the tested materials to formulate cheap diets for sheep.

Corresponding author

Hamed A.A. Omer

Animal Production Department, National Research Center, Dokki, Giza, Egypt
hamedomer2000@yahoo.com

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Outcome of Sublingual Immunotherapy with Multiple Allergens in Asthmatic Patients with and without Allergic Rhinitis

Emara M.M.¹, Mansour H.A.², Shehata M.T.³ and Zakia Abu-Zahab⁴

Departments of ¹Thoracic Medicine and ²Clinical Pathology, Mansoura University

³Department of ENT, Ain Shams university

⁴Department of Clinical Pathology, Faculty Of Medicine For Girls, Al Azhar University, Egypt.

omima468@yahoo.com

Abstract: Background: Asthma is a chronic inflammatory pulmonary disorder that is characterized by reversible obstruction of the airways. Allergic rhinitis and allergic asthma are chronic inflammatory conditions that frequently co-exist, both with hallmark eosinophils. Immunotherapy is an established treatment of allergic diseases. Non-injectable routes for immunotherapy such as the sublingual route are thought to be valuable therapeutic options for respiratory allergy and have the primary aim of minimizing the risk of adverse events and of improving the compliance of the patients. Sublingual immunotherapy is now officially accepted as a viable alternative to the traditional subcutaneous route. **Aim of the work:** In the present study, a trial has been made to administer the sublingual immunotherapy using multiple allergens in allergic asthmatic Patients with and without allergic rhinitis and to evaluate the clinical efficacy, safety, and changes in allergen-specific antibodies during sublingual immunotherapy (SLIT). **Patients and methods:** This study was conducted at Kingdom of Saudi Arabia. The present study comprised two groups; group I included 20 asthmatic patients (13 males and 7 females) with a mean age of (29.05± 8.27 years). Group II included 20 male asthmatic patients with allergic rhinitis with a mean age of (33.61 ± 6.43 years). All patients were subjected to careful history taking and careful clinical examination, routine laboratory investigations, chest X ray PA, X ray paranasal sinuses, eosinophilic blood count and total IgE in serum by ELISA technique before start, after 6 months and after one year of the course of the sublingual immunotherapy, skin prick test and specific IgE to food and inhalants, Pulmonary function testing (spirometry) before start and after one year of the course of the sublingual immunotherapy. **Results:** Our results revealed that 8 out of 20 asthmatic patients group (40%) had nocturnal asthma and 11 patients (55%) had asthmatic attacks. On the other hand, 12 patients (60%) of asthmatic patients with allergic rhinitis had nocturnal asthma and asthmatic attacks. Our study revealed that, there were statistically significant decreases in blood eosinophils one year after SLIT compared to that before SLIT in both asthmatic patients with and without allergic rhinitis. Our study showed there were statistically insignificant decrease in total IgE in asthmatic patients group and statistically significant decrease in total IgE in asthmatic patients with allergic rhinitis one year after SLIT compared to that before SLIT. Results of specific IgE to food and inhalants revealed that, there were statistically significant reduction of number of allergens from 3.65±1.60 to 1.55±1.27 in asthmatic group and from 3.95±2.11 to 1.35±1.34) in asthmatics with allergic rhinitis group ($P<0.05$) one year after SLIT compared to that before SLIT. Results of skin prick test revealed that, there were statistically significant reduction of number of allergens from (3.30±1.30 to .55±1.19) in asthmatic group and from (4.1±2.1 to 1.1±1.33) in asthmatics with allergic rhinitis group ($P<0.05$) one year after SLIT compared to that before SLIT. The majority of asthmatic patients group were sensitive to mites (60%), followed by mixed grass pollens (30%), *Penicillium notatum* (25%), house dust (20%), Cockroach (20%) respectively. On the other hand, the majority of asthmatic patients with allergic rhinitis group were sensitive to mites (75%), house dust (40%), mixed grass pollens (40%), mixed pollens (30%), cat epithelium (30%), *Penicillium notatum* (25%), Cockroach (25%), dog epithelium (20%), and sheep wool (20%). Results of Pulmonary function in both asthmatic patients group and asthmatic patients with allergic rhinitis showed statistically significant increase in FVC, FEV1, PEF, FEF25%, FEF50% and MVV one year after SLIT compared to that before SLIT. As regard the duration of sublingual immunotherapy one patient (5%) of asthma group discontinued treatment after one year, two (10%) after 18 months, 3 (15%) after 2 years, and 14 (70%) continue > 2 years. Two patients (10%) of asthma allergic rhinitis group discontinued treatment after one year, 2 (10%) after 18 months, 4 (20%) after 2 years, and 12 (60%) continue > 2 years. Local reverse reactions (throat itching) were reported in one (5%) patient of asthma group. No other local side effects or systemic side effects were reported in both asthmatic patients and asthmatic with allergic rhinitis group. From the twenty asthmatic group, 11 patients (55%) tolerated sublingual immunotherapy therapy very well, 7 (35%) good, 2 (10%) moderate. On the other hand, 10 asthmatic patients with allergic rhinitis (50%) tolerated therapy very well, 6 patients (30%) good, and 4 patients (20%) moderate. Our results revealed that 13 out of 20 (65%) asthmatic patients group had reduction of symptoms, 7 out of 8 patients (87.5%) had reduction of nocturnal asthma, 7 out of 11 patients (63.63%) had reduction of asthmatic attacks and 14 out of 20 patients (70%) had reduction of need to rescue treatment one year after the course of sublingual immunotherapy. On the other hand, 15 out of 20 (75%) asthmatic patients with allergic rhinitis group had reduction of

symptoms, 11 out of 12 patients (91.66%) had reduction of nocturnal asthma, 9 out of 12 patients (75%) had reduction of asthmatic attacks 15 out of 20 patients (75%) had reduction of need to rescue treatment, and 13 patients (65%) had reduction of nasal symptoms one year after the course of sublingual immunotherapy. **Conclusion:** From this study we concluded that sublingual immunotherapy is a safe treatment which significantly reduces symptoms and medication requirements, improves lung function in both asthmatic patients with and without allergic rhinitis. SLIT using multiple allergens lowered the allergen burden in both asthmatic patients with and without allergic rhinitis.

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Key words: Sublingual immunotherapy (SLIT) multiple allergens, Bronchial asthma, and Allergic rhinitis.

Abbreviations: Sublingual immunotherapy (SLIT), Allergic Rhinitis (AR).

1. Introduction:

Asthma is a chronic inflammatory pulmonary disorder that is characterized by reversible obstruction of the airways⁽¹⁾. Allergic rhinitis is a common condition which, at its most severe, can significantly impair quality of life despite optimal treatment with antihistamines and topical nasal corticosteroids⁽²⁾. Allergic rhinitis and allergic asthma are chronic inflammatory conditions that frequently co-exist, both with hallmark eosinophils. Rhinitis or rhino sinusitis usually occurs in more than 75% of patients with allergic asthma and in more than 80% of patients with non allergic asthma, with reported percentages varying from 30 to 99%⁽³⁾. It is postulated that rhinitis and asthma represent the manifestations of one syndrome in two parts of the respiratory tract, the upper and lower airways, respectively. At the low end of the severity spectrum, rhinitis may occur alone; in the middle range of the spectrum, rhinitis and AHR may be present; and, at the high end, rhinitis and asthma may both be present, with the severity of each condition tracking in parallel. Disease manifestations in the upper and lower airways may be linked via a systemic inflammatory response⁽⁴⁾. Immunotherapy is an established treatment of allergic diseases. Subcutaneous allergen immunotherapy is clearly beneficial in the treatment of select patients with allergic rhinitis or asthma. However, this therapy is underused, partly because it requires administration in a medical facility⁽⁵⁾. Non-injective routes for immunotherapy such as the sublingual route are thought to be valuable therapeutic options for respiratory allergy and have the primary aim of minimizing the risk of adverse events and of improving the compliance of the patients⁽⁶⁾. Sublingual immunotherapy is gaining widespread attention as a viable alternative to subcutaneous immunotherapy for the treatment of allergic rhino conjunctivitis. In addition, sublingual immunotherapy has been studied in other allergic disorders including asthma⁽⁷⁾. Sublingual immunotherapy (SLIT) is a form of allergen immunotherapy that involves administration of the allergen under the tongue. It appears to be associated

with fewer serious adverse effects than SCIT, which would allow for home administration⁽⁵⁾. The mechanism of action of both injection and sublingual immunotherapy remain under investigation, and injection immunotherapy has been proven to lead to long-term changes in the immunological response to allergen that may persist for years following discontinuation⁽⁸⁾.

Aim of the work:

The purpose of this study was to evaluate the clinical efficacy, safety, and changes in allergen-specific antibodies during sublingual immunotherapy (SLIT) in asthmatic patients with and without allergic rhinitis.

2. Subjects and Methods:

This study comprised two groups; group I included 20 asthmatic patients with a mean age of (29.05± 8.27 years). Group II included 20 asthmatic patients with allergic rhinitis with a mean age of (33.61 ± 6.43 years) documented in a two-year follow-up study. The performance of the sublingual specific immunotherapy should last at least for 12 months. All patients were subjected to the following:

1. Careful history taking and clinical examination including age; sex; smoking habits; associated comorbidity; onset of asthma, nocturnal worsening of asthma symptoms, frequency of asthmatic attacks, asthma medication needed, and asthma symptoms; dyspnea, cough, wheezy chest, nasal obstruction, rhinorrhea, sneezing, tolerability and duration of the sublingual immunotherapy, reduction of symptoms, asthmatic attacks and nocturnal asthma, and side effects experienced by the patients.
2. Chest X ray PA and x ray paranasal sinuses for asthmatic allergic rhinitis patients.
3. Routine laboratory investigations.

Eosinophilic blood count and total IgE in serum before start, after 6 months and after one year of the course of the sublingual immunotherapy. Total IgE: was determined using the By ELISA technique using a kit supplied by Bender Med Systems diagnostics (Bender Med systems Diagnostics, Vienna, Austria).

Expected range between 29-87 iu/ml according to age.

4. Skin-prick testing using battery containing 25 allergens was performed before start and after one year of the course of the sublingual immunotherapy. Patients should stop steroids and antihistaminic 48 hours before the test. Skin prick test was performed on the internal side of the forearms, with needle As follows:

- Clean arm with soap and water or alcohol
- The forearm is coded with a skin marker pen corresponding to the number of allergens being tested. Marks should be at least 2cm apart.
- A drop of allergen solution is placed beside each mark
- A small prick through the drop is made to the skin using a sterile prick lancet. A new lancet must be used for each allergen tested.
- Excess allergen solution is dabbed off with a tissue

Observe skin reactions – if a reaction occurs it should do so within 20-30 minutes In addition to the allergens tested, there should be a positive and negative control. The positive control, usually a histamine solution, should become itchy within a few minutes and then become red and swollen with a “wheal” in the centre. The negative control, usually a saline solution should show no response.

Skin prick testing results: There are a couple of grading scales used but the size of the wheal is most accurate. The size of the wheal does not indicate the severity of the symptoms but shows us the degree of sensitivity to the allergen.

Wheal size (mm)	Old “+” scale	Interpretation
<4	0+	Negative
5 – 10	2+	Mildly sensitive
10 – 15	3+	Moderately sensitive
>15	4+	Very sensitive

For skin prick tests to be informative they must be interpreted in conjunction with the patient's history and physical examination. The doctor must also be aware of the many reasons for a false-positive and false-negative reaction to properly interpret test

5. Specific IgE to food and inhalants in serum by RAST before start and after one year of the course of the sublingual immunotherapy. RAST by Using UniCAP 100e , Pharmacia, for analyzing specific IgE in human serum detection limit of the CAP System is 0.35 kU/L and values greater than 0.35kU/L were considered positive

In principal, The CAP System FEIA employs a type of “architecture” whereby the allergen of interest is covalently bound to a hydrophilic carrier polymer, encased in a capsule, which catches all allergen-specific IgE in the sample. Allergen-specific IgE is

detected directly with a combination of polyclonal and monoclonal anti-IgE (Fc) antibodies labeled with beta-galactosidase, generating fluorescence.

The specific IgE antibody in the sample is connecting to the allergens in the immunoCAP after buffer solution has been washed away (Pre-wash), all not connected sample is washed away (sample wash). Antibodies in the conjugate connects to the IgE during conjugate incubation, excess conjugate is washed away in the conjugate wash. Development solution is added and reacts with the conjugate connected to the ImmunoCAP, After development incubation stop solution is added to halt the process. The volume is high (3x200µl), this is to flush the fluorescent product down to the elution well for measurement.

6. Pulmonary function testing using computerized spirometry before start and after one year of the course of the sublingual immunotherapy.

All asthmatic patients had paroxysmal attacks of wheezy chest, dyspnea, cough and expectoration or documented reversible airway obstruction as determined by a 20% improvement in FEV1 after bronchodilator administration or Peak expiratory flow rate variability (>20%). Oral consent was taken from all patients before inclusion in this research. The choice of the allergen to be employed for SLIT should be made in accordance with the combination of clinical history and results of skin prick tests. Polysensitisation, i.e. the occurrence of multiple positive responses does not exclude SLIT, which may be done with the clinically most important allergens⁽⁶⁾. All significantly positive antigens (end point of ≥ 3) were included in each patient's SLIT treatment regimen. The performance of the sublingual specific immunotherapy should last at least for 12 months. No former specific immunotherapy was documented in any of the studied patients. Maintenance dosage was reached after six months.

Proportions of the various allergens used were specified on each immunotherapy set. Thus, each treatment was individually formulated. The extract suspended in extracting fluid (Coca's solution) containing 50% glycerin I.P. was standardized according to w/v ratio of native material to the extracting fluid. Each course was provided in multi-dose vial of allergens, with color code in graded strengths as follows:

Strength 1 Black label 0.01% w/v

Strength 2 Green label 0.1% w/v

Strength 3 Blue label 1% w/v

Maintenance Set: 1% w/v

Maintenance dose (strength 3) was recommended to be continued for three years. Dosage patterns were devised according to patient's sensitivity and tolerance. Care was taken to increase the dose at

regular intervals however; it could be increased provided the previous dose has been tolerated without any reaction. In case there was gap in treatment for more than two weeks, therapy was re-initiated (for safety reasons) with half of the dose last given. In the event of interruption of more than 4 weeks, the therapy was resumed from the initial dose. The patients received increasing doses of the extract, starting with 1 drop from vial 1 and increasing by one drop daily to 10 drops on the tenth day, following the graded course up to vial 4, the drops being taken sublingually in the morning before breakfast and being kept sublingually for 1-2 minutes and then swallowed with 1/2 cup of water. Maintenance therapy consisted of 10 drops daily and was reduced to three times per week after 6 months of therapy. The number of used allergens for immunization ranged from 1 to 7 allergens.

3.Results:

This study comprised two groups; group I included 20 asthmatic patients (13 males and 7 females) with a mean age of (29.05± 8.27 years). Group II included 20 male asthmatic patients with allergic rhinitis with a mean age of (33.61 ± 6.43 years) (Table 1). As regard the clinical presentation of the studied patients, 20 asthmatic patients (100%) had cough & expectoration, 17 patients (85%) had dyspnea, 11 patients (55%) had wheezy chest, 8 patients (40%) had nocturnal asthma and 11 patients (55%) had asthmatic attacks. On the other hand, 20 asthmatic patients with allergic rhinitis (100%) had cough & expectoration, 14 patients (70%) had dyspnea, 9 patients (45%) had wheezy chest, 12 patients (60%) had nocturnal asthma and asthmatic attacks, 5 patients (25%) had nasal obstruction, 13 patients (65%) had sneezing and 16 patients (80%) had rhinorrhea (Table 2). Our study revealed that, there were statistically insignificant decrease in blood eosinophils 6 months after SLIT and statistically significant decrease in blood eosinophils one year after SLIT compared to that before SLIT in both asthmatic patients and asthmatic patients with allergic rhinitis (Table 3). Our study revealed that, there were statistically insignificant decreases in blood eosinophils one year after SLIT in asthmatic patients with allergic rhinitis compared to asthmatic patients group (Table 4). Table (5) showed, there were statistically insignificant decrease in total IgE 6 months and one year after SLIT compared to that before start of SLIT in asthmatic patients group. On the other hand, there were statistically insignificant decreases in total IgE 6 months after SLIT and statistically significant decrease in total IgE one year after SLIT compared to that before SLIT in asthmatic patients with allergic rhinitis. Our study showed statistically insignificant decrease in total IgE one year after SLIT in asthmatic patients with allergic rhinitis group compared to asthmatic patients group (Table 6).

As regard to distribution of specific IgE to food and inhalants, the majority of asthmatic patients group before start of SLIT were sensitive to 3 allergens (30%) followed by 6 allergens (15%), 2 allergens (15%), and one allergen (15%) respectively. One year after SLIT, the majority were sensitive to 0 allergen (35%) followed by one allergen (35%). On the other hand, the majority of asthmatic patients with allergic rhinitis group before start of SLIT were sensitive to 6 allergens (25%) followed by one allergen (25%), 7 allergens (10%), 5 allergens (10%), 4 allergens (10%), and two allergens (10%) respectively. One year after SLIT, the majority were sensitive to 0 allergen (45%) followed by one allergen (20%). There were statistically significant reduction of number of allergens from 3.65±1.60 to 1.55±1.27 in asthmatic group and from 3.95±2.11 to 1.35±1.34 in asthmatics with allergic rhinitis group ($P<0.05$) one year after SLIT compared to that before SLIT (Table 7). The results of skin prick test revealed that, the majority of asthmatic patients group before start of SLIT were sensitive to 3 allergens (60%) followed by 2 allergens (20%) respectively. One year after SLIT, the majority were sensitive to 0 allergen (40%) followed by one allergen (40%). On the other hand, the majority of asthmatic patients with allergic rhinitis group were sensitive to 3 allergens (25%) followed by 4 allergens (20%) respectively. One year after SLIT, the majority were sensitive to 0 allergen (55%) followed by 2 allergens (20%). There were statistically significant reduction of number of allergens from (3.30±1.30 to .55±1.19) in asthmatic group and from (4.1±2.1 to 1.1±1.33) in asthmatics with allergic rhinitis group ($P<0.05$) one year after SLIT compared to that before SLIT (Table 8). Our results revealed that the majority of asthmatic patients group were sensitive to mites (60%), followed by mixed grass pollens (30%), *Penicillium notatum* (25%), house dust (20%), Cockroach (20%) respectively. On the other hand, the majorities of asthmatic patients with allergic rhinitis group were sensitive to mites (75%), followed by house dust (40%), mixed grass pollens (40%), mixed pollens (30%), cat epithelium (30%), *Penicillium notatum* (25%), Cockroach (25%), dog epithelium (20%), and sheep wool (20%) (Table 9). As regard the distribution of drug therapy in asthmatic patients 20 patients (100%) received combined LABA and inhaled steroids, 4 patients (20%) received theophylline, 5 patients (25%) received leukotriene modifiers and one patient (5%) received systemic steroids. 20 patients (100%) of asthmatic allergic rhinitis group received combined LABA and inhaled steroids, 4 patients (20%) received leukotriene modifiers and two patients (10%) received systemic steroids and 20 patients (100%) received topical nasal steroids (Table 10). As regard the results of pulmonary function in both asthmatic patients group and asthmatic

patients with allergic rhinitis, there were statistically significant increase in FVC, FEV1, PEF, FEF25%, FEF50% and MVV and statistically insignificant increase in FEF75% one year after SLIT compared to that before SLIT (**Table 11**). Our study revealed that, there were statistically significant increase in FEV1, and MVV and statistically insignificant increase in FVC, PEF, FEF25%, FEF50% and FEF75% in asthmatic patients with allergic rhinitis group one year after SLIT compared to that in asthmatic patients group (**Table 12**). As regard the duration of sublingual immunotherapy one patient (5%) of asthma group discontinued treatment after one year, two (10%) after 18 months, 3 (15%) after 2 years, and 14 (70%) continue > 2 years. Two patients (10%) of asthma allergic rhinitis group discontinued treatment after one year, 2 (10%) after 18 months, 4 (20%) after 2 years, and 12 (60%) continue > 2 years (**Table 13**). Local reverse reactions (throat itching) were reported in one (5%) patient of asthma group. No local side effects were reported in asthmatic patients with allergic rhinitis group. No systemic side effects were reported

in both groups (**Table 14**). From the twenty asthmatic group, 13 patients (65%) tolerated sublingual immunotherapy therapy very well, 6 (30%) good, 1 (5%) moderate. On the other hand, 11 asthmatic patients with allergic rhinitis (55%) tolerated therapy very well, 7 patients (35%) good, 2 patients (10%) moderate (**Table 15**). Our results revealed that 13 out of 20 (65%) asthmatic patients group had reduction of symptoms, 7 out of 8 patients (87.5%) had reduction of nocturnal asthma, 7 out of 11 patients (63.63%) had reduction of asthmatic attacks and 14 out of 20 patients (70%) had reduction of need to rescue treatment one year after the course of sublingual immunotherapy (**Table 16**). On the other hand, 15 out of 20 (75%) asthmatic patients with allergic rhinitis group had reduction of symptoms, 11 out of 12 patients (91.66%) had reduction of nocturnal asthma, 9 out of 12 patients (75%) had reduction of asthmatic attacks, 15 out of 20 patients (75%) had reduction of need to rescue treatment, and 13 patients (65%) had reduction of nasal symptoms one year after the course of sublingual immunotherapy (**Table 17**).

Table (1): Age and Sex distribution among the studied patients.

GROUP	AGE M ± SD	Minimal	Maximal	SEX			
				Males		Females	
				No.	%	No.	%
Asthmatic patients. No. = 20	27.9 ± 11.63	14	50	13	65	7	35
Asthmatic patients with allergic rhinitis. No. = 20	31.75 ± 9.16	15	49	20	100	0	0

Table (2): Clinical presentation of the studied patients.

Symptoms	Asthmatic patients. No= 20		Asthmatic patients with allergic rhinitis. No=20	
	No.	%	No.	%
Cough & expectoration	20	100	20	100
Dyspnea.	17	85	14	70
Wheezy Chest.	11	55	9	45
Nocturnal asthma.	8	40	12	60
Asthmatic attacks	11	55	12	60
<input type="checkbox"/> Nasal symptoms:				
• Nasal obstruction.	0	0	5	25
• Sneezing.	0	0	13	65
• Rhinorrhea.	0	0	16	80

Table (3): Distribution of blood eosinophils in asthmatic patients and asthmatic patients with allergic rhinitis before, 6 months and one year after the course of sublingual immunotherapy.

	Before SLIT. M ± SD	6 months after SLIT. M ± SD	One year after SLIT. M ± SD
Asthmatic patients. No= 20	321.45 ± 125.64	272.2 ± 86.74 <i>P</i> > 0.05	232 ± 106.27 <i>* P</i> < 0.05
Asthmatic patients with allergic rhinitis. No=20	345.65 ± 138.45	271.6 ± 141.79 <i>P</i> > 0.05	217.05 ± 128.65 <i>* P</i> < 0.05

Table (4): Comparison of blood eosinophils in asthmatic patients versus asthmatic patients with allergic rhinitis one year after the course of sublingual immunotherapy.

	Asthmatic patients. No= 20; M ± SD	Asthmatic patients with allergic rhinitis. No=20 M ± SD
Blood eosinophils	232 ± 106.27	217.05 ± 128.65; <i>P</i> > 0.05

Table (5): Results of total IgE in asthmatic patients group and asthmatic patients with allergic rhinitis before, 6 months and one year after the course of sublingual immunotherapy.

	Before SLIT. M ± SD	6 months after SLIT. M ± SD	One year after SLIT. M ± SD
Asthmatic patients. No= 20	949.73 ± 1275.4	588.5 ± 635.89 <i>P</i> > 0.05	469.8 ± 615.04 <i>P</i> > 0.05
Asthmatic patients with allergic rhinitis. No=20	629.8 ± 485.48	461.19 ± 465.88 <i>P</i> > 0.05	312.76 ± 380.99 * <i>P</i> < 0.05

Table (6): Comparison of total IgE in asthmatic patients group versus asthmatic patients with allergic rhinitis one year after the course of sublingual immunotherapy.

	Asthmatic patients. No= 20; M ± SD	Asthmatic patients with allergic rhinitis. No=20 M ± SD
Total IgE	469.8 ± 615.04	312.76 ± 380.99; <i>P</i> > 0.05

Table (7): Results of specific IgE to food and inhalants in asthmatic patients and asthmatic patients with allergic rhinitis before and one year after the course of sublingual immunotherapy.

Specific IgE to food and inhalants.	Asthmatic patients				Asthmatic patients with allergic rhinitis			
	Before SLIT.		One year after SLIT.		Before SLIT.		One year after SLIT.	
	No.	%	No.	%	No.	%	No.	%
Specific IgE to 7 allergens	1	5	0	0	2	10	0	0
Specific IgE to 6 allergens	3	15	0	0	5	25	0	0
Specific IgE to 5 allergens	2	10	0	0	2	10	0	0
Specific IgE to 4 allergens	2	10	1	5	2	10	1	5
Specific IgE to 3 allergens	6	30	3	15	1	5	3	15
Specific IgE to 2 allergens	3	15	2	10	2	10	3	15
Specific IgE to one allergen	3	15	7	35	5	25	4	20
Specific IgE to 0 allergen	0	0	7	35	1	5	9	45
Total	20	100	20	100	20	100	20	100
Mean ± SD	3.65±1.60		1.55±1.27 * <i>P</i> < 0.05		3.95±2.11		1.35±1.34 * <i>P</i> < 0.05	

Table (8): Results of skin prick test results in asthmatic patients and asthmatic patients with allergic rhinitis before and one year after the course of sublingual immunotherapy.

Skin prick test	Asthmatic patients				Asthmatic patients with allergic rhinitis			
	Before SLIT.		One year after SLIT.		Before SLIT.		One year after SLIT.	
	No.	%	No.	%	No.	%	No.	%
Positive test for 7 allergens	1	5	0	0	4	20	0	0
Positive test for 6 allergens	1	5	0	0	1	5	0	0
Positive test for 5 allergens	1	5	0	0	3	15	0	0
Positive test for 4 allergens	1	5	1	5	4	20	1	5
Positive test for 3 allergens	12	60	1	5	5	25	2	10
Positive test for 2 allergens	4	20	2	10	1	5	4	20
Positive test for one allergen	0	0	8	40	2	10	2	10
Positive test for 0 allergen	0	0	8	40	0	0	11	55
Total	20	100	20	100	20	100	20	100
Mean±SD	3.3±1.30		.55±1.19 * <i>P</i> < 0.05		4.1±2.1		1.1±1.33 * <i>P</i> < 0.05	

Table (9): Results of allergen sensitivity in asthmatic patients and asthmatic patients with allergic rhinitis.

	Asthmatic patients No =20		Asthmatic patients with allergic rhinitis No =20	
	No.	%	No.	%
House dust	4	20	8	40
Mites	12	60	15	75
Mixed grass pollens	6	30	8	40
Palm tree pollens	1	5	2	10
Rye pollens	2	10	2	10
Mixed pollens	3	15	6	30
Hay dust	0	0	1	5
<i>Candida albicans</i>	1	5	1	5
<i>Aspergillus fumigates</i>	3	15	0	0
<i>Penicillium notatum</i>	5	25	0	0
Mixed moulds	2	10	0	0
Tobacco	1	5	2	10
Sheep epithelia	1	5	0	0
Goat epithelia	3	15	2	10
Camel epith.	1	5	2	10
Cow epith.	0	0	1	5
Dog epith.	2	10	4	20
Cat epith.	6	30	6	30
Horse hair	0	0	3	15
Pigeon	0	0	1	5
Shrimps	0	0	2	10
Cockroach	4	20	5	25
Sheep wool	0	0	4	20
Peanuts	1	5	1	5
Hazelnuts	1	5	2	10
Milk	0	0	1	5
Egg yolk	1	5	1	5
Soya bean	1	5	1	5
Fish	1	5	2	10
Egg white	0	0	1	5
Shelfish	1	5	0	0
Banana	2	10	2	10

Table (10): Distribution of drug therapy in asthmatic patients group and asthmatic patients with allergic rhinitis.

Duration of treatment	Asthmatic patients. No= 20		Asthmatic patients with allergic rhinitis. No=20	
	No.	%	No.	%
• Combined (LABA) and inhaled steroids.	20	100	20	100
• Theophylline.	4	20	0	0
• Leukotriene modifiers.	5	25	4	20
• Systemic steroids.	1	5	2	10
• Topical nasal steroids.	0	0	20	100

Table (11): Results of pulmonary function in asthmatic patients and asthmatic patients with allergic rhinitis before and one year after SLIT.

Pulmonary Function Data.	Asthmatic patients No. = 20		Asthmatic patients with allergic rhinitis No. = 20	
	Before SLIT. M ± SD	One year after SLIT. M ± SD	Before SLIT M ± SD	One year after SLIT. M ± SD
FVC.	78.68±6.04	88.35 ± 5.01 * <i>P</i> < 0.05	79.45 ± 15.74	89.10 ± 5.39 * <i>P</i> < 0.05
FEV1.	67 ± 13.21	81.15 ± 7.53 * <i>P</i> < 0.05	70.4 ± 15.37	86.5 ± 5.88 * <i>P</i> < 0.05
PEF.	58.1 ± 13.45	78.1 ± 9.97 * <i>P</i> < 0.05	64.6 ± 18.81	79.85 ± 9.19 * <i>P</i> < 0.05

FEF25%	57.65 ± 15.49	69.6 ± 9.57 * <i>P</i> < 0.05	64.55 ± 18.43	74.9 ± 11.29 * <i>P</i> < 0.05
FEF50%	59.15 ± 15.05	69.65 ± 11.84 * <i>P</i> < 0.05	63.9 ± 19.89	76.9 ± 11.99 * <i>P</i> < 0.05
FEF75%	60.3 ± 20.25	68.8 ± 14.84 <i>P</i> > 0.05	68.4 ± 25.08	77.75 ± 15.08 <i>P</i> > 0.05
MVV	63.25 ± 12.57	80.05 ± 7.27 * <i>P</i> < 0.05	71.85 ± 16.6	86.6 ± 7.56 * <i>P</i> < 0.05

Table (12): Comparison of pulmonary function data in asthmatic patients group versus asthmatic patients with allergic rhinitis one year after the course of sublingual immunotherapy (SLIT).

Pulmonary Function Data.	Asthmatic patients. No. = 20; M ± SD	Asthmatic patients with allergic rhinitis. No. = 20; M ± SD
FVC.	88.35 ± 5.01	89.10 ± 5.39 <i>P</i> > 0.05
FEV1.	81.15 ± 7.53	86.5 ± 5.88 * <i>P</i> < 0.05
PEF.	78.1 ± 9.97	79.85 ± 9.19 <i>P</i> > 0.05
FEF25%	69.6 ± 9.57	74.9 ± 11.29 <i>P</i> > 0.05
FEF50%	69.65 ± 11.84	76.9 ± 11.99 <i>P</i> > 0.05
FEF75%	68.8 ± 14.84	77.75 ± 15.08 <i>P</i> > 0.05
MVV	80.05 ± 7.27	86.6 ± 7.56 * <i>P</i> < 0.05

Table (13): Distribution of duration of sublingual immunotherapy (SLIT) in asthmatic patients and asthmatic patients with allergic rhinitis.

Duration of treatment	Asthmatic patients		Asthmatic patients with allergic rhinitis	
	No.	%	No.	%
One year.	1	5	2	10
18 months	2	10	2	10
2 years.	3	15	4	20
> 2 years	14	70	12	60
Total	20	100	20	100

Table (14): Distribution of side effects of sublingual immunotherapy (SLIT) in asthmatic patients and asthmatic patients with allergic rhinitis.

Side effects	Asthmatic patients. No= 20		Asthmatic patients with allergic rhinitis. No=20	
	No.	%	No.	%
• Nausea.	0	0	0	0
• Mouth itching or burning.	0	0	0	0
• Throat itching.	1	5	0	0
• Systemic reaction.	0	0	0	0
Total	1	5	0	0

Table (15): Distribution of tolerability of sublingual immunotherapy (SLIT) in asthmatic patients and asthmatic patients with allergic rhinitis.

Tolerability	Asthmatic patients.		Asthmatic patients with allergic rhinitis.	
	No.	%	No.	%
Very well	13	65	11	55
Good	6	30	6	30
Moderate	1	5	1	20
Bad	0	0	0	0
Total	20	100	20	100

Table (16): Distribution of reduction of clinical symptoms one year after (SLIT) in asthmatic patients group.

	Asthmatic patients.	
	No.	%
• Reduction of symptoms No= 20.	13	65
• Reduction of nocturnal asthma No = 8.	7	87.5
• Reduction of asthmatic attacks No= 11.	7	63.63
• Reduction of need to rescue treatment No = 20.	14	70

Table (17): Distribution of reduction of clinical symptoms one year after (SLIT) in asthmatic patients with allergic rhinitis group.

	Asthmatic patients with allergic rhinitis.	
	No.	%
• Reduction of symptoms No= 20.	15	75
• Reduction of nocturnal asthma No = 12.	11	91.66
• Reduction of asthmatic attacks No= 12.	9	75
• Reduction of need to rescue treatment No = 20.	15	75
• Reduction of nasal symptoms No = 20.	13	65%

4. Discussion:

Asthma and allergic rhinitis are characterized by common histopathological and inflammatory cellular processes and appear to be manifestations of the same underlying disorder⁽⁹⁾. The common features of the two diseases suggest that symptoms of one may impact symptoms of the other. In fact, the presence of concomitant allergic rhinitis in patients with asthma is associated with higher rates of asthma-related resource utilization and worsened asthma control⁽¹⁰⁾. Moreover, therapy for allergic rhinitis can have a beneficial effect on asthma-related outcomes: clinical trials have shown that treatment of allergic rhinitis can reduce asthma symptoms⁽¹⁰⁻¹²⁾, and emergency care for asthma⁽¹³⁾. Immunotherapy is the treatment that modifies the response of the immune system to allergens. It is considered a cornerstone in the management of respiratory allergy. Sublingual immunotherapy (SLIT), which is administered in the form of drops underneath the tongue, has been widely utilized in Europe for the past 10 years. Sublingual immunotherapy is now officially accepted as a viable alternative to the traditional subcutaneous route⁽¹⁴⁾. Sublingual immunotherapy (SLIT) has received approval from WHO working group and the international ARIA (Allergic rhinitis and its impact on asthma) consensus group for use in patients with allergic rhinitis and asthma. The aim is to alleviate symptoms during exposure to the allergen. It is an FDA-approved, clinically effective method and induces long-term remission of allergic rhinitis and allergic asthma, with improvement in clinical symptoms^(15,16).

Successful immunotherapy results not only in the increase of allergen concentration necessary to induce immediate or late-phase reactions, but also in the decreased responses to nonspecific stimulation⁽¹⁷⁾. Therefore, in contrast to symptomatic treatment, it can

reduce the likelihood of developing additional sensitizations by interrupting the so-called “atopic march” and patients may benefit from persistence of alleviation of clinical symptoms^(15,17,18). SLIT induces ten to hundred fold increase in IgG1 and -4 and a modest increase in IgG2. It has been observed that IgG4 exerts inhibitory effects on binding of IgE-FcεRII complexes on B cells⁽¹⁹⁾. SLIT affects T-cell responses to allergen by employing several mechanisms, including the following: by increasing the allergen-induced ratio of TH1 cytokines to TH2 cytokines, by inducing epitope-specific T-cell anergy that can be blocked by neutralization of IL-10, by generating allergen-specific T reg cells that can suppress the responses of effector T cells and by increasing the production of cytokines with regulatory activity⁽²⁰⁾. The studies have shown that sublingual immunotherapy exerts a long-lasting effect up to 5 years after discontinuation and that it is able to prevent the onset of new sensitizations⁽²¹⁾. In the present study, a trial has been made to administer the sublingual immunotherapy using multiple allergens in allergic asthmatic Patients with and without allergic rhinitis and to evaluate the clinical efficacy, safety, and changes in allergen-specific antibodies during sublingual immunotherapy (SLIT). Our study revealed that (40%) of asthmatic patients group and (60%) of asthmatic patients with allergic rhinitis group had nocturnal asthma (**Table 2**). This is in agreement with **Storms et al., 1994**⁽²²⁾, who reported that a total of 204 out of their 304 studied patients (67%) had nocturnal symptoms of asthma. Our study revealed that there were statistically significant decrease in blood eosinophils one year after SLIT compared to that before SLIT in both asthmatic patients group and asthmatic patients with allergic rhinitis group **Table (3)**. **Kim et al., 2010**⁽²³⁾ reported that there were

significant decrements in peripheral blood eosinophil counts and ECP ($p = 0.025$ and $p = 0.048$, respectively) in their allergic rhinitis patients treated with SLIT. Also, **La Grutta et al., 2007**⁽²⁴⁾ reported that the reduction of nasal eosinophils was statistically greater ($p < 0.05$) only in the SLIT group. Our study revealed that, there were statistically insignificant decrease in total IgE 6 months and one year after SLIT compared to that before SLIT in asthmatic patients group. On the other hand, there were statistically insignificant decrease in total IgE 6 months after SLIT and statistically significant decrease in total IgE one year after SLIT compared to that before SLIT in asthmatic patients with allergic rhinitis group (**Table 5**). This is in accordance to **Abd Elwadoud, and Salem, 2005**⁽²⁵⁾ who reported that the total IgE level (iu/ml) in allergic rhinitis patients before and after immunotherapy decreased from 789.24 ± 426.49 to 341.24 ± 227.15 iu/ml. The difference was found to be highly significant ($P < 0.001$). On the other hand, **Kim et al., 2010**⁽²³⁾ reported that total IgE did not change significantly before and after SLIT of their studied patients. Our studied asthmatics with and without allergic rhinitis had positive skin prick test ranged from 1 to 7 allergens. Sixty percent of asthmatic group before start of SLIT were sensitive to 3 allergens with a mean number of 3.30 ± 1.30 allergens., and (25%) of asthmatic patients with allergic rhinitis group were sensitive to 3 allergens with a mean number of 4.1 ± 2.1 allergens. This is similar to the study of **Al-Shehri, 2002**⁽²⁶⁾ who reported that the number of used allergens for immunisation ranged from 1 to 7 allergens. For most of the patients the use of 2, 3 or 4 allergens was reported (39 patients = 21.4 %, 58 = 31.9 %, 39 = 21.4 %). For 17 patients (9.3 %) only one allergen was needed, 10 patients (5.5 %) used 5 allergens and 3 patients used 6 and 7 allergens respectively. **Wise et al., 2009**⁽²⁷⁾ reported that the mean number of antigens included in SLIT regimens in their patient group was 11.6 (range, 3-21 antigens). Also, **Tripathi et al., 2008**⁽²⁸⁾ reported that allergens in graded strength having not more than 5 allergens were administered sublingually in all the patients. Our results revealed that the majority of asthmatic patients group were sensitive to mites (60%), followed by mixed grass pollens (30%), *Penicillium notatum* (25%), house dust (20%), Cockroach (20%) respectively. On the other hand, the majority of asthmatic patients with allergic rhinitis group were sensitive to mites (75%), followed by house dust (40%), mixed grass pollens (40%), mixed pollens (30%), cat epithelium (30%), *Penicillium notatum* (25%), , Cockroach (25%), dog epithelium (20%), and sheep wool (20%) **Table(9)**. **Abd Elwadoud, and Salem, 2005**⁽²⁵⁾ reported that the dust mites allergens represented (44%) of the group; fungus allergens alone represented (12%) of them while Mixed

Dust mites and Fungus allergens represent 44% of cases. Also, **Tripathi et al., 2008**⁽²⁸⁾ reported that the most common allergens responsible for allergic asthma treated with SLIT using multiple allergens were house dust, house dust mites, pollen and fungi. Results of specific IgE to food and inhalants and skin prick test revealed that there were statistically significant reduction of number of allergens one year after SLIT compared to that before start of SLIT in both asthmatic group and asthmatic with allergic rhinitis group ($P < 0.05$). This is in agreement with **Tripathi et al., 2008**⁽²⁸⁾ who reported that allergen specific IgE tested by skin prick test showed significant reduction at the end of three years of SLIT. Also, **Bahceciler et al., 2005**⁽²⁹⁾ reported that total eosinophil count and specific IgE decreased significantly after treatment with SLIT compared to that of healthy controls. Results of Pulmonary function in both asthmatic patients group and asthmatic patients with allergic rhinitis showed statistically significant increase in FVC, FEV1, PEF, FEF25%, FEF50% and MVV one year after SLIT compared to that before SLIT (**Table 11**). This is in accordance to **Wise et al., 2009**⁽²⁷⁾ who reported that increases in forced expiratory volume in 1 second (FEV1), mean expiratory flow at 25% of forced vital capacity (FVC), and methacholine threshold dose in adult patients treated with SLIT for birch pollen allergy. Also, **Calamita et al., 2006**⁽³⁰⁾ reported in a systematic review of randomized-clinical trials using the Cochrane Collaboration method that among the respiratory function tests evaluated (FEV1, FEV1%, PEF, and FEF25–75%), FEV1% showed a significant improvement (SMD 1.48; 95% CI: 0.13–2.82), among 144 patients in four studies (54, 55, 60, 66) and FEF25–75% (SMD 1.06; 95% CI: 0.40–1.72) among 42 patients in two studies (54, 66); the values in these studies, which are greater than zero, indicated that treatment by SLIT was favored. As regard the duration of sublingual immunotherapy one patient (5%) of asthma group discontinued treatment after one year, two (10%) after 18 months, 3 (15%) after 2 years because they felt free of symptoms, and 14 (70%) continued > 2 years. Two patients (10%) of asthma allergic rhinitis group discontinued treatment after one year, 2 (10%) after 18 months, 4 (20%) after 2 years because they felt free of symptoms, and 12 (60%) continued > 2 years (**Table 13**). **Pajno et al., 2005**⁽³¹⁾ reported that the discontinuation rate for SLIT after 12 months was 8.2%. Also, **Steiner et al., 2009**⁽³²⁾ reported that results of SLIT were equal 1, 3 and 5 years after termination of SLIT. Side effects of SLIT in our study was very few and negligible. Local reverse reactions (throat itching) were reported in one (5%) patient of asthma group. No local side effects were reported in asthmatic patients with allergic rhinitis group. No systemic side effects were reported in both

groups (**Table 14**). This is similar to the results of **Wise et al., 2009**⁽²⁷⁾ who reported that there were no serious adverse events reported with the initiation of SLIT at their study. Similarly, **Girado et al., 2005**⁽³³⁾ reported that the overall rate of all adverse events associated with the use of SLIT was very low at 1.4 to 4.9 events per 1000 SLIT doses. Also, **Al-Shehri, 2002**⁽²⁶⁾ reported reverse reactions were reported in 4 cases (2.1 %), one case with systemic side effects and 3 cases with local intolerance. Adverse reactions were described as burning sensation and itching in the mouth as well as nausea, severe throat smarting and scraping. Upon passing through a too fast dosistitration one patient reported allergic symptoms like conjunctivitis, rhinitis and aggravated asthma, which longed for a reduction and adjusted doses titration in future. Dilution of 1:10 and a slow doses titration resulted in good tolerance. For one patient the adverse reactions were not specified. From the twenty asthmatic group, 13 patients (65%) tolerated sublingual immunotherapy therapy very well, 6 (30%) good, 1 (5%) moderate. On the other hand, 11 asthmatic patients with allergic rhinitis (55%) tolerated therapy very well, 7 patients (35%) good, 2 patients (10%) moderate (**Table 15**). These results were similar to that of **Al-Shehri, 2002**⁽²⁶⁾ who reported that from the 182 documented patients 125 (68.7 %) tolerated therapy very well, 50 (27.5 %) good, 4 (2.2 %) moderate and one patient (0.5 %) bad. Also, **Steiner et al., 2009**⁽³¹⁾ reported that tolerability of SLIT were 85% rated "excellent", 12% "good", 3% of patients reported moderate side effects but no patient rated "bad". The following score was offered for selection: 1. excellent, no side effects; 2. good, slight side effects; 3. moderate side effects; and 4. bad, not acceptable. Our results revealed that 13 out of 20 (65%) asthmatic patients group had reduction of symptoms, 7 out of 8 patients (87.5%) had reduction of nocturnal asthma, 7 out of 11 patients (63.63%) had reduction of asthmatic attacks and 14 out of 20 patients (70%) had reduction of need to rescue treatment one year after the course of sublingual immunotherapy (**Table 16**). On the other hand, 15 out of 20 (75%) asthmatic patients with allergic rhinitis group had reduction of symptoms, 11 out of 12 patients (91.66%) had reduction of nocturnal asthma, 9 out of 12 patients (75%) had reduction of asthmatic attacks 15 out of 20 patients (75%) had reduction of need to rescue treatment, and 13 patients (65%) had reduction of nasal symptoms one year after the course of sublingual immunotherapy (**Table 17**). These results were similar to that of **Bahceciler et al., 2005**⁽²⁹⁾ who have reported significantly reduced asthma symptoms and medication use, reduced number of asthma exacerbations, increased FEV1, and increased peak expiratory flow rate with SLIT. Similarly, **Tripathi et al., 2008**⁽²⁸⁾ who had reported

that results of sublingual immunotherapy using multiple allergen showed significant reduction in symptoms, medication, and improvement in PEFr by modifying the natural history of the disease and preventing the onset of new sensitization. Also, **Abramson et al., 2003**⁽³⁴⁾ reported in a meta-analysis of allergen immunotherapy that included 75 prospective, randomised controlled trials of immunotherapy for asthma showed a reduction in the need for medication, a reduction in bronchial hyper-responsiveness, and improvement in forced expiratory volume in 1 second (FEV1). Another meta-analysis of SLIT efficacy in the treatment of adult and pediatric allergic rhinitis by **Wilson et al., 2005**⁽²⁾ assessed 22 studies and found a statistically significant symptom reduction and a statistically significant reduction in medication use, supporting the efficacy of SLIT in allergic rhinitis. **Lue et al., 2006**⁽³⁵⁾ and **Bahceciler et al.**⁽³⁶⁾ have reported significantly reduced asthma symptoms and medication use, reduced number of asthma exacerbations, increased FEV1, and increased peak expiratory flow rate with SLIT. On The other hand, **Ferrés et al., 2010**⁽³⁷⁾ reported that as for the long-term effect of SLIT on asthma symptoms, they did not find any reduction in the consumption of treatments for asthma between baseline and the six month visit. Moreover, they did not find any temporal improvement in asthma severity, assessed using the GAS, or in respiratory function. The clinical condition of the patients selected in their study might explain the lack of a significant improvement in asthma severity: peak flow and FEV1 at baseline were markedly high, indicating that asthma was well controlled in these patients when they initiated the treatment with SLIT. **Wilson et al., 2006**⁽¹⁴⁾ reported that in the patients who received SLIT, researchers observed a significant reduction of nasal obstruction, itching and cough, and a decreased need for medications for symptom relief. They also discovered that the patients who received SLIT made fewer trips to the physician's office and missed fewer days of work than those patients treated with only standard allergy/asthma medication.

There are several limitations to our study. First, the relative small number of patients and limited follow up period of the study about 28 months. Second most SLIT studies have used single allergen monotherapy to evaluate efficacy whereas a few studies have included more than one allergen in the treatment regimen. So, studies on the efficacy of monotherapy vs polytherapy in SLIT treatment regimens are lacking.

Third, loss of follow up of patients after 28 months to evaluate long term outcome of SLIT in these patients.

Conclusion:

From this study we concluded that sublingual immunotherapy is a safe treatment which significantly reduces symptoms and medication requirements, improves lung function in both asthmatic patients with and without allergic rhinitis. SLIT using multiple allergens lowered the allergen burden in both asthmatic patients with and without allergic rhinitis.

Corresponding author

Zakia Abu-Zahab,
Department of Clinical Pathology, Faculty of Medicine
For Girls, Al Azhar University, Egypt.
omima468@yahoo.com

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Novel Antisickling, Antioxidant and Cytotoxic Prenylated Flavonoids from the Bark of *Morus alba* LK.M. Meselhy¹, Lamiaa N. Hammad² and Nahla Farag³¹Department of Pharmacognosy, Faculty of Pharmacy, Cairo University & Misr International University, Egypt²Department of Biochemistry, Faculty of Pharmacy, Misr International University, Egypt³Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Misr International University, Egyptkmeselhy@hotmail.com

Abstract: Two novel prenylated flavonoids and quercetrin were isolated from extract of the bark of *Morus alba* L. The structures of these compounds were established based on physicochemical data, UV spectral data, ¹H-NMR, ¹³CNMR, ¹H-¹H COSY, HMBC and EIMS. Extract and isolated compounds exhibited a significant antisickling activity, a powerful antioxidant activity and remarkable cytotoxic activity. Both antioxidant & cytotoxic activity were supported & evidenced by docking structure of isolated compounds in the receptor binding site and estimation of binding affinity into 17beta-hydroxysteroid dehydrogenase type 1 (17beta-HSD1) (3HB5) and glutathione reductase (1XAN) as a standard docked model.

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Keywords: prenylated flavonoids, flavonol, *Morus alba*, antisickling activity, antioxidant activity, cytotoxic activity, docking

1. Introduction

The White Mulberry (*Morus alba*) is a short-lived, fast-growing, small to medium sized tree to 10–20 m tall, native to northern China known as Tuta in Sanskrit and Tuti in Marathi, and widely cultivated elsewhere. It is also known as Tuta in Egypt (Bailey, 1953; Leung & Foster, 1977 & Taylor *et al.*, 2006). Bark is characterized by light tannish brown colour while stems are yellowish brown with sweet taste. White substance appears when stem is broken in warm conditions (Bailey, 1953). Trees are extensively grown (e.g. southern Europe, India) for their leaves as food for silkworms. Fruits may be eaten raw or cooked. Fruits are an ingredient of a particularly seductive drink known as Mulberry Wine. Stem bark is fibrous and used in China and Europe for paper making. Twigs are used as binding material and for making baskets. Trees often used as ornamentals, roadsides or boundary markers (C.S.I.R., 1948 & Reed, 1976)

In Traditional Chinese Medicine, the fruit is used to treat prematurely grey hair, to "tonify" the blood, and treat constipation and diabetes. The bark is used to treat cough, wheezing, edema, and to promote urination. It is also used to treat fever, headache, red dry and sore eyes, as well as cough (Taylor *et al.*, 2006).

Medicinally, fruits are laxative, refrigerant in fevers, and used locally as remedy for sore throat, dyspepsia. Roots and bark are purgative, anthelmintic, and astringent; leaves considered diaphoretic and emollient; a decoction of leaves being used as a gargle for inflammation of throat (Reed, 1976).

Drug design is an important tool in the field of drug discovery where new compounds are synthesized by molecular or chemical manipulation of the lead

moiety in order to produce highly active compounds with minimum side effect (Cavasotto & Abagyan, 2004). Search for new ligands and the assessment, improvement and extension of the lead is a very important step in identification of new chemical entities (Borges *et al.*, 2002). Nowadays, the use of computers to predict the binding of libraries of small molecules to known target structures is an increasingly important component in the drug discovery process (Schoichet, 2004 & Koppen, 2009). Docking of small molecules in the receptor- binding site and estimation of binding affinity of the complex is a vital part of structure based drug design (Seeliger & Groot, 2010).

Natural Drugs are usually discovered by means of high-throughput screening approaches that use *in vitro* experiments to evaluate the activity of a large number of compounds against a known target. This procedure is very costly, time-consuming and consume large amount of natural isolated compounds. If the crystallographic structure of the protein target is available, then molecular docking simulations can be a helpful computational approach in the drug-discovery process. This computer simulation process allows for faster and cheaper identification of promising drug candidates using structure-based virtual screening (Bellows & Floudas, 2010; De Azevedo, 2010; De Azevedo, 2010 & Kim *et al.*, 2010; Hernandez *et al.*, 2010; Krystof & Uldrijan, 2010; Mitrasinovic, 2010 & Rizzolio *et al.*, 2010).

Subsequently, *in vitro* tests can be performed to further evaluate the drug candidates found by the virtual screening process (Thomsen & Christensen, 2006).

Reviewing the available current literature, different organs of *Morus alba* species have been

investigated previously (Deshpande, 1968; Anca Maier *et al.*, 1997; Shin-Ichi & Setsuko, 1997; Jiang *et al.*, 2003; Park *et al.*, 2003; Sohn *et al.*, 2004; Chen *et al.*, 2005; Singab *et al.*, 2005; Chen *et al.*, 2006; El-Beshbishy *et al.*, 2006; Katsube *et al.*, 2006; Ercisli & Orhan, 2007; Kaushik *et al.*, 2008; & Zheng *et al.*, 2008).

Few works were done on root bark of some *Morus* species (Jiang *et al.*, 2003; Park *et al.*, 2003 & Singab *et al.*, 2005;). Little was reported concerning the study of flavonoids of *Morus alba* root bark in the available literature (Jiang *et al.*, 2003).

Preliminary phytochemical screening of bark of the plant revealed the presence of different phenolics. Therefore, it was deemed of interest to isolate and identify these constituents; as well as to evaluate certain biological activities of the plant with evidence based approach by docking structure of isolated compounds in the receptor binding site and estimation of binding affinity.

We found two novels prenylated flavonoids 1-2, as well as known compound 3. In addition some biological activities of these compounds were carried out & These activities were proved by docking module. Now we report our results of this study in detail

2. Material and Methods

2.1. Plant material:

Plant material was collected from bark of *Morus alba* L. was collected from plants cultivated in the private farm, Zagazig, EL-Sharkya, Egypt. The identity of the plant was kindly confirmed by Prof. Dr. Mohamed Nabil Attia, Head of Cultivated Plants Department, Faculty of Agriculture, Zagazig University. The plant was air-dried, reduced to fine powder and kept in tightly closed amber coloured glass containers. Voucher specimens are kept in the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

2.2. Chemicals:

2.2.1. Reference samples:

Flavonoids from Sigma (USA) and Aldrich (Germany)

2.2.2. Material for chromatography:

Silica gel G (60 mesh) for TLC, silica gel (70-230 mesh) for CC, precoated TLC plates (silica gel 60 GF254) from E. Merck (Darmstadt, Germany), sephadex LH-20 from Pharmacia (Uppsala, Sweden)

2.2.3. Solvent systems:

S1: Hexane-ethyl acetate-methanol (in different ratios v/v)

S2: Chloroform - Methanol (in different ratios v/v).

Spray reagents; Sulphuric acid (50%) and P-anisaldehyde were used.

2.3. Material for biological evaluation:

2.3.1. Plant extracts:

The biological evaluation was performed on the three isolated compounds in addition to the Methanol (95 %) extract of the air-dried powdered bark. Bark of *Morus alba* L. were air-dried, milled and extracted with methanol for 24 hrs by maceration. The MeOH extract was evaporated in rota-vapor to yield a semisolid residue (650 g). The solvent-free residue was kept for investigation

2.3.2. Animals:

Male albino rats of Sprague Dawley Strain (120-150g) were used. Animals were obtained from the animal house, of the National Research Center, Dokki, Giza, Egypt. The animals were fed on a standard laboratory diet composed of vitamin mix (1 %), mineral mix (4 %), corn oil (10 %), sucrose (20 %), cellulose (0.2 %), casein (10.5 %) and starch (54.3 %)

2.3.3. Tumor cell lines:

Tumor cell lines (cervix, HELA), (liver, HEPG2) and (breast, MCF7) from National Cancer Institute of Egypt were used for cytotoxic screening

2.3.4. Reference drugs and kits:

Glutathione Kit (Wak, company – Germany), and Vitamin E (Pharco Pharmaceutical Co, Egypt) in addition to Testosterone propionate (4µg/ml TC199) were used in the pharmacological screening

2.4. Apparatus:

UV-visible spectrophotometer, Shimadzu UV 240 (P/N 204-58000); Mass spectrometer: Varian 90 NMR spectrophotometer; NMR Jeol GLM, Jeol TMS Route instrument (¹H-NMR, 300 MHZ, ¹³C, 75 MHZ, Japan); Koffler's heating stage microscope

2.5. Software for docking:

ChemDraw 3D structures were constructed using Chem 3D ultra 8.0 software (Molecular Modeling and Analysis; Cambridge Soft Corporation, USA (2010)), and then they were energetically minimized by using OPAC (semi-empirical quantum mechanics), Jop Type with 100 iterations and minimum RS gradient of 0.01, and saved as DL MolFile (mol)

There is a wide range of software packages available for the conduct of molecular docking simulations like, **Molsoft** (Murcko & Stouten, 1997; Anderson & Weng, 1999; Halperin, 2002 & Wang *et al.*, 2003;); as flexible docking program enable us to predict favorable protein-ligand complex structures with reasonable accuracy and enhanced docking speed.

All docking studies were performed using "Internal Coordinate Mechanics (Molsoft IC 3.4-8C)". ICM docking is probably the most accurate predictive tool of binding geometry today (Murcko & Stouten, 1997; Anderson & Weng, 1999; Halperin, 2002 & Wang *et al.*, 2003).

2.6. Phytochemical study:

2.6.1. Investigation of flavonoid content:

2.6.1.1. Extraction, isolation and Identification:

Bark of *Morus alba* L. were air-dried, milled and extracted with methanol for 24 hrs by maceration. The

mark left was repeatedly extracted five times similarly, for complete extraction. The MeOH extract was evaporated in rota-vapor to yield a semisolid residue (650 g)

The methanolic residue (650g) was successively fractionated over silica gel VLC (silica for TLC packed on column; 10 cm i.d. × 20 cm) with *n*-hexane, ethyl acetate, methanol. The solvents were removed to give fractions of *n*-hexane (3.2 g), ethylacetate (2.6 g) and methanol (9.6 g).

The ethylacetate fraction (2.6 g) (was subjected to a silica gel column chromatography (SiO₂ column; 20 mm i.d. × 25 cm) using solvent system of CHCl₃-MeOH (9:1). The active fraction of CHCl₃:MeOH (9:1) eluate (115 mg) was further separated & purified by Sephadex LH-20 column chromatography (30 mm i.d. × 50 cm, MeOH) to give compound 1(23 mg) & compound 2(21 mg).

The methanolic fraction of VLC (9.6 g) was column chromatographed over silica gel (SiO₂ column; 40 mm i.d. × 20 cm) using chloroform and methanol, step gradient as eluents to yield compound 3 (15 mg) from fraction eluted by CHCl₃-MeOH (8:2).

The structure elucidation of compounds (1-3) was established based on physico-chemical data, UV spectral data, ¹H-NMR, ¹³C-NMR and EIMS

2.7. Biological study:

2.7.1. Antioxidant activity:

The antioxidant activity of the tested samples (Methanolic extract and isolated compounds) was assessed by measuring the glutathione level in blood samples collected from alloxan-induced diabetic rats as compared to Vitamin E (12 mg / kg b.wt., positive control) and adopting the procedure described by Beutler (Beutler *et al.*, 1963). The restoration of blood glutathione levels (reduced due to induction of diabetes) was taken as a measure of antioxidant activity. The percentage change observed after dose administration was, in each case, calculated according to the following equation: % of change = $(G_c - G_t) \times 100 / G_c$. The results are recorded in Table (1) and illustrated in Figures (2)

2.7.2. Antisickling activity:

Sickle cell anaemia (SCA) is a hereditary anaemia that results from an abnormal β-haemoglobin molecule (valine is substituted for glutamic acid), forming haemoglobin AS instead of normal one (haemoglobin AA). Sickle cell crisis occurs when an individual homozygous for the sickle cell gene is exposed to an adverse state (hypoxia, dehydration), causing the cell to take on a sickle shape, with subsequent occlusion of small vessels (Pousada *et al.*, 1996).

Antisickling activity was determined as described (Sofowara & Isaac, 1971). Tested samples were redissolved in tissue culture medium no. 199 (T.C. 199). The final suspensions were filtered, the volume made up to 180 ml with T.C. 199 and the resulting solutions autoclaved. Tested solutions were similar, in pH, to T.C.

199. Testosterone propionate was diluted with T.C. 199 to give a 4 μg/ml suspension which was used for comparison.

Blood sampling and preparation for the test samples of haemoglobin AA and haemoglobin AS blood were obtained by veinpuncture from males aged 25 and 36, respectively. Both donors were in good health and the blood was collected in acidic citrate-dextrose-saline. The blood was added either to a well, formed by sealing a drilled sterile microscope slide (diameter of hole = 3 mm) with cover slips, or to a sterile Bijou bottle. To haemoglobin AA blood, an equal volume of tested samples or testosterone propionate suspension (4μg/mL) was added. To the haemoglobin AS sample, an equal volume of sterile 2% sodium metabisulphite was first added until sickling was observed in 95% of the red cells (usually after one hr incubation at 37°C) and the same volume of tested samples or testosterone propionate suspension was then added.

A control containing a volume of T.C. 199 in place of the tested samples. All solutions were sterilized before use and aseptic techniques were used throughout the experiments. The drilled slides were observed directly under the microscope, while samples from the Bijou bottles were put on slides and sealed over with cover slips just before examination. Observations consisted of estimates of the effect of control (TC199) and tests (methanolic extract and isolated compounds.) on haemoglobin AA cells which crenated and on haemoglobin AS which sickled after incubation at 37°C for 18 hrs. At this stage, maximum effect occurred and could be compared with the effect of testosterone propionate (concentration 4μg /mL) as standard. The results are recorded in Table (2) and illustrated in Figure (3)

2.7.3. Evaluation of Potential Cytotoxicity by SRB

Assay:

Potential cytotoxicity of the tested samples (Compounds 1, 2 and 3) was tested at the National Cancer Institute of Egypt adopting the method of Skehan (Skehan & Strong, 1990). Cells were plated in a 96-wells plate (10⁴ cells/well) for 24hrs before treatment with the tested sample to allow attachment of the cells to the wall of the plate. Different concentrations of each of the tested samples under study (0, 1, 2.5, 5 and 10 μg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose and were incubated for 48hrs at 37°C in an atmosphere of 5% CO₂. After 48hrs, cells were fixed, washed and stained with Sulphorodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris-EDTA buffer. Colour intensity was measured in an ELISA reader. The survival curves of each of the tumor cell lines (cervix, liver and breast) were plotted and IC₅₀ was calculated for each of the tested samples table (3)

2.8. Docking of Isolated compounds in the receptor-binding site:

2.8.1. Generation of Ligand and Enzyme Structures:

The crystal structures of target protein compounds 1, 2, 3 into 17 β -hydroxysteroid dehydrogenase type 1 (17 β -HSD1) (3HB5) and glutathione reductase (1XAN) active site were retrieved from the Protein Data Bank (<http://www.rcsb.org/pdb/welcome.do>). All bound waters, ligands and cofactors were removed from the protein. The amino acids of the binding site were defined using data in `pdbsum` (<http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/>).

2.8.2. Docking using Molsoft IC 3.4-8C program:

2.8.2.1. Convert our PDB file into an IC object.

This conversion involves addition of hydrogen bonds, assignment of atom types, and charges from the residue templates.

2.8.2.2. To perform IC small molecule docking

- a) Setup Docking Project:
 - Set Project Name
 - Setup the Receptor
 - Review and Adjust Binding Site
 - Make Receptor Maps
- b) Start docking simulation

2.8.2.3. Display the result:

IC stochastic global optimization algorithm attempts to find the global minimum of the energy function that include five grid potentials describing interaction of the flexible ligand with the receptor and internal conformational energy of the ligand, during this process a stack of alternative low energy conformations is saved (Tables 4 & 5). The mode of interaction of compounds 1, 2, 3 into 17 β -hydroxysteroid dehydrogenase type 1 (17 β -HSD1) (3HB5) and glutathione reductase (1XAN) was used as a standard docked model. All inhibitors were compared according to the best binding free energy (minimum) obtained among all the run.

3. Results

The structure elucidation of compounds (1-3) Figure 1, was established based on physico-chemical data, UV spectral data, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and EIMS.

Compound 1: 23 mg, yellow crystals

MP: 235-240 °C.

Rf: 0.53 (CHCl_3 -MeOH, 9:1).

UV/Vis λ_{max} (MeOH) nm (log ϵ): 228 sh, 293, 339 + NaoMe, 248, 285, 333; + AlCl_3 , 221, 316, 392; + NaoAc, 287, 297, 334 + H_3BO_3 .

$^1\text{H NMR}$ (300 MHz, DMSO- d_6): 9.08 (1H, s, OH-3'), 8.86 (1 H, s, OH-4'), 6.73 (1 H, d, J= 1.5 Hz, H-2'), 6.70 (1 H, d, J= 1.6 Hz, H-6'), 5.88 (1 H, d, J=2.4 Hz, H-8), 5.70 (1 H, d, J=2.4 Hz, H-6), 5.2 (1H, m, H-2''), 5.1 (1H, m, H-2'''), 3.60 (1 H, dd, J= 7.1 Hz, J= 9.2 Hz H- β H-3), 2.8 (1 H, dd, J= 7.1 Hz, J= 9.2 Hz H- α H-3), 2.65(2H, d,

J= 4.7 Hz, H-1''), 2.60(2H, d, J= 4.7 Hz, H-1'''), 1.25(6H, s, H-5'', H-5'''), 1.06(6H, s, H-4'', H-4''')

$^{13}\text{C NMR}$ (75 MHz DMSO- d_6): 197 (C-4), 157 (C-7), 156 (C-9), 145.6 (C-3'), 145.5 (C-4'), 140.1 (C-3'''), 138.2 (C-3''), 131 (C-1'), 126.5 (C-2''), 126.5 (C-2'''), 124.5 (C-5), 119 (C-6'), 115.5 (C-5'), 115 (C-2'), 100 (C-10), 96 (C-6), 94.5 (C-8), 82 (C-2), 43 (C-3), 30.5 (C-1'''), 29.5 (C-1''), 26.7 (C-5''), 26.7 (C-5'''), 13.1 (C-4''), 13.1 (C-4''').

MS (EI, 70 eV): m/z (%) = 408 (M^+ , 88%), 324 (45%).

Compound 2: 21 mg, yellowish white crystals

MP: 246-248 °C.

Rf: 0.82 (CHCl_3 -MeOH, 9:1).

UV/Vis λ_{max} (MeOH) nm (log ϵ): 229 sh, 298, 333 + NaoMe, 248, 276, 333; + AlCl_3 , 220, 392; + NaoAc, 284, 295, 336 + H_3BO_3 .

$^1\text{H NMR}$ (300 MHz, DMSO- d_6): 9.08 (1H, s, OH-3'), 8.86 (1 H, s, OH-4'), 6.73 (1 H, d, J= 1.5 Hz, H-2'), 6.70 (1 H, dd, J= 8 Hz, J= 1.6 Hz, H-6'), 6.62 (1 H, d, J=9.1 Hz, H-5'), 5.88 (1 H, d, J=2.4 Hz, H-8), 5.70 (1 H, d, J=2.4 Hz, H-6), 5.88 (1 H, d, J=2.4 Hz, H-8), 5.70 (1 H, d, J=2.4 Hz, H-6), 5.2 (1H, m, H-2''), 4.8 (1H, d, J=7.5, H-2), 4.41 (1H, m, H-3), 2.65(2H, d, J= 4.7 Hz, H-1''), 1.25(3H, s, H-5''), 1.06(3H, s, H-4'')

$^{13}\text{C NMR}$ (75 MHz DMSO- d_6): 196.5 (C-4), 157.2 (C-7), 155.8 (C-9), 145.8 (C-3'), 145.7 (C-4'), 138.4 (C-3''), 131.3 (C-1'), 126.5 (C-2''), 124.5 (C-5), 118.8 (C-6'), 115.6 (C-5'), 115.1 (C-2'), 99.7 (C-10), 95.8 (C-6), 94.6 (C-8), 81.7 (C-2), 67.1 (C-3), 29.5 (C-1''), 26.4 (C-5''), 13.4 (C-4'').

MS (EI, 70 eV): m/z (%) = 356 (M^+ , 10%), 326 (9%).

Compound 3: 15 mg, yellow powder

MP: 181-183 °C.

Rf: 0.95 (CHCl_3 -MeOH, 9:1).

UV/Vis λ_{max} (MeOH) nm (log ϵ): UV/Vis λ_{max} (MeOH) nm (log ϵ): 259, 299sh, 358; + NaoMe, 272, 320sh, 415; + AlCl_3 271, 300sh, 328sh, 430; + NaoAc, 275, 324sh, 395; + H_3BO_3 260, 324sh, 377

$^1\text{H NMR}$ (300 MHz, DMSO- d_6): Aglycone: 12.5-(S, OH), 7.4-(2H, d, J=8Hz, H-2',6'), 6.85-(1H, d, J=7.5Hz, H-5'), 6.4-(1H, d, J= 2.5Hz, H-8), 6.23-(1H, d, J=2.5Hz, H-6)

Sugar: 5.3-(1H, d, J=2.5Hz, H-1''), 3.1-3.7-(m, other protons of sugar), 1.1-(3H, d, J=6.5Hz, Me)

MS (EI, 70 eV): m/z (%) = 448 (M^+ , 18%), 302((38%), 146 (18%).

The different biological activities evaluated (antioxidant, antisickling and cytotoxic) for the aforementioned extract and isolated compounds, represented in (Tables 1-3), revealed variable although significant efficacy & potency for all the samples when compared to standard and suggest their incorporation in herbal formulations after necessary clinical trials.

Concerning docking study results; It was reported that (Mazumdar *et al.*, 2009) Oestradiol is a well-characterized sex hormone that stimulates breast cancer and other oestrogen-related diseases. 17 β -

hydrosteroid dehydrogenase type 1 (17 β -HSD1) catalyses the last step in the synthesis of oestradiol and androstenediol in breast tumour tissue. The enzyme's high expression and activity after simultaneous blockade of oestrogen receptors and inhibition of aromatase in the tumour shows the necessity for its inhibition as a requirement for breast cancer therapy.

On the other hand Glutathione reductase is an important glutathione reductases housekeeping enzyme for redox homeostasis both in human cells. It showed that inhibitor to bind noncovalently with some amino acid residues explains the reduction at the flavin of glutathione reductase (Savvides & Karplus, 1996).

In the present study, we have screened 3 flavonoides (Figure 1) for cytotoxic effect in (breast & cervix cancer) cell lines and the antioxidant activities. So, we docked the isolated compounds 1, 2 & 3 from

plant under study into high affinity crystal Structure of 17 β -hydrosteroid dehydrogenase type 1 (17 β -HSD1) (3HB5) (Mazumdar *et al.*, 2009) in complex with a natural inhibitor E2B {3-(3',17 β -dihydroestra-1',3',5'(10')-trien-16 β -methyl) benzamide} and into the crystal Structure of glutathione reductase (1XAN) (Savvides & Karplus, 1996) active sites in order to predict their binding modes, their binding affinities and orientation of these compounds at the active site of the enzymes. The ICM score values and hydrogen bonds formed with the surrounding amino acids obtained by docking our titled compounds shows good agreement and predicted binding affinities obtained by molecular docking studies on (3HB5) as verified by antiproliferative (Table 4, Fig. 4), and on 1XAN as verified by antioxidant screening (Table 5, Fig. 5).

Table 1. Antioxidant effect of methanol extract and isolated compounds obtained from the bark of *Morus alba* L. grown in Egypt.

Tested Samples	Blood glutathione (mg%)	
	Mean \pm S.E	Potency ²
Control (Saline)	35.2 \pm 0.5	-
Diabetic	23.9 \pm 0.1*	-
Methanol extract	32.2 \pm 0.4	92.2
Compound 1	34.3 \pm 0.9	98.1
Compound 2	31.1 \pm 1.3	89.1
Compound 3	33.9 \pm 1.1	97.1
Diabetic + Vit E.	34.9 \pm 0.5	100

1: Blood glutathion level expressed in mg % as mean \pm S.E. 2: Percentage of Potency as compared to standard.

* Significantly different from the control group at $p < 0.01$

Table 2. Antisickling effect of methanol extract and isolated compounds obtained from the bark of *Morus alba* L. grown in Egypt.

Tested Samples	Effect on RBCs	
	% of crenated cells after 18	% of sickled cells after 18 hrs
Control (T.C. 199)	74%	91%
Methanol extract	45%	65%
Compound 1	49%	59%
Compound 2	41%	52%
Compound 3	43%	54%
Testosterone propionate (4 μ g/ml TC199)	40%	51%

Table 3. Cytotoxic activity of compounds 1 & 2 isolated from the bark of *Morus alba* L. grown in Egypt.

Cell Line	IC ₅₀ (μ g/ml)		
	Compound 1	Compound 2	Compound 3
Cervix (MCF7)	0.71	0.92	0.89
Breast (HELA)	0.56	1.41	0.97
Liver (HEPG2)	0.52	0.45	0.68

Table 4. ICM Scores of inhibitor ligand E2B, NADP, the compounds, and hydrogen bonds formed with amino acid residues and their lengths.

Tested Samples	ICM score	No. of hydroge bonds	Involved group of ami acid	Atom of lig involved	Length of hydrogen bo (A)
Ligand	-133.03	7	S142 hg -- m	o3	2.56 A
			V188 hn -- m	n1	2.78 A
			C185 o -- m	h04	2.26 A
			V188 o -- m	h11	2.52 A
			V188 o -- m	h12	1.47 A
			T190 og1 -- m	h02	1.88 A
			E282 oe1 -- m	h03	2.11 A
NADP	-303.59	41	S11 hn -- m	o9	2.58 A
			S11 hn -- m	o10	1.85 A
			S12 hn -- m	o9	1.29 A
			I14 hn -- m	o1	2.24 A
			G15 hn -- m	o1	1.56 A
			R37 hn -- m	o11	1.68 A
			R37 he -- m	o13	2.02 A
			R37 he -- m	n6	2.65 A
			R37 hh21 -- m	o13	1.61 A
			R37 hh21 -- m	o12	2.71 A
			R37 hh22 --m	o13	1.02 A
			T41 hg1 -- m	o12	2.59 A
			R67 he -- m	o14	2.38 A
			R67 hh22 -- m	o14	2.32 A
			N90 hn -- m	n3	2.54 A
			N90 hd22 -- m	o5	1.94 A
			A91 hn -- m	n3	1.87 A
			K159 hz1 -- m	o16	2.23 A
			K159 hz1 -- m	o15	2.79 A
			K159 hz2 -- m	o16	2.69 A
			K159 hz2 -- m	o15	1.81 A
			T8 og1 -- m	h06	2.72 A
			G9 o -- m	h09	1.92 A
			G9 o -- m	h10	1.21 A
			G9 o -- m	h13	2.43 A
			S11 og -- m	h14	2.04 A
			S12 og -- m	h11	2.73 A
			S12 o -- m	h11	1.89 A
			S12 o -- m	h01	1.53 A
			S12 o -- m	h13	1.75 A
			G13 o -- m	h01	2.74 A
			D65 od1 -- m	h16	1.65 A
			C89 o -- m	h07	1.39 A
N90 od1 -- m	h10	2.15 A			
N90 o -- m	h32	1.43 A			
A91 o -- m	h06	2.61 A			
A91 o -- m	h32	2.45 A			
A91 o -- m	h05	1.25 A			
T140 o -- m	h08	2.34 A			
G141 o -- m	h19	2.79 A			
Y155 oh -- m	h20	1.56 A			
Comp.1	-111.44	9	S11 hn -- m	o4	1.91 A
			S11 hn -- m	o3	2.5 A
			S12 hn -- m	o4	1.44 A
			I14 hn -- m	o2	2.36 A

			K159 hz1 -- m	o5	1.63 A
			K159 hz2 -- m	o5	2.45 A
			S11 og -- m	h10	1.99 A
			S12 o -- m	h11	2.09 A
			Y155 oh -- m	h12	2.22 A
Comp. 2	-91.71	9	I14 hn -- m	o5	1.99 A
			G15 hn -- m	o5	2.27 A
			T118 hg1 -- m	o3	2.48 A
			K159 hz1 -- m	o6	1.67 A
			K159 hz2 -- m	o6	1.58 A
			K159 hz3 -- m	o4	1.97 A
			N114 o -- m	h9	2.07 A
			T118 og1 -- m	h8	2.49 A
			Y155 oh -- m	h18	2.54 A
Comp. 3	-99.36	14	I14 hn --m	o8	2.51 A
			N90 hd21 --m	o11	1.56 A
			N90 hd22 --m	o11	1.23 A
			T140 hg1 --m	o11	2.26 A
			S142 hg --m	o6	1.79 A
			G9 o -- m	h19	1.57 A
			N90 od1 --m	h18	2.58 A
			N90 od1 --m	h20	2.72 A
			N90 o --m	h18	1.45 A
			N90 o --m	h20	1.74 A
			A91 o --m	h7	1.38 A
			T140 o --m	h8	1.31 A
			C185 o --m	h9	2.36 A
			G186 o --m	h9	2.70 A

Table 5. ICM Scores of FAD, the compounds, and hydrogen bonds formed with amino acid residues and their lengths.

Tested Samples	IC scores	No. of hydrogen bo	Involved group of amino acid	Atom of ligand involved	Length of hydrogen bond (Å)
FAD	-281.49	31	G29 hn -- m	o5	2.42 A
			G31 hn -- m	n5	1.93 A
			T57 hn -- m	o6	2.37 A
			T57 hg1 -- m	o3	2.12 A
			T57 hg1 -- m	o6	2.46 A
			T57 hg1 -- m	o7	2.57 A
			K66 hz3 -- m	n9	2.37 A
			G158 hn -- m	o1	2.33 A
			G158 hn -- m	o3	2.29 A
			S177 hg -- m	o10	1.76 A
			R291 hh11 -- m	o12	2.60 A
			R291 hh12 -- m	o9	1.84 A
			R291 hh12 -- m	o10	2.44 A
			R291 hh21 -- m	o7	2.02 A
			R291 hh21 -- m	o9	2.22 A
			N294 hd21 -- m	o1	2.18 A
			N294 hd21 -- m	o2	2.72 A
			D331 hn -- m	n3	2.75 A
			D331 hn -- m	n4	1.50 A
			E50 oel -- m	h41	2.56 A
			T57 og1 -- m	h22	1.77 A
			V61 o -- m	h15	1.56 A
			V61 o -- m	h18	2.36 A
G62 o -- m	h07	1.54 A			
D331 od1 -- m	h51	1.74 A			

			G158 o -- m	h11	1.31 A
			G158 o -- m	h01	2.28 A
			S177 og -- m	h16	1.40 A
			D178 od1 -- m	h14	1.90 A
			D178 od2 -- m	h13	2.30 A
			D331 od1 -- m	h07	2.28 A
Comp. 1	-112.05	6	T57 hn -- m	o2	1.96 A
			Y197 hh -- m	o3	2.13 A
			D331 hn -- m	o5	2.13 A
			T339 hn -- m	o4	2.41 A
			V329 o -- m	h12	2.08 A
			L337 o -- m	h11	1.53 A
Comp. 2	-92.09	8	T57 hn -- m	o6	2.68 A
			C58 hn -- m	o2	2.22 A
			G157 hn -- m	o4	2.46 A
			V332 hn -- m	o4	2.23 A
			L338 hn -- m	o5	1.76 A
			A155 o -- m	h8	1.46 A
			V329 o -- m	h9	1.67 A
			L337 o -- m	h10	1.39 A
Comp. 3	-101.46	18	G29 hn -- m	o11	2.57 A
			T57 hn -- m	o2	2.46 A
			T57 hn -- m	o8	1.57 A
			T57 hg1 -- m	o2	2.54 A
			T57 hg1 -- m	o8	2.31 A
			T156 hn -- m	o5	2.19 A
			T156 hn -- m	o4	1.91 A
			L338 o -- m	o5	1.22 A
			G157 hn -- m	o4	2.56 A
			D331 hn -- m	o1	2.07 A
			L338 hn -- m	o6	2.51 A
			T339 hn -- m	o6	2.58 A
			E50 oel -- m	h19	2.72 A
			I154 o -- m	h7	2.10 A
			A155 o -- m	h18	1.72 A
			T156 o -- m	h8	1.50 A
			V329 o -- m	h7	2.55 A
			G157 hn -- m	h9	1.79 A

Figure 1. Isolated compounds from methanol extract of *Morus alba* L. bark.

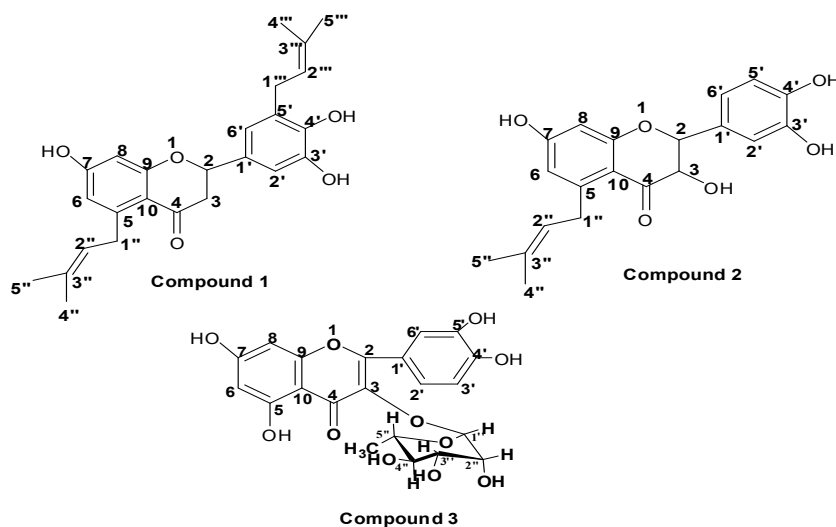
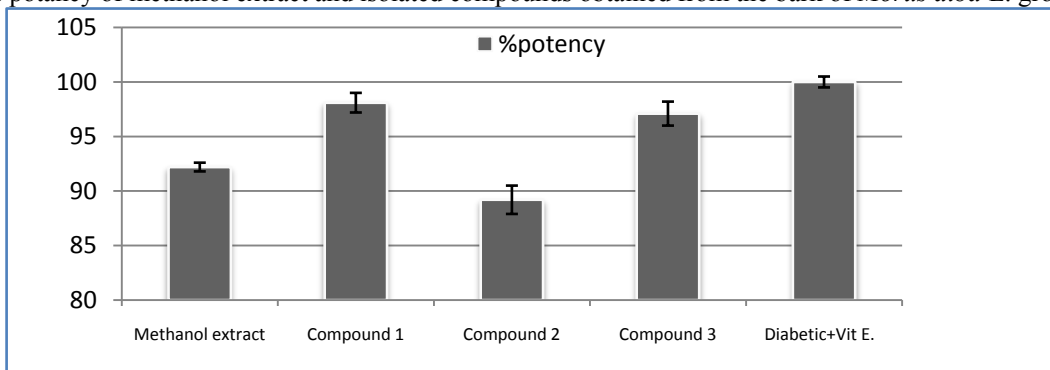
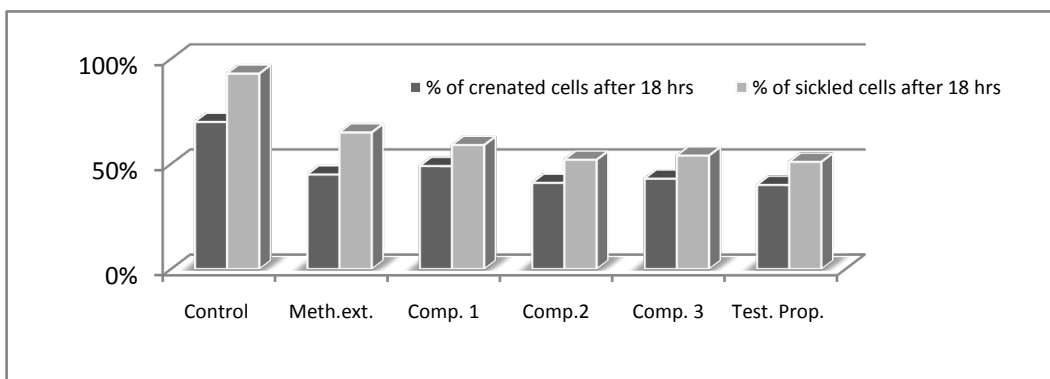
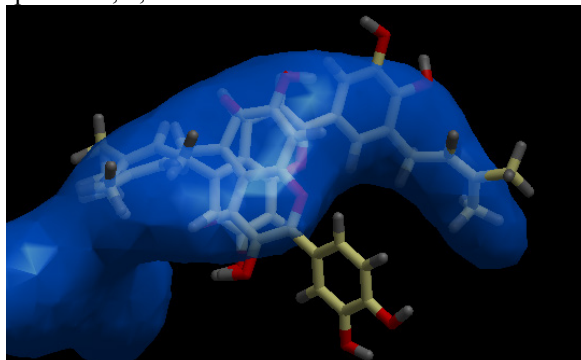
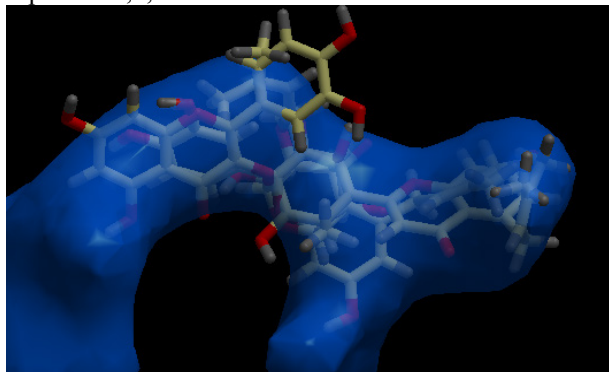


Figure 2. % potency of methanol extract and isolated compounds obtained from the bark of *Morus alba* L. grown in Egypt.**Figure 3.** Antisickling effect of methanol extract and isolated compounds obtained from the bark of *Morus alba* L. grown in Egypt.**Figure 4.** Orientation of active compounds 1, 2, 3 at 3HB5 active site.**Figure 5.** Orientation of active compounds 1, 2, 3 at 1XAN active site.

4. Discussion

Compound **1** designated as 5, 5'-diprenyl-7, 3', 4'-trihydroxy flavanone. Its molecular formula was established as C₂₅H₂₈O₅ due to parent ion at m/z 408 (M) + and (M + 1) at 409. The characteristic U.V absorption bands (λ_{\max} MeOH nm = 228 sh, 293,339 + NaOMe, 248, 285, 333; + AlCl₃, 221, 316, 392; + NaOAc, 287, 297,334 + H₃BO₃) suggested a flavanone structure (Ercisli & Orhan, 2007; Kaushik *et al.*, 2008 & Zheng *et al.*, 2008). ¹HNMR spectrum showed the signals characteristic for flavanone (Geissman, 1962; Harborne *et al.*, 1975 & Mabry *et al.*, 1996). 5,7,3',4',5' substituted derivative (absence of 5,7,3',4' and 5' protons). The chemical shifts of aromatic carbon signals and the molecular weight of compound **1** showed that the A-ring and the B-ring were substituted by one hydroxyl group and by two ortho hydroxyl groups, respectively. The presence of a pair doublet of doublet of the two geminal protons at H-3 β at 2.8 & α at 3.2 ppm respectively with one proton integration, each due to geminal coupling & coupling with H-2. ¹HNMR showed a doublet of doublet at 4.8 ppm assigned for β H-2, due to coupling with α & β protons at 3. The geranyl group was observed at δ 1.06, 1.2 (3H, each, s, for 4", 4" Me & 3", 3" Me), 2.6 and 2.65 (2H, each, d, for 1", 1" CH₂), 5.1, 5.2 (1H, each, m, 2", 2" CH). ¹³CNMR (ppm) showed 25 signals characteristic for flavan nucleus substituted by two isoprene units. 5 signals of oxygenated aromatic carbons at δ : 186.2 (C-4) 158 (C-7), 157 (C-9), 146.8 (C-3') and 146.5 (C-4'), 7 signals of nonoxygenated aromatic carbons at 131.3 (C-1'), 119.07 (C-6'), 115.7 (C-5'), 115.2 (C-2'), 99.7 (C-10), 95.8 (C-6) and 94.5 (C-8) and 3 signals of central ring carbon at 81.7 (C-2) and 67.1 (C-3).

Geranyl carbons were recorded at δ : 139.2, 139 (C-3"', 3"', respectively), 125.6, 125.5 (C-2"', 2"', respectively), 30.2, 28.5 (C-1"', 1"', respectively), 24.1, 24.2 (C-5"', 5"', respectively) and 12.4, 12.5 (C-4"', 4"', respectively). The two geranyl groups were attached to C5 and C5' this was confirmed from ¹H-¹H COSY and HMBC correlation between methylene signals at δ H 2.65 (H-1") & δ H 2.60 (H-1'") and carbon signals at δ C 124.5 (C-5) & δ C 115.5 (C-5'); respectively. In addition 6, 8 and 2',6' meta coupling and geminal coupling of 2H on C3 were confirmed from 1H-1H COSY correlation between these protons. Mass spectrum showed a molecular peak at m/z = 408 ascribed structure formula C₂₅H₂₈O₅.

Compound **2** designated as 5-prenyl-3, 7, 3', 4'-tetrahydroxy flavanonol. Spectral data of compound **2** is different from compound **1** in absence of geranyl moiety in ring B and this was confirmed by the presence 20 carbons only in ¹³CNMR. ¹HNMR spectrum revealed the presence of H3' proton at 6.60 (1H, d, J =9.1 Hz, ortho coupled with 2') presence of 3-OH assigned for flavanonol nucleus and this was confirmed by the presence of one proton at 4.41 (1H, d,

J =7.2 Hz due to coupling with H-2) and absence of geminal coupling. The geranyl group was attached to C5 and this was confirmed from ¹H-¹H COSY and HMBC correlation between methylene signal at δ H 2.65 (H-1") and carbon signal at δ C 124.5 (C-5). In addition 6,8 and 2',6' meta coupling were confirmed from 1H-1H COSY correlation between these protons. Mass spectrum showed a molecular peak at m/z = 356 ascribed structure formula C₂₀H₂₀O₆.

Compound **3** exhibited a purple colour in UV light changing to yellow on exposure to ammonia vapours and AlCl₃ reagent. Properties and spectra were identical to those reported earlier (24, 25) for Quercetin-3-O- α -L-rhamnoside (Quercetin)

From the previously mentioned physicochemical and spectral data, as well as, comparison with the published data (26-28), compounds **C1-C3** (Figure 1) could be identified as 5, 5'-diprenyl-7, 3', 4'-trihydroxy flavanone, 5-prenyl-3, 7, 3', 4'-tetrahydroxy flavanonol and Quercetin-3-O- α -L-rhamnoside (Quercetin). Compounds C1 and C2 were isolated for the first time from *M. alba* L.

The variation in biological activities of the tested samples may be attributed to the difference in chemical nature of tested sample. As a matter of fact, all tested samples exerted high potency as antioxidant (Table 1 & Figure 2); which may be due to synergetic effect of methanol extract content and phenolic nature of isolated compounds (Stephen & Duke, 1996).

The experiments tested with haemoglobin AA blood which crenated in acidic citrate-dextrose-saline (Table 2 & Figure 3) showed that the tested samples caused appreciable reversal of crenation (% of crenated cells decreased from 74% in control to about 41-49% in tested samples). Haemoglobin AS samples also exhibited reversal of sickling in the presence of the tested samples (% of sickled cells decreased from 91% in control to 52-65% in tested samples).

The results in all cases were more or less equal to testosterone propionate in the concentrations 4 μ g/mL (decrease % of crenated and sickled cells to 40% & 51%, respectively). Effect may be due to direct effect of phenolics on RBCs membrane (Gurib *et al.*, 1992).

Considering cytotoxic activity a high potency of compound **1** was noticed especially against cervix & breast tumor cell lines. On the other hand compound **2** recorded higher activities against liver tumor cell line than compound **1**

To understand the biological data on structural basis, we evaluate the isolated compounds **1**, **2** & **3** through docking techniques using Molsoft ICM 3.4-8C program, by docking our titled compounds on the crystal structures of 17 β -hydroxysteroid dehydrogenase type 1 (17 β -HSD1) in complex with its natural inhibitor E2B {3-(3',17 β -dihydroxyestra-1',3',5'(10')-trien-16 β -methyl)benzamide} available

through the RCSB Protein Data Bank (PDB entry 3HB5) and on another crystal structures of peroxiredoxin available through the RCSB Protein Data Bank (PDB entry 1XAN) (<http://www.rcsb.org/pdb/welcome.do> & <http://www.ebi.ac.uk/thornton-srv/databases/pdbsum>).

Docking studies on 3HB5 reveals that E2B (the original ligand) has ICM score of -133.03 and form two hydrogen bonds with Ser-142, and C-185 and docking of NADP reveals ICM score of -303.59 and form three hydrogen bonds with Ser-11, and three bond with Ser-12, another four bonds with K-159, four bonds with N-90, four bonds with A-91, one bond with Thr-140, and another bond with Tyr-155 (Table 4, Fig. 4). Compound 2 exhibits relatively weak binding affinity with ICM score of -91.71 but form three hydrogen bonds with with K-159, another one bond with Tyr-155 (Table 4, Fig.4). Compound 3 possess ICM scores of -99.36 and form one bond with Ser-142, and another bond with C-185, and form six bonds with N-90, and one bond with A-91, and another bond with Thr-140 (Table 2, Fig. 4). Compound 1 which is the most active compound as antiproliferative possesses ICM scores of -111.44 and form three hydrogen bonds with Ser-11, and two bond with Ser-12, two bonds with K-159, and another bond with Tyr-155 (Table 4, Fig. 4).

In addition, docking studies on 1XAN reveals that FAD has ICM score of -281.49, forms five hydrogen bonds with Thr-57, four bonds with Asp-331, and another bond with G-29 (Table 5, Fig. 5). Compound 2 exhibits relatively weak binding affinity with ICM score of -92.09 and form one hydrogen bond with Thr-57 (Table 5, Fig. 5). Compound 3 possess ICM scores of -101.46 and form four bonds with Thr-57, one bond with Asp-331, and another bond with G-29 (Table 5, Fig. 5).

Compound 1 which is the most active compound as antioxidant possesses ICM score of -112.05 and forms one hydrogen bond with Thr-57, and another bond with Asp-331 (Table 5, Fig. 5), where compounds 1 is the most active as antitumor against tumor cell lines (breast, MCF7) and (cervix, HELA) of IC_{50} 0.56 μ g/ml, and 0.71 μ g/ml respectively, also, shows maximum potency as antioxidant of 98.1%, and shows good affinity with the receptor that reveals ICM score value of -111.44.

Compound 3 has less antioxidant activity than compound 1 with antioxidant potency of 97.1%, while compound 2 which reveal less activities as antiproliferative against tumor cell lines (breast, MCF7) and (cervix, HELA) of IC_{50} 1.41 μ g, 0.92 μ g respectively, and shows least potency as antioxidant of 89.1%, and shows least binding affinity with ICM score value of -91.71.

The data reported herein indicates that prenylated flavonoid represents a new interesting class

of potentially useful compounds as antiproliferative and antioxidant. Di prenylated derivative compound 1 represents the most active than the monosubstituted analogue compound 2 of flavonoid systems and may enhances its binding affinity with enzymes, it was found that hydrogen bonds formation with Ser-142, C185, K-159, Ser-11, Ser-12, N-90 amino acid residues may be responsible for the antiproliferative activity as referred to natural inhibitor E2B, and NADP, while to be active as antioxidant it may need formation of hydrogen bonds with Thr-57, Asp-331.

Conclusions

According to this article, we can conclude that compound 1 appears to be the most interesting compound among the newly isolated and seem potentially attractive as antiproliferative and antioxidant candidates. (3HB5) and (1XAN) enzymes, which are potential, target of hormone dependent cancer & antioxidant activity; respectively were considered for docking and screening studies. This work finds application using these targets to design drugs for the treatment of hormone dependent cancer & act as preventive to risk factors of cancer. Consequently, the approach is useful in designing novel molecules for treatment & prevention of hormone dependent cancer

Corresponding Author:

Khaled Meselhy Ibrahim

Pharmacognosy & Medicinal Plants, Faculty of Pharmacy, MIU & Cairo Universities
kmeselhy@hotmail.com

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Assessment of correlation between Brain Natriuretic Protein test and early prognosis in Acute Coronary Syndrome

Ashkan Tabibzadeh¹, Gholamreza Hemassi², Hamidreza Mahboobi^{3,4}, Reza Yazdani¹, Oveis Salehi¹, Mehrdad Jalalian⁵

¹. Trauma and Medical Emergencies Research Center, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandarabbas, Iran

². Department of Emergency Medicine, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

³. Research Center for Behavioural and Neurosciences, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandarabbas, Iran

⁴. Payame Noor University, Tehran, Iran

⁵. M.D., Editor In-Chief, Electronic Physician Journal, Mashhad, Iran

hamidrezamahboobi@yahoo.com

Abstract: The aim of this study was to determine; the relationship between Brain Natriuretic Protein (BNP) and early prognosis in Acute Coronary Syndrome (ACS). In this analytical study, 158 patients with ACS admitting to Emergency Department of Rasul Akram hospital in Tehran entered in study. For each patient, information include of demographic, past medical history, drug therapy, presenting symptoms and signs, results of serum chemistry test, radiographic studies, electrocardiography and NT-proBNP test that measured using immunoassay were collected. Totally 158 patients with mean age of 59.7 ± 0.95 that 86(54.4%) of them were male included in study. The ROC curve showed that BNP more than 612 (pg/ml) had sensitivity and specificity of 98% in predicting the outcome (discharged vs. CCU admission) which had a Positive predictive value (PPV) of 99% and Negative predictive value (NPV) of 96%, while in another ROC curve analysis BNP greater than 3200(pg/ml) had a sensitivity of 81%, specificity of 92% in predicting heart failure, fatal arrhythmia or death as an outcome with a PPV of 37.5% and NPV of 98.5%. Results demonstrate NT-proBNP for strongly predicted short-term outcomes in subjects with chest pain, with 1.2 increases in risk for death by 7 days among those with marked elevation in NT-proBNP concentration. Other studies have found similar results.

[Ashkan Tabibzadeh, Gholamreza Hemassi, Hamidreza Mahboobi, Reza yazdani, Oveis Salehi, Mehrdad Jalalian.

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Keywords: Acute Coronary Syndrome; Brain Natriuretic Protein (BNP); Prognosis

1. Introduction

The significance of contribution of Laboratory Medicine to clinical cardiology has grown in importance over the years (1, 2). Until 20 years ago, the clinical laboratory only placed at cardiologist's disposal a few assays for the retrospective detection of cardiac tissue necrosis, such as enzymatic methods for creatin Kinase (CK) and lactate dehydrogenase catalytic activities (3). However, in the latter part of 20th century, highly sensitive and specific assays for detection of myocardial damage, such as cardiac troponins, as well as assays for reliable markers of myocardial function, such as cardiac natriuretic peptides, have become available, assigning to laboratory a pivotal role in the diagnosis and follow up of patients with cardiac disease. This is witnessed by the incorporation of these markers into international guidelines and the redefinition of myocardial infarction. BNP is a 32 amino acid polypeptide initially found in brain but later found abundant in cardiac ventricle tissue. Baseline NT-BNP

measurements, regardless of sample timing (on presentation; early 12-24 hours and sub-acute > 3days), during the index clinical presentation, have consistently been shown to be of utility as a predictive for short and long term mortality in patients with acute heart failure (4), but the importance of this marker as an independent predictor of ACS in debate.

The aim of this study was to determine the relationship between Brain Natriuretic Protein (BNP) and early prognosis in Acute Coronary Syndrome (ACS).

2. Material and Methods

This analytical study was done on patients admitting to Emergency Department of Rasul Akram hospital in Tehran. Totally 158 patients with ACS entered in our study. All four component data sets had comparable information available, including standard demographics, past medical history and drug therapy, presenting symptoms and signs of Physical examination, results of serum chemistry tests,

radiographic studies, (typically plain chest radiographs), electrocardiography results and finally, the results of NT-proBNP testing. Glomerular Filtration Rate (GFR) was estimated using modified diet in renal disease (MDRD) equation. For each trial, blood was collected into EDTA tubes and NT-proBNP was measured using validated, commercially available immunoassay (Elecsys® ProBNP, Roche Diagnostics, Indianapolis), using established methodology. This assay has been reported to have < 0.001% cross-reactivity with bioactive BNP, and had inter-run coefficients of variation ranging from 0.9 to 5.5%. NT-proBNP levels were expressed in pg/ml. In the next few days, the outcome of patients in 7 days after admission surveyed. Outcome of patients was: Discharge, CCU admission, fatal arrhythmia, Heart failure, expired.

Exclusion criteria were: signs and symptoms of heart failure, and inability to provide informed consent, chronic renal failure. We obtained written informed consent from all study participants. Mean and standard deviation was used for description of the data. X² analysis, with appropriate correction, was used to compare the categorical variables. Standard parametric tests were used for continues

variables (T test, ANOVA) and spearman correlation was done as a non parametric test. Logistic regression analysis was used for performing multivariate analysis for prediction of related variables with categorized outcome. (End outcome, discharge)

BNP values followed log normal distributions, so correlation and regression analysis were done using natural log transformed BNP. ROC curve used to find cutoff point and the sensitivity and specificity and predictive values to predict the short term outcome. The analysis was done by SPSS 15 software. A p value of < 0.05 was considered statistically significant.

3. Results

Totally 158 patients included in this study with mean age of 59.7 ± 0.95 that 86(54.4%) of them were male. Normal ECG finding found in 55(34.8%) of patients at presentation. The mean and median of BNP were respectively 1607 ± 154 and 1100. There was no significant difference of BNP between males versus females (1753 ± 218 versus 1433 ± 215) ($P > 0.05$).

Table 1. Comparing the factors at the presentation of patients with chest pain in two categorized outcome (Discharged group versus End group)

Variables		Discharged group	End group outcome	P value
Age		59.96 ± 1.420	61.5 ± 1.253	N.S
Sex	Male	23(44.2%)	63(59.4%)	N.S
	Female	29(55.8%)	43(40.6%)	
Quality of pain	Pressure	36(69.2%)	84(59.4%)	N.S
Pain duration	>20 min	36(69.2%)	84(79.2%)	N.S
Pain referring	Yes	39(75%)	82(78.1%)	N.S
Exertional pain	Yes	21(40.4%)	60(56.6%)	0.01
Associated symptoms	Yes	51(98.1%)	103(98.1%)	N.S
PMH	Positive	40(67%)	90(85%)	N.S
DH	Positive	36(69%)	92(87%)	0.01

NS: Not significant

The outcome of patients in next 7 days was as below: 52(32.9%) discharged, 95(60.1%) CCU admission, 3(1.9%) heart failure, 4(2.5%) fatal arrhythmia and 4 (2.5%) expired. The comparing variables into two categorized outcome has been shown in Table 1 and 2. The mean of BNP is significantly more in End group than discharge group outcome (2327 ± 194 versus 138 ± 25) ($P = 0.001$) (Table 2).

The mean of BNP according to the ECG finding is as below: Normal ECG findings (526.93 ± 105.89), ST elevation (2472.50 ± 311.56), ST Depression (2040.30 ± 424.97), T inversion (1861.59

± 377.64). There was significant difference of mean among four ECG groups. (ANOVA $P = 0.001$). This difference was not significant between ST depression, T inversion (Post Hoc $P > 0.05$), but all of them had significant difference with normal ECG findings. (Post Hoc $P = 0.001$). There was a negative correlation between BNP and Ejection fraction of left ventricle. (Spearman correlation $P = 0.001$, $r = -0.499$).

The multivariate analysis showed that BNP was the independent predictive value for 7 days categorical outcome in patients admitting with chest pain. (Logestic regression $P = 0.001$, R Square =

0.741, Exp (B) = 1.8) and also in another multivariate analysis, predicted the outcome of heart failure, fatal arrhythmia and mortality in next 7 days more strongly than other variables in the model. (P = 0.001, R square = 0.721, Exp (B) = 1.2). The ROC curve showed that BNP, greater than 612 pg/ml had sensitivity and specificity of 98% in predicting of End group outcome and the predictive values were:

PPV = 99% and NPV = 96%. Also in another ROC curve analysis BNP, greater than 3200 pg/ml had sensitivity and specificity of 81% and 92% respectively in predicting of outcome which the heart failure, fatal arrhythmia and death included and the predictive values were: PPV = 37.5% and NPV = 98.5%.

Table 2. Comparing the tests done at the presentation of the patients with chest pain in two categorized outcome (Discharged group versus Ed group)

Variables		Discharged group outcome	End group outcome	P value
BNP		138.35±25.698	2327.46±194.566	0.001
Troponin I	Positive	2(3.8%)	52(49.1%)	0.001
CPK		116.80±27.902	271.96±88.510	N.S
CPK MB		24.22±2.146	156.74±79.104	N.S
Ejection fraction		76.90±1.140	55.63±1.251	0.001
ECG findings	Normal	38(73.1%)	17(16%)	0.001
	ST elevation	0(0%)	47(44.3%)	
	ST Depression	5(9.6%)	20(18.9%)	
	T inversion	9(17.3%)	22(20.8%)	

NS: Not significant

4. Discussions

A reliable biomarker or group of biomarkers that would provide incremental data to predict risk for cardiovascular morbidity and death would certainly enhance clinical care in the outpatient setting. An enhanced appreciation of risk may lead to adequate up-titration of medical therapy and improve patient compliance in high risk subsets. The biomarkers would be most useful if it further guided targeted treatment strategies that would in turn optimize patient outcome. Our results demonstrate NT-proBNP for strongly predicted likelihood for short-term outcome in subjects with chest pain, with 1.2 increases in risk for death by 7 days among those with marked elevation in NT-proBNP concentration. Other studies have found similar results as well (5-10). NT-BNP has been shown to be an independent predictor of mortality in these studies. However, a significant proportion of predictive ability of increasing levels of NT-BNP in acute setting due to its association with other well established clinical risk factors: Age, Female gender, diabetes mellitus, hypertension, previous MI, heart failure, resting heart rate, and ST-Segment depression, renal dysfunction and inflammatory markers like CRP (5, 6). We solved this problem by including almost all of these variables in the model as covariates to find the predictive potential of NT-BNP. The admission NT-BNP concentration was so strongly predictive of short-term outcome among our patients with chest pain that its presence in multi-variable models

overwhelmed the prognostic impact of other traditional risk factors. NT-BNP measurement, have also been correlated with myocardium at risk, infarct size, and extent and complexity of coronary artery disease (11-14). It is well known that among healthy subjects, NT-proBNP levels are higher in older female when compared with age-matched malesubjects, possibly due to a higher prevalence of diastolic abnormalities or more significant age-related reductions in GFR in women. No significant gender-related difference in mean of NT-proBNP and BNP appear to deliver important diagnostic and prognostic information in a wide variety of patient types; the choice of which marker to use should be based on difference in analytical performance, the individual clinician comfort with the results from the assays (16). In summary, the findings in this study and the results of other clearly indicate the remarkable ability of NT-BNP to predict short term outcome. It appears to be effective in patients with an ACS. Its value appears to be incremental to that obtained from standard clinical variables. In addition, it seems to integrate risk from an array of clinical variables that may be relatively easy to comprehend.

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Corresponding Author:

Dr. Hamidreza Mahboobi,
 Research Center for Behavioral and Neurosciences,
 Faculty of Medicine,
 Hormozgan University of Medical Sciences,
 Bandarabbas, Iran
 Tel: +98.9172121995
 Fax: +98.7643273311
 E-mail: hamidrezamahboobi@yahoo.com

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6/6/2012

Design of a Reversible Ripple Carry Adder for Excess-3 Code

Vida Abdolzadeh¹, Nasser Lotfivand², Siamak Haghypour³

¹ Department of Computer Engineering, Tabriz Branch, Islamic Azad University, Tabriz, Iran

² Department of Electronic Engineering, Tabriz Branch, Islamic Azad University, Tabriz, Iran

³ Department of Biomedical Engineering, Tabriz Branch, Islamic Azad University, Tabriz, Iran

S.V.Abdolzadeh@iaut.ac.ir, Lotfivand@iaut.ac.ir, Haghypour1@yahoo.com

Abstract: One of the main characteristic in VLSI circuits is power dissipation. Due to the information loss, conventional logic circuits result in energy dissipation. Reversible circuits because they do not lose information, have zero internal power dissipation. This paper proposes a reversible 4-bit parallel adder for Excess-3 code. Excess-3 is an unweighted and self-complementing code. Excess-3 coding over BCD coding has various advantages. The primary superiority is that a decimal number can be nines' complemented (for subtraction) as facily as a binary number can be ones' complemented by inverting all bits. The proposed Excess-3 adder in the number of reversible gates and garbage outputs, allowing high-speed and low-power reversible circuits, covers all favorable characteristics of reversible circuits.

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Keywords: Excess-3 adder; Parallel Adder; Reversible Excess-3 adder; Reversible adder

1. Introduction

High growth rate in portable systems have caused requests for power-sensitive designs increase. As a result, new power-efficient design techniques have been considerable subject for researchers. In 1966, Landauer's researches demonstrated that the minimum energy dissipation for every irreversible bit operation is $kT \ln 2$ joules, where $k = 1.3806505 \times 10^{-23}$ is Boltzmann's constant, and T is the environment's temperature [1, 2]. Bennett found $kT \ln 2$ energy consumption can be avoided, when the system regenerates inputs base viewed outputs [3]. Recently, several technologies subsuming, low power CMOS technologies, optical technologies, quantum computing, DNA computing and nanotechnology for reversible logic have been studied [4].

In a reversible circuit each input is mapped to a singular output, and this mapping must be bijective. The number of inputs and outputs, in a reversible circuit, are the same. These circuits make possible inputs denominate from outputs [5, 6, and 7]. Fan-out or feedback in reversible logic is not allowed, so the synthesis of a reversible circuit is principally more hard set in contrast with conventional logic [8].

Excess-3 is an unweighted and self-complementing code. Excess-3 coding over BCD coding has various advantages. The primary superiority is that a decimal number can be nines' complemented (for subtraction) as facily as a binary number can be ones' complemented by inverting all bits [9]. In this paper, we propose an Excess-3 Adder design using reversible logic gates. The design is based on Feynman Gate and Haghparast--Navi gate.

The rest of the paper is organized as follows: Section 2.1 provides the basic concepts. Section 2.2 discusses the proposed circuits. Section 3 concludes our work.

2. Material and Methods

2.1 Basic Concepts

An α -input and β -output function $f: B^\alpha \rightarrow B^\beta$ is a reversible function if and only if:

- $\alpha = \beta$, where α and β are the number of inputs and outputs, and
- it maps each input to a unique output

With a cascade of reversible gates g_i we can synthesize a reversible circuit $G = g_0 g_1 \dots g_{k-1}$ over inputs $X = \{x_1, x_2, \dots, x_n\}$. We can represent a reversible gate with $g(C, T)$, where C contains the Control lines, and may be empty. T represents the Target lines. If and only if the control lines satisfy the control conditions; the gate operation is then applied to the target lines [10]. Many reversible gates exist, and we present some common reversible gates below:

Figure 1.a shows a 2*2 Feynman Gate, also known as 1-CONT [11]. Figure 1.b depicts a 3*3 Toffoli Gate [8]. The inputs 'A' and 'B' are passed as first and second outputs, respectively. The first and second inputs control the third output to invert the third input. Figure 1.c shows a 3*3 Peres Gate (PG) [12], also known as a New Toffoli Gate; a Peres gate combines a Toffoli and Feynman Gate. Figure 1.d shows a 3*3 Fredkin Gate [13]. Here, input 'A' is passed as the first output. Inputs 'B' and 'C' are swapped to obtain the second and third outputs, controlled by 'A'. If $A = 0$, the outputs are simply

duplicates of the inputs; otherwise, if $A = 1$, the two input lines (B and C) are swapped.

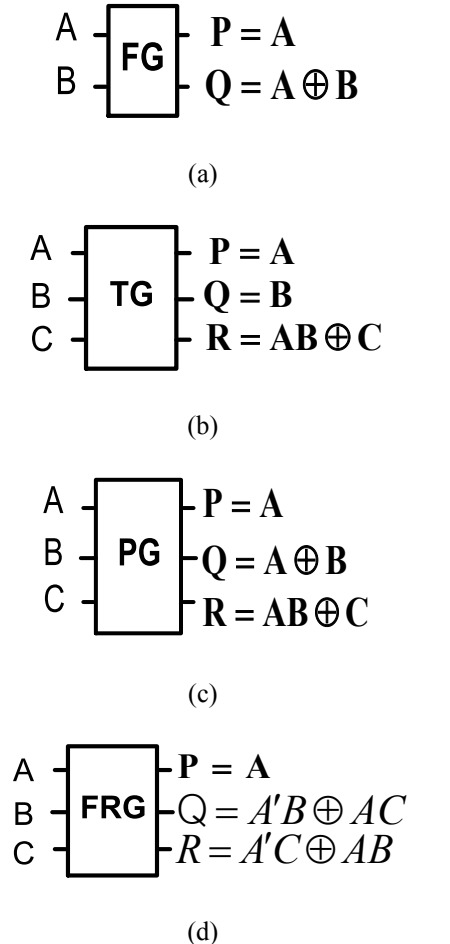


Figure 1: Commonly used reversible gates. a) Feynman gate; b) Toffoli gate; c) Peres Gate; d) Fredkin Gate

2.2 The proposed Reversible 4-bit Adder for Excess-3 codes

One of the widely used elements in digital circuits, are full adders. Researchers have introduced various designs for a reversible full adder [2, 4, 14, 15, 16], but to our knowledge, no research has presented a design for a reversible 4-bit parallel Excess-3 adder. Our design uses Haghparast-Navi gates (HNG) (as in [2]), which can work as a reversible full adder unit, shown in Figure 2.

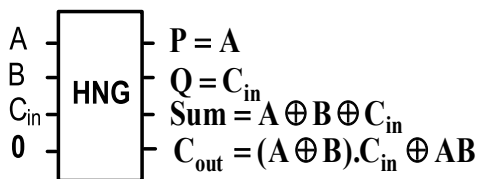


Figure 2: HNG gate as Reversible Full Adder

An Excess-3 adder circuit adds two Excess-3 numbers and converts the results into the equivalent Excess-3 number. Figure 3 illustrate the block diagram of the proposed Excess-3 adder. The circuit uses two 4-bit binary adders. The first adder's inputs are Excess-3 numbers, producing an excess-6 sum. Conversely, when the two decimal numbers sum to 9, the first adder's output is 15; if their sum is 10, the output of the first adder is 0, and the output carries a bit if the first adder goes high.

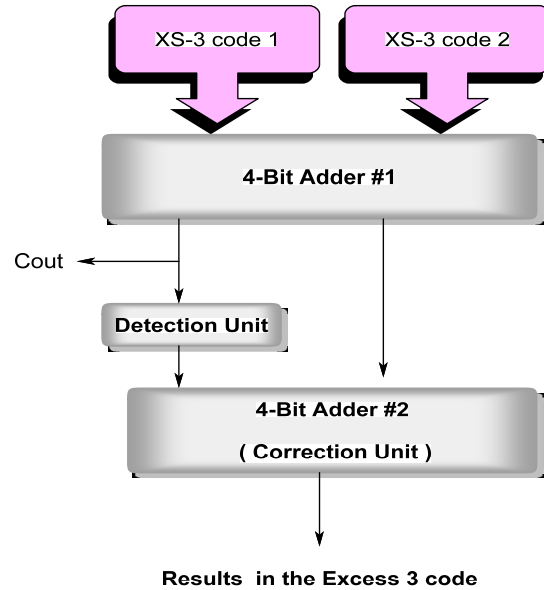


Figure 3: Block diagram of the proposed 4-bit Excess-3 adder

If and only if the two decimal digits' sum is greater than 9, $C_{out}=1$. These are the cases for which a carry must be activated. Achieving an Excess-3 sum in the circuit requires some correction in the first adder's output. The second adder performs this correction.

To convert an Excess-6 value to an Excess-3, if $C_{out}=0$ in the first adder, correction occurs by subtracting 3 from the sum of the first adder. If there is a carry, correction occurs by adding 3. When the two decimal numbers sum to 10, the output of the first adder is 0000, and $C_{out}=1$. By the second adder 3 is added to 0000 that creates 0011 in the result (the Excess-3 representation for 0).

If $C_{out}=0$, it subtracts 3 by adding 2s complement of 3. This subtraction occurs in two steps. It first adds the bit-wise complement of 0011 (e.g., 1100) to the output of the first adder and then adds 0001 to this result, by inputting 1 to the C_{in} of the second adder.

An implementation of proposed circuit by FPGA showed good performance of circuit. Figure 4 depict RTL schematic of the circuit and Figure 5 shows simulation results.

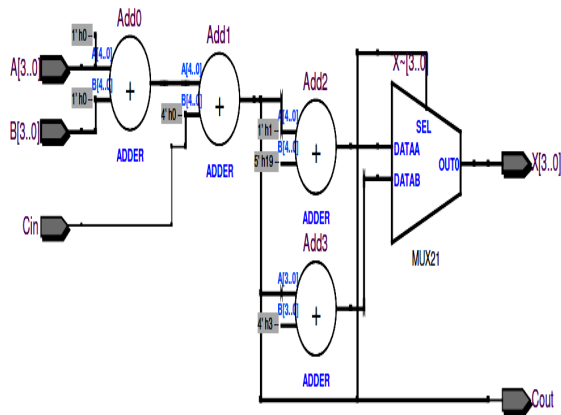


Figure 4: RTL schematic of implemented proposed adder by FPGA

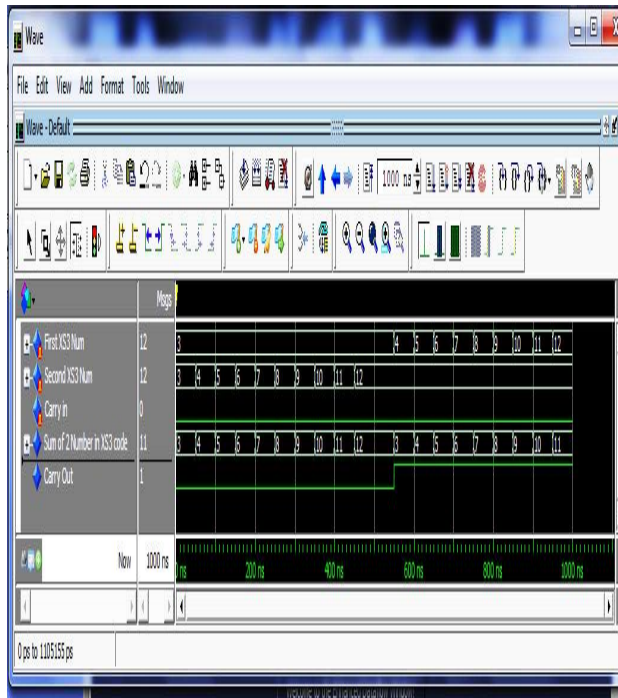


Figure 5: Simulation results

Figure 6 represents the proposed reversible 4-bit parallel Excess-3 adder using HNG gates. The proposed Excess-3 adder has two reversible 4-bit parallel adders implemented by eight HNG gates. It also requires five Feynman gates to avoid bit fan-out. Therefore, the total number of gates required to construct the reversible 4-bit Excess-3 adder is $2*4+5=13$.

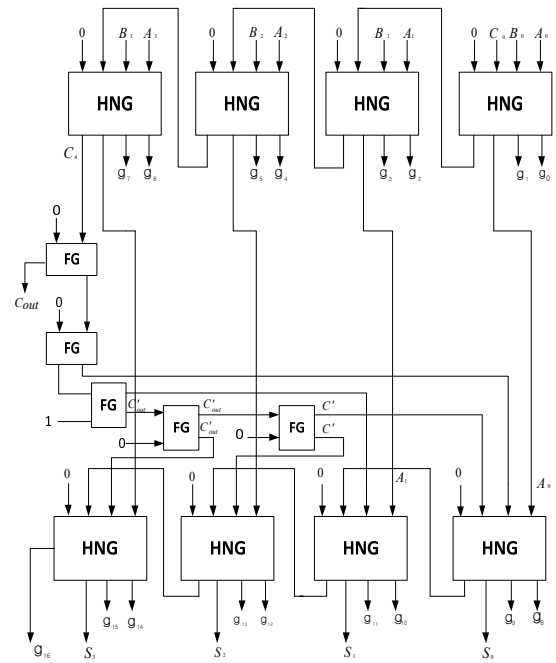


Figure 6: The proposed reversible 4-bit Adder for Excess-3 code

The proposed Excess-3 adder uses four reversible full adder circuits to construct a reversible 4-bit parallel adder; each full adder circuit produces two garbage outputs. The total number of garbage outputs generated from the 4-bit reversible adder is eight. The five Feynman Gates, which copy bits, do not produce any garbage outputs. The last carry in the second 4-bit adder is garbage. The total number of produced garbage outputs is thus $2*8+1=17$ (the circuit has two 4-bit parallel adders).

Hardware complexity is a major representative for a digital circuit's evaluation. With the four main factors of circuit complexity defines as: α is the number of two-input EX-OR gate, β is the number of two-input AND gate, δ is the number of NOT gate, and T is Total logical calculation. The total logical calculation of this circuit is $T= 45\alpha + 16\beta$, and it uses only 13 gates. Decreasing of the number of garbage outputs is another definitive criterion in designing a reversible full adder. Garbage output is an output that is not input to other gates or is not a primary output. The purposed circuit in the hardware complexity, number of reversible gates and garbage outputs, demonstrates the good features of reversible circuits.

3. Conclusion

In this paper, we proposed a reversible 4-bit parallel Excess-3 adder by using HNG and FG gates. Table 1 shows features of this circuit.

Table 1: features of reversible 4-bit parallel Excess-3 adder

Number of Reversible Gates	13
Number of Garbage Outputs	17
Total Logical Calculation	$45\alpha + 16\beta$

For quantum computers and nanometric-based systems, a reversible arithmetic unit is a compulsory need. The proposed circuit can be utilized as a building block for synthesizing a reversible arithmetic unit.

The proposed Excess-3 adder in the number of reversible gates and garbage outputs, allowing high-speed and low-power reversible circuits, covers all favorable characteristics of reversible circuits.

Corresponding Author:

Nasser Lotfivand

Department of Electronic Engineering, Tabriz Branch, Islamic Azad University, Tabriz, Iran E-mail: Lotfivand@iaut.ac.ir

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Training Periodization in Lower Limb Performance and Neuromuscular Controlling in taekwondo athletes

Yen Ke-tien

Department of sports, health and leisure, Cheng Shiu University, Kaohsiung, Taiwan. kyen2006@gmail.com

Background: Taekwondo is a powerful sport in which the maximal performance relies on anaerobic metabolism and explosive power. **Aims:** to determine the dedication of different strength and power training programs (off-season, pre-season and in-season) to lower limb performance and physiological modulation during a 20-week training period. **Methods:** Eight male collegiate taekwondo athletes completed 20-week systemic training programs divided into a linear training mesocycle (general conditioning, muscular recruitment, and hypertrophy) from 1 to 12 weeks, and two microcycle (maximum strength, explosive power, agility, speed) from 13 to 20 weeks in periodized fluctuation. Subjects were evaluated biochemical index, forearm total vascular occlusion test and muscular stiffness test six times during Training program: at the beginning (week 0, date1, T1), in the middle (week 2, Date 13, T2; week 8, Date 55, T3; week 14, Date 97, T4; week 18, Date 125, T5) and at the end (week 21, Date 143, T6) of the training program. Squat jump (SJ), countermovement jump (CMJ) and continuous jump bent leg (CJb) were tested before and after the systemic strength training period. **Results:** There were significantly increasing in the SJ (7.8(2.7)%), CMJ (18.3(4.1)%) and CJb (8.7(4.7)%) after the totally training programs. Training increased creatine kinase levels from T1 to T4 (327.8%) and recovered at T6 (99.4%). Muscle damage and muscular recruitment function recovered at T6 after taper. **Conclusion:** The conjunction of systemic periodized 20 weeks training programs would increase lower limb performance and strengthen neuromuscular controlling in taekwondo athletes. [Yen Ke-tien. **Training Periodization in Lower Limb Performance and Neuromuscular Controlling in taekwondo athletes.** *Life Sci J* 2012;9(3):850-857]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 120

Key words: taekwondo, periodization, neuromuscular controlling, muscle mechanical properties

1. Introduction

Taekwondo is a powerful sport in which the maximal performance relies on the specific technical, tactical and physical developments. Taekwondo is a periodic intense sport that demands athletes to compete in sequence of short duration attack (from 1 to 5 s) and waiting for another opportunity of frequent short attack of high intensity exercise, consisted of both moving play and contact play. The anaerobic metabolism and explosive power are needed.

Sports scientists have evaluated joint mobility, muscle conditioning [1], neuromuscular complex [2] and soft tissue characteristics [3]. The muscle mechanical properties have also been used to study training effects on muscle conditioning by recording the damped mechanical oscillations.

Although, most elite athletes consecrate themselves to training programs that force them to the extreme of their exercise performance. Optimal performance is only reachable if athletes can resume after optimally balance training stress and energetic recovery. Coaches should organize modifications in training load and compensation in regularly practice. However, when the athlete is not well improved, mild hurts could develop into tissues damages and is characterized by falling performance in spite of an expanded rest period, associated by physiological, biochemical, and psychological stress symptoms.

The assessment and monitoring of stress markers during training programs would assign the individualization of an athlete's training workload [4]. Therefore, the best management to evaluate the training adaptation and to avoid overreaching and overtraining during training programs is the regular monitoring of selected biochemical and physiological markers.

Blood levels of creatine kinase (CK) have been used as an index of training-induced physiological stress. CK is a muscle enzyme that occasionally increases in the blood following strenuous exercise, altering permeability of muscle cell membranes. Factors that influence the degree of CK efflux into the blood include exercise duration and intensity, exercise mode, and fitness level of the individual [5].

The role of peripheral microcirculation including microvascular perfusion, tissue oxygenation and oxygen utilization is the major parts of the physiological mechanisms in different conditioning [6]. Moreover, ensuring tissue oxygenation, evaluating the entirety and adequacy of microvascular to apply tissue needs play important physiological regulatory functions. Since Jobsis (1977) first used Near Infrared Spectroscopy (NIRS) in monitoring cerebral and myocardial oxygen. It has been used to investigate tissue perfusion and oxygenation noninvasively, such as oxygen saturation in athletic muscles [7].

It is noteworthy that NIRS provides an average of saturation of hemoglobin in all vascular compartments

(arterioles, capillaries and venules) within the tissue, termed as tissue oxygen saturation (StO₂).

However, different metabolic stress indices should have various time courses in response to the periodization of intense training and following effective recovery. This suggests that training monitoring in athletes should be effected not only in a multi-level approach using estimations of performance but various physiological indices as well.

Thus, to provide a better understanding of the martial art periodization, the aim of the study was to determine the dedication of different strength and power training programs (off-season, pre-season and in-season) to lower limb performance and physiological modulation during a 20-week training period. The training programs divided into a linear training mesocycle from 1 to 12 weeks, and two microcycle from 13 to 20 weeks in periodized fluctuation. Our hypothesis was that the conjunction of systemic periodized 20 weeks training programs from strengthening soft tissue and muscular recruitment in off-season/pre-season, to maximum explosive strength and peaking optimal specific power performance during in-season would increase lower limb performance and strengthen neuromuscular controlling in taekwondo athletes.

2. Methods

2.1. Subjects

Eight male collegiate taekwondo athletes were recruited in this study. The study protocol and aim were explained and informed consent obtained from all subjects before the study. Approval for the study was obtained from the Human Ethics Committee of the Cheng Shiu University. In a pre-study interview, information on routine use of vitamins and other nutritional supplements were obtained from each participant. Subjects were informed to avoid exercise

or strenuous physical activity for 3 days prior to the before and after lower limb performance tests. In the twenty-four hour period preceding the study, subjects recorded all food and drink intake and this dietary pattern were duplicated in other parts of monitoring. Squat jump (SJ), countermovement jump (CMJ) and continuous jump bent leg (CJb) were tested before and after the systemic strength training period.

2.2. Experimental design and procedures

Subjects were evaluated six times during Training program (TP) I to IV: at the beginning (week 0, date1, T1), in the middle (week 2, Date 13, T2; week 8, Date 55, T3; week 14, Date 97, T4; week 18, Date 125, T5) and at the end (week 21, Date 143, T6) of the training program. Diets or lifestyles were not controlled during the course of the different seasons (Table 1).

The tests were always performed in the same order in the testing session. On the day of the experimental test, measurements were executed at 6:20 am- 7:20 am before breakfast on Friday morning. Athletes did not perform strenuous physical activities in the 12 hours before recordings. No subject was taking drugs at the time of the recording sessions. Subjects reported to the laboratory following a 10-h overnight fast. Subjects were instructed to consume 240 ml of water to increase hydration when they arrived at the laboratory. The experiments were performed in the room at comfortable ambient temperature (22°C to 24°C) and relative humidity (55-60%). The athletes lay supine for 5 minutes before experiments to relax in the room made noiseless. Firstly, subjects were measured forearm total vascular occlusion test (FTVOT) and muscular stiffness test (MST). Blood samples were collected in a rest position from the earlobe in order to analyze hemoglobin (HB) and creatine kinase (CK).

Table 1. Evaluations during training program (I-IV) of the study

Training period	Before-training		Training program				After-training		
			I	II	III	IV			
Aim	Base line		general conditioning	muscular recruitment	maximum strength	explosive power			
Week			1-6	7-12	13-16	17-20	21		
periodization			Off-season	Pre-season	Pre-season	In-season			
Test	before	T1	T2	T3	T4	T5	T6	after	
Testing Date	-7 th - -3 rd	0	13 th	55 th	97 th	125 th	143 th	144 th -146 th	
Testing parameters	SJ, CMJ, CJb		HB, CK, NIRS, STIFFNESS					SJ, CMJ, CJb	

2.3. Training program

This study was 20 weeks including a total of 1002.2 hours of taekwondo training. The taekwondo training program included of taekwondo related basic physical, tactical and specific technical training, and recreational training (Table 2).

The first 6 weeks of training program I consisting general conditioning and strengthen soft tissue (36 h)

(Table 3). The 7th to 12th weeks of training program II including muscular recruitment, strengthen muscle tissue and hypertrophy (44 h) (Table 4). The 13th to 16th weeks of training program III containing maximum strength and strengthen power (24 h) (Table 5). The 17th to 20th weeks of training program IV including explosive power, agility and speed (40 h) (Table 6).

Table 2. Training programs (I-IV) characteristics

Type of Training	Duration of a session (min)	Training programs weekly frequency			
		I	II	III	IV
Recovery	30	6	6	10	7
Endurance	30	12	8	3	3
Specific taekwondo	30	12	18	16	18
Specific speed	40	9	12	9	9
Tactical	30	3	10	13	10
Technical	40	10	12	10	10
Strength training	40	9	11	9	10
Simulated match	40	3	6	6	7
Recreational	30	3	1	2	1

Table 3. Training program I (general conditioning) used between weeks 1 and 6.

AIM: general conditioning, strengthen soft tissue (Monday- Wednesday- Friday)

Exercises	Sets x repetitions	Intensity
Warm-up	15 min of general activity + stretch	
Abdominal work	1-3 Sets-maximum 50 reps	
Back hyperextension	1Sets-maximum 10 reps	
Side bend with dumbbell	1Sets-maximum 10 reps	
Bar twists	1Sets-maximum 10 reps	
Upper body exercises		
Bench press	3x25-40	45-25% 1RM
Triceps extensions	3x25-40	45-25% 1RM
Wide-grip lat pull-downs	3x25-40	45-25% 1RM
Shoulder dips	1-3x 10reps	
Bicep curls	3x25-40	45-25% 1RM
Lower body exercises		
Body weight squat	3x25-40	45-25% 1RM
Half-squat	3x25-40	45-25% 1RM
Leg press	3x25-40	45-25% 1RM
Hamstring curl	3x25-40	45-25% 1RM

Table 4. Training program II (muscular recruitment) used between weeks 7 and 12

AIM: muscular recruitment, strengthen muscle tissue, hypertrophy (Monday- Wednesday- Friday)

Exercises	Sets× repetitions	Intensity
Warm-up	15 min of general activity + specific	
Abdominal work	1-3 Sets-maximum 50 reps	
Back hyperextension	1Sets-maximum 10-20 reps	
Side bend with dumbbell	1Sets-maximum 10-15 reps	
Bar twists	1Sets-maximum 10-15 reps	
Upper body exercises		
Incline bench press	3x8-10	75-85% 1RM
Dumbbell lat row	3x8-10	75-85% 1RM
Bicep curls	3x8-10	75-85% 1RM
Low pulley seated lat row	3x8-10	75-85% 1RM
Lower body exercises		
Standing calf (heel) raise	3x8-10	75-85% 1RM
Power clean	3x5-7	20-40% 1RM
Hang clean	3x5-7	25-45% 1RM
Leg press	3x8-10	75-85% 1RM
Hang snatch	3x5-7	20-45% 1RM
Leg extensions	3x8-10	75-85% 1RM
Standing calf (heel) raise	3x8-10	75-85% 1RM
Hamstring curls	3x8-10	75-85% 1RM

Table 5. Training program III (maximum strength) used between weeks 13 and 16

AIM: maximum strength, strengthen power (Monday- Wednesday- Friday)		
Exercises	Sets x repetitions	Intensity
Warm-up	15 min of general activity + specific	
Abdominal work	3-5 Sets-maximum 40 reps	
Back hyperextension	1-2Sets-maximum 10-20 reps	
Side bend with dumbbell	1-2Sets-maximum 10-15 reps	
Bar twists	1-2Sets-maximum 10-15 reps	
Upper body exercises		
Power drops	4x8	Medicine ball (3 kg)
Dumbbell lat row	4x3	90% 1RM
Push press	4x3	90% 1RM
Single arm throw	4x8	Medicine ball (2 kg)
Low pulley seated lat row	4x3	90% 1RM
Lower body exercises		
Standing calf raise	4x3	90% 1RM
Power clean	4x3	45% 1RM
Hang clean	3x5	45% 1RM
Leg press	4x3	90% 1RM
Hang snatch	3x5	40% 1RM
Leg extensions	4x3	90% 1RM
Hamstring curls	4x3	90% 1RM

Table 6. Training program IV (explosive power) used between weeks 17 and 20.

AIM: explosive power, agility, speed (Monday- Wednesday- Friday)		
Exercises	Sets x repetitions	Intensity
Warm-up	15 min of general activity + specific	
Abdominal work	3-5 Sets-maximum 40 reps	
Back hyperextension	2 Sets-maximum 15 reps	
Side bend with dumbbell	2 Sets-maximum 15 reps	
Bar twists	2 Sets-maximum 15 reps	
Upper body exercises		
Bench press + medicine ball throws	3x (8 + 8 reps)	80% 1RM
Dumbbell lat row	3x 8	80% 1RM
Push press + medicine ball throws	3x (8 + 8 reps)	80% 1RM
Bicep curls	3x 8	80% 1RM
Low pulley seated lat row	3x 8	80% 1RM
Lower body exercises		
Jump and reach	4x7	90% 1RM
Power clean + (standing triple jump)	3x (5 + 4 reps)	40% 1RM
Lateral box jump + (5-m lateral shuttle)	3x (5 + 4 reps)	90% 1RM
Leg press + (standing long jump)	4x(3+ 2reps)	90% 1RM
Hang snatch + (tuck jumps)	3x (5 + 10 reps)	40% 1RM
Leg extensions + 5-m sprint	4x3	90% 1RM

2.4. Blood Collection and Analysis

Earlobe blood samples were collected from each subject for determination of HB and CK. The serum CK concentration was measured immediately with the Ektachem DTSC chemistry analyzer (Johnson and Johnson, Rochester, NY, USA). The hemoglobin was measured with Analyzer (HemoCue® Hb 201+, Ängelholm, Sweden).

2.5 Forearm total vascular occlusion test (FTVOT)

The subject should then be placed sub supine with slight head-of-bed elevation for comfortable resting and to prevent the influence of gravity on blood flow and the accumulation of venous blood. The TSNIR-3 (TSNIR-3, Anheng Inc., Hefei, China) probe should be placed on the thenar muscle ensuring adequate sealing to prevent ambient light. The dominant thenar

muscle with relatively superficial and easy to access characteristics was chosen for FTVOT. During the first 5min, the athletes were in a comfortable supine position [8]. The sleeve-pressing method was used to perform the forearm venous occlusion. The pressure was increase 30-50 mmHg over the systolic pressure to ensure the artery and venous blood flow of the forearm was impeded completely, and this complete occlusion was maintained for 2 min. The pressure was then released and allowed to recover to the normal state. Adequate time should be allowed for the recording of the reactive hyperemia until the return of tissue oxygen saturation (StO₂) to baseline values [9].

After the vascular occlusion measurement, the inflation of the cuff is followed by a rapid pressure decreasing. The StO₂ decrease has been used extensively in the literature as an estimate of oxygen consumption rate (Oxygen consumption rate, %/min) of the muscle tissue[8]. The oxygen consumption rate reflects the basic metabolism of skeletal muscle at rest, but is also influenced by the adequacy of microcirculation to provide necessary oxygen to the tissue. The rate of StO₂ increase after cuff release reflects the resaturation rate of hemoglobin (reperfusion rate, %/min), which relies on integrating function of the vascular endothelium.

2.6. Muscle mechanical properties

The evoked mechanical oscillations are clearly visible. If there is no neural activation, the condition of the muscle is considered to be characterized mainly by the viscoelastic properties [10].MyotonPRO (MyotonPRO, Myoton AS, Tallinn, Estonia) enables us to observe mechanical oscillation of the tissue provoked by mechanical impact with an effort to avoid neurological reactions and non-elastic deformations in the tissue and measures the distortion properties of natural damped oscillations produced following a short (15 ms) mechanical tap to the surface of the skin. Stiffness was created of the underlying muscle, based on equations calculated from the acceleration of the testing probe during oscillations [11]. Rectus femoris (RF) of dominate leg was measured two occasions at the same time. The subjects lay with their knees extended and hips in neutral to rest for 5 min prior to measurements. During this time, room and skin surface temperature were recorded via an electro-thermometer, and the site for measurement on the RF muscle was identified by measuring two-thirds distally from the most anterior aspect of the anterior superior iliac spine to the superior border of the patella, following the direction of muscle fibers. After 5 min, the testing end of MyotonPRO was placed on the skin perpendicular to

the surface of the muscle belly over the site located. The contraction of RF muscle was evoked by raising the leg to an angle of 45°. Stiffness reflects the resistance of the tissue to the force that changes its shape. The higher this value is, the more energy is needed to modify the shape of the tissue. During contraction the stiffness of the muscle increases linearly with the increase in the contraction force [12].

2.7. Lower limb performance

Lower limb performance is a determinant factor for success in many sports. A mixture of explosive force, endurance and coordination is trained and very carefully optimized for each particular sports type. Gymmy Jump provides an objective measurement of force, power and jump height. Gymmy Jump consists of a portable Kistler force plate on which different jump types are performed. The force plate measures the vertical jump force which is analyzed with the computer connected to the system. The Bosco Protocol evaluates 3 different jump types and calculates a variety of parameters: (a) Squat jump (SJ) : Single jump starting from knees bent at 90 degrees; (b) Countermovement jump (CMJ): Single jump starting with straight legs with a natural flexion before takeoff; (c) Continuous jump bent legs (CJb): Series of 0s to 60s jumping with knees bent. Performance in a squat jump describes jumping ability and explosive (maximal) force production of the lower extremities.

2.8. Statistical analysis

Standard statistical methods were used to calculate the mean and standard deviations (SD). The haemocrit level was used to adjust the other parameters for different dehydration. The differences between the Before- and After training program values were statistically examined by paired *t* test. ANOVA with repeated measures was used to determine the differences between tests. When a significant *F* value was achieved, appropriate LSD *post hoc* tests procedures were used to locate the difference between means. The P<0.05 criterion was used to establish statistical significance.

3. Results

3.1. Anthropometric characteristics and lower limb performance values

Table 7 shows the variables concerning the anthropometric and metabolic characteristics of the athletes examined before-training and after-training programs period. There were significantly increasing in the SJ, CMJ and CJb after the training programs period.

Table 7 Anthropometric characteristics and lower limb performance of the taekwondo athletes at the before and after the end of the training period

Characteristics	unit	BEFORE-training	AFTER-training	Change (%)
Age	y	22.7(2.3)	22.7(2.3)	
Height	cm	175.4(5.7)	175.4(5.7)	
Weight	kg	76.5(6.3)	77.2(6.8)	0.92(.67)
Hemoglobin	g/dL	13.3(0.5)	13.8(0.7)	3.7(3.5)
Squat jump	cm	37.9(6.0)	40.8(5.1)*	7.8(2.7)
Countermovement jump	cm	41.4(4.2)	42.8(4.9) *	18.3(4.1)
60s Continuous jump bent legs	cm	31.8(5.3)	34.6(5.7)*	8.7(4.7)

Values are expressed as means(SD). *: vs. Before-training period, $P < 0.05$.

3.2. Creatine kinase

Training increased creatine kinase levels (Fig. 1) from T1 to T2 (120.5%), T3 (197.7%) and T4 (327.8%). In contrast, CK levels tend to recover at T5 (289.2 %) and incline to baseline significantly ($P < 0.05$) at T6 (99.4%) thereafter. Blood levels of CK have been used as an index of training-induced physiological stress.

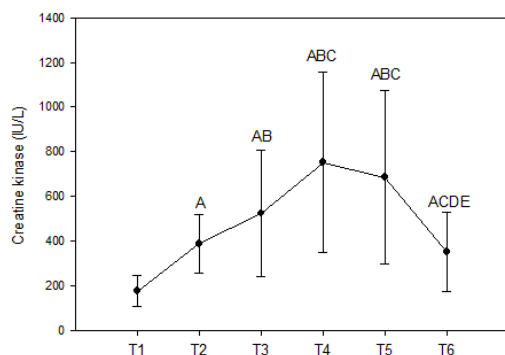


Figure 1. Creatine kinase responses with training. T1, baseline; T2–T5, the four training programs (I–IV); T6, After-training. A: vs.T1, $P < 0.05$; B: vs.T2, $P < 0.05$; C: vs.T3, $P < 0.05$; D: vs.T4, $P < 0.05$; E: vs.T5, $P < 0.05$.

3.3. Forearm total vascular occlusion test

Changes in muscle tissue oxygen consumption rate status are represented in Fig. 2. oxygen consumption rate decreased significantly ($P < 0.05$) from T4 (53.1 %), in contrast, oxygen consumption rate restored at T5 (53.3 %) and incline to T4 significantly ($P < 0.05$) at T6 (86.7 %). The trend of reperfusion rate was the same as oxygen consumption rate. Reperfusion rate significantly decreased ($P < 0.05$) at T4 (46.30 %) and elevated at T6 (74.1%). The lower oxygen consumption rate of FTVOT after the training programs was, the less basic metabolism of skeletal muscle performed. FTVOT could be as an index of training-induced metabolic stress.

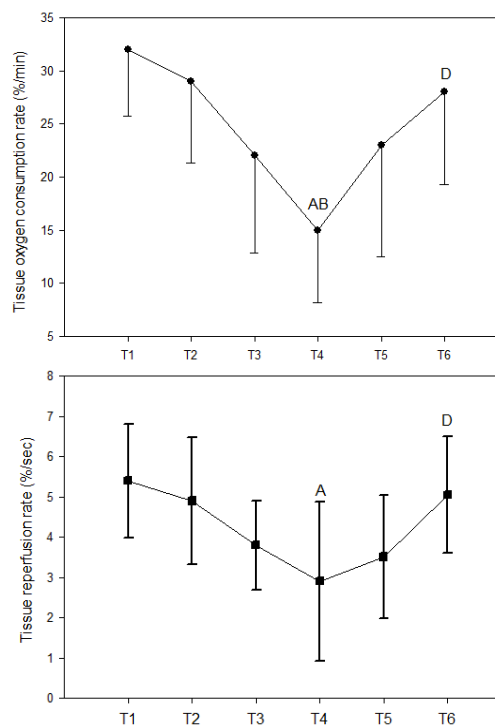


Figure 2. Indices of forearm total vascular occlusion test during training programs. T1, baseline; T2–T5, the four training program periods (I–IV); T6, After-training period. A: vs.T1, $P < 0.05$; B: vs.T2, $P < 0.05$; D: vs.T4, $P < 0.05$.

3.4. Muscle mechanical properties

Fig3 illustrates stiffness changes. There were the same significantly changes trend between contraction and relaxation. Stiffness of contraction were increased from T1 to T4 (T2, 15.0%; T3, 41.8%; T4, 62.1%) and recovered from T4 to T6 (T5, -11.8%; T6, -25.9%). Stiffness of relaxation were increased from T1 to T4 (T2, 22.6%; T3, 44.7%; T4, 53.2%) and recovered from T4 to T6 (T5, -8.5%; T6, -13.0%). Responses of muscle stiffness could be as a quantified index of training load and training intensity.

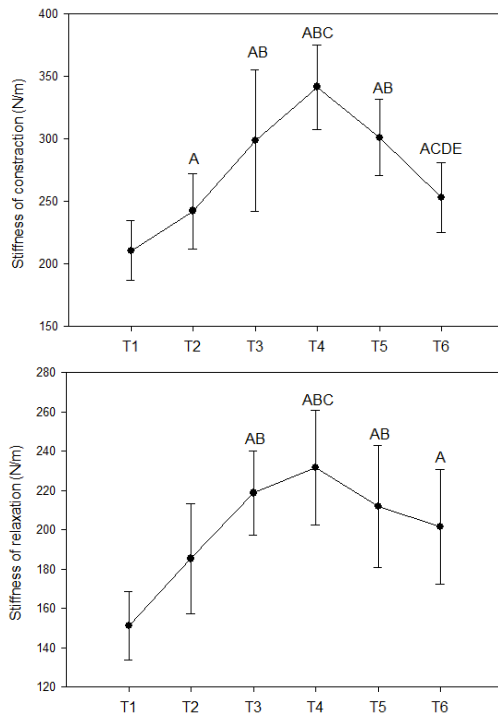


Figure 3. Indices of muscle mechanical properties (stiffness of contraction; stiffness of relaxation reperfusion rate) responses during training. T1, baseline; T2–T5, the four training programs (I–IV); T6, After-training. ^A: vs.T1, $P<0.05$; ^B: vs.T2, $P<0.05$; ^C: vs.T3, $P<0.05$; ^D: vs.T4, $P<0.05$; ^E: vs.T5, $P<0.05$.

Discussion

Taekwondo can be identified as an intermittent exercise alternating dominantly anaerobic metabolism. We believe that athletes must arrange basic and specific muscular fitness to conditioning their performance.

The training load in competitive sport can be characterized as a combination of training intensity, volume, and frequency [13]. This training load is extremely decreased during the taper in an attempt to reduce accumulated fatigue, but reduced training should not be hurtful to training-induced adaptations [14]. During taekwondo high-intensity bouts of activity, athletes are involved in defensive and offensive speeding attacks underlie the anaerobic metabolism contribution that strength, power, agility, and sprint are needed. Therefore, athletes and coaches must judge the content to which the training load can be regulated at the amount of the training variables while reserved or slightly improving adaptations. The study establishes the scientific bases for well monitoring different seasons (TP-I~IV) changed training loads, supporting athletes, coaches, and sports scientists accomplish the optimum training conjugation during the periodization, leading to peak

performances at the planned point during the seasons.

The training load is clearly depressed during a taper (TP-IV) after that athletes regain from intensive training (TP-III to TP-IV) and energized before major events (After-training or competition). Research easily displays controlling conditioning (TP-IV) to avoid detraining risks, training intensity should be sustained during the taper, because the organized training periodization is important to reserving performance and explosive power. In addition, high-quality training during the taper can further enhance physiological and performance adaptations (TP-IV). Conversely, if training intensity is diminished, some training-induced adaptations may be lost, leading to suboptimal competition performance.

CK is an important enzyme in muscle energy production that is generally confined to the inside of muscle cells. The presence of elevating values of CK in the blood from TP-I to TP-III suggested that muscle cell membranes had suffered some kind of damage, allowing CK to escape the cells. Following tapering and reducing intensive training, the blood concentration of CK was remarkably decreased, suggesting TP-III increased levels of muscle tissue breakdown.

Thomas and colleagues (2008) present that an overload before the taper is essential to maximize performance but imposes specific requirements during the taper. We prepared to strengthen soft tissue (TP-I), muscular recruitment and hypertrophy (TP-II) firstly. Then, Athletes were potentiated maximum strength applied to explosive power (TP-III) and intensified functional performance (TP-IV). Our study from TP-I to TP-III reflected increasing CK level (327.8%), reducing oxygen consumption rate (-53.1 %) and elevating muscular stiffness responses (62.1%). The study developed a progressive modulation being preferably to taper after prior overload training. Our findings confirm the relevance of the original modeling between TP-I to TP-IV approach in the study of individual responses to training and the optimization of tapering strategies and selected monitoring index.

Near and colleagues (2005) used near-infrared spectroscopy (NIRS) to examine the effects of a taper on muscle oxygenation during cycling. These authors examined muscle oxygenation of the right vastus medians during a simulated 20 km/cycling time trial before and after a 7-day taper consisting of either a 30%, a 50%, or an 80% reduction in weekly training volume. Mean tissue oxygenation during the cycling test was significantly greater (i.e., increased muscle deoxygenation) following the 50% training volume reduction taper. In addition, there was a moderately high correlation between the change in tissue oxygenation and 20 km time trial performance, which

suggests that metabolic changes that occur in the vastus medialis muscle are in part responsible for performance changes at the whole-body level. In other words, the taper induced increased oxygen extraction, which was associated with increased performance [15]. We detected FTVOT responses by monitoring StO₂ changes reacting neuromuscular conditioning during TP-I to TP-IV. Increases in the StO₂ during the taper might contribute to taper-induced performance gains. A lower oxygen cost of exercise after the taper from TP-I to TP-III could also contribute to improved performances. Any observed change seems to be a reflection of the athletes' adaptation reflecting the resaturation rate of hemoglobin to preceding training rather than to the taper itself.

The extraordinary plasticity of skeletal muscle tissue allows it to adapt to variable levels of functional demands, neuromuscular activity, and biochemical signals and reversibly change its functional characteristics and structural composition[16]. A in-season/precompetition taper presumably reduces the demands placed on the neuromuscular system compared with previous phases of a training program. Increased strength and power as a result of a taper have been a common observation in different athletic activities. Costill and colleagues (1985) were among the first researchers to describe such gains in swimmers. These authors described an 18% improvement in swim bench power and a 25% gain in actual swim power in a group of 17 collegiate swimmers undergoing a 2-week taper. Swim power improvement correlated with a 3.1% competition performance gain ($r = .68$). In the study, there were recoveries from reducing in CK levels (99.4%), elevating reperfusion rate (74.1%) and decreasing stiffness of contraction (-25.9%) at T6. The reduced training may have allowed for an increase in maximal tension development through changes in the contractile mechanisms or neural controls on fiber recruitment.

In conclusion, the results of this study indicate that systemic periodised strength training programs elevated biological stress increasing CK, oxygen consumption rate and muscular stiffness modulation. According to taper in periodization, there are strong correlations among training load and physiological stress. The conjunction of systemic periodised 20 weeks training programs would increase lower limb performance and strengthen neuromuscular controlling in taekwondo athletes.

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The Impact of Good Governance in Increasing Efficiency of Fars Economic and Finance Organization, Iran

¹Fatemeh Farhadi Ayoubloo, ²Saeed Mazloomiyan, ²Mohammad Hassan Seif, ³Mehrzad Saeedikiya

¹MA of Public Management, Payame Noor University, I.R.IRAN

²Department of Educational Psychology, Payame Noor University, I.R.IRAN

³MSC of Management

Abstract: To explore the relationship between components of good governance and improving the efficiency of the administrative system in Fars Economic and Finance Organization, 125 employees from this organization were selected as the sample regarding the general purposes of the present study and they were asked to complete two questionnaires; one dealing with good governance model while the other was related to the efficiency of the administrative system. The results obtained through investigating the research hypotheses, generally, indicated that there is a significant and positive relationship between good governance model along with its indicators and the efficiency of the administrative system in Fars Economic and Finance Organization ($p < 0.001$). The result derived from these findings will be discussed at the end of this study.

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Keywords: good governance, efficiency of the administrative system, control of corruption, political stability, freedom of speech and accountability

1. Introduction

Good governance versus bad governance is regarded as one of the most effective factors of development in different societies especially in developing communities. Rapid spread of good governance and the attention paid by international institutions to this issue have caused many large international organizations to insert much pressure on governments to improve their reformation attitudes (Lambsdorff, 2004).

On the other hand, some of researchers and scientific societies have emphasized the instrument of realization of good governance and have numerated macroeconomic policies, efficient allocation of resources, the quality of administration, and bureaucracy as main characteristics of good governance (Landman, 2003).

The significance of good governance and the efficient administrative system arises from the fact that according to known international standards, public participation in government decisions, government accountability towards the public, the country's political stability, improving government effectiveness, improvement of regulations quality for regulating economic affairs, people's and statesmen's respect for law, and corruption control, all being indicators of good governance, are the most important factors in improving the country's economic performance and

achievement of sustainable development to the extent that this index (i.e. good governance) and its components have been introduced by the World Bank (as the main centre for analyzing countries' economic development) as a major issue in the development

strategy for countries with poor performance (World Bank, 2000).

UN's Development Program has viewed governance as the exercise of administrative and political power (authority) for management of the country's affairs at all levels. Good governance, based on the definition proposed by UNDP, includes mechanisms, processes, and institutions through which citizens, civil groups, and civil societies pursue their own interests, meet their obligations and legal rights, and minimize their differences (UN-ESCAP, 2003).

Kaufman, a World Bank researcher, employs the following indices to measure these triple issues in the above classification (Kaufman, 2002):

Corruption: factors affecting the measurement of corruption: The prevalence of corruption across the country, the spread of relationships of so-called nepotism (favoritism) at different level throughout the country, and the dominance of paperwork in bureaucracy can result in the prevalence of corruption. Corruption also includes amount of monies paid illegally in order to accelerate work process (as called bribery), the level of public trust in politicians' financial honesty, illegal evasions of taxes and obtaining licenses.

Political stability and chaos: refer to transparent and peaceful processes used in power transfer and political tranquility (UNDP, 2002).

Government Effectiveness: refers to government effectiveness and success in achieving the entrusted objectives. Factors affecting government effectiveness: the utilization rate of technical and

working skills by middle and upper level managers, the effectiveness of government bureaucracy, government effectiveness in collecting tax revenues and other governmental revenues, the government's ability to implement national-political innovations, on time compilation of annual budget, the quality of civil services and the ability of bureaucracy in providing civil services without being influenced by political changes and the movements made in the structure of political power (Kaufman et al, 1999).

Rule of Law: The existence of general mechanisms and strong determination in law making and law enforcement. (Kaufman et al, 2007).

Quality of Rules and Regulations: This means excessive and debilitating regulations and additional costs (Diouf et al, 1989).

Freedom of speech and accountability: refers to public participation and authorities' accountability

Reasons for paying attention to good governance: Governments are faced with new challenges at the turn of the 21st century, including:

1. In the era of globalization, governments should actively respond to positive economic opportunities and show defensive reactions to the negative economic pressures.
2. Demographic changes have influenced governments as employers and service providers in most countries. Populations in most countries are getting older and this older populations are demanding more social services. Besides, government agencies need to compete with nonprofit organizations and private employers to hire skilled and motivated individuals, especially given that wages in the public sector are lower than what is in the private sector. So, government agencies must recognize non-monetary incentives to hire and maintain high performance employees which is perceived as a big challenge for them (Gholipour, 2995).
3. The public sector will also face new expectations of educated citizens, meaning that citizens will expect to receive higher quality services. Therefore, governments must provide capacities for citizens and stakeholders so that they can participate more actively in public issues.
4. Finally, expectations from the part of organizations personnel have changed and to respond to these expectations demands new conditions. Responding to these challenges requires sound proper governance at the macro level in the society (Sanei, 2006). The administrative system is one of the most complex levels in open systems. The purpose and mission of an administrative system as a

factor for formation and survival of individual components of the system is completely dynamic and variable that can change according to needs priority. Human resources are the main decision making body, deriving force, and decision making element in every administrative system, as there is no approach not being dependent on human beings (Homburg, 2002).

Issazadeh and Ahmadzadeh (2009) investigated the effects of institutional factor alongside the other factors on the economic growth. Results obtained through various estimates indicate that public institutions such as rule of law, political stability, control of corruption, bureaucracy quality, and government effectiveness may positively and significantly affect economic growth. In addition, the effect of freedom of speech and accountability (democracy index) was positive but not significant. Furthermore, it was noted that the more dominant good governance in a country, the higher the rate of economic growth.

Pahlavan Zadeh (2010) in a study entitled "Review of Iran's employment legal system in the light of good governance theory" tried to find out how much new laws and regulations are in line with good governance and how much these regulations are influenced and inspired by indicators of good governance.

Virmani et al., (2006) explored the effects of various economic and non-economic variables on indicators of good governance and concluded that indicators used as good governance variables have a positive and significant effect on the quality of services rendered by the government and on per capita income (Virmani et al., 2006).

Deininger and Empuga (2007) examined the data obtained from Uganda and pointed out that corruption and lack of accountability in executive institutions are the most important factors that may reduce the quality of services provided by the government.

Given the broad objectives of the Fars Economic and Finance Organization in categories such as: monitoring financial payments and receipts in governmental organizations and affiliations, the search for scientific and codified approaches in recognizing financial, tax, and economic capacities in Fars Province is, generally, directed at application of rule-governed practices and strategies for development of such capacities. Naturally, since this organization is in charge of collecting, handling and protestation of financial and economic affair it demands a very comprehensive solutions. As a result, concerning what has been mention above with regard

to increasing efficiency and effectiveness in the management of financial resources suppliers with a focus on structures, methods, and equipment employed for financial resources and human resource management, good governance is a missing link for our country's development (Fars Economic and Finance Organization Portal, 2010).

2. Research Methodology

1.2. Determining the sample size

The census method was employed in the present study due to small size of the population under study. The total number of employees working for Fars Economic and Finance Organization (including official and contractual staff and contract for performing certain works) are 137 individuals (36 females and 89 males) consisting of managers, assistants, specialists, accountants, and their deputies and staff. Of 137 questionnaires distributed among employees, 125 questionnaires were filled in and returned to collect the needed data.

2.2. Instruments

In order to collect the required data, questionnaire and library methods were used. The main instrument used in the study to test the hypothesis was compiled by combining two closed questionnaires. All items in the questionnaire were measured based on Likert scale.

The questionnaire measuring good governance included 4 questions asking for respondents' personal profiles (i.e. gender, age, work experience, and education) and 32 questions dealing with aspects of good governance including 6 aspects of corruption control, political stability, government effectiveness, rule of law, quality of regulations and rules, and freedom of speech and accountability. Another questionnaire including 12 items was used to measure the efficiency of the administrative system. The validity of the questionnaires was content-validated while the reliability of questionnaires was measured through Cronbach's alpha method.

3. Results of the study

Table 1: Pearson correlation coefficient for good governance model and efficiency of administrative system

As Table 1 indicates, there is a positive and significant relationship between good governance model and the efficiency of the administrative system of Fars Economic and Finance Organization at significance level of $p < 0.001$. In other words, an increase in good governance model will lead to an increase in the efficiency of the administrative system of Fars Economic and Finance Organization.

Table 2: Pearson correlation coefficient between corruption control and administrative system efficiency

Based on Table 2, there is a positive and significant relationship between corruption control as a component of good governance model and the efficiency of the administrative system of Fars Economic and Finance Organization at significance level of $p < 0.001$. In other words, an increase in the component of corruption control will lead to an increase in the efficiency of the administrative system of Fars Economic and Finance Organization.

Table 3: Pearson correlation between political stability and efficiency of administrative system

As it is suggested by Table 3, there is a positive and significant relationship between political stability as a component of good governance model and the efficiency of the administrative system of Fars Economic and Finance Organization at significance level of $p < 0.001$. In other words, an increase in the political stability will lead to an increase in the efficiency of the administrative system of Fars Economic and Finance Organization.

Table 4 - Pearson correlation coefficient between government effectiveness and efficiency of administrative system

As Table 4 indicates, there is a positive and significant relationship between government effectiveness as a component of good governance model and the efficiency of the administrative system of Fars Economic and Finance Organization at significance level of $p < 0.001$. In other words, an increase in the government effectiveness will lead to an increase in the efficiency of the administrative system of Fars Economic and Finance Organization.

Table 5: Pearson correlation coefficient between the rule of law and efficiency of administrative system

As shown in Table 5, there is a positive and significant relationship between rule of the law as a component of good governance model and the efficiency of the administrative system of Fars Economic and Finance Organization at significance level of $p < 0.001$. In other words, an increase in rule of the law will result in an increase in the efficiency of the administrative system of Fars Economic and Finance Organization.

Table 6: Pearson correlation coefficient between quality of laws and regulations and efficiency of administrative system

According to Table 6, there is a positive and significant relationship between the quality of laws and regulations as a component of good governance model and the efficiency of the administrative system of Fars Economic and Finance Organization at significance level of $p < 0.001$. In other words, an increase in the quality of laws and regulations will result in an increase in the efficiency of the

administrative system of Fars Economic and Finance Organization.

Table 7 - Pearson correlation coefficient between freedom of speech and accountability and efficiency of administrative system

As it can be noted in Table 7, there is a positive and significant relationship between freedom of speech and accountability as a component of good governance model and the efficiency of the administrative system of Fars Economic and Finance Organization at significance level of $p < 0.001$. In other words, an increase in freedom of speech and accountability will result in an increase in the efficiency of the administrative system of Fars Economic and Finance Organization.

4. Discussion and conclusion

Overall, the results of the present study indicate that the characteristics of good governance model directly and significantly affect the efficiency of administrative systems in Fars Economic and Finance Organization. This means that improvement of good governance model in this organization leads to the enhancement of the efficiency of administrative systems ($p = 0.702$). these findings are in line with those observed by Pahlavan Zadeh (2010) and Issazadeh and Ahmadzadeh (2009). As a result, it is possible to promote the efficiency of the administrative system in the organization in order to optimize services offered, absorption and allocation of resources, and also to optimize organizational units and reduce administrative costs so that to maintain the organization's health in the society through identification of good governance characteristics in the organization and providing favorable conditions to increase good governance characteristics in workplace. Generally, it can be said that since all hypotheses proposed in this studies have been confirmed and there are positive and significant

correlations between variables examined through the study which can be used as a basis for other studies and research in the field of good governance. It is believed that good governance is established if the government is more responsive, more efficient with greater political stability and if excessive regulations and costs are reduced, rule of law is more dominant, and if corruption is more limited.

The results obtained through t-test results suggest that women are slightly more efficient than men so that their mean score with regard to efficiency was 53.2 while that of men was 52.3. But this difference was not significant ($t = 1.04$) and therefore it cannot be generalized to the whole population under study. As a result, there is not a significant relationship between gender and efficiency of the administrative system. It should be noted that this result is based on the equality of variances, as f value obtained through Levine test is not significant ($f = 2.47$).

The correlation between age and efficiency of the administrative system is 0.075 because the obtained level of significance is greater than the assumed level of significance of 0.05. Therefore, it can be said with 95% confidence that there is not a significant relationship between participants' work experience and efficiency of the administrative systems.

The observed f value (0.27) at alpha level of 0.05 is smaller the value given in the table. As a result, there is not a significant relationship between participants' level of education and efficiency of the administrative system. These findings indicate that none of the respondents' demographic characteristics, including gender, age, work experience, and level of are significantly related to efficiency of the administrative system.

Table 1: Pearson correlation coefficient for good governance model and efficiency of administrative system

Variable	Indicators	Model of good governance
Efficiency of the administrative system	Pearson correlation coefficient	0.702
	Level of significance	0.001
	Total	125

Table 2: Pearson correlation coefficient between corruption control and administrative system efficiency

Variables	Indicators	Components of corruption control
Efficiency of the administrative system	Pearson correlation coefficient	0.651
	Level of significance	0.001
	Total	125

Table 3: Pearson correlation between political stability and efficiency of administrative system

Variables	Indicators	Political stability
Efficiency of the administrative system	Pearson correlation coefficient	0.574
	Level of significance	0.001
	Total	125

Table 4 - Pearson correlation coefficient between government effectiveness and efficiency of administrative system

Variables	Indicators	government effectiveness
Efficiency of the administrative system	Pearson correlation coefficient	0.574
	Level of significance	0.001
	Total	125

Table 5: Pearson correlation coefficient between the rule of law and efficiency of administrative system

Variables	Indicators	Rule of the law
Efficiency of the administrative system	Pearson correlation coefficient	0.501

Table 6: Pearson correlation coefficient between quality of laws and regulations and efficiency of administrative system

Variables	Indicators	Quality of laws and regulations
Efficiency of the administrative system	Pearson correlation coefficient	0.355
	Level of significance	0.001
	Total	125

Table 7 - Pearson correlation coefficient between freedom of speech and accountability and efficiency of administrative system

Variables	Indicators	freedom of speech and accountability
Efficiency of the administrative system	Pearson correlation coefficient	0.247
	Level of significance	0.001
	Total	125

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6/5/2012

Karyological Study of Marsh Frogs (*Rana Ridibunda*)

* Ashraf Jazayeri, Forogh Papan, Amin Ismaili

Shahid Chamran University of Ahwaz

Corresponding author: jazayeriashraf@Ymail.com

Abstract: Tailless amphibians, including frogs, the dead are an important part of food chains and networks in most ecosystems. This special place, further studies are necessary to identify a more complete way to prove this. Currently, few studies in the field survey in Iran biosystematics and tailless amphibian biodiversity has been done. Accordingly, in this research study Karyological a dead frog in the city of Ahwaz - Iran has taken place. Study based on conventional methods on bone marrow tissue was performed in both sexes. Results showed that the chromosome number of this species in the study area has 26 chromosomes. Chromosomes based on arm ratio and the locations of the centromere in the two groups were including seven pairs of chromosomes and 6 pairs of sub metacentric were. Furthermore, based on giemsa staining clearly recognizable sex chromosomes and two sex chromosomes that males Homomorphous XY and XX sex chromosomes in females Homomorphous and had two. Also check the number of chromosomal arms (F_n) of this species, 52 showed the arm. [Ashraf Jazayeri. **Karyological Study of Marsh frogs (*Rana ridibunda*)**. *Life Sci J* 2012;9(3):864-866]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 122

Keywords: *Karyological, Rana ridibunda*

1. Introduction

Tailless amphibians as part of the Food Network, Many important ecosystems are in equilibrium (16). Unfortunately, today marked the global amphibian population been declining rapidly, so these animals are facing extinction (6 and 16 and 5). Report Disappeared Be Some Of Species Frogs, Toads And Salamanders At Areas South, And Center North America, Europe, Asia, Africa And Australia Do The (1 and 5). The first studies in this regard was conducted in 1980 found that about 42 percent of all known amphibians are facing extinction (15). The cause of population change and water conditions Earth air, as they are. Among the important factors in this context, Warming and increased CO_2 The atmosphere is (15). Amphibian tails due to the outbreak, Karyological diversity issues are appropriate for Cytogenetic Studies (3). Chromosome studies in amphibians began in 1932 when MAKINO There is 22 chromosomes in BUFO SACHALINENSIS Using tissues that do divide mitosis described (14). Despite the high phenotypic similarities among species of tailless amphibians very clear methods of identifying species can impose (2) in Iran and other the world, especially on the tail of amphibian species *Rana* Karyological various studies have been done (12, 9, 11 and 2). But in some cases there is still a shortage (4) In order to accurately identify the species present study examined the chromosomal structure of frog *Rana ridibunda* is in Ahwaz.

2. Materials and Methods

After collecting samples from the city of Ahwaz, first, and then the solution was carefully

weighing kolshicin (mg / ml 1), per gram of body weight of animal, ml 0.01 was injected into the abdominal area.

Table1. Long arm of chromosome 1 frog species (*Rana ridibunda*) per micrometer

Centro mere of chromosome type	Total length (p + q)	During the long arm (p)	During the short arm (q)	Number of chromosomes
Metacentric	87.15	86.9	01.6	1
Metacentric	13	32.8	68.4	2
Metacentric	89.11	90.6	99.4	3
Metacentric	82.9	05.5	77.4	4
Metacentric	73.8	51.4	22.4	5
Metacentric	30.6	86.3	44.2	6
Submetacentric	19.5	72.3	47.1	7
Submetacentric	21.4	46.3	75.0	8
Submetacentric	82.3	95.2	87.0	9
Submetacentric	59.3	83.2	76.0	10
Submetacentric	48.2	92.1	56.0	11
Submetacentric	20.2	58.1	35.0	12

After 24 h samples, were killed and bone marrow tissue was immediately explained to the Chinese plant contained KCl (0.075 M) was transferred and was maintained for 30 min at room temperature (twice during this period of hypotonic solution KCl Was replaced), then the sample was transferred to a centrifuge tube and 10 minutes away rpm Was centrifuged at 1500. After discharging the supernatant soup, a plate of fresh and cold fixator solution of acetic acid - methanol ratio (1 to 3) was added and then 10 minutes away from the homogenized rpm the second centrifugation step was repeated once again in 1500.

Table 2. Diploid haploid chromosomal number of frogs arm *Rana ridibunda*

The number of autosomal chromosomes FN ^a	FN	No. Diploid (n 2)	No. Haplo (n)	Type of Chromosome
24	28	14	7	Metacentric
24	24	12	6	Submetacentric
48	52	26	13	Total

The Sex Chromosome has not been counted

So completely sediment was washed in the end, ml One solution fixation cooled slowly and the drop to the sediment added and by following Pat uniform, then the solution obtained from a distance of 50 cm on the lam clean and cool, it slides right onto the ramp was dry, the color slides stained by Giemsa solution were made, stained slides, labeling and by microscope Optical studies and the best metaphase plates were photographed. After the image of the metaphase plate, on each chromosome Necessary measures such as total length, length of the long arm, short arm length was then the separation and identification of homologous chromosomes, the Karyological was arranged according to standard methods (8).

3. Results

This study details the frog Karyological *Rana ridibunda* Collected from different areas of Ahvaz, is. On the basis of investigation, chromosome numbers of species detected (*Rana ridibunda*) As $2n = 26$ Was observed (Figure 1 and 2). Identify chromosomes classified according to class rules leevan Is (13) Of these chromosomes, 7 pairs of type Metacentric and 6 pairs of submetacentric type (Table 1), sex chromosomes were clearly visible and identifiable so in female form. XX, In male form XY The smell Ned, the number of chromosomal arms (FN) 52, the sex chromosomes X Of the total, compared with 3 pairs of chromosomes and sex chromosomes Y The total length of chromosome pair 6 were comparable.]

4. Discussion

Studies worldwide have shown that sex *Rana* There is a number of chromosomes 26 (10-17). In this study confirmed that the number of chromosome 26 for this species research also is consistent with other researchers.

Existence of sex chromosomes X and Y That sexual differentiation may reflect this species in this study. Was proved, studies of chromosomal markers showed that these chromosomes in the two groups and sub met centric Contract, These results with similar studies in other regions of the world's scientists (7).

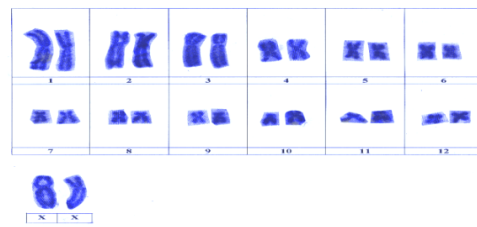


Fig 1. Male metaphase plate Marsh frogs

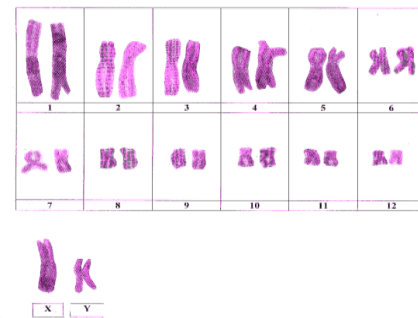


Fig2. Male Karyological Marsh frogs

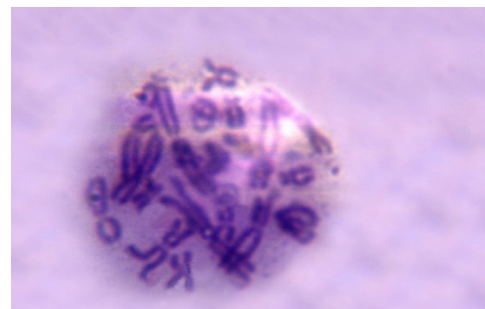


Fig3. A female Marsh frog's metaphase plate

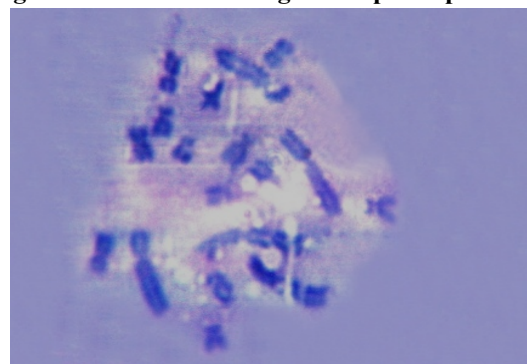


Fig4. Marsh frogs female Karyological

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7/13/2012

Comparing the Incidence of Pulmonary Edema in Anesthesia (*with or without use of morphine and lasix*) in Children Undergoing PDA Surgery

A. Ebadi¹, M. Deghani Firooz abadi², M. Soltan zade³, A. Emmami Moghadam⁴, SH. Nesioonpour⁵, R.Akhondzadeh⁶, K. Behaien⁷, A. Ghorbani⁸, SH. Beladi behbahani⁹

1,3. Associate Professor of Cardiac Anesthesiology MD, Department of Cardiac Anesthesiology, Golestan Hospital, Ahvaz Jundishapour University, Ahvaz, Iran

2. Assistant Professor of Cardiac Anesthesiology MD JundiShapour University, Ahvaz,

5, 6- Assistant Professor of Anesthesiology MD JundiShapour University, Ahvaz.

7- Assistant Professor of JundiShapour University, Ahvaz

4- Assistant Professor of Cardiac pediatric MD, Of Jundishapour University, Ahvaz

8, 9. Resident of Anesthesiology MD of Jundishapour University, Ahvaz

Abstract: Introduction: Patent ductus arteriosus (PDA) is a congenital cardiovascular disorder found in patients of all ages, from tiny premature infants to older adults (1). Persistent patent ducts arteriosus often cause hemodynamic and respiratory disorders, which require use of inotropic drugs and respiratory support in the full term infants. Surgical ligation should be considered for patients when medical therapy fails (2). The aim of this study was two anesthesia techniques evaluation for pulmonary edemas reduce in PDA surgery. **Methods and Materials:** In this study, 120 patients underwent PDA surgery, Patients were divided into two groups of 60, the patients in first group received morphine and lasix during anesthesia but second group received nothing. **Results:** The average age of the patients in the case and control groups was 32.8 and 31.36 months. Frequency of Male and female in case and control groups was 38.3%, 61.7% and 45%, 55%, respectively. In control group, from 60 patients, 18.3% had pulmonary edema and 5% of them had loss of consciousness. There was significant difference between case and control groups about patients with pulmonary edema. Also, RR (0.001), Pao₂ (p=0.005), PH (p=0.01) and PR (P=0.003) had significant difference in cases and controls. **Conclusion:** The findings of this study suggest that the use of morphine and Lasix is useful for reduction of pulmonary edema after PDA surgery. Further studies are needed to find the better management method.

[A. Ebadi. **The Comparing the Incidence of Pulmonary Edema in Anesthesia (*with or without use of morphine and lasix*) in Children Undergoing PDA Surgery.** *Life Sci J* 2012;9(3):867-870]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 123

Key words: patent ductus arteriosus, pulmonary edema, morphine, lasix, anesthesia

1. Introduction

The patent ductus arteriosus (PDA) is a vascular structure that connects the proximal descending aorta to the roof of the main pulmonary artery near the origin of the left branch pulmonary artery. This essential fetal structure usually closes spontaneously after birth (1). Permanent closure of PDA usually occurs within a few weeks after birth however; the failure of closure within 72 hours after birth will result in persistent PDA (3).

Clinical significant and physiological damages that PDA can do depend on PDA size and patient cardiovascular status. Although PDA often diagnosed in infants, it may not be discovered until childhood or sometime even adulthood (asymptomatic PDA), therefore PDA may also be discovered by accident during Echo cardiography for other reason and it can be in different sizes (small/medium/large). Regardless of the size, it is important that pediatric and adult Cardiologist know about the clinical presentation of a PDA, pathophysiology and control of the PDA (1).

The incidence of PDA in term neonates has been estimated 1 in 2000 births (4-5) that include 5-10% of all congenital heart disease although; by considering the asymptomatic PDA the estimated incidence will rise to 1 in 500 births(6). In most report the ratio of girls to boys is 2:1. (1)

Cause permanent stay open ductus arteriosus, at 24 to 48 hours of neonatal life is not well known. Clearly premature birth, increased incidence of PDA is mostly due to physiological factors related to prematurity rather than hereditary disorder of PDA (7). In term infants often it appears to be sporadic, but increasing evidence shows that genetic factors play an important role in many patients with PDA. In addition, other factors such as prenatal infection appear to play a role in some cases. (1). Persistent patent ductus arteriosus often cause hemodynamic and respiratory disorders, which require use of inotropic drugs and respiratory support in the full term infants. When medical therapy failed, surgical ligation should be considered for patients (2). The aim of study is to assess the effect of two

anesthesia techniques on reduction of pulmonary edema in PDA surgery.

2. Methods

120 children from 1 to 12 years old with PDA and pulmonary hypertensions participate in this study. The children were divided into two groups of 60; surgical ligation of PDA was performed in the operating room of Golestan Hospital. Following drugs used for induction of anesthesia in all patients:

- I. Midazolam: 0.1 mg/kg
- II. fentanyl 3-5µg/kg
- III. Sodium thiopental: 4-6 mg/kg
- IV. Atracurium 0.5 mg/kg

Then anesthesia was maintained with intermittent doses of fentanyl (0.5-1.5 µg/kg). During anesthesia monitoring of ABG, urinary Out put control and aggressive control of BP was performed for all patients. The first group as prophylaxy for pulmonary edema received morphine (0.1 mg / kg) and lasix (0.5 mg / kg) with regard to the hemodynamic after clamping off the ductus.

At the end of operation intubated patients transferred to ICU and specific parameters like Blood Pressure, ABG changes, Heart rate, intubation time and level of consciousness were recorded.

Then, the classic stages of extubation from IPPV to SIMV mode and afterwards Spontaneous mode with monitoring vital signs and Controlling ABG and heating patients were Wean until 24 hours after extubation (When the patient is in ICU), symptoms like dyspnea, pulmonary rales and reduce and control Out put together the results of both groups were compared.

3. Results

The average age of the patients in the case and control groups was 32.8 and 31.36 months. Frequency of Male and female in case and control groups was 38.3%, 61.7% and 45%, 55%, respectively. In the control group, 11 out of the 60 patients (18.3%) had pulmonary edema and 5% of them had loss of consciousness. Only 1 patient from those patients who had pulmonary edema required re-intubation and 5% of them were suffering from decreased level of consciousness. Significant differences between groups was found in terms of developing pulmonary edema after closure of PDA, ($P = 0.0001$). Also significant differences between groups in terms of respiratory rate ($p = 0.001$), arterial oxygen ($p = 0.005$), PH ($p = 0.01$) and heart rate ($P = 0.003$) was found. However, there was not significant different between the two groups in the mean arterial pressure ($P = 0.839$), mean pressure in ICU ($P = 0.096$) and after extubation ($P = 0.238$).

One case of death in patients with pulmonary edema was observed. Average transfer time from the ICU for case group was 24 hours after surgery and for control group was 36.5 hours which has significant different with case group ($p = 0.01$).

4. Discussion

During intrauterine life, 10% of cardiac output passes through the lungs and 90% shunt through the ductus arteriosus to the aorta and systemic circulation. After birth, most of right ventricle outflow should be able to pass through the lungs for gas exchange, thus ductus arteriosus should be closed right after the birth in full term infants.

In 80% of full term infants ductus arteriosus close within 48 hours after birth and in 100% of them close in 96 hours after birth. Failure of ductal closure causes special problems in infants are pre term (10, 8, and 9).

From hemodynamic point of view, significant shunt of PDA present in 40% of infants with weight less 1 kg and in 20% of infants with weight between 1-5/1 kg (10, 8, and 9).

Pre-term infant with respiratory distress syndrome requires surfactant and ventilation, usage of surfactant itself associated with an increased risk of PDA development. The indomethacin and ibuprofen are used for treatment of PDA (11, 12) PDA treatment has negative correlation with fetal age and birth weight (10, 8, and 9). Surgical indications include contraindications for medical therapy and failure of the second indomethacin course. Two forms of surgical therapy are performed for closure of PDA, the traditional surgical approach, Thoracotomy or Video-assisted thoracoscopic surgery (VATS) (13). Shunting blood form systemic to pulmonary circulation because congestive heart failure that associates with widened pulse pressure and bounding peripheral pulses. Overload of lung blood vessels, leading to pulmonary edema / hemorrhage and predispose the Infant to chronic lung disease. (10, 8, and 9).

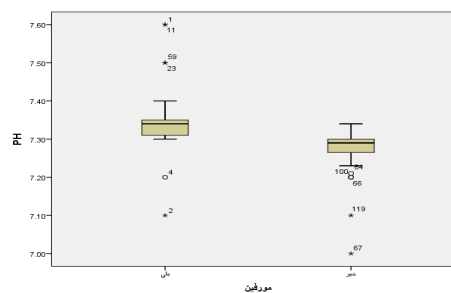


Figure 1: Comparison of PH and control groups

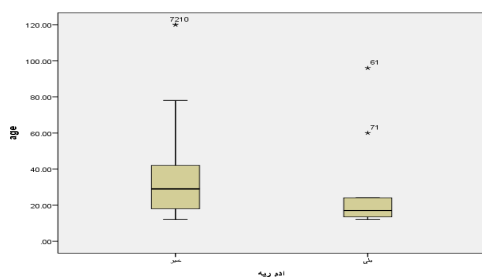


Figure 2: Average age of patients developing pulmonary edema

Although morphine has been used for years in treatment of acute pulmonary edema but its mechanism of this action are still largely unknown. Studies on animals show that morphine is significantly causes venous dilatation and that moves the blood from the central to the peripheral circulation 'medical phlebotomy' term describes this phenomenon. (14). There are several definitions on beneficial results of morphine administration in pulmonary edema:

1. *Morphine to calm anxiety and agitation by central venous pressure helps.*
2. *In respiratory distress, Intrathoracic negative pressure causes alveolar collapse and impaired blood vessel formation is oxygen, morphine eliminates this condition by maintaining normal breathing (15).*

Morphine effects on the peripheral venous system in patients with pulmonary edema may be quite different. These patients had increased venous capacity, which is caused by sympathetic nervous system activity, Shown that the vasodilatation caused by morphine in normal cases, secondary to reduced sympathetic nervous system activity, probably is at the level of central system.(14)

Lasix (furosemide) is a loop diuretic that lower blood pressure (16). In acute pulmonary edema it is given as single dose of intravenous or intramuscular (1mg/kg). If the diuretic response to the initial dose is not satisfactory and edema resist, dosage may be increased by 1 mg/kg (18, 17).

In 2008 a study was conducted by Andrew and colleagues, in their study 23patients With PDA participated, divided into two groups, underwent surgical PDA ligation without any mortality (19). But in our study we had one case death and it was due to pulmonary edema.

Janvier and colleagues in 2010 studied the anesthesia techniques and postoperative outcomes in full term

infants. These infants had PDA ligation surgery. This study was conducted at the University of Montreal, Canada.

All infants underwent surgery within 21 months period and they were studied retrospectively, altogether 33 infants were examined. Average weights of infants were 0.29 ± 1.031 kg. All infants, except one, during the operation, had received the opioid. Unstable breathing period after surgery and postoperative hypotension were the variables that were studied in all infants. The study found that 8 patients after surgery have shown unstable postoperative respiratory course (UPRC). Average fentanyl dose in patients with UPRC, 2.6 ± 5.3 $\mu\text{g} / \text{kg}$ and in patients without UPRC, 16.6 ± 22.6 $\mu\text{g} / \text{kg}$, respectively. This study reveals that proper dose of opioid associated with diuretic has significant effect in reduction of pulmonary edema.

Afzali and colleagues in 2009 studied the patients with patent ductus arteriosus in Golestan Hospital, Ahwaz University of Medical Sciences. In Golestan Hospital in Ahwaz 107surgical PDA ligation performed from January 1995 to January 1999, from 107 patients 70% were female and 30% male the age range of 4 months to 38 years with a mean of 8.5 years (21). In our study female to male ratio was 2:1 (1) which is same compare to above however, the average age of patients in our study is 3.5 years less than average age above study.

In a study conducted in 1976 by Lewis and colleagues, the effect of morphine on the reduction of pulmonary edema was studied. Following injection of morphine, dyspnea of patients and their respiratory rates improved and decreased from 24.3 ± 2.5 to 17.2 per min (22). In our study group also those patients who had received morphine during surgery, compared to control group, had significantly lower respiratory rate per minute ($p = 0.01$).

5. Conclusion

Very few studies have been conducted on beneficial effect of morphine and lasix administration during PDA ligation surgery. To achieve more definitive results and gaining more solid evidence there is need of doing more research in this field. In this study the use of morphine and Lasix has been supported as part of treatment. This technique appears to be safe and effective in reduction of pulmonary edema after surgery. (23)

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Contact us:

JundiShapour University of Medical Sciences,
Golestan Hospital Cardiovascular Anesthesia,
Ahwaz, Khuzestan, IRAN,
Dr Ahmad Ebadi Telephone: 09161110099
Work Fax: 06113743017
E-mail: Ebadi1959@ajums.ac.ir

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Effects of acupressure on nausea and vomiting after gynecological laparoscopy surgery for infertility investigations

Mansoor Soltanzadeh, Associate Professor of Anesthesiology, Ahwaz Jundishapur University of Medical Golestan Hospital, Iran, Ahvaz, Fax:+98-611-3743017 [Tel:+98-611-3743050](tel:+98-611-3743050)

Kaveh Behaen: Assistant professor of anesthesiology, Ahvaz Jundishapur University of Medical Sciences, Imam Khomeini Hospital, Iran, Ahvaz, Tel: +98-611-2222114

Zahra Pourmehdi: Assistant professor of anesthesiology, Ahvaz Jundishapur University of Medical Sciences, Razi Hospital, Iran, Ahvaz, Tel: +98-611- 3335936

Abdolah Safarimohsenabadi: Anesthesia Resident, Ahwaz Jundishapur University of Medical Sciences, Imam Khomeini Hospital, Iran, Ahvaz, Tel: +98-611-2222114

Corresponding author:

Mansoor Soltanzadeh, Associate Professor of Anesthesiology, Ahwaz Jundishapur University of Medical Sciences, Golestan Hospital, Iran, Ahvaz, Golestan St.

Email: sultanman84@yahoo.com

Tel: +98611-3743050; Fax: +98611- 3743017

Abstract: Background: Laparoscopic gynecological procedures have nearly an 80% incidence of Postoperative nausea and vomiting. Acupressure is a non-invasive and non-pharmacological method of preventing nausea and vomiting. In this study we used of Korean hand acupressure method for treatment of postoperative nausea and vomiting. **Materials and Methods:** One hundred and twenty patients were randomized into three groups of 40 female for laparoscopic gynecological procedures base on type of interventions prior operation: Group I (control), Group II (acupressure) and Group III (metoclopramide). **Results:** The incidence of nausea within 24 hours after operations were 45%, 27.5% and 30% in control, acupressure and metoclopramide groups respectively which were significant between three groups with minimum incidence in acupressure group ($p=0.005$). **Conclusion:** Acupressure is a non-pharmacological method for PONV with no side effects and cost benefit advantages.

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Key Words: Acupressure, Nausea, Vomiting, Laparoscopy, Gynecology

1-Introduction:

One of the important problems after anesthesia and surgery is postoperative nausea and vomiting (PONV) [1,2]. It is frequent in patients undergoing general anesthesia and is a distressing experience for patients.

The serious complications may ensue, including tension on suture line, increased blood pressure and bleeding under skin flaps affecting quality of surgical outcome. The patients are at increased risk of pulmonary aspiration of vomitus as the airway reflexes are depressed postoperatively. PONV occurs in approximately 30% of all patients undergoing general anesthesia (3,4). The incidence of PONV is influenced by various patient related factors, type of surgery, anesthesia technique and postoperative factors such as pain, use of opioids for pain relief, dizziness and ambulation (5,6). The type of surgery is an important influence factor for PONV (7,8). Interestingly,

laparoscopic gynecological procedures have nearly an 80% incidence of PONV (9). Although the exact reasoning is unknown it has been suggested that in addition to the female gender risk factor that the increased intra-abdominal pressure used during laparoscopic procedures may be partly responsible for this dramatic increase in PONV (10).

Different kind of pharmacological and non-pharmacological management has been proposed for postoperative nausea and vomiting. Various pharmacological means are available to alleviate PONV, but may cause many side effects such as lethargy, restlessness, tachycardia, extrapyramidal effects, cardiac arrhythmias and dystonic reactions (11, 12, 13). Acupressure is a non-invasive type of acupuncture that has reported as a potential non-pharmacological method of preventing nausea and vomiting. In acupressure, manual stimulation is applied, unlike acupuncture where the skin is pierced with a needle. Studies have shown

that acupressure can decrease nausea caused by morning sickness (14), general anesthesia (15) and chemotherapy (16). But other studies of this technique have had unfavorable results (17, 18).

In contrast to Chinese acupuncture, Korean hand acupuncture is a new method that was first developed and described with the Korean physician T-W Yoo (19). However, a very limited number of studies on the efficacy of Korean hand acupuncture are available. The Korean hand acupuncture point K-K9 is located on the middle phalanx of the fourth finger on both hands. In this study we used of Korean hand acupressure instead of acupuncture for prevention of PONV as a non-pharmacological and noninvasive method for treatment of PONV.

2-Materials and Methods:

After obtaining approval from the institutional ethics committee of our university, and written informed consent, we conducted a prospective, randomized and double-blind study.

We studied ASA I-II female patients in the fertile ages, undergoing laparoscopy for infertility investigations and treatment in our hospital. Criteria for exclusion included obesity (BMI >35), prolong and severe manipulations during surgery, diabetes mellitus and a previous history of PONV. Patients with diabetes mellitus are predisposed to peripheral vascular disease and we therefore excluded because of the risk of blood flow impairment to the digits (site for placement of acupressure band). One hundred and twenty patients were randomized into three groups of 40 each using a table of random numbers: Group I (control), Group II (acupressure) and Group III (metoclopramide).

In the group II acupressure band (special acupressure seed with 2-mm diameter), and in the other two groups (I, III) a dummy band were placed on the middle phalanx of the fourth finger on both hands (toward palms), 15 min prior to induction of anesthesia and bands were held on the fingers for 24 hours. Group III patients received metoclopramide 10 mg I.V. just before induction. The patients in groups I, II received normal saline 1 mL I.V. just before induction of anesthesia to maintain blinding.

General anesthesia for all patients were induced with thiopental 5 mg/kg iv, atracurium 0.5 mg/kg iv, morphine 0.1 mg/kg iv, midazolam 0.01-0.02 mg/kg iv and were maintained with O₂ and N₂O. Residual neuromuscular block were antagonized in all patients

with neostigmine 2.5 mg and atropine 1.25 mg I.V. at the end of operations. All procedures were carried out with experienced surgeons.

The patients received petidine 0.3 mg/kg for pain relief and metoclopramide 0.15 mg/kg for PONV in the recovery room as required. The patients received analgesic and antiemetic for pain and PONV in the ward as required. Patients and nurses were informed that an antiemetic should be given in the presence of intolerable nausea or vomiting. Both patients and nurses were unaware of patient group allocation. The incidence of nausea and vomiting during the first 24 h was determined. The results were scored in a manner similar to that of Allen, Kitching and Nagle (20) as none, nausea, retching/vomiting. If a patient experienced both nausea and vomiting, they were recorded as having vomiting. To examine the severity of nausea and vomiting, nausea was classified as none, mild and moderate or severe. Vomiting and retching were not distinguished and severity was classified with the number of episodes within 24 hours: none, mild (1 episode), moderate (2 episodes) or severe (≥ 3 episodes).

The parametric data of the patients were compared using the student t-test for the continuous variables and the chi-square test for the categorical variables. A P-value < 0.05 was considered significant.

3-Results:

Patients were comparable in all three groups with regard to age, weight and duration of surgery (Fig 1, 2, 3).

The incidence of nausea within 24 hours after operations were 45%, 27.5% and 30% in control, acupressure and metoclopramide groups respectively which were significant between three groups with minimum incidence in acupressure group ($p=0.005$) (table 1).

The incidence of vomiting within 24 hours after operations were 37.5%, 20% and 27.5% in control, acupressure and metoclopramide groups respectively with minimum incidence in acupressure group ($p=0.219$) (table 2). The incidence of severity of vomiting at severe score (≥ 3 number of episodes) was in acupressure group zero and 10.5%, 2.5% in control and metoclopramide groups respectively (table 3).

No side effects or complications were observed due to the placement of acupressure finger bands.

Table 1: Incidence of nausea within 24 hours after operations

Groups	Numbers	Percentage
Control (n=40)	18	45%
Acupressure (n=40)	11	27.5%
Metoclopramide(n=40)	12	30%

Abbreviations: n Number P Value = 0.005

Table 2: The incidence of vomiting within 24 hours after operations

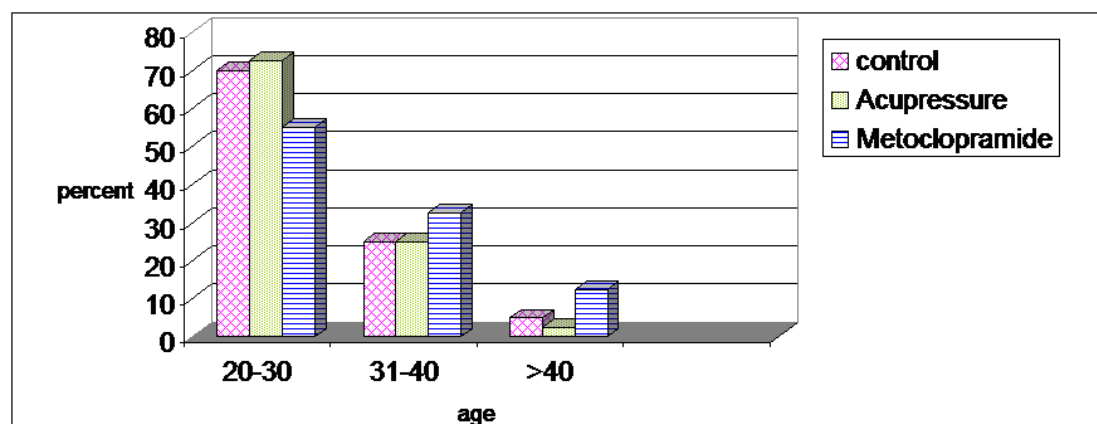
Groups	Numbers	Percentages
Control (n=40)	15	37.5%
Acupressure (n=40)	8	20%
Metoclopramide(n=40)	11	27.5%

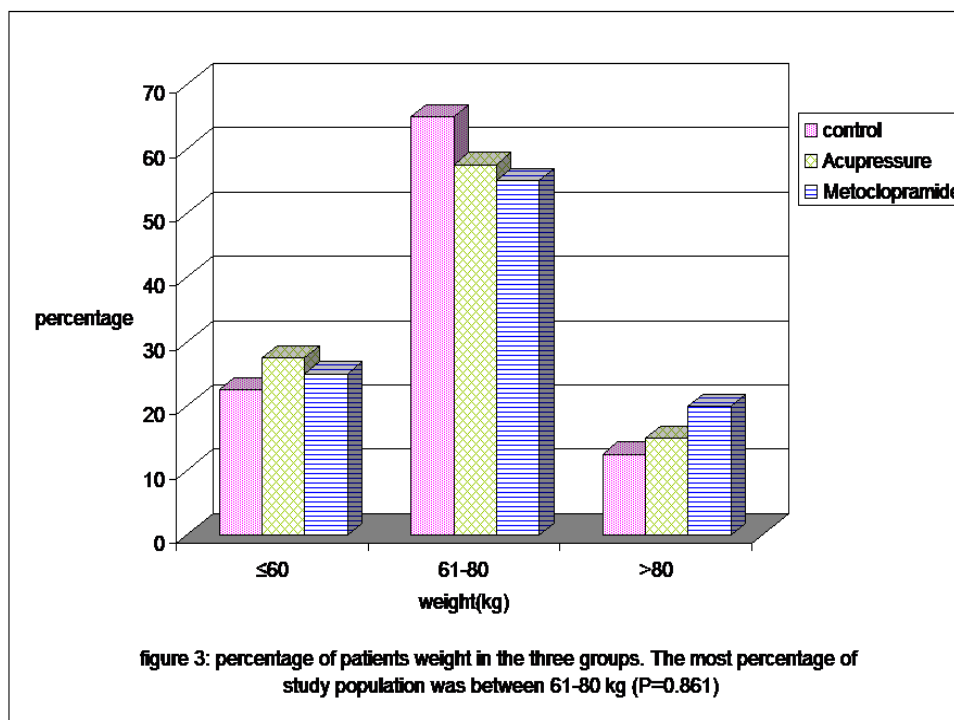
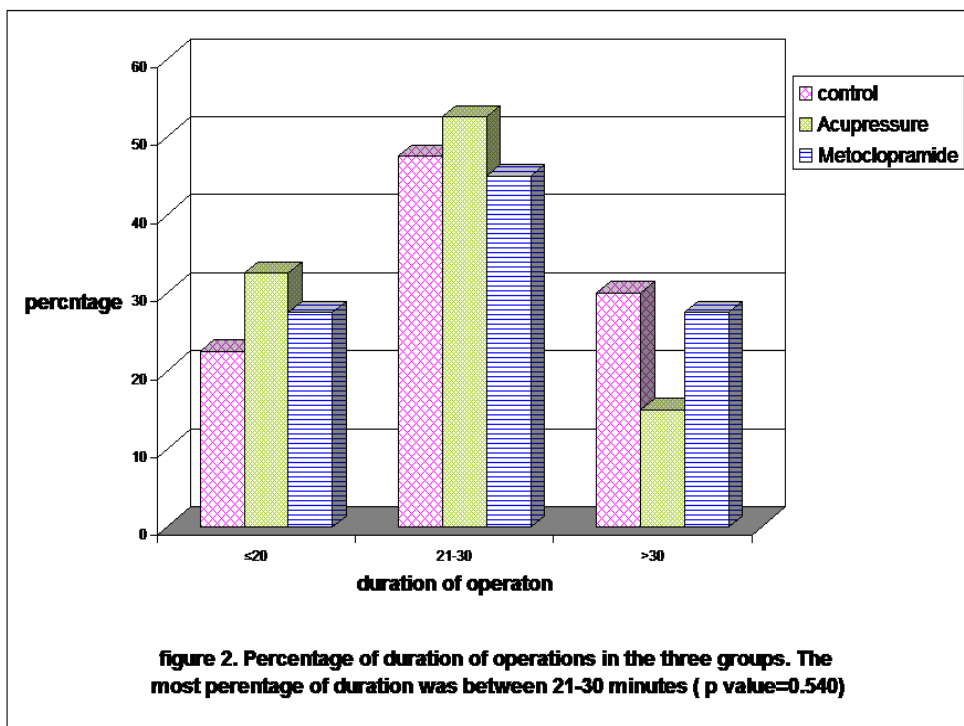
Abbreviations: n Number P Value = 0.219

Table 3: The incidence of severity of vomiting within 24 hours after operations

Severity	Control (n)	Acupressure (n)	Metoclopramide (n)
No	25 (62%)	32 (80%)	29 (72.5%)
Mild	6 (15%)	5 (12.5%)	9 (22.5%)
Moderate	5 (12.5%)	3 (7.5%)	1 (2.5%)
Severe	4 (10.5%)	0	1 (2.5%)

Abbreviations: n Number Outside the range of p value

**Figure 1:** Percentage of patients age in the three groups. The most percentage of study population was between 20-30 years old(P value-0.299)



4-Discussion:

PONV can delay recovery room discharge by 47-61 minutes (21). The time and resources required to treat PONV add to the institutional costs of the procedure. Acupressure is a non-pharmacological

method for PONV with no side effects and cost benefit advantages. The mechanism of action of acupressure is not clear. It is postulated that acupressure causes low frequency electrical stimulation of the skin sensory receptors in which may activate A β and A δ fibres.

These fibres synapse within the dorsal horn and may cause release of endorphins from the hypothalamus.

Increased levels of β -endorphin concentration have reported in human cerebrospinal fluid after acupuncture stimulation (22). In addition, serotonergic and norepinephrinergic fibres may activated and a possible change in serotonin levels has a role in prevention of PONV. Acupressure has shown to enhance gastric motility (23).

In our study in the acupressure group, incidence of nausea (27.5%) and vomiting (20%) within 24 hours after operations were lower than control and metoclopramide groups so acupressure significantly reduced nausea but vomiting was reduced to a lesser degree. Interestingly the incidence of severity of vomiting at severe score (≥ 3 number of episodes) was in acupressure group zero.

5-Conclusion:

Acupressure is a non-pharmacological method for PONV with no side effects and cost benefit advantages.

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7-Corresponding author:

Mansoor Soltanzadeh, Associate Professor
Anesthesiology, Ahwaz Jundishapur University of
Medical sciences, Golestan Hospital, Iran, Ahvaz,
Golestan St.

Email: sultanman84@yahoo.com

Tel: +98611-3743050; Fax: +98611- 3743017

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INPUT USE EFFICIENCY AND IRRIGATION IN AGRICULTURE: THE CASE OF IRAN

HADI GHAFARI

Assistant Professor, Department of Economics, Payame Noor University, Arak, Iran
P. O. Box 38135-1136, GhafariH@gmx.com

Abstract: The present paper attempts to study the resource use efficiency over a cross section of sample farms drawn from two development blocks (one from highly irrigated region and other from less irrigated region) in Arak district of Markazi province, Iran; with the purpose to find out whether the increase in irrigation facilities leads to increase the efficiency of other inputs in the crop production, identify the inputs which are not efficiently utilised in the production function and draw policy implications. Using a Cobb-Douglas type of function and computing marginal value productivity of each input, according to the findings of the regression model, irrigation and fertilizers & manure are efficiently used in both the blocks, while labor is efficiently utilized only in highly irrigated area and bullock labor, farm implements & machinery are inefficiently used in both the blocks. Comparative study of wheat and barley crops in both the blocks exhibits that wheat farming is profitable only in highly irrigated block while barley farming is profitable in both the blocks.

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Keywords: Efficiency, Input Efficiency, Irrigation, Agriculture.

1. Introduction

Like other productive enterprises, resources used in agriculture are also scarce and need to be used and managed in an efficient manner to enhance farm output and income (FAO, 2002; Seckler et al., 2003). Irrigation is one of the crucial inputs for obtaining higher growth in agriculture (Gowing, 2002). It is a deciding factor in augmenting the efficiency of other complementary inputs. This factor along with its complementary inputs increases the productivity of land with the existing inputs and also induces the other inputs for their increased use (IWMI, 2002a, b). Raising thereby, the level of production and employment still further. Various studies have been conducted on different aspects of input use efficiency in agriculture (Howell, 1994; Skewes & Meissner, 1998; Shannon & Raine, 1996; Cai et al., 2001). The studies of Saini (1969), Sharma and Rathi (1985) and Kaushik and Gangwar (1985) show higher input use efficiency in small farms while the findings of Singh (1973) divulge the higher input use efficiency in large farms. The study of Begi (1980) which shows the nexus between irrigation and resource use efficiency indicates higher input use efficiency on irrigated farms in comparison to unirrigated farms (Machibya, 2003; Molden et al., 2003). Dhawan (1983) and Thakur and Kumar (1984) found higher input use efficiency on private tube well irrigated farms as compared to other sources of irrigation (Lankford, 1998). The results of the study conducted by Gajja et al. (1994) indicate that as the land irrigability class and soil degradation level deteriorate, the marginal value productivity and allocative efficiency of all inputs decline. The study

of Das (1993) evinces higher resource use efficiency on farms having new dug well with pumpsets. The present paper attempts to study the resource use efficiency over a cross section of sample farms drawn from two development blocks (one from highly irrigated region and other from less irrigated region) in Arak district of Markazi province, Iran; with the purpose to find out whether the increase in irrigation facilities leads to increase the efficiency of other inputs in the crop production, identify the inputs which are not efficiently utilised in the production function and draw policy implications. The study is confined only to two main crops (wheat and barley) of the district.

2. Material and Methods

The present study is based on primary data collected through pre-tested questionnaire schedule for the agriculture year 2009-2010. On the basis of available irrigation facilities, the study area has been divided into two regions, viz. highly irrigated and less irrigated. The highly irrigated region which comprises 9 development blocks covers 81% of net sown area (NSA) of the district. The less irrigated region includes 2 development blocks and covers only 19% of NSA. The net irrigated area in highly irrigated and less irrigated regions was 93.14% and 42.65% respectively. Stratified random sampling technique of survey has been adopted to collect the primary data. At the first stage, Khondab block from highly irrigated region and Farahan block from less irrigated region are selected. In the second stage, five villages from each block are selected and at the third stage 150 operational holdings (75 holdings from each block) are selected. The operational holdings are

categorised as small (0-2 ha), medium (2-4 ha) and large (4 ha and above).

3. Results

Wheat and barley crops have been selected for the analysis of resource use efficiency mainly because these two crops are the common crops of both the blocks and they occupy a sizable area in the district. In highly irrigated block, i.e. Khondab block, wheat and barley occupy 55% and 23% of cropped area (GCA) respectively. While in less irrigated block, i.e. Farahan block, they cover 20% and 31% of GCA respectively. In highly irrigated block, the major sources of irrigation are canal, diesel operated tube wells (DOTW), electric operated tube wells (EOTW) and conjunctive (canal plus DOTW). The net irrigated area by all sources of irrigation on sample farms was 99.65%. The irrigated area under canal, EOTW, DOTW and conjunctive was found 18.38%, 18.49%, 26.95% and 36.19% respectively. Farahan block has spare irrigation facilities. This block has no canal irrigation. Minor irrigation is the only source of irrigation in the block. The net irrigated area on sample farms was observed only 18% of which DOTW and EOTW constitute 83% and 17% respectively.

The input elasticity's along with their standard errors for wheat and barley, marginal value productivities (MVPs) and geometric mean of gross income and all inputs have been worked out to know the input use efficiency. Cobb-Douglas type of function has been fitted to work out the inputs elasticities with respect to farm income. The choice of the function is based on its theoretical fitness to agriculture.

$$Y = A \cdot X_1^{b_1} \cdot X_2^{b_2} \cdot X_3^{b_3} \cdot X_4^{b_4} \cdot X_5^{b_5} \cdot X_6^{b_6} \cdot X_7^{b_7} \cdot U$$

By taking logarithms of both sides the above function is linearised as follows:

$$\log Y = \log A + b_1 \log X_1 + b_2 \log X_2 + b_3 \log X_3 + b_4 \log X_4 + b_5 \log X_5 + b_6 \log X_6 + b_7 \log X_7 + \log U$$

Where:

Y = Gross income (GI) per hectare / 1000 Rials

X₁ = Value of seeds per hectare / 1000 Rials

X₂ = Value of fertilizers and manure per hectare / 1000 Rials

X₃ = Standard hours of irrigation per hectare (one standard hour of irrigation = 10000 gallon of water)

X₄ = Labor absorption in man days / hectares

X₅ = Bullock labor days per hectare

X₆ = Per hectare cost of machines in 1000 Rials. It includes charges for owned as well as hired implements and machinery for the purpose of ploughing, threshing, transportation etc.

X₇ = Per hectare cost of rest of inputs in 1000 Rials.

In order to know the input use efficiency, marginal value productivity (MVP) of each input is computed. The ratio of MVP of an input to its cost must be either equal to one or more than one if the resource is to be utilized efficiently. MVP of X_i, the ith input, is worked out by the following equation:

$$MVP(X_i) = b \cdot Y / X_i$$

Where:

b is the elasticity of GI of ith resource

Y is Geometric mean of GI

X_i is Geometric mean of ith resource

1.3. Resource Productivity in Wheat

The value of regression coefficients, their standard errors and R² for different categories of farms in both the blocks is given in Table 1. In Khondab block, the values of R² reveal that 6% to 28% variations in GI per hectare from wheat are explained by the explanatory variables. The magnitudes of regression coefficients which have positive and significant impact on GI are: 0.207 for fertilizers & manures for small farms, 0.184 for fertilizers & manure and 0.329 for irrigation on medium farms; 0.296 for fertilizers & manures, 0.657 for human labor on large farms and 0.231 for fertilizers & manure, 0.115 for irrigation, 0.413 for human labor on over all categories of farms. The bullock labor on large and over all farms has not been efficiently utilized as its coefficients have significant negative values. The regression coefficients for all other inputs are not found statistically significant.

In Farahan block, 6% to 84% variations in GI are explained by the explanatory variables. The magnitudes of regression coefficients which have significant negative impact on GI are seeds, irrigation, human labor, bullock labor, farm implements & machinery and other cost on small farms. Except irrigation on medium and over all farms, all the inputs have either negative values or insignificant positive values. A close look on the table indicates that wheat is not found a profitable crop in less irrigated block. All the inputs used in wheat production show general inefficiencies. In highly irrigated block, fertilizers & manure, irrigation and human labor are the inputs which are found efficient to raise the GI from wheat while all other inputs are not judiciously applied.

2.3. Marginal Value Productivity in Wheat

The MVPs of the resources and their standard errors for different categories of farms of wheat are presented in Table 2. In Khondab block, the resources whose MVPs are significantly higher than their acquisition cost are: fertilizers & manure on all categories of farms, irrigation on medium and over all categories of farms, human labor on large and over all categories of farms. The MVPs of bullock labor are found negative and significant

which exhibit that reduction in the use of bullock labor in wheat would increase GI. The MVPs of all other inputs are not found significantly different from zero. A perusal of the table reveals that except fertilizers & manure, irrigation and human labor in highly irrigated block, rest of the inputs are not

efficiently utilized by the farmers in wheat. The bullock labor and farm implements & machinery are excessively used. Therefore, expenditure on these inputs must be curtailed to raise GI from wheat.

Table 1: Results of Regression analysis for Wheat

Size of farms	No. of farms	Value of Intercept	Regression Coefficients							
			Log A	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇
Khondab block										
Small	25	3.103* (0.970)	0.057 (0.140)	0.207*** (0.138)	-0.086 (0.242)	0.253 (0.363)	0.094 (0.154)	-0.069 (0.105)	0.60 (0.059)	0.06
Medium	25	2.175* (0.853)	0.051 (0.076)	0.184*** (0.129)	0.329* (0.163)	0.342 (0.286)	-0.026 (0.042)	0.027 (0.029)	0.013 (0.092)	0.21
Large	25	2.619* (0.835)	-0.138 (0.111)	0.296** (0.168)	-0.038 (0.277)	0.657** (0.297)	0.085*** (0.060)	-0.028 (0.057)	0.048 (0.078)	0.28
Overall	75	2.595* (0.415)	-0.004 (0.046)	0.231* (0.065)	0.115*** (0.084)	0.413* (0.130)	0.034*** (0.025)	-0.006 (0.018)	0.013 (0.029)	0.25
Farahan block										
Small	11	15.440* (3.132)	-1.097 (0.256)	-0.398 (0.321)	-0.598** (0.183)	1.608*** (0.264)	-1.126** (0.371)	-1.419* (0.361)	-.676** (0.284)	0.84
Medium	10	1.958 (2.850)	0.241 (0.246)	0.103 (0.234)	0.611** (0.207)	-0.519 (0.571)	0.499 (0.462)	-0.240 (0.365)	0.336 (0.240)	0.52
Large	15	5.843* (1.981)	-0.781 (0.823)	0.002 (0.336)	0.146 (0.130)	-0.007 (0.488)	0.454 (0.985)	0.362 (0.424)	-0.496 (0.429)	0.06
Overall	36	4.389 (0.894)	-0.075 (0.130)	0.112 (0.153)	0.184* (0.091)	-0.408 (0.259)	-0.003 (0.208)	-0.086 (0.102)	0.001 (0.134)	0.18

Figures in parentheses are standard errors of regression coefficients.

* Significant at 1 percent level of significance.

** Significant at 5 percent level of significance.

*** Significant at 10 percent level of significance.

In less irrigated block, wheat is not found profitable as MVPs of all inputs except irrigation on medium and overall size of farms are either negative or positive but insignificant. All the resources are severely under used in wheat in this block. The inadequate availability of water in this block might be the main reason of inefficient utilization of scarce resources. A comparative study of these blocks evinces that resources are relatively more efficiently used in wheat in the highly irrigated block.

3.3. Resource Productivity in Barley

In Khondab block, the values of R² show that 11% to 70% variations in GI on different categories of barley farms are explained by the combined effect of exogenous variables (Table 3). The magnitudes of regression coefficients which are found to have significant positive influence in explaining variability in GI are: 0.144 for irrigation, 0.366 for human labor, 0.071 for farm implements & machinery and 0.062 for other cost for small farms; 0.165 for irrigation and

0.228 for human labor for medium farms and 0.168 for fertilizers & manure and 0.107 for irrigation for large farms.

For over all categories of farms, the values of regression coefficients which turn out significant are: 0.133 for irrigation, 0.141 for human labor, 0.042 for farms implements & machinery and 0.056 for other cost. It is clear from the table that irrigation, human labor and farm implements & machinery turn out significant in causing variations in the level of GI. The values of regression coefficients which are found insignificant indicate that further increase of these variables would not make any beneficial effect on GI. In Farahan block, the values of R² indicate that 48% to 84% variations in the level of GI on different categories of barley farms are explained by the explanatory variables. The values of regression coefficients which are found to have significant positive effect on GI are: 0.456 for fertilizers & manure and 0.244 for irrigation for small farms, 0.415 for irrigation on medium farms and 0.225 for

fertilizers & manure; 0.449 for irrigation and 0.145 for bullock labor on large farms; 0.127 for fertilizers & manure, 0.303 for irrigation and 0.122 for other cost on overall size of farms. It is evident from the table that irrigation and other cost in both the blocks, fertilizers & manure in Farahan block, human labor and farms implements & machinery in Khondab block are found the main income generation variables for barley crop. Bullock labor, seeds and fertilizers & manure in Khondab block and seeds, farms

implements & machinery in Farahan block are not efficiently used. The reduction in the use of these inputs would not do any harm to GI.

On the basis of the comparative study of wheat and barley, it is concluded that wheat is profitable crop in highly irrigated block while barley is equally profitable in both the blocks. In general, the resources are better utilized in barley crop than wheat crop.

Table 2: Marginal Value Productivities for Wheat

Size of farms	Variables						
	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇
Khondab block							
Small	1.399 (3.437)	4.245* (2.830)	-16.083 (45.257)	34.060 (48.867)	43.618 (71.458)	-7.393 (11.251)	3.382 (3.325)
Medium	1.331 (1.983)	3.776* (2.647)	68.495** (33.935)	47.039 (39.337)	-13.800 (21.032)	1.795 (1.928)	0.732 (5.184)
Large	-3.897 (3.135)	5.762*** (3.264)	-6.748 (49.192)	104.76*** (47.357)	-97.278*** (68.667)	-0.585 (1.192)	2.549 (4.142)
Overall	-0.105 (1.208)	4.634** (1.304)	21.90* (16.003)	59.250** (18.650)	-21.865* (16.077)	-0.318 (0.954)	0.718 (1.602)
Farahan block							
Small	-12.790** (2.980)	-6.030 (4.870)	-95.360*** (29.180)	-49.500*** (21.490)	-332.300*** (109.490)	-30.390** (7.730)	-12.400*** (5.210)
Medium	3.900 (4.070)	1.380 (3.140)	112.230*** (38.020)	-42.020 (46.230)	164.960 (152.720)	-9.760 (14.840)	8.950 (6.390)
Large	-16.310 (17.190)	0.031 (5.190)	31.510 (28.060)	-0.660 (45.910)	207.730 (450.680)	12.170 (14.250)	-16.280 (14.080)
Overall	-1.220 (2.110)	1.660 (2.260)	34.470** (17.050)	-35.170 (22.330)	1.090 (75.610)	2.650 (3.150)	0.030 (3.440)

Figures in parentheses are standard errors of regression coefficients.

* Significant at 1 percent level of significance.

** Significant at 5 percent level of significance.

Table 3: Results of Regression analysis for Barley

Size of farms	No. of farms	Value of Intercept	Regression Coefficients							R ²
			Log A	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	
Khondab block										
Small	25	3.239** (0.653)	-0.127 (0.154)	-0.051 (0.154)	0.144 (0.048)	0.144** (0.054)	0.366* (0.264)	0.027 (0.034)	0.062** (0.023)	0.70
Medium	25	2.932** (0.716)	0.129 (0.224)	-0.015 (0.079)	0.165*** (0.083)	0.228* (0.138)	-0.046 (0.058)	0.017 (0.050)	0.061 (0.059)	0.11
Large	25	3.435** (0.419)	0.080 (0.098)	0.168** (0.063)	0.107* (0.068)	-0.067 (0.212)	-0.047 (0.049)	-0.027 (0.054)	0.019 (0.035)	0.20
Overall	75	3.305** (0.262)	-0.001 (0.068)	0.022 (0.031)	0.133** (0.037)	0.141*** (0.075)	-0.014 (0.022)	0.042*** (0.020)	0.056** (0.018)	0.34
Farahan block										
Small	14	-2.201 (4.330)	0.951 (1.629)	0.456* (0.278)	0.244*** (0.093)	0.536 (0.423)	-0.328 (0.241)	0.411 (0.302)	0.048 (0.140)	0.84

Medium	12	0.881 (9.266)	-0.351 (0.521)	-0.039 (0.225)	0.415*** (0.124)	-1.317 (1.259)	0.088 (0.724)	-0.706 (2.676)	0.064 (0.224)	0.48
Large	15	5.483** (1.214)	-0.003 (0.419)	0.0225* (0.148)	0.449** (0.132)	-0.864* (0.513)	0.145* (0.071)	-0.313 (0.262)	-0.222 (0.221)	0.50
Overall	41	4.241 (0.614)	-0.423* (0.231)	0.127*** (0.069)	0.303** (0.051)	-0.233 (0.051)	0.027 (0.046)	0.029 (0.125)	0.122*** (0.067)	0.53

Figures in parentheses are standard errors of regression coefficients.

* Significant at 1 percent level of significance.

** Significant at 5 percent level of significance.

*** Significant at 10 percent level of significance.

Table 4: Marginal Value Productivities for Barley

Size of farms	Variables						
	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇
Khondab block							
Small	-4.211 (5.107)	-0.618 (0.581)	25.560** (9.584)	51.287 (36.994)	24.021 (30.248)	1.569 (0.707)	1.662** (0.616)
Medium	3.946 (6.852)	-0.171 (0.899)	28.525*** (14.349)	33.231* (20.114)	-43.240 (54.520)	0.277 (0.815)	1.852 (1.792)
Large	2.259 (2.676)	2.085 (0.782)	19.561* (12.431)	-11.640 (36.837)	-71.257 (74.289)	-0.349 (0.698)	0.431 (0.793)
Overall	-0.031 (2.081)	0.263 (0.371)	23.636** (6.575)	21.510*** (11.441)	-14.071 (23.812)	0.701*** (0.334)	1.480** (0.476)
Farahan block							
Small	24.310 (41.640)	5.950* (3.630)	42.400*** (16.160)	79.320 (62.600)	-241.870 (177.710)	5.060 (3.710)	1.000 (2.920)
Medium	-9.080 (13.480)	-0.460 (2.630)	70.240*** (20.990)	-179.220 (171.320)	-54.840 (451.200)	-8.980 (34.030)	1.700 (5.930)
Large	-0.090 (12.120)	2.590* (1.700)	69.570** (19.060)	-138.820* (44.250)	139.020* (44.250)	-8.020 (7.980)	-8.020 (7.980)
Overall	-11.350* (6.200)	1.530*** (0.830)	50.100** (8.430)	-34.670 (32.590)	20.860 (35.540)	0.390 (1.680)	3.340* (1.830)

Figures in parentheses are standard errors of regression coefficients.

* Significant at 1 percent level of significance.

** Significant at 5 percent level of significance.

*** Significant at 10 percent level of significance.

4.3. Marginal value Productivity in Barley

The MVPs of resources and their standard errors for different categories of farms are given in Table 4. In Khondab block, the estimated MVPs of factors of production which are significantly higher than their respective acquisition cost are Rials 25560, 1570 and 1660 for irrigation, farms implements & machinery and other cost respectively on small farms; Rials 28530 and 33230 for irrigation and human labor respectively on medium farms; Rials 2090 and 19560 for fertilizers & manure and irrigation respectively on large farms and Rials 23640, 21510 and 1480 for irrigation, human labor and other cost respectively on overall size of farms. The MVPs for seeds and bullock labor for all farms, fertilizers & manure on small, medium and overall size of farms and other cost, farm

implements & machinery on medium and large farms are not found higher than their respective acquisition cost. This indicates that further investment on these resources would not contribute to GI. In Farahan block, the MVPs of resources which are found significantly higher than their acquisition cost are: Rials 5950 for fertilizers & manure and Rials 42400 for irrigation on small barley farms; Rials 70240 for irrigation on medium farms; Rials 2590 for fertilizers & manure, Rials 69570 for irrigation, Rials 139020 for bullock labor on large farms and Rials 1530 for fertilizers & manure, Rials 50100 for irrigation and Rials 3340 for other cost on overall size of farms. The resources which have significant negative MVPs are seed on overall size of farms and human labor on large categories of farms. The expenditure on these

resources must be reduced to raise the level of GI. The MVPs of all other inputs are not found significantly different from zero. Therefore further increase in investment on these inputs in barley would not enhance GI. The comparative analysis of the MVPs of inputs indicates that MVPs for irrigation and fertilizers & manure are found encouraging in less irrigated block. This reveals that increase in irrigation and fertilizers would contribute a lot to barley growing farmers in less irrigated block. The negative MVPs for human labor in less irrigated block evince that human labor is disguisedly unemployed while the MVPs for human labor in highly irrigated block indicate the absence of unemployment of unskilled labor in the block.

4. Discussions

The study of the resource use efficiency in two blocks brings out the following conclusions:

- High MVPs of labor in highly irrigated area reveal that this factor is efficiently utilized in this block while in less irrigated block, MVPs of labor are either negative or positive but insignificant which reflect the acute problem of disguised unemployment in this block.
- Relatively in both the blocks, it is observed that irrigation and fertilizers & manure are efficiently used. Generally, bullock labor and farms implements & machinery are inefficiently used in highly irrigated block. In less irrigated block, besides, bullock labor, farm implements & machinery and human labor is also used inefficiently. It is suggested that expenditure on these resources may be reduced for their rational use.
- Comparative study of wheat and barley crops in both the blocks exhibits that wheat farming is profitable only in highly irrigated block while barley farming is profitable in both the blocks.

Above empirical evidences suggest certain policy implications. They could cover sum of the main resources used in the production of crops, but primarily attention needs to be given to human labor in less irrigated block as negative MVPs of this factor indicate the presence of under employment and disguised unemployment. To overcome this problem, concerted efforts will have to be made to generate extra employment opportunities in the rural sector itself. For this, crop activities should be integrated to complementary farm activities such as livestock, fishery, and horticulture etc. on the one hand and agro-based rural industries such as fruit, food and vegetable processing small scale industries on the other. Besides, ongoing rural development programs will have to orient towards generating employment

opportunities in non-farm sectors. Farm implements & machinery also requires serious attention. This factor is severely underutilized in both the blocks. Therefore, the expenditure on this input may be curtailed to increase farm income. The provision of subsidy on small tractors and power tillers may further aggravate situation. Irrigation and fertilizers have been efficiently utilized in most of the cases in both the blocks. However, to make the better utilization of scarce water resource in less irrigated block, in ongoing water-development program, people's participation should be encouraged.

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Corresponding Author:

Dr. HADI GHAFARI

Assistant Professor, Department of Economics,
Payame Noor University, Arak, Iran

P. O. Box 38135-1136

E-mail: GhafariH@gmx.com

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Folk Elements and Signs in the Poems of Hafez

Fariba Raeisi

Department of Persian Literature and Language, Islamic Azad University, Marvdasht branch, Marvdasht, Iran.
Fraeisi@gmx.com

Abstract: Undoubtedly, there is direct relationship between literature and folklore by which can relate the history of this relation to the invention of alphabet. Through alphabet invention, human being could transfer their inner willingness, influence each other, teach their experience, record their custom and then present them skillfully and artistically to the next generation with literary form. So interaction of literature and folklore plays important role in independent of writers and poets' works. Regarding that, origin of the Persian literati's poems is social and cultural theme, also regarding Persian literature extension and independence, variety of social issues and discussions, can ascertain that literary valuable texts are kind of artistically recreation of truth especially social and cultural truth. So literary masterpieces especially Saadi, Hafez, Rumi are accepted for they are integrated with people and society. They have created such beautiful works by using folkloric genre by which their works have specific popularity among people and society. Custom and behavioral traditions and utterance of folkloric elements existence in the most of their sonnet is picture of society of that time. Research of folkloric element in Hafiz's poem, in addition understanding his role in independence of formal literature of 8th century, enable us to ascertain his influence on oral literature.

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1. Introduction

First time "folklore" term was introduced in 1885 by Ambrose Morton to Europe literary society. In Persian it means populace knowledge, common culture; etc. In fact folklore is a science which investigates all activity of person from birth to death.

Alan Dundes calls folklore as a myth, legend, proverb, invocation, curse, oath, vituperation, josh, repartee and even vitriol. He believes that most of non-literary written words such as wall posts, signs, words of tombstones, overstatements and even verbal endearment in dandling children is a part of folklore. (Dundes. 1995.p:3). With this totality, folklore not only posses history but even in myths has a tangible presence. Most of the aspects of popular culture is a memorial of our fathers' high culture which keeps its place in our memory until now. Although some of the aspects has been rejected scientifically and we called them superstitions, they have had a specific place in last time and in our ancient culture, so because today generation do not understand its origin and emergence, in their opinion it is worthless. Meanwhile the Western countries have shown a special care to collect aspects of folk and even assigned a special major in universities. C.S. Bern, folklore researcher, has included folklore subjects to three major categories and several sub-categories. These three categories are:

A) Beliefs, custom and habit concern with earth and sky, world of plants, animals world, human world, human artifact creature and objects, self spirit and

other world, superhuman beings, necromancy, miracles and munificences, magic and medicine.

B) Custom concern with political, social and economic institutions, ritual and rite of human life, jobs and career, calendar and celebrations, games and entertainment leisure.

C) Tales, songs and proverbs, stories (real and fun), chansons, conundrum. (bayhagi .1985.p:21)

All three categories mentioned, are reflected in Hafez poem and shown directly. Hafez beside enjoying people's oral traditions, uses official literature which makes his poetry be pleasant. He makes a pleasant link between people and literature through use of literary and popular values and innovative array and also sociology. From today's perspective, Hafez is a sociologist who could depict his society and put most Walks in visibility range of his works reader. Researchers investigate Hafez poem as an important part of social sense not a fancy subject. Cultures and subcultures invisible in Hafez poem have considerable reflection among people. Hafez scholars not only know him as a nice speaker, but as a sociologist who show the society of his time with realism. If we understand Hafez's sonnets depth, we will notice that in addition to social, political and mystical poetry, he has referred common traditions of the period which can discover valuable point by searching them in cultural aspect. Custom such as: wearing papery garment, standing under justice flag, to fold up tent, burning rope, breaking lute, turning on light, pouring the wine on the soil, washing out

paper, taking blood-price from slain, opening banquet-cloth of plunder, donating glorious vestment, filling mouth by gold,sacrificing,sending to hostage, ascending the throne of Norouzi King,etc.

Also through the view of culture can see the slang and ironic in his sonnets, such as to have wind in the hand (tobe proud), standing on the wall (to ignore people),boats,ignore,etc.

Almost all of folk elements are observable in Hafez poem. Except for the above; some of these elements can be divided into:

1) Myth: In Hafez poem myth is included several groups:

A) National myths: Jamshid, Siyawash, Rostam, Keykavous,Afrasiyab.

B) Religious myths: Solomon,Moses,Jesus,Adam, Noah, Khizar.

C) Historical myths:Alexander,Plato,Sheikh Snan

D) Love myths: Leili and Majnoon, Farhad and Shirin and Khosrow, Owrang and Golchehre, Vafa and Mehr, Joseph and Zlykha.

E) Mythical creatures: demon, fairy, antichrist, ascetic cat, desert giant, haroot and maroot, gryphon and phoenix.

2) Games: Glass game, Backgammon, Polo, Chess.

3) Medications: Rosewater and Sugar Syrup, Opium, Flowers Forums, Mandragora, Sulphate, Ruby fun, Kohl al-jvahr.

4) Diseases: Months of sickness, Peri sickness, Burner liver, the eye disease

5) Clothing: Cloak, Golden Cassock, Felt hat, Turban, Lakhlakhe,Woolen reed, Veiling(mask).

6) Tools: Tin cup, Carboy.

Proverb as a part of folklore has allocated a special part of Divan of Hafez to them.

Although Hafez's sonnets have examined by researcher in aspect of text, specially describe and explain the verses, aspect of version, poetic language and style, rhyme and literary array, there has not been a research to examine his poem only in folklore traditions and people culture. This article tries to answer to questions existing between literary traditions and folklore ,also tries to show the link between people, their customs ,official literature and Hafez's poetry

1.1. HAFEZ and People

Shams-al-din Mohammad Hafez Shiraz ,the famous poet in 8th century, is a man of genius in literary world whose poetry after passing centuries is transferring person to person and land to land. From Indian parrot , Kashmiry blacked eyes , Samarkand Turkish,Anatolian,Far and Near East till Egypt, China, Rome, Kharazm, Khudzhand and Rey ,all admire his pleasant nature. In addition to people in ground, the Saints recite his poems and Heaven fling

her clustered Pleiades upon his poetry .He has influenced not only Shiraz, Fars and past Iran ,but today's world by his pleasant poetry. Today, Hafez does not belong to particular geography. He belongs to any enlightened man who has restless heart .He is the poet of all places and times; he has swept literary geography and has became universal. German Goethe, Bosnian Soodee,Indian Tagore, Egyptian Mohammad Ali Pasha,Lahori Khatmi,Italian Danila Mancini, English Wilberforce Clarke, Turkish Abdoulbaki Gulpinarli and hundreds researchers, translators and scholars in the world adore Hafez's poem. His poem from Adam's time decorated flowers' notebook papers. Hafez (Divan) is a house having been decorated with sorts of literal and spiritual array encouraging inquirer to read and learn. Hafez's life located in ambiguities, is a part of folklore. Some scholars draw a picture of his life according to their guess or by virtue of his own poem. Also people make myths and stories of his private life because of their love to Hafez and his poem. This myths are abundant about poets and mystics such as Rumi,Shams,Sa'adi and Hafez. Meanwhile there is not such stories about mentioned persons' contemporaneous like Zakani, Bokharayi, Ohadi Esfahan etc. About Hafez, the most trivial events are important and interesting. On the assumption that the stories be pure legend, they are important for tell fame and glory of Hafez and prove in any time how people with different thoughts pay attention to him and make such myths. Hafez's life and his livelihood- divination,shakh-e-nabat, wiseman, children and totally all his spiritual and physical dimensions ,are attractive for people. Of folklore experts all these stories and myths considered and quoted stiltedly by people, are a part of the Iranian folklore. Not only Iranian, but Persian language countries like Tajikistan and Afghanistan admire Hafez and his poem. For instance, he has a privileged position among Former Soviet Union as far as they put Hafez (Divan) at the bedside of children to keep them from injury and harm and Hafez (Divan) is located among scripture. Most of Hafez's sonnets is sung with music and lyric by famous singer.Tajik people are accustomed to Hafez poem since fourth grade textbooks and recite most of them forever.

“Over hundreds of years, Hafez(Divan) like Rumi,sa'adi,Jami and Mirza Bidel was considered as an important curriculum book in Iranian, Afghanistan, Central Asia schools. Thesedays Hafez (Divan) is favorite book among families with Persian language. Hafez's divination is current among Persian-speaker in the world. People believe in result of his divination and use it in different cases. It plays important role in Persian literature and culture.

Collecting and teaching of popular beliefs through Hafez divination was very important. It can be valuable source for researches about Hafez and his connection to culture of people. (Rahim Ov, 2008, 65). Hafez's divination which draw people attention to itself, is the most important factor in link and connection between Hafez and people. People consider him as their confidant and have intimacy and affection with him. So he comes to their house and is their confidant, they tell him their secrets and expect him a proper response and sometimes despite disbelief, they receive pleasant response which recounts it for others. This divination whether or not will place in people memory and anecdotes about it will transfer chest to chest and generation to generation. Hafez's divination is printing as a published production with abundant circulation. For instance, in 2006 more than 200 divination books printed with different patterns, images and interpretations which show the people believed in Hafez.

2.1. Folklore in Hafez's Poem

Among dozens of cases identical with folklore theoretical foundations in Divan of Hafez, we express some most important elements and signs of folklore in Hafez poem. They are as following:

A) Myths: Knowing myth and mythological character is one of the indicators which plays important role in investigating folklore works. Myth always has been a source of creation in literary works especially in poetry and its world of mystery have stimulated most of the poets and writers' imagination to create poetry and stories based on myth. Hasan Anoushe, calls myth as a tale within Mythology "myth means the congruent system and set of old tales hereditary which were true in point of view of people at the time. Tales which explain why it is in this way, based on the goals and activities of supernatural beings." (Anoushe.1997.P:91). He believes that the most important function of myth is discover and reveal the typical patterns of all customs and all man's meaningful activity: "if the hero be one with natural character not supernatural, this tale usually will be called a myth, also if the hero be supernatural and the story be non-mythological, it will be called folk tale." (Anoushe.1997.P:91). "Poets' treatment about myths deepens on their skill and imagination in addition to historically the political and social atmosphere and their living environment. Hint to myths whether Persian or not, varies in poets' imagination. The study of evolution Persian poetry has shown that poets' enjoyment about myths has had more thanks and these mysteries have increased to the exact boundary of human and divine meaning. Totally there is two kinds of myths among myths which poets intended :First, the lyric myths and the

other epic myths. These myths can be divided to two kinds: Semitic myths and Persian myths which have existed the period before the Islam and have Semitic character." (Shafiee Kadkani. 2006. P:241-242). Mythologists according to function of myths divided them to 5 categories: Ritual myth, Origin myth, Religion myth, Character myth and World myth. Among this function Character myth is more visible in Hafez poem. Character myth function is that the important activity of hero is covered by an aura of mystery. Hafez, like other poets, has used myths in allusion. A poet like Hafez knows that artistically use of myths and tales causes reader to make new link with his distant past culture and history and tie two periods to a moment, in addition to exact comprehension of meaning. In part of national myths Hafez paid attention to characters who were mentioned in Firdausi's Shah-Name. The existence of such characters in Hafez show that he was familiar with mythological, heroic and historic characters of Shah-Name, but there is no certain idea whether he has studied Shah-Name or not. The tales of Shah-Name are so famous among Persian language people that there is hundreds tales and myths for its character. People manipulate tales and make new tales from story of Hakim-E-Toos. Hafez may has heard this tales from people and has used Shah-Name mythological subjects about that time society environment in his poems. Although he mentions a famous character only in one verse, he calls him so artistically that make reader to study biography of mentioned character completely and demand reading or listening verbal or non-verbal tales about the character to discover the meaning of the verse.

"Frowsy is a storyteller and is careful in detail while Hafez is sonnet teller and briefs tales so renders the results briefly by a sentence or a verse. Ferdowsi recounts tales and Hafez mentions the result. For example, Bijan and Manije in Shah-Name has ten verses but Hafez brifes it only on one verse:

*When the King of Turkan decided to put me in the prison,
If the favored of Tahamtan does not help me, what should do?*

Or recounts Siyawah tale in such way:

*The King of the Turkan heard the speech of adversaries,
Of the oppression of Siyawash, his a great shame be."* (Rastegar Fasayi. 2009.p:142)

Skalmowski supposes that old kings whose name is in Hafez's poem suggests his attention to Ferdowsi's epics. However, some of these names hint

to his attention. In a verse of Hafez (Divan), he hints to metrical historian directly:

*The glory of Pashang's son, and his mythic sword,
Becometh the tale of each group in all
Kings' book*

As Skalmowski said, Hafez's multiple mentions to Iran before Islam in his poems is around in 1325-1390 cases which can be divided to three categories of several themes:

A) Zoroastrian Religion Theme.
B) Ancient History Theme. (Whether legendary and mythological or real history)
C) Subjects adopted from epic tales and their theme.
Hafez has used the name of old king ironical, rather than real historical events expression.

Function of name of Kawos, Parviz, Qobad, Bahram, Bahman in Hafez's poems is to show glory gone with the wind symbolically. For example:

*The up-lifted sky! Is it not the sieve blood-splattering,
Whose scattering, the head of Kasra and the crown of
Parviz is? (41/6)*

On the other hand, the old kings are occasionally prominent as an index and symbol of the highest manifestation of earthly glory to show them nothing in front of sweetheart.

*Hafez! utter not again the tale of pomp of Parviz,
Whose lip, the draft-drinker of the sweet Khosro of
mine is. (52/8)*

In Hafez's poem the symbolic value of hero like Fereydoon, Afrasiyab, Siyamak, Zo and Siyawash is that they are the manifestation of the break of world glory. Only there is name of Rostam that is used as a savior who is able to change the bitter destination miraculously:

*When the King of Turkan decided to put me in prison,
If the favor of Tahamtan does not help me, what
should do? (349/5)*

Also magical factors are considered as a part of symbolic functions of mythological character. For example, Jamshid and Salmon were so attractive for people, since they have had superhuman power and abundant wealth and tools like magic ring by which they dominated human being and demons also the existence of characters like demon and evil spirit. So in folklore, many myths and tales are told about their life. Hafez has attended to this matters but he follows another goal by reminding them. In Hafez poetry, like Prop's pattern, both hero (Jamshid and Solomon) counter false hero (demon and evil) through generosity and obtain magical factors, but none of them resist death. Variable factors in the tale of these characters are in the service of poet because of cultural and social changes and influence of new thought, for example, whenever Hafez mentions their character, after mention their power and glory

immediately reminds invalidity of their power and wealth.

*Isaid: "O throne of Jamshid! thy cup world-
displaying, where?
It said: "Alas! wakeful fortune slept." (81/6)*

*As naught, I take Sulaiman's seal-ring,
On which, sometimes, Ahriman's hand shall be. (160/2)*

*In thy hand is only wind, if thou place thy heart on
any thing:*

*In a meeting-place where to the wind, Soleiman's
throne goth. (100/4)*

Religious myths form another part of Divan of Hafez. Any character in Divan of Hafez has assigned a tale in Qur'an. Illiterate or low literate people who have studied in school (Maktab-Kane) and were less familiar with Qur'an, manipulated tales. Solomon's tale was very famous among people for its attractiveness. This tale has allocated many verses in Hafez poem. Tale of ant and Solomon, knowing birds' language, Solomon's throne and property, adventure of stealing his ring, queen of sheba tale, hoopoe and his dominance over wind, are most commonly use theme in tale and common among people and Hafez has mentioned them in his poem.

*When the path of dust-kissing of this door is not for
kings,*

*How, the favor of an answer to the salutation of ours
falleth? (114/4)*

*O bird of morning! prolong the melody of Dawood,
For from the quarter of the air, the Soleiman of the
rose hath come back. (174/2)*

*The pomp of being an Asaf, the wind-steed, and the
language of bird,*

*Went to the wind, and from them, the Khwaja obtained
no profit. (25/7)*

*He who doubt about Solomon's wisdom,
Fish and bird will laugh at his wisdom and
knowledge. (491/4)*

*Fix not a knot on the wind though, on thy object, it
favorably blow,*

*For the Suleiman this speech, as a proverb, the wind,
uttered. (88/7)*

*Have a mercy on Dervishes is not against the dignity,
Solomon, who was a great man, did ant a
favor. (278/6)*

*O heart! Glad tidings that the morning breeze hath
come back,
From the quarters of Saba the lap-wing of good news
hath come back.(174/1)*

*Thy small sweet mouth is perchance Soleiman`s seal;
For, the world beneath the seal- stone,the picture of
the seal of its ruby hath.(121/3)*

*Because of his ruby seal I boast Solomon`s stories,
When with such greatness I deal, I put Satan on
parole. (327/6)*

*Zuhra`s singing should bring to dancing the
Mahsiha.(8/4)*

*The single Masiha, is deserved;
For he makes balance with the sun.*

*If thou depart the world with integrity and pure heart
like Christ,*

*Thy brightness will give a new start to the sun, even
shining at its crest. (409/3)*

In Divan of Hafez other religious characters like Adam, Noah, Khazr and Moses have an special place which is beyond of this article. In Divan of Hafez mythological creature, historical character and lovers that are the source of many folklore tales, have obvious manifestation. As said previously these tales have been mingled with poetical imagination and lead mind of curious reader to follow the origin of the tale.

3.1. Proverbs

Proverbs are the sentences which imply great wisdom and pure thought and their simplicity and clarity causes to be acceptable and admirable for all people, so that later have became common and placed in folklore like Saer proverb. Ahmad Bahmanyar has called proverb as an old invention of human "Human has invented proverb before composes poem and calligraphy and has used them in conversation. (Bahmanyar .1982). Large part of researches of folklore experts is allocated to search about proverb to realize social history of a nation included: cultural, social, scientific, artistic and subsistence situation. Since, proverb frequently has entered to official literary through non-verbal literary, so the works of poets who have took inspiration from people thought and experience, are more pleasant and more immortal. The large part of Persian apophthegm includes proverbs that have a historical past and originated from literary texts and famous poetry. The influence of Hafez poetry on culture and language of Persian is somewhat that makes myths about his life and causes many verses of his poetry be common proverb which people cite by them in proper times. The apophthegm hidden in Hafez poetry so

influences on people that make fictitious tales and strange dignity for some of them. The proverbs in Hafez poetry are divided totally into several kinds:

A) Some of them is based on an custom, tale or historical event which narrative form of them is kept till poet period and Hafez has used the wisdom thoughts existed on them:

*The tale of claimants and the fancy of thy fellow-
worker;*

*Resemble the tale of the gold-stitcher and the mat-
weaver.(44/6)*

The tale of gold-stitcher and mat-weave is this:"A King invited all gold-stitcher to prepare a golden cloth, all mat-weaver came too,the King astonishingly asked them:"why do you come?"they resposed:"If you mean weaver,we are weaver too."(Qani.Hafez with Qani`s notes. P: 109). Known proverb "Live beyond one`s means" is among proverbs that hints an old custom and is used in poetry of poet who lived before Hafez. This proverb calls about a custom in past that any child who was going to school carried a Kulim (kelim) for himself and sat on it.If any of them stretched his leg from Kilim and encroach on others', was contested, it is why "Live beyond one`s means", means encroach on others` right. Mentioned proverb is used in poetry of Nezami, Abou Shakour and A`atar:

*Do not search more degree than thy dignity,
Do not stretch leg more than thy killim.(Nezami)*

*Do not out of deal thy place,
Do not stretch thy leg more than thy killim.(Abou
shakor Balkhi)*

*Go and do not search useless deal,
Do not out of plac over thy dignity. (A`tar)*

*And Hafez says too:
Hafez!Why would the beloved,blame thou;
Did thou stretched leg over thy killim?(494/6)*

*Hafez!It is not in our dignity to boast so much;
Why exceed more than ours? (377/8)*

B) Some proverbs were used by poets like Ferdowsi, Sa`adi, Molavi and Nezami before Hafez. But there is no doubt that speech subtlety of Hafez has led to his poetry be more known and people use them. There are common subjects in these proverbs:

*Men of God did and said the truth;
O`Jurisprudent!first advise yourself.(Sa`adi)*

*They teach people to forsake the world,
Whilst themselves accumulate property.(Sa`adi)*

O` Thou who teach people the science,

Do, whatever thou say to them.(Sa`adi)

*What thou advise me? First,
Remain loyal to what thou say.(Sanayi)*

*If thou do not act what say,
Thy advice will be lie and trick. (Naser khosro)*

*The admonishers who, in the prayer-arch and the
pulpit, grandeur make;
When into their chamber they go, that work of
another kind they make. (199/1)*

Ali Akbar Dehkoda has called "Practice what one
(you) preach" synonymous of above verses.

Another slangy proverb Hafez used is
"Fall/jump out of frying-pan into the fire." which is
used in Farsi.Fakhr-o-din Asa`ad Gorgani has
mentioned to it before Hafez:

*So he became disappointed from his door,
Getting tired of snare, in pit, fell.*

*From the pit of Thy chin, in the curl of Thy trees, my
heart clung:
Alas. Forth from the pit, it came; and into the snare,
fell.(111/7)*

Following verses are official literary forms of the
proverb:

*"A stitch in time saves nine."
Today, the flowery is in spring,
Do not pick flower tomorrow that is
useless.(Shahname)*

*O` Comrade;Sufi is Ibnu l-waqt,
It is not the rule of Way to say
"Tomorrow".(Masnavi)*

*I was young, when master told me,
Time is a prize should not be waste.(Sa`adi)*

*O` thou who are able to do,Do it!
Before be disable to do nothing.(Sa`adi)*

*Gain,gain,the time of happy heartedness;
For,in the shell,ever the jewel is not.(162/2)*

Another proverb saying:"Bury everybody in his own
grave. "is written in Hafez:

*If I be good, or If I be bad, Go thou: be thyself;
In the end everyone reaped that work that he sowed.
(80/2)*

Literary form of the proverb "Carry coals to
Newcastle", in this verse is following:

*Nobody talks of Egyptian sugar,
It surpasses all, in sweetness is great.(279/5)*

Egypt of old has been one of the main place of
sugarcane cultivation and Egyptian sugar is known
in Persian and Arab literature .Sa`adi has said:

*Any commodity comes from a mine,
Sugar from Egypt and Sa`adi from Shiraz.*

Before Sa`adi and Hafez, Ibn_e_Yamin had
composed:

*I sacrificed myself for thou, but;
Nobody carry cumin to Kerman.(Nobody carry coal
to Newcastle.)*

C) Another part of proverb including meaningful and
wise words which is written by Hafez ,has took place
in mind of people and has transformed into proverb
according to the proverb "What comes from heart,
goes to heart", and wisely thought on them. For
example, the second hemistich of following has such
reputation that was used in The Islamic Revolution as
a slogan and could be heard in songs and be used as
apophthegm:

*Solitude is not a place to speak opposite,
When The beast goes, the Fairy will come. (232/2)*

*Or:
Thou cannot take place of great men,
Unless thou prepare all basis of greatness.(483/4)*

The proverb:"Be wet behind the ear "is mentioned in
this verse:

*The milk was flowing from thy lips when I told:
This sugar is not what salt I am gathering. (75/2)*

Another proverb like this is following:

*The day of union of friends remember;
Those times, remember, remember.(103/1)*

*I am in shocked bound from arrogance of rival,
God! Lest a day that pauper be wealthy.(226/7)*

*There is no agreement to show you, on the stand of
nifties
O` wise man, speak wisely or be quiet. (286/8)*

*To him, whose last sleeping-place is with two
handfuls of earth?
Say"Thine what need to exalt the turrets to the
sky?"(9/8)*

*Whoever wisheth,say:"Come."Whoever
wisheth,say:"Speak":
In this court is neither arrogance nor
haughtiness;chamberlaine,or door-keeper, is
none.(71/7)*

*Be hold the perfection of love`s mystry,not sins
defect;*

*For, whoever skill-less is, glance at the defect
maketh. (188/2)*

*If I be good, or if I be bad. Go thou: be thyself:
In the end, every one reapeth that work that he
sowed.(80/2)*

*In the end, every one reapeth that work that he
sowed.(80/2)*

There are many verses which are transformed to proverb but to prevent of prorogation of speech, it is forgone mention. There is a proverb among people is used when they tell that all have heard certain topic and certain secret is disclosed .It says "He who is an aware, is khwaja Hafez Shirazi." "As said by Shirazi, it is not clear that the origin of this proverb belongs to whom, when mad where. It is understood that people believe that Hafez was so sunk in spiritual realms and course in the world that when news is spread and all have heard, he is excluded. As Mr.Qazvini has deduced that when people want to show the fame of a rumor, they attribute its unawareness to a famous person and because of Hafez's fame is more than others this proverb has become widespread."Moen.1990.Sweet Talk Hafez.p:696). To examine other proverb of Hafez and its role in folklore, leads to two results: The role of former poets in forming the proverb which has had independence in Hafez poetry and also Hafez has heard this subjects from people(Influenced by popular culture).Second, the role of Hafez in creation adage and also in folklore(Effectiveness in folklore).

4.1. Customs

Undoubtedly, Hafez's attention to custom of his land leads to connect people with his poetry. In new age understanding some forgotten customs which was performed by specific procedure, can reveal many ambiguous points of anthropology knowledge. Mention to old customs has had high frequency. It is worth mentioning that some of this customs is still common among people. Like harmful burning to prevent the evil eye or reading *Va_en Yakad*(A verse from the Quran)which is due to beliefs. Some of these customs is related to people with different level in Hafez age, like Sufis customs, the drunk, the joy and musical, physicians, etc. Now some of customs which has been implemented in Hafez era, is forgotten and belong to the age before Hafez that show the society of that time to its readers. Among dozens of cases identical with folklore theoretical foundation in Divan of Hafez, we have expressed some of common customs:

1.4.1. Ascending the throne of Nourozi King:

"It was a custom in Iran to ascend the throne an impermanent king in Nouroz for public recreation

and ridicule and after expire the celebration, he abdicated the throne. As if real king for general conformance apparently dethrone himself from reign and assigned kingdom and all its accessories, like absolute rule and obedience of government agents, to a false king who ruled for recreation, fun and ridicule, and issued warrants like depose, assign, incarcerate, etchant after a few day his lost the thorn and everything was like previous. So Norouzi King is an allegory of a king whose kingdom is not temporary."(Qazvini,Yadegar Magazine,No:3,P:70-72). In first chapter of *Jahangosha_ye Jovini* book,it is said about victory of Kharazm by Mongolian armies:"since there was countless person among the best to have recourse to him and prevent from conflict time by him,they chose Nourozi King."(Jovini, 1999:98) this custom was common in Iran and its effects have remained in parts of Iran until recently.

*I told thou this words secretly that come out like
flower from veil,
There is no more than few days on Nourozi King.
(456/7)*

2.4.1. Sending to Hostage:

Dr.Harvi has written about this custom "it was a custom in past to make the king sure from obedience of governors or enemies, they sent a person, who was very respectable, as a hostage to ensure the king that they will not attack to his territory suddenly."(Harvi.1999.p:405)

*So long as grief's army ruined not the heart's
country,
Words and odes, with melody and modulation, I send
thee.*

3.4.1. Filling mouth by gold:

It was a tradition in kings meetings to encourage person who tell outstanding and proper sentence or composed nice poem in eulogy of kings, his mouth was fill by gold.

*The basis of verse is lofty, Jahangir, tell:
The King to fulfill my mouth by gem.*

*Wearing papery garment and staying under justice
flag:*

Papery garment was made by paper which complainant was wearing and was going to meet the king, and then the king understood that he is complainant. This tradition was performing before Hafez era. As Khaqani said in following verses:

*I wear a papery garment from the oppression of my
beloved,
For, she withheld from me the paper and pen.
(Khaqani)*

*We wear papery garment to gain goal,
To change the face by sword of dawn.*

Ata`ar also has mentioned this custom:

*We are in the door of Righ(justice),
All we pour soil over head for petition
We all wear papery garment
To gain our master finally.*

Sirous Shamisa according to a tale of Siyasat Name, calls the papery garment in red color. (Shamisa.1999. Allusion Culture. Vol2. 929). "I have heard that a king was hard of hearing, and believed that men who restate, do not tell real will of complainant and he make a decision which not be right, so he ordered only complainant wear red garment to be identifiable. (1992. Siyasat Name. 13) Hafez in following verse tried to wash papery garment in bloody water, so we can be sure that its color was red.

*The peppery garment, we wash in bloody water. For,
the sky,
My guidance to the standard of justice, made not.*

Apparently, it may be that complainant can not to give their petition to the king, so they wrote its content on paper and wore and stay under a flag called justice flag in front of the king house to see them. (Harvy.1999.578)

Rumi also has mentioned to this tradition in Shams sonnets:

*Be quiet for, I am such pressing that I went under the
justice flag,
Put paper down, break pen, the cupbearer came.*

Rumi has written to a king in his letter: "It is hoped that come back glad from the forgiveness house where flag of justice is." (Written.126) It was done to show petition and implore. As another tradition like to daub head with mud or to shed straw on head was done in order to petition. Naser Khosrow has called rubbing mud to sheepskin as a sign of petition:

*If thou want to petition from justice,
So, must rub mud on sheepskin.*

Sirous Shamisa reminds that: "the rubbing mud was common till our time." (Shamisa.1999. Vol2.928).

Now in Iran, sometimes people wear a winding sheet and come to street to show their objection and outcry.

4.4.1. To open the banquet-cloth of plunder:

There was a custom among Turkish Sultans and princes that to open the banquet-cloth in parties and holidays and guests had to plunder what is edible in short time. In Qate`e Borhan has been explained about this custom: "Tray of plunder is one that is opened by benevolent men and invite all people,

because plunder means to steal large amount of property." Some scholars have called Plunder (Yaqma) a city where its people belonged to Turkish race. Aboufazi Mosafa believes that "plunder (Yaqma) something of" means that Turkan of city or tribe of Yqma in their attack to their neighbour countries whatever they found as a trophy, brought to their city or tribe Yaqma. Plunder was a habit which primitive tribes could not be indifferent to it." (Mosafa.1987.42)

Dr. Adolhossein Zarrinkob in his book, Rascal Alley, says about this custom: "Plunder tray has been a general banquet-cloth which mostly was opened by Sultans and Princes in holidays specially Eid Al-Qurban and the poor have plundered it. Such tradition has described in Baghdad for Passover, a large banquet-cloth was opened in length of three 300*7 measure plundered after Passover prayer. (Zarrinkob.1985.220)

Sa`adi also has mentioned to such custom in his poem:

*The hide of earth, His open banquet-cloth:
As such a free-for-all, enemies and friend are one.*

In Hafez poetry, he has mentioned this custom several times:

*Alas! These saucy dainty ones sweet of work, the
torment of the city,
Take patience from the heart even as the man of
Turkistan the tray of plunder. (3/3)*

*Come; for plunder of tray of fasting, the Turk of the
sky hath made:*

*Hint at the circulation of the cup, the new crescent
moon hath made.*

*The science and the eloquence that .in forty years, my
heart acquired;*

*I fear that, as plunder, that intoxicated narcissus
taketh.*

*If intention be Thine against our life, there is no need
of pretence:*

*When the chattels are Thine, of plunder, is what
need?*

5.4.1. Donating glorious vestment:

Glorious vestment or dress of honor is clothing which is donated by the king as a gift. "Donating glorious vestment for appreciation is an old custom among the East. According to Maqrizi the first person among Muslims who performed this custom, was Harun al-Rashid when donate this clothing to his minister, Jafar bin Yahya Barmaki. Honor dress is called "glorious vestment". Its prevalence of it went so far that sometimes the

prince undressed himself and donated it to a person who liked. (Dezi.1980:147)

*Whatever unfitness there is is by reason of our unfit,
formless form;
If notion a person's stature, thy dress of honor, short
is none.*

6.4.1. Burning lute /Breaking wineglass:

As it is obvious that Hafez has mentioned the custom of burning lute and breaking the wineglass to show leaving sins. When it was repent of carouse, the wineglass and musical instruments were broken. Following verse support above sentence:

*No sweet melody maketh Zohre.Perchance she hath
consumed her lute:*

*Intoxication,none desireth.To the wine-drinkers,
what hath happened?(169/8)*

*As the cup of my heart broke from the repentance
that I made,*

*My liver, like a wine flagon, without wine and the
tavern, consumed.(17/6)*

*Yesterday, the distraught Sufi who broke the cup and
the goblet;*

*Yester-night, by one draught of wine, wise and
learned became.(170/2)*

*The foundation of penitence that, firm as a rock,
appeared,*

How the crystal cup hath shattered it, behold! (25/2)

*Like aloe-wood, how long can one consume in the
torment of repentance?*

*Give wine. For life in the essence of raw madness
hath passed. (82/8)*

7.4.1. Taking the blood-price from the slain:

It mentions to a custom among Dervishes.This means that if there was a dispute between two dervishes from sect, led to murder one of them,it was slain's fault.For there was a belief that the slain has caused the dervish be considered as a murderer and sinner."(Hervi.1999:405)

*Darvish!Lament not of the sword of friends;
For this band taketh the blood-price for the slain.*

8.4.1. Pitching a tent by dervishes:

It was a tradition that dervishes pitch a tent in front of the rich` house till their need be met .but sometimes it happened that the rich sent his agents with wood and stick and asked them to cut the tent ,instead of meeting their needs. "(Hervi.1999:189)

*Perchance, with death's sword, I may up-pluck the
tent. If not;*

*Shunning the door of fortune is not the custom of
mine.(53/5)*

9.4.1. Washing out paper (To eliminate knowledge):

Usually Sofia after reach high degree of conduct, reject and became enemy of formal science and whatever learnt in school even some of them buried their books that were the symbol of formal science.In Asrar al-Tohid:"After that Sheikh Abo-Saeed turned to Conduct Science from Smelting Science, collected all his book and bury them in basement while was saying:"You was great guideline and leader, but it is impossible to pay attention to guide after reach goal."Another mystics like, Ahmed Ibn abi-Alhevari,Abubakr Varaq and Suhrawardi,had done too."At first, this matter was a personal matter, thereafter became the manifestation of fighting with science philosophy.Suhrawardi, author of Avaref al-Ma`aref,in Adale al-A`ayan mentioned Ibn al-Marestani who burnt all philosophic books as Khalifa ordered.Suhrawardi is proud about himself tp wash out ten volum of Avicenna book(Shefa book)."(Safiee Kadkani.1987.11)

*For, Saba washed flowers with water of favor,
Call me crooked heart if I look at papers.(347/7)*

*If, our fellow student, thou remain, wash white the
leaves;*

For, in the book, love's art is not.(162/6)

10.4.1. Pouring wine on the soil:

There is a custom in Farsi and Arab poetry, called pouring wine on the dead men grave originated from Greece. Such custom was common among ancient people who pour wine or oil or water and honey or milk or other drinks on the sculpture or tombs, before sacrifice in the honor of gods and dead. Mohammad Moeen ia quoted by Qazvini : "Pouring wine on the grave of a dear has been common among Iranian and Arab originated from ancient Greece and attributed to Bacchanal wine goddess.Since Grecian believe that the grapevine is Heavenly that bear by soil and render the most delicate extract of plant to humanbeings,so they pour wine on the soil to appreciate of His bounty.(Qazvini.1988:156) Mohammad Moeen believes that the influence of Grecian habit and thought on Iranian happened in two phases: the first, after victory of Alexander and in kingdom of Seleucids and Parthian, the second, in Abbasid caliphate when Greek books has translated to Arabic and transferred to Iran. The acceptance of this custom by Iranian is a sign of their magnanimity not sacrifice.Manoucheri has said:

*I pour a sip on the soil from wineglass,
For, scholar men pour a sip on the soil*

It will be unfairly, if there is no share for soil from tun.

The content of (pouring wine on the soil) was common among poets and writers. Dehkhoda in his book *Apophthegm* (Amsal va Hekam) has called this custom "the share of land from tun of chivalric".

Hafez says:

*If thou drink a wine, let a drop spill,
A sin that helps another, courageously fulfill.*

*The angel of love does not know what is it, O`cup bearer,
Ask a cup and pour a drop on Adam soil.*

It has been mentioned several times in *Masnavi*. Sometimes it has mentioned to spraying wine to the sky that show happiness and disdain the Heaven.

*Drink a goblet and spray a sip to the sky,
How more is sorrowful from the grief of fate.*

11.4.1. Drinking:

It has been a specific way among chivalry. First step to entrance among them, is drinking. Parto Alavi explains: "This act is equivalent with promise, obey. New chivalrous has to get up and pick up tun of wine, raise it and call the name of master and drink the tun all at once. They call the leader of chivalry as a master. They is expected to obey him. The word of drinking means to be student, devoted and ministrant. (Parto Alavi.112). In *Samak-e-Ayar* tale this custom is mentioned: "After abounding tun several times, he got up and take the tun of wine and said "It is for he whose name is known in chivalry and his name is *Samak_e_Ayar*, then drank the wine." (Parto Alavi). Hafez has said:

*O disciple of the tavern! give me the heavy reward:
The joy of Shaikh, that the cloister hath not. (127/6)*

That idol of young Christian, the wine-seller, well said;

"Enjoy the joy of that person`s face, that purity, hath." (123/8)

12.4.1. Handing wine around:

It was another custom in Hafez age, hand the wine around in which drinkers hand the cup of wine around. It has specific discipline. Hafez has mentioned it frequently:

O`Saki until when the delay is in passing around of goblet?

The passing must have sequence when time is with lovers. (276/7)

Or

Ho! O Saki, pass around and offer the bowl:

For love at first appeared easy, but difficulties have occurred. (1/1)

*At time`s banquet, enjoy one or two cups; and go:
Verily desire not perpetual union. (7/4)*

In this ceremony the drinkers hand wine cup around from right and deprecated to hand it around from left, methinks it was a custom of ignorance Arab or at least was common among them. (Shamisa. 1999.p:112)

13.4.1. Jewish drink:

Hossein Ali Heravi says about a custom in Jewish drinking party: "It was a custom in Jewish party, after drinking too much wine, they skirmished at late night and break all dishes and whatever was close at hand, as whoever came in the morning, found that there was Jewish drinking wine." (Heravi. vol2.p:1199)

Hafez has composed about this custom:

*Mood of Sheikh and Bencher and their Jewish drink,
I asked from old vintner in the morning. (285/3)*

It is no exaggeration to say that Hafez has recounted his humanity messages not only in his age but also during human future history.

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Corresponding Author:

Fariba Raeisi
Faculty Member of Islamic Azad
University, Marvdasht branch. Department of Persian
Literature and Language. Marvdasht. Iran.
Fraeisi@gmx.com

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CLIMATE CHANGE AND URBAN CHILDREN'S HEALTH: A CASE STUDY OF IBADAN SOUTH WEST LOCAL GOVERNMENT, NIGERIA

A.S. Oyekale* and Y. A. Adesanya**

*Department of Agricultural Economics and Extension, North-West University Mafikeng Campus, Mmabatho, 2735 South Africa.

**Department of Agricultural Economics, University of Ibadan, Ibadan, Nigeria.

Corresponding author's e-mail: asoyekale@yahoo.com

Abstract: Climate change and human health are intricately linked. The impact on children's health is particularly enormous because of the uniqueness of their bodies' physiology and dependence on adults for protection. This study determined the perception of women about the impact of climate change on health status of their children and analyse the factors influencing children's medical bills. Data were collected with the use of simple random sampling and analysis was done using descriptive and Tobit regression methods. Results show that 49.45% of the children normally fall ill once in at least 4 months while 31.87 were falling sick at least one time in 3 months. Skin infection is the common health problems among children during dry season, which catarrh was most reported (71.43%) during wet season. Tobit regression results show that age (-ve), household size (+ve), per capita expenditure on food (+ve), per capita other expenditure (-ve), falling sick often (+ve), wet season sickness (+ve), cough (+ve), catarrh (-ve) and malaria (+ve) significantly influenced children's medical expenses ($p < 0.10$). It was recommended that subsidy for children malaria treatment, provision of more mosquito nets and awareness creation on preparedness of households for adverse climatic situations.

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1. Introduction

Climate change is any significant change in measures of climate such as temperature, precipitation, or wind that last for an extended period of time {Intergovernmental Panel on Climate Change (IPCC), 2001}. Global temperature and the frequency of extreme weather events have been significantly altered by climate change. However, though overall variability of weather patterns cannot be denied, some regional projections have shown some variations in amount and timing of precipitation which will increase in some places and decrease in others (Bernstein *et al.* 2007).

Conservative environmental estimates of the impact of changes in climate indicate that they will result in numerous health effects on children. The nature and extent of these changes will be greatly affected by actions that are taken at the global level (Paediatrics, 2007). Available estimates reveal that 66.5 million children around the world were affected by disasters in the 1990s (Penrose and Takaki, 2006). However, in the future, the estimates are as high as 175 million children per year (Save the Children UK, 2007).

Children are always hardest hit by environmental hazards because of their physical, physiologic, and cognitive immaturity (Etzel and Balk, 2003). It had been

emphasized that increasing environmental hazards resulting from climate changes will disproportionately affect children's health (Shea, 2003). It had been submitted that spread of some infectious diseases categorized as vector-borne, food-borne, and water-borne diseases will increase due to climate change. Malaria and dengue fever stand out among the vector-borne illnesses that would be promoted by climate change (Sheffield and Landrigan, 2011).

Children represent a particularly vulnerable group that will suffer disproportionately from both direct and indirect adverse health effects of climate change. Paediatric health care professionals understand these threats, anticipate their effects on children's health and advocate for strong mitigation and adaptation strategies (Bunyavanich, 2003). Children are at increased risk of death and injury from adverse climatic events such as drought, flood and extreme heat because they depend totally on adults for protection. The World Health Organization (WHO) recently estimated that 34% of all childhood illness in the world (compared to 24% of all age illness) and 36% of deaths in children under age 14 are due to modifiable environmental factors. Because of physical, physiological and cognitive immaturity, children are more sensitive than adults to harm

from environmental hazards. Poor children in developing countries will particularly suffer disproportionately because of limited access to health care facilities and insurance (Sheffield and Landriga, 2011).

In many parts of Nigeria, the pattern of rainfall is changing with cases of flooding in many urban areas. Because of poor drainage system and inadequate urban housing development and planning, adverse climatic event in the form of flooding disproportionately affects urban children. However, heavy rainfall is highly correlated with outbreaks of waterborne diseases because of contamination of surface and ground water. About 68% of outbreaks of waterborne disease morbidity in Nigeria over the past 45 years were associated with rainfall above the 80th percentile (WHO, 2007). Infants are at higher risk for complications and hospitalizations from such infections.

Furthermore, extremely high temperature favours development of mosquitos, which are carriers of malaria causing vector, *Plasmodium falciparum*. Specifically, malaria causes 350-500 million morbidity annually, and more than one million deaths, mostly in young children. World Health Organization (WHO) (1997) also submitted that 5 million children die annually from diseases linked to air pollution. Compared with adults, children breathe more rapidly and more often play outdoors, leading to greater exposure to pollutants per unit mass. Epstein (2002) states that the potential child health impacts of severe weather include drowning, gastrointestinal disease, malnutrition, and psychological trauma.

The main objective of the study is to determine the impact of climate change on children's health. We specifically assess respondents' perception of climate change impact as a risk or challenge to children's health, identify prevalent health problems, estimate costs associated with children's health problems attributable to climate change and determine the implications of children's health problems on parental income. In the remaining paper, we have presented the materials and methods, results and discussions and recommendations.

2. Materials and Methods

Area of study

The study area is Ibadan South West Local government. Ibadan has a population of 6,617,720. Ibadan, city in south-western Nigeria, capital of Oyo State, located about 110 km (about 70 miles) northeast of Lagos. Ibadan is a major transit point between the

coast and areas to the north. Ibadan South West Local Government is located at Oluyole.

Sources and method of data collection

Primary data were obtained through questionnaires and personal interviews. The questionnaires were administered to parents. The designs of the questions were such that information relating to income of parents, pattern of expenditure on children's health, and other socioeconomic characteristics of the households were collected. Other information captured in the questionnaire include children health problem associated with different weather conditions such as wet and dry season. The sampling population include individual parents in various households across the local government area. The sampling unit is the household that were randomly sampled. A total of 100 households were sampled.

Analytical procedures

The data were analysed with descriptive methods such as frequency counts, percentages and mean. Tobit regression analysis was also carried out to determine the impact of climate change on children's medical expenses. Tobit was preferred because some households recorded zero (0) as children's medical expenses. If ordinary least square method is applied, the estimates will be biased and even inconsistent (Gujarati and Sabgeetha, 2007). Estimated model can be stated as:

$$Y_i = \alpha + \beta_i \sum_{i=1}^n X_i + v_i \quad \dots 1$$

Where α and β_i are the constant coefficient and slope coefficients of the variables. v_i is the error term. Y_i is the total household's monthly expenditure on children's health (₦). The independent variables (X_i) are age of household heads (years), household size, trading (dummy) (yes=1,0 otherwise), per capita food expenditure (₦), per capita education expenditure (₦), per capita other expenditure (₦), fall sick often (dummy) (yes=1,0 otherwise), wet season sickness (dummy) (yes=1,0 otherwise), temperature affects health (dummy) (yes=1,0 otherwise), skin rashes (dummy) (yes=1,0 otherwise), measles (dummy) (yes=1,0 otherwise), smallpox (dummy) (yes=1,0 otherwise), cough (dummy) (yes=1,0 otherwise), catarrh (dummy) (yes=1,0 otherwise), malaria (dummy) (yes=1,0 otherwise), number of children affected by climate change, protects children (dummy) (yes=1,0 otherwise), use first aid (dummy) (yes=1,0 otherwise) and access to free medication (dummy) (yes=1,0 otherwise).

3. Results

Socio-economic characteristics of the respondents

Table 1 shows that majority (42.86%) of the women were traders, while 27.47% were salary earners. Also, the largest percentage (51.65%) of the women was between 28 and 37 years. This is followed by women that were between 18 and 27 years that constitute 23%. Average age is 32.09 years with standard deviation of 6.70. The table shows that women with tertiary education had the highest percentage of 48.35%. This is followed by those with secondary education with 32.97%. Average years of education is 12.91, with standard deviation of 5.039. This implies that the respondents, being urban residents, were largely educated. Also, household sizes of between 2 to 5 had the highest percentage of 87.91%. This is followed by household size 6 to 9 which accounts for 10.99%. Average household size is 3.91 with a standard deviation of 1.396. The majority (80.22%) of the households were earning less than ₦30000. Average monthly income is ₦19979.65 with standard deviation of 18388.19.

Table 1: Distribution of women's socio-economic characteristics

Socio-economic variable	Frequency	%
Occupation		
Traders	39	42.86
Salary Earners	25	27.47
Self Employed	14	15.38
Professionals	5	5.49
Students	4	4.40
Farmer	1	1.10
Others	3	3.30
Age		
<18	5	5.49
18-27	21	23.08
28-37	47	51.65
38-47	16	17.58
>47	2	2.20
Education		
None	6	6.59
Primary	11	12.09
Secondary	30	32.97
Tertiary	44	48.35
Household size		
2-5	80	87.91
6-9	10	10.99
10-13	1	1.10
Monthly income (₦)		
<30000	73	80.22
3000<50000	13	14.29
50000<70000	3	3.30
>=70000	2	2.20

Source: Field survey, 2008

Perception about climate change and child morbidity

The study probed into the frequency of children morbidity, the season in which they fall sick and perception of mothers on contributions of climate change to children morbidity in the different seasons. Table 2 shows that 49.45% of the children were sick once in 4 months and above. Those who were sick once in 3 months were 31.87%. The table further shows that in dry season, 45.05% of the children fell ill as against 42.86% in wet season. Table 2 further shows the perception of mothers on the contribution of climate change to children's health problem. It indicates that 64.84% of the respondents believed that climate change contributed to their children's health problem, while 26.37% indicated otherwise.

Table 2: Children's sickness time, season and types

	Frequency	%
Morbidity frequency		
Monthly	12	13.19
Once in 3 months	29	31.87
Once in 4 months and above	45	49.45
No response	5	5.49
Seasonal morbidity		
Dry Season	41	45.05
Wet Season	39	42.86
No Response	11	12.09
Morbidity influenced by climate change		
Affected	59	64.84
Not Affected	24	26.37
No response	8	8.79
Common Sickness in dry season		
Skin Rashes	40	43.96
Measles	21	23.08
Small Pox	9	9.89
Common sickness in rainy season		
Catarrh	53	71.43
Cough	65	49.45
Malaria	45	12.09

Source: Field Survey, 2008

The table also indicates that skin rashes are common health problems among children during dry season. This is followed by measles and then smallpox that represent 23.08% and 9.89% respectively. However, during wet season, catarrh was most reported with 71.43%. This is followed by cough and malaria that were indicated by 49.45% and 12.09% of the respondents respectively.

Access to free medication

Table 3 shows that majority of the respondents did not have access to free medication. The table also reveals that cost of treatment does not reduce 64% of the respondent's eagerness to hospital treatment.

Table 3: Access to free medications and restrictions due to hospital bills

Access Availability	Frequency	%
<i>Access to free treatment</i>		
Available	22	24.18
Not Available	69	75.82
<i>Affected by cost</i>		
Affected	33	36.26
Not Affected	58	63.74

Source: Field Survey, 2008

Factors influencing households' medical bills on children

The results of Tobit regression are presented in table 4. The results indicate that the model produced a good fit for the data since the log likelihood function parameter is statistically significant ($p < 0.01$). The computed sigma is also statistically significant ($p < 0.01$). We will Focus only on the parameters that are statistically significant, which are age ($p < 0.10$), household size ($p < 0.01$), per capita expenditure on food ($p < 0.01$), per capita other expenditure ($p < 0.01$), falling sick often ($p < 0.10$), wet season sickness ($p < 0.05$), cough ($p < 0.05$), catarrh ($p < 0.05$) and malaria ($p < 0.05$).

The parameter of age is with negative sign (-160.61) showing that as the age of household heads increases by one year, their children's health expenses significantly decrease by ₦160.61 This is expected because the as the household heads grow older, they are expected to have fewer number of children and spend less on their medical expenses. The results also indicate that as household size increases by one person, medical expenses on children increases by ₦1295.86. This is also expected because increase in the size of household members may imply having more children in that household. Also, infection of one child by one ailment may imply that every other child or person in that household will be infected and require some medication. This will no doubt increase the expenses on medical care.

Also, as households' per capita expenditure on food increases by ₦1.00, their children's medical expenses significantly increase by ₦2.14. It should be noted that conventionally, food expenditure is a proxy of

income, which should be positively correlated with children's medical expenses because wealthier households are able to spend more on their children health. However, increasing the per capita amount of money spent on other households' needs ₦1.00 will lead to ₦2.12 reduction in children's medical bills. This is also expected because if there is the need to treat a child for illness, some other not so essential needs of the households can be forgone.

Households that indicated that their children were falling sick often have their medical bills on children being higher by ₦2420.89. This is expected because for every episode of illness, money has to be incurred. Households that indicated that their children were falling sick more during wet season also incurred medical bills that are higher by ₦2811.74 when compared with those without such experience. This may have resulted from the nature of prevalent sickness during that season, especially cough and malaria, which could sometimes require consultations with medical personnel and expensive drugs.

On the nature of sickness, households with children that were diagnosed to have cough spent significantly higher amount of money (₦2926.33) on children's medical bills. This shows that children's infection with cough requires huge medical bills. This may be as a result of the nature of treatment that is sometimes required. For instance, coughing among children may result from malaria infection and sometimes requiring some expensive antibiotics drugs depending on the nature of sickness. However, households with children that had catarrh spend an amount that is lower by ₦2908.90 on medical bills. This may have resulted from the fact that infection with catarrh requires less expensive drugs. In many instances, mere consultation with a chemist may be all that is required. Previous experience may also inform administration of the right treatments by the mothers. However, households with Children that were infected with malaria have their medical bill being higher by ₦2623.05. This is as a result of the nature of treatment that is required to treat malaria. Because of development of resistance by some malaria parasites to some drugs, malaria sometime requires a combination of two drugs for effective treatment. This always requires a lot of money per episode because in such a case, visiting a doctor is inevitable in order to check the child's case notes for proper prescriptions.

Table 4: Tobit regression results of factors determining children's medical expenditure

Variables	Coefficient	Standard Error	t-value	Probability
Age of household head	-160.61	83.37	-1.93	0.058
Household size	1295.86	443.22	2.92	0.005
Trading (dummy)	-463.74	1023.48	-0.45	0.652
Per Capita Food expenditure	2.14	0.31	6.80	0.000
Per Capita Education expenditure	-0.20	0.23	-0.88	0.380
Per Capita Other expenditure	-2.12	0.77	-2.74	0.008
Fall sick often (dummy)	2420.89	1412.32	1.71	0.091
Wet season sickness (dummy)	2811.74	1119.88	2.51	0.014
Temperature affects health	1378.24	1126.94	1.22	0.225
Skin rashes (dummy)	418.78	1131.05	0.37	0.712
Measles (dummy)	-1745.90	1402.46	-1.24	0.217
Smallpox (dummy)	-404.57	1760.82	-0.23	0.819
Cough (dummy)	2926.33	1120.35	2.61	0.011
Catarrh (dummy)	-2908.90	1317.04	-2.21	0.030
Malaria (dummy)	2623.05	1058.38	2.48	0.016
Number of children affected by climate change	-116.11	701.78	-0.17	0.869
Protect children (dummy)	2077.42	1536.69	1.35	0.181
Use first aid (dummy)	-1246.62	1623.02	-0.77	0.445
Access to free medication (dummy)	-388.53	1209.50	-0.32	0.749
Constant	-3342.17	2877.34	-1.16	0.249
Sigma	4302.60	332.87		

4. Recommendations

The study examined the impact of climate change on children's health in Ibadan southwest local government. Climate change threatens children's health, their welfare, and future. Findings from this study have pinpointed some vital policy issues. First, there is the need to educate the people on the implications of climate change on the health of their children. This is very important because some situations that children are exposed to which often deteriorate their health could be prevented. It was found that some households were scared of seeking medical treatments for their children because of the envisaged large amount. There is therefore the need to ensure functionality of the National health Insurance Scheme (NHIS) and expansion of its coverage to whoever wants to be part of it. This will start with awareness creation and ensuring adequacy of existing health facilities to deliver the needed services. There is also the need for government to provide subsidized treatments for diseases like malaria among children given the upward trend of its incidences as climate changes. This is going to provide some form of relieve to poor households that seek medical

care. There is also the need to ensure that mosquito nets are distributed to households in order to protect children from mosquito bites. This will reduce malaria morbidity among children and save their families some medical bills.

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Study of Syrphid fly in Ilam province and the first report of *Merodon hirtus* (Hurkmans, 1993) for Iranian fauna

Mostafa Bedoreh¹, Amir Ansari pour^{2*}

1. Agriculture faculty, Department of Plant protection, Ilam University, Ilam, Iran.

2. Agriculture faculty, Islamic Azad University, Khorramabad branch, Khorramabad, Iran.

Amir.ansari2010@gmail.com

Abstract: Syrphid fly in Ilam province fauna were studied during 2006 and 2007. In this study a total of 21 species belonging to 13 genus and 2 subfamilies were collected and identified. Samples were collected and identified by characteristics of their appearance and their genitalia using a valid key. The identified species were identified are as follows: *Chrysotoxum parmense* (Rondani, 1845), *Episyrphus balteatus* (De Geer, 1776), *Eristalinus aeneus* (Scopoli, 1763), *Eristalinus sepulchralis* (Linnaeus, 1758), *Eristalinus taeniops* (Wiedemann, 1818), *Eristalis arbustorum* (Linnaeus, 1758), *Eristalis tenax* (Linnaeus, 1758), *Eumerus ahmadi* (Barkalova & Gharaei, 2004), *Eupeodes corolla* (Fabricius, 1794), *Eupeodes nuba* (Wiedemann, 1830), *Ischiodon aegypticus* (Wiedemann, 1830), *Melanostoma melinum* (Linnaeus, 1758), *Merodon hirtus** (Hurkmans, 1993), *Paragus bicolor* (Fabricius, 1794), *Paragus compeditus* (Hull, 1949), *Scaeva albomaculata* (Macquart, 1842), *Scaeva dignota* (Rondani, 1857), *Sphaerophoria rueppelli* (Wiedemann, 1830), *Sphaerophoria turkmenica* (Bankowska, 1964), *Sphaerophoria scripta* (Linnaeus, 1758), *Syrirta pipiens* (Linnaeus, 1758). From the top species, *Merodon hirtus* (Hurkmans, 1993) new species in Iranian fauna were identified and confirmed by Dr. Ante Vujic from Serbia.

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1. Introduction

Diptera is the one of the largest orders of insects, Insects in this order are identified by the first pair of wings that are membranous and hind wings that are reduced and known as halteres, which are used for stability (Triplehorn & Johnson, 2004).

Syrphid fly is one of largest families of the Diptera order and there are 6000 species (Vockeroth, 1969), known as flower flies or hover flies that have been identified within the family. Adult insects of the Syrphid fly family are important pollinators that eat the pollen and sap of flowers. The larvae of this family have a varied diet and based on morphological and other features are divided into two subfamilies Syrphinae and Milesiinae (Vockeroth, 1969). Most larvae of the subfamily Syrphinae are predators of Aphididae, Coccidae and the larvae of some Coleoptera (Buprestidae, Chrysomelidae) Due to their large appetite and the high fertility rate of the female insects, they can be considered for biological pest control. While the subfamily Milesiinae includes species that are saprophagous, herbivorous and producing Myiasis (Vockeroth, 1969). Some Syrphid flies eat other insects such as Coccidae, Aleyrodidae and larvae of Coleoptera and Cicadellidae (Sommaggio, 1999).

Flowers are a favorite location for Syrphid flies. Different species of flower flies tend favor different species of flowering plants. Insects have mouthparts of various different structures, and as such are attracted to plants with a shape that best fits their physical characteristics (Gilbert, 1988). In this family, adult insects of each different species tend to be attracted to flowers of a specific height (Coe, 1953); some species tend toward short-stemmed flowers and others to tall ones. One way of locating a specific family of these insects is to search in places where specific plants favored by these insects grow. Some species feed on nectar and others on pollen and these species specific feeding habits provide a way to distinguish between the Syrphid fly and other families within the order of Diptera (Gilbert, 1988). Feeding on prey and pollen is essential for gametogenesis in syrphid flies (Gilbert, 1988). Syrphid flies, due to small size and short snout often feed on flowers with shallow corolla such as Apiaceae and Common Ivy (Coe, 1953). Flies of this family show a keen interest in red and yellow colored flowers (Coe, 1953). Fauna studies on Syrphid flies have been done in many different regions of the world. In 1758 Linnaeus for the first time placed all species within this family in the genus of *Musca*. Fabricius in 1775 placed this insect in the genus

Syrphus. In 1805, species of the genus were divided into two groups by Fabricius; the first group with plumose arista and the other group without plumose arista, this researcher called the first group *Syrphus* (it had previously had been named by *Volucella* by Geoffroy) and the second group were named *Scaeva*. In 1921 a review was published by Metcalf that stressed the importance of genitalia for observing difference among species. Genitalia of different species of *Syrphus* in the Palearctic were studied by Hippa in 1968 and 13 genera close to it were identified. In 1968 these genera were examined and the desired species were identified. Insects of this family were classified in to the following seven subfamilies by Verral in 1969; Cerinae, Microdontinae, Chrysotoxinae, Volucellinae, Eristalinae, Milesiinae and Syrphinae. In another classification, done by Vockeroth in 1969, the family was divided into two subfamily groups Milesiinae and Syrphinae. The most important subfamily identified was that of the flower fly Syrphinae, among which are the aphid-eating species (Coult, 1997), this subfamily in the Palearctic region has 5 tribes Paragini, Bacchini, Chrysotoxini, Melanostomini and Syrphini. So far about 6000 species in 180 genera of this family have been reported, of which 590 species from 120 genera are in the Palearctic region (Vockeroth & Thompson, 1981).

In Iran there have been numerous studies on the insect fauna of this family, Radjabi introduced species of the subfamily Syrphinae as predators that damage aphid populations in cold fruit trees (Radjabi, 1986). Farahbakhsh reported on *Metasyrphus corolla* from Tehran province and Markazi province (Madarrese et al., 1997). Golmohamad Zadeh khyaban *et al.*, reported 35 species of Syrphid fly in 23 genera from Nazlu area and surrounding villages in western Azarbaijan province and there were studies on the biology of the two species, *Eupeodes corollea* and *Episyrphus balteatus* (Golmohamad Zadeh khyaban *et al.*, 1998). The review of Syrphidae of Ahwaz by Doosti introduced two species *Scaeva dignota* and *Paragus azureus* for the first time in Iran. Lotfalizadeh and Gharali in a review of Marand Syrphids reported 20 species of the subfamily Syrphinae (Lotfalizadeh and Gharali, 2000). In a study of Syrphid fly in Gorgan fauna, Goldasteh *et al.*, 2002 reported *Chrysotoxum ceraerum* for the first time in Iran. Sadeghi, 2003 added *Paragus antioionetta*, *P. aegypticus* and *Scava selenitica* to the list of Iranian Syrphid. Considering that studies of fauna provide the foundation for further biological, demographic and ecological studies, this research aimed to investigate the Syrphid fly fauna in Ilam district and its suburbs, paving the

way for further research in the field of biological pest control in the province.

2. Material and method

Sampling Syrphid flies was done in February 2007 until May 2007 with regular daily and weekly samples taken. Standard nets were used with a 30 cm diameter metal ring and Malaise traps. A Malaise trap is tent-like construction with one side higher than the other. The function of this trap is consistent with the behavior of these insects, in that when they encounter an obstacle they fly upward, therefore on encountering one of these traps the fly will pass through it to the top. At the top of the trap used in this study was installed a plastic canister with a hole through which the flies entered. Under this plastic canister cyanide was placed in a glass container. When insects were at the highest point of the tent area, from the hole in the bottom of the trap they entered a plastic canister and then the glass of alcohol. A number of samples was collected by special steel needles and insects were kept as samples with an attached label containing information on the location of the collection site, the host and the date of collection. Samples collected with the Malaise trap were kept in a glass vessel containing 90 percent alcohol. Species of Syrphid fly were identified by their genitalia using the method cited below:

Dried samples were placed for 24 hours in a desiccator to soften. After softening the samples' male genitalia, that are located outside the body of the insect, were separated with a fine needle. Genitalia were boiled for a few minutes at 20-15 percent solution of potassium, once they had become transparent they were then rinsed in distilled water and placed in 75% alcohol or glycerin solution and then onto a single cavity slide for observation. Adult insects were placed on prepared slides for observation of their organs and then identified by existing valid keys to genus level and then in terms of species. Images of new species of Iranian fauna were sent to Dr. Ante Vujic of Serbia for approval.

3. Results and discussion:

Insect samples were taken from February 2007 until May 2007 to identify species of flower fly in Ilam province. A total of 21 species belonging to two subfamilies were collected, species that marked with symbol * are newly identified species for Iran fauna, they are as follows:

Chrysotoxum parmense (Rondani, 1845), *Episyrphus balteatus* (De Geer, 1776), *Eristalinus aeneus* (Scopoil, 1763), *Eristalinus sepulchralis* (Linnaeus, 1758), *Eristalinus taeniops* (Wiedemann, 1818), *Eristalis arbustorum* (Linnaeus, 1758), *Eristalis*

tenax (Linnaeus, 1758), *Eumerus ahmadi* (Barkalova & Gharaei, 2004), *Eupeodes corolla* (Fabricius, 1794), *Eupeodes nuba* (Wiedemann, 1830), *Ischiodon aegypticus* (Wiedemann, 1830), *Melanostoma melinum* (Linnaeus, 1758), *Merodon hirtus** (Hurkmans, 1993), *Paragus bicolor* (Fabricius, 1794), *Paragus compeditus* (Hull, 1949), *Scaeva albomaculata* (Macquart, 1842), *Scaeva dignota* (Rondani, 1857), *Sphaerophoria rueppelli* (Wiedemann, 1830), *Sphaerophoria turkmenica* (Bankowska, 1964), *Sphaerophoria scripta* (Linnaeus, 1758), *Syrirta pipiens* (Linnaeus, 1758).

***Episyrphus balteatus* De Geer, 1776:**

Flower flies with a length of 10 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 61♂, 57♀.

This species had also been reported from Savadkooh, Babol, Sari, Amol, Ghaemshahr, Joibar, Behshahr, Neka and noshhr (Ghahari *et al.*, 2008), Marand, Ardabil, Qazvin, Andimeshk, Gorgan, Gilan, Khorramabad, Hamedan and Kerman (Gilasian, 2007).

***Ischiodon aegypticus* Wiedemann, 1830**

Flower flies with a length of 8-9 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 68♂, 51♀.

This species had also been reported from urmia (Khiaban & Parchami Araghi, 2001).

***Eupeodes corolla* Fabricius, 1794**

Flower flies with a length of 8-10 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 62♂, 39♀.

This species had also been reported from Savadkooh, Babol, Sari, Amol, Babolsar, Joibar and Neka (Ghahari *et al.*, 2008), Ardabil, Golpayegan, Kalibar, Maku, Damavand, Varamin, Andimeshk, Dezful, Shahrood, Zabol, Eghlid, Kazeroon, Qom, Qasr-e Shirin, Gonbad, Gorgan and Khorramabad (Gilasian, 2007).

***Eupeodes nuba* Wiedemann, 1830**

Flower flies with a length of 8-10 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 48♂, 52♀.

This species had also been reported from Varamin, Dezful, Zabol and Eghlid (Gilasian, 2007).

***Scaeva albomaculata* Macquart, 1842**

Flower flies with a length of 10-16 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 49♂, 53♀.

This species had also been reported from Ghaemshahr (Ghahari *et al.*, 2008), Natanz, Damavand, Absard and Dizin (Gilasian, 2007).

***Scaeva dignota* Rondani, 1857**

Flower flies with a length of 10-13 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 37♂, 32♀.

This species had also been reported from Savadkooh, Babol, Sari, Amol, Babolsar, Ramsar and Behshahr (Ghahari *et al.*, 2008), Shiraz, Tonekabon, Ramsar (Gilasian, 2007).

***Sphaerophoria rueppelli* Wiedemann, 1830**

Flower flies with a length of 5-8 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 41♂, 32♀.

This species had also been reported from Shiraz and Shirgah (Gilasian, 2007).

***Sphaerophoria turkmenica* Bankowska, 1964**

Flower flies with a length of 8-9 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 75♂, 35♀.

This species had also been reported from Kalibar, Shiraz, Tonekabon and Ramsar (Gilasian, 2007).

***Sphaerophoria scripta* Linnaeus, 1758**

Flower flies with a length of 9-12 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 25♂, 11♀.

This species had also been reported from Rostam abad (Moetamedian *et al.*, 2004).

***Melanostoma melinum* Linnaeus, 1758**

Flower flies with a length of 7-8 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 36♂, 27♀.

This species had also been reported from Fars province (Gharali, 2004) and Khuzestan province (Rezaei *et al.*, 2006).

***Chrysotoxum parmense* Rondani, 1845**

Flower flies with a length of 7-8 mm, this species was recorded in the following locations:

Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 54♂, 32♀.

***Paragus compeditus* Hull, 1949**

Flower flies with a length of 4-5 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 37♂, 25♀.

This species had also been reported from Urmia (Khiaban & Parchami Araghi, 2001).

***Paragus bicolor* Eabricus, 1794**

Flower flies with a length of 5-6 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 37♂, 40♀.

This species had also been reported from Neka (Ghahari *et al.*, 2008), Urmia (Khiaban & Parchami Araghi, 2001), Keleyber (Khaghaninia *et al.*, 2011).

***Eristalis arbustorum* Linnaeus, 1758**

Flower flies with a length of 7-8 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 32♂, 41♀.

This species had also been reported from Amol, Behshahr, Noshahr, Noor, Chalus and Neka (Ghahari *et al.*, 2008), Keleyber (Khaghaninia *et al.*, 2011).

***Eristalis tenax* Linnaeus, 1758**

Flower flies with a length of 14-15 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 52♂, 37♀.

This species had also been reported from Sari, Joibar, Ramsar, noor and Amol (Ghahari *et al.*, 2008), Keleyber (Khaghaninia *et al.*, 2011).

***Eristalinus taeniops* Wiedemann, 1818**

Flower flies with a length of 12-15 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 48♂, 29♀.

This species had also been reported from Neyshabur (Sadeghi Namaghi & Hussein, 2009).

***Eristalinus aeneus* Scopioil, 1763**

Flower flies with a length of 10-12 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 34♂, 28♀.

This species had also been reported from Keleyber (Khaghaninia *et al.*, 2011), Neyshabur (Sadeghi Namaghi & Hussein, 2009).

***Eristalinus sepulchralis* Linnaeus, 1758**

Flower flies with a length of 7-11 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 50♂, 29♀.

This species had also been reported from Keleyber (Khaghaninia *et al.*, 2011).

***Syrirta pipiens* Linnaeus, 1758**

Flower flies with a length of 7-11 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 50♂, 29♀.

This species had also been reported from Keleyber (Khaghaninia *et al.*, 2011), Ghaemshahr, Babol, Noor, Amol and Sari (Ghahari *et al.*, 2008), Neyshabur (Sadeghi Namaghi & Hussein, 2009).

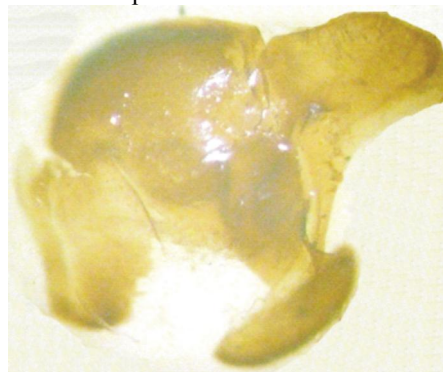
***Eumerus ahmadi* Barkalova & Gharaei, 2004**

Flower flies with a length of 7-11 mm, this species was recorded in the following locations: Eyvan, Sarabele, Dareh Shahr, Abdanan, Mehran, Dehloran. Numbers were reported as 55♂, 29♀.

***Merodon hirtus* Hurkmans, 1993**

This species was first reported from Iran, this hoverfly from Ilam (Dalab forest park) has been collected and reported. Records identified 10♂, 4♀.

The species has large eyes, clear and with long hairs. The legs are completely black with yellow spots, hind part of the second coxa is without hair, the trochanter is simple and without any appendage. The thorax is filled with orange hair and hair the front of the anepisternum is reduced. The second section of the abdomen is without the red and orange spots. The third section of the tergite has two narrow bands, the fourth segment has bands that appear at the front margins of the tergite. The posterior part of the male genitalia (surstyli) has two hairy lumps and two lumps without hair are found, the hypandrium is without any indentation at the base and at the end it has two shoulder plates.



Male genitalia in *Merodon hirtus* Hurkmans, 1993



Merodon hirtus Hurkmans, 1993 (Adult)

Corresponding Author

Amir Ansari pour
Department of agriculture
Islamic Azad University, Khorramabad branch, Iran
Email: Amir.ansari2010@gmail.com
Tel: +989166674902

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Quantitative Comparison of Blood and Blood Products Requirement between Two Groups with and without Auto-transfusion following Coronary Artery Bypass Grafting Surgery

- 1-Mansoor Soltanzadeh MD, Cardiac Anesthesia, Associate Professor of Anesthesiology, Ahwaz Jundishapur University of Medical sciences, Golestan Hospital, Iran, Ahvaz, Golestan St.
- 2-Ahmad Ebadi MD, Cardiac Anesthesia, Associate Professor of Anesthesiology, Ahwaz Jundishapur University of Medical sciences, Golestan Hospital, Iran, Ahvaz, Golestan St.
- 3-Seyed Kamaladin Tabatabaiee MD, Cardiac Anesthesia, Assistant Professor of Anesthesiology, Ahwaz Jundishapur University of Medical sciences, Golestan Hospital, Iran, Ahvaz, Golestan St.
- 4-Mehdi Dehghani Firoozabadi MD, Cardiac Anesthesia, Assistant Professor of Anesthesiology, Ahwaz Jundishapur University of Medical sciences, Golestan Hospital, Iran, Ahvaz, Golestan St.
- 5-Shahriar Mali MD, Cardiac Surgeon, Assistant Professor of Cardiac Surgery, Ahwaz Jundishapur University of Medical sciences, Golestan Hospital, Iran, Ahvaz, Golestan St.
- 6- Mohammad ali Sheikhi MD, Cardiac Surgeon, Assistant Professor of Cardiac Surgery, Ahwaz Jundishapur University of Medical sciences, Golestan Hospital, Iran, Ahvaz, Golestan St.
- 7-Farshid Ravanshadi MD, Cardiac Anesthesia Fellowship, Ahwaz Jundishapur University of Medical sciences, Golestan Hospital, Iran, Ahvaz, Golestan St.
- 8-Vahid Nezamabadi MD, Anesthesia Resident, Ahwaz Jundishapur University of Medical sciences, Golestan Hospital, Iran, Ahvaz, Golestan St.

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Ebadi1959@ajums.ac.ir

Abstract: The objective of this study was to assess the effectiveness of auto-transfusion in reducing need to blood products transfusion after open-heart surgery. **Design:** A randomized, controlled, double-blind, prospective study. **Setting:** An academic, tertiary and referral hospital. **Participants:** One hundred male patients scheduled for coronary artery bypass grafting surgery. **Interventions:** Patients divided in two equal groups. In group (A) Donated 500ml of patient's blood after induction of general anesthesia and saved at room temperature and transfused it to patient at the end of surgery. The control group (Group C) received the same anesthesia method and surgery without any transfusion. **Measurements and Main Results:** In the group (A) need to transfusion of packed cell, FFP and platelet significantly decreased in compare with group (C). **Conclusions:** Saving the patient's blood and auto-transfusion will improve hemostasis after CABG surgery.

[F.Sabeti, M.Soltanzadeh, Sh.Mali, N.Akbari Nassaji, T.Mahvar, A.Ebadi. Quantitative Comparison of blood and blood products requirement between two groups with and without auto-transfusion following coronary artery bypass grafting surgery. Life Sci J 2012;9(3):905-907] (ISSN:1097-8135). <http://www.lifesciencesite.com>. 129

Key words: Transfusion, CABG, Platelet, FF

1. Introduction

Despite improvement of surgery and cardiopulmonary methods in open-heart surgery, nonsurgical bleeding is still of serious complications after this kind of operation. Coagulation abnormalities that are created during cardiopulmonary bypass surgery is often multifactorial and because of a set of factors such as Fibrinolysis, Platelet dysfunction, inadequate reversal of heparin and Coagulopathy induced by blood exposure to artificial surfaces of bypass machine.(1,2)

In some studies the incidence of life threatening bleeding after open-heart surgeries are between 5-25%.(3) Moreover after adult open-heart surgery reopening for bleeding is required in 2-7 %

of patients, being associated with increased rates of morbidity and mortality(~10%) (4,5); Although in about 50-80% reopening causes there were no specific source of bleeding would be found(6).

The increasing in bleeding tendency during open-heart surgery will cause augment in blood and its products usage after surgery and subsequently increase side effects of transfusion such as transmission of infectious diseases, suppression of immune system and hemolytic or non-hemolytic reactions (7, 8).

In order to decrease in blood transfusion after open-heart surgery a great number of techniques such as auto-transfusion, hem dilution and anti-fibrinolytic drugs has been used. Auto-transfusion means taking patient's blood before surgery; replace

it by isotonic fluids and finally take it back to the patient at the end of operation (9, 10, 11, and 12). In order to clarifying auto-transfusion benefits and ambiguities, this study has been designed.

2. Methods

After approval by the institutional ethics committee of our university, the male patients candidate for coronary artery bypass grafting by cardiopulmonary bypass method (open-heart), enrolled in a period of six months for our study. Inclusion criteria were male gender, being elective surgery, being first open-heart surgery, age <70 years old, ejection fraction (E.F.) > 35% and body mass index (B.M.I.) <24.

In this study exclusion criteria were involving left main coronary artery stenosis, Hb (hemoglobin) <12 g/dL, Platelet<100,000 μ l, taking antiplatelet drugs like clopidogrel in last 9 days and pervious hemostatic disorders.

Patients randomly divided into two groups; Auto-transfusion group (Group A) underwent general anesthesia and before taking heparin, in sterile situation donated 500ml blood from central line by standard routine blood bag containing CPD(Citrate, Phosphate, Dextrose) and at the same time replaced it with ringer lactate solutions in proportion 3:1(ringer / blood) . The blood would save in the room temperature for preservation of platelet in the saved blood and take back to the patient after administrating Protamine sulfate at the end of operation. Control group (Group C) underwent the same induction of general anesthesia and the same surgery without donating blood and transfusion during surgery.

The surgeon and surgery team were the same and core body temperature during surgery was 30-32 centigrade.

The study data were expressed as mean \pm standard deviation for the quantitative variables percentages for the categorical variables. The parametric data of the patients were compared using the student t-test for the continuous variables and the chi-square test for the categorical variables. A P-value < 0.05 was considered significant.

3. Results

Each of the both groups contained 50 male patients. Mean volume of used packed cells in the group C (control group) was 1.48 \pm 1.07 liter and at group A (auto-transfusion group) 0.4 \pm 0.67 liter (table 1). Mean volume of used FFP at group C was 2.02 \pm 2.34 liter and at the group A 0.82 \pm 1.67 liter (table 2). Mean volume of used platelet at group C was 0.76 \pm 2.046 liter and at group A zero (table 3). Group A significantly taking less packed cell, FFP

and platelet in compare with group C. (P-Value < 0.05)

4. Discussion

In our study transfusion of packed cell, FFP and platelet to the patients decreased significantly in auto-transfusion group.

In Marberg and colleagues study, bleeding rate during 12 hours after open-heart surgery and need to blood products had no significant differences between two groups; which is different with our study. In Schmidtz and colleagues study, auto-transfusion decreased blood transfusion approximately 50%; which is significant and justifiable with our results.

Although in our study transfusion of all blood products reduced in the group (A) but the main point is reduction in platelet transfusion in this group; That it shows platelets may be more prone to damage during cardiopulmonary bypass. Each unit of fresh saved blood is equivalent about ten units of platelets; which it can effectively help to hemostasis. This research determined that auto-transfusion improves hemostatic performance and suggest that this method may be better for control of hemostasis during cardiopulmonary bypass surgery.

Table1. Descriptive statistics for received packed cells between two groups

Group	N	Mean	SD	T	df	P-value
Control	50	1.48	1.07	6.034	82.144	<0.001
Auto-transfusion	50	0.4	0.67			

Note: N=number, SD=standard deviation, T=t-test value, df =degrees of freedom, L=liter

Table2. Descriptive statistics for received fresh frozen plasma between two groups

Group	N	Mean	SD	T	df	P-value
Control	50	2.02	2.34	2.947	88.69	0.004
Auto-transfusion	50	0.82	1.67			

Table3. Descriptive statistics for received platelets between two groups

Group	N	Mean	SD	T	df	P-value
Control	50	0.76	2.046	2.627	49	0.011
Auto-transfusion	50	0.000	0.000			

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Corresponding Author:

Dr. Ahmad Ebadi MD: Cardiac Anesthesia, Associate Professor of Anesthesiology, Ahvaz Jundishapur University of Medical sciences, Golestan Hospital, Iran, Ahvaz,
Telephone: 09161110099, Work Fax: 06113743017
E-mail: Ebadi1959@ajums.ac.ir

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Effects of Preoperative Oral Gabapentin in Reduction of Intraocular Pressure and Cardiovascular Changes Following Laryngoscopy and Tracheal Intubation

Mansoor Soltanzadeh¹, Farhad Soltani², Ahmadrza Mohtadi², Mehdi Dehghani Firoozabadi², *Ahmad Ebadi¹, Siamak Taheri Tabatabaee³

¹ Associate Professor of Anesthesiology, Ahwaz Jundishapur University of Medical sciences, Golestan Hospital, Iran, Ahvaz.

² Assistant professor of anesthesiology, Ahwaz Jundishapur University of medical sciences, Imam Khomeini hospital, Iran, Ahvaz.

³ Anesthesia Resident, Ahwaz Jundishapur University of Medical Sciences, Imam Khomeini Hospital, Iran, Ahvaz. Ebadi1959@ajums.ac.ir

Abstract: Laryngoscopy and tracheal intubation are associated with hypertension, tachycardia and increased circulating catecholamines. They are also associated with increase in intraocular pressure. Various techniques have been studied to prevent increase intraocular pressure. Also there were used various techniques for attenuate the hemodynamic response to laryngoscopy and intubation. Gabapentin is a multimodal perioperative drug. We investigated whether the pre-treatment with gabapentin attenuates the intraocular pressure in addition to a hemodynamic response to tracheal intubation. **Methods:** One hundred patients, 15-50 years of age with ASA class I, II undergoing elective surgery with general anesthesia and endotracheal intubation were divided in two groups. Fifty patients received placebo and fifty patients received 900 mg (capsule) gabapentin two hours before surgery. **Results:** Intraocular pressure and heart rate in 1, 3, 5 and 10 minutes after laryngoscopy and intra-tracheal intubation in the gabapentin group were significantly lower than placebo group. In addition in our study mean arterial pressure in 1, 3, 5 minutes after laryngoscopy and tracheal intubation in the gabapentin group were significantly lower than placebo group. **Conclusion:** preoperative premedication with oral gabapentin is effective in attenuating the hemodynamic response and prevention of increase IOP to laryngoscopy and endotracheal intubation.

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Keywords: Gabapentin , Laryngoscopy , Tracheal intubation , Intraocular pressure , Blood pressure

1. Introduction

Laryngoscopy and tracheal intubation are associated with hypertension, tachycardia and increased circulating catecholamines (1, 2, and 3).

Hemodynamic changes are usually transient and without sequel. However, in patients with pre-existing coronary artery disease, hypertension or cerebrovascular disease, these changes may precipitate myocardial ischemia, arrhythmias, myocardial infarction and cerebral hemorrhage (4, 5).

Laryngoscopy and tracheal intubation is associated with increase in intraocular pressure (6).

Brief elevation of IOP is of little consequence in normal or even glaucomatous eyes, as long as the eyeball is intact. However, in a patient with an ocular laceration, perforation, or recent surgical ocular intervention, even a transient increase in IOP may be hazardous (7, 8)

Various techniques have been studied to prevent or attenuate the hemodynamic response to laryngoscopy and intubation, such as omitting cholinergic medications, deepening of anesthesia, pretreatment with nitroglycerine, beta-blockers, calcium channel blockers, gabapentin and opioids like

fentanyl and remifentanyl (9-16). Again various techniques have been studied to prevent increase in the intraocular pressure following laryngoscopy and tracheal intubation (17, 18)

Gabapentin, a structural analogue of γ -aminobutyric acid, is used as an anticonvulsant drug (19). Pretreatment with gabapentin can prevent the development of hyperalgesia (20). Also, gabapentin has a selective effect on the nociceptive process relating central sensitization (21).

Gabapentin is a multimodal perioperative drug. It has a favorable side effect profile and has less interaction with other drugs (22, 23). Gabapentin was shown to be effective in decreasing post-operative analgesic consumption and pain (24).

Memis and colleagues showed that oral administration of gabapentin 800 mg one hour before induction of anesthesia can attenuate the pressor response to the laryngoscopy and tracheal intubation (25). We investigated whether the pre-treatment with gabapentin attenuates the intraocular pressure in addition to a hemodynamic response to tracheal intubation.

2. Methods

After obtaining approval from the institutional ethics committee of our university, and written informed consent, we conducted a prospective, randomized and double-blind study. One hundred patients, 15-50 years of age with ASA class I, II undergoing elective surgery with general anesthesia and endotracheal intubation were divided in two groups. Fifty patients received placebo and fifty patients received 900 mg (capsule) gabapentin two hours before surgery. Exclusion criteria were: anticipated difficulty in intubation (Mallampatti Grade 3 and 4), more than one attempts to intubation, patients on chronic neuroleptic medications and taking tricyclic antidepressants or serotonin and norepinephrine reuptake inhibitors, patients with history of allergy to gabapentin, patients with hypertension, ischemic heart disease, severe renal or hepatic disease. Patients with amblyopia or history of eye surgery and hemodynamically unstable patients (heart rate >120/min, heart rate <50/min, systolic blood pressure <90 mmHg, systolic blood pressure > 140mmHg, diastolic blood pressure <50 mmHg, and diastolic blood pressure >100mmHg) were excluded of our study.

On arriving to the operating room and following insertion of intravenous catheter, all patients were infused with 5 ml/kg normal saline. Routine monitoring comprised, ECG, pulse oximetry, and non-invasive blood pressure. 2µg/kg Fentanyl and 0.05 mg/kg midazolam intravenous was administered before induction of anesthesia. Patients were pre-oxygenated for 3 minutes with oxygen 100% and anesthesia was induced with 5 mg/kg thiopental sodium and 0.5 mg/kg atracurium. Three minutes later, laryngoscopy using Macintosh blade size 3 and intubation using intratracheal tube (size 7.5-8) were performed by an anesthetist or by a two-year trained resident in anesthesiology.

Intraocular pressure (measure by Schiotz Tonometer), heart rate, systolic, diastolic and mean arterial blood pressure were recorded before induction of anesthesia, one minute before laryngoscopy, and 1, 3,5,10 min after intubation.

Data were expressed as mean (SD). Comparison between the groups was performed using the unpaired student's t-tests. Chi-squared test or Fisher's exact test, when proper, was utilized for analysis of categorical data. Statistical analyses were done with SPSS 17.0 package program for Windows. A p-value of less than 0.05 was considered statistically significant.

3. Results

In gabapentin group of the 50 patients, 10 patients were excluded from study due to cancellation

of operation (n=5), vertigo (n=2) and decreasing in systolic blood pressure lower than 90 mmHg (n=3).

Demographic variables in term of age and male: female ratio did not differ significantly between the two groups (table 1). The baseline hemodynamic variables (SBP, DBP, MAP and HR) and intraocular pressure were similar between two groups (table 2).

Table 1: distribution of gender and mean of age in the study population

P Value	Placebo n=50	Gaba n=40	Variable
0.28	Mean ±SD 27.6±7.5	Mean ±SD 29.3±6.6	Age(Y/O)
0.39	M:27 F:23	M:18 F:22	Gender

Note: Gaba: Gabapentin, n: number, M: Male, F: Female, Y/O: Years Old, SD: Standard Deviation

Hemodynamic variables (SBP, DBP, MAP and HR) and intraocular pressure one minute after laryngoscopy in the gabapentin group were lower than placebo group. There were significant difference for SBP, MAP and H.R. but there was not significant for DBP (table 2).

Hemodynamic variables (SBP, DBP, MAP and HR) and intraocular pressure three and five minutes after laryngoscopy in the gabapentin group were lower than placebo group. There were significant difference for DBP, MAP and H.R. but there was not significant for SBP (table 2).

Hemodynamic variables (SBP, DBP, MAP and HR) and intraocular pressure ten minutes after laryngoscopy in the gabapentin group were lower than placebo group. But there were only significant for H.R. and intraocular pressure (table 2).

4. Discussion

There are recent evidences that preoperative administration of oral gabapentin is efficacious for attenuation of hemodynamic response to laryngoscopy and intubation. In our study intraocular pressure and heart rate in 1, 3, 5 and 10 minutes after laryngoscopy and intra-tracheal intubation in the gabapentin group were significantly lower than placebo group. Patients with an ocular laceration, perforation, or recent surgical ocular intervention particular with co-existing ischemic heart disease may benefit of oral gabapentin 900mg two hours before operation as a premedication because control of heart rate and IOP are essential in these patients.

In addition in our study mean arterial pressure in 1, 3, 5 minutes after laryngoscopy and tracheal intubation in the gabapentin group were significantly lower than placebo group and patients with cerebrovascular disease and ischemic heart disease that are at risk of cerebral hemorrhage and myocardial

infarction respectively may benefit of preoperative oral gabapentin.

Table 2. Comparison of intraocular and hemodynamic variables after tracheal intubation.

P-Value	Group placebo Mean \pm SD	Group gaba Mean \pm SD	
			H.R.(beats/min)
0.01	94.24 \pm 12.98	86.73 \pm 6.276	1 Min
0.01	88.74 \pm 9.4	83.18 \pm 5.1	3 Min
0.03	85.1 \pm 8.3	80.5 \pm 5.2	5 Min
0.01	82.2 \pm 5.8	77.6 \pm 11.9	10 Min
			SBP (mmHg)
0.03	129.2 \pm 11.7	121.9 \pm 10.1	1 Min
0.09	124.2 \pm 10	118.7 \pm 9.1	3 Min
0.06	120.6 \pm 9.2	115.5 \pm 7.3	5 Min
0.7	114.6 \pm 16.3	113.6 \pm 6.6	10 Min
			DBP (mmHg)
0.06	80.68 \pm 10	76.7 \pm 10	1 Min
0.03	79.9 \pm 9.3	75.5 \pm 10	3 Min
0.04	78.7 \pm 10	74.5 \pm 9	5 Min
0.1	73.5 \pm 8.7	72.2 \pm 8.4	10 Min
			MBP(mmHg)
0.02	95.3 \pm 12.4	90.23 \pm 7.8	1 Min
0.05	93.4 \pm 9.1	88.05 \pm 8.1	3 Min
0.04	92.1 \pm 9.6	86.6 \pm 7.3	5 Min
0.08	87.2 \pm 7.9	84.4 \pm 7.1	10 Min
			IOP(mmHg)
0.001	15 \pm 2.6	11.4 \pm 1.1	1 Min
0.001	14.02 \pm 2.4	10.2 \pm 1.1	3 Min
0.001	13.05 \pm 2.1	9.9 \pm 0.6	5 Min
0.001	12.3 \pm 1.5	9.96 \pm 1.3	10 Min

Note: gaba =gabapentin, Min=minute, IOP=intraocular pressure, SBP=systolic blood pressure, DBP=diastolic blood pressure, MBP=mean blood pressure, HR=heart rate

A.Fassoulaki & colleague (26) found that oral gabapentin used as premedication attenuate the hemodynamic response to laryngoscopy & intubation. In their randomized placebo-controlled trial gabapentin-treated patients (1600 mg in four divided doses, at 6 h intervals starting the day before surgery) had significantly lower systolic ($p<0.004$) and diastolic arterial pressure ($p<0.004$) during the first 10 min after endotracheal intubation when compared with placebo. Nevertheless, gabapentin had no effect on heart rate changes that was differ with our results. In our study heart rate in 1, 3, 5 and 10 minutes after laryngoscopy and intra-tracheal intubation in the gabapentin group were significantly lower than placebo group.

Memis D and colleague (25) found that patients receiving 800 mg of gabapentin 1 h before surgery had significantly decreased mean arterial pressure and heart rate during the first 10 min after endotracheal intubation compared with either 400 mg gabapentin or placebo ($p<0.05$). Serhat Koc and colleague (27) also observed the same response. In

Usha Bafna and colleagues study(28), oral gabapentin 1000 mg given 1 h prior to operation resulted in significant decreases in MAP and HR during study period ($p<0.05$). In our study also MAP and heart rate after laryngoscopy and intra-tracheal intubation in the gabapentin group were significantly lower than placebo group. Results of these recent studies indicate preoperative premedication with oral gabapentin is effective in attenuating the hemodynamic response to laryngoscopy and endotracheal intubation. It acts by decreasing the synthesis of neurotransmitter glutamate and by binding to $\alpha 2\delta$ subunit of voltage dependent calcium channel (29). Action similar to calcium channel blockers may be responsible for blunting hemodynamic response to laryngoscopy and intubation (30).

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*Corresponding Author:

Dr. Ahmad Ebadi MD: Cardiac Anesthesia, Associate Professor of Anesthesiology, Ahvaz Jundishapur University of Medical sciences, Golestan Hospital, Iran, Ahvaz,
Telephone: 09161110099, Work Fax: 06113743017
E-mail: Ebadi1959@ajums.ac.ir

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Efficiency of Web-Based Education versus Counseling on Diabetic Patients' OutcomesFathia A. Mersal¹; Naglaa E. Mahday² and Nahed A. Mersal³

Departments of ¹Community Health Nursing and ²Medical Surgical Nursing, Faculty of Nursing, Ain Shams University, Egypt.
khomarkh@yahoo.com

Abstract: Background: Diabetes mellitus is one of the most common non-communicable diseases globally, and its related complications result in increasing disability, reduced life expectancy and enormous health costs. Diabetes management education is a critical element of care for all people with diabetes and it is necessary to improve patient outcomes through traditional diabetes patient education strategy and/or through internet based education that has the opportunity to expand the to the massive individuals with diabetes. **Objectives:** The aim of this study was to compare the efficiency of Web-Based education versus counseling on diabetic patients' outcomes including patients' diabetic knowledge, level of self-efficacy, self-care activities and blood glucose level. **The design** of this study was a quasi-experimental research design. **Setting:** The study was conducted at the outpatient clinic for diabetes in Ain Shams University hospitals, Cairo, Egypt. **The Subjects:** Purposive sample of patients were included in the study. Patients for this study were adult and diagnosed with type 2 diabetes. Patients divided randomly into two equal groups (45 patients each) to constitute the web-based group and counseling group. **Instruments:** Patient's assessment and clinical data sheet, Diabetes Management Self-efficacy Sale (DMSES), diabetic patients' knowledge questionnaire sheet and a Summary of Diabetes Self-Care Activities Scale (SDSCA) were used. **Results:** The majority of counseling and web-based group had unsatisfactory knowledge, low level of self efficacy, inadequate self care activities and abnormal glucose level with no statistically significant difference between them pre-intervention. While, post-intervention, The majority of counseling and web-based group had satisfactory knowledge, high level of self efficacy, adequate self care activities and normal glucose level with. Alao, Counseling group had more satisfactory knowledge, high level of self efficacy, adequate self care activities and normal blood glucose level than web-based group with no statistically significant difference in all items except for self care activities. **Conclusion:** It was concluded that, both of counseling and web-based diabetic patients' education improve patient outcome however counseling was more effective than web-based education strategy with no statistically significant difference between them in all items except for self care activities. This study recommended further research into the full use of the available technology is imperative for improving the quality of nursing intervention.

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Keywords: Diabetes, Self Care Management, Self Efficacy, Web-based education, Counseling, patient's outcome.

1. Introduction

Diabetes mellitus is one of the most common non-communicable diseases globally, and its related complications result in increasing disability, reduced life expectancy and enormous health costs for virtually every society (IDF, 2009). Egypt had been estimated to be the 9th country in the prevalence of diabetes. Recent changes in physical activity and dietary patterns have promoted the development of diabetes but if different preventive and control activities are not adopted by the year 2025 more than 9 million Egyptians (13% of the population above 20 years old) will have diabetes (Abdo and Mohamed, 2010).

People with diabetes can lead a full life, while keeping their diabetes under control. However, this illness requires a life-long management plan, and persons with diabetes have a central role in this plan. Diabetes management plan can be a very effective way to keep diabetes under control. Nevertheless they are

not always easy but they can be very effective for controlling diabetes. They can improve blood glucose control and prevent or slow the progression of long-term complications. In many cases, numerous small changes add up to surprising improvements in diabetes control, including a decreased need for medication (Evans and Pinzur, 2005).

Patients play a central role in diabetes care because of their daily responsibility for a large number of behavioral choices and activities (Heinrich *et al.*, 2010). Effective education is one component of chronic illness' broader management through traditional diabetes patient education strategy that change knowledge and attitude through information transfer and instruction and/or through internet based education that has the opportunity to expand the to the massive individuals with diabetes (Kennedy *et al.*, 2007 ; WU *et al.*, 2007).

Diabetes management education is a critical element of care for all people with diabetes and it is necessary to improve patient outcomes. It is important in promoting health practices and in reducing risks of complications (ADA, 2007 ;Funnell *et al.*, 2009). Diabetic management intervention has emerged as a resource to assist patients in managing daily diabetic care through dissemination of information and facilitation of diabetic management behaviors that has effect on diabetes related self efficacy (Channon *et al.*, 2003; Viner *et al.*, 2003). Education, counseling, skill building, and support through behavioral interventions offered by health care providers used to enable diabetic patients to manage their diabetes (Al-Khawaldeha *et al.*, 2012).

With the increased prevalence of diabetes, there is increasing need for diabetic management support that has the ability to reach large numbers of adults (IDF, 2009; Song, 2010). Traditional clinical approaches, such as counseling and group-based diabetic education programs have inadequate reach, and have not been sufficient to support long-term behavior changes, widespread use of the Internet provides an opportunity to expand the reach to massive individuals with diabetes (Khattab *et al.*, 2010).

Internet provides continuous support and tools for achieving necessary changes in multiple behaviors, such as healthful eating, regular physical activity, and managing medications (Glasgow *et al.*, 2012). The general public is beginning to seek medical information and support online. There are currently many Web sites providing general information on diabetes, its treatments, knowledge and skills of diabetic management (Leea *et al.*, 2007). Internet-based intervention programs for diabetes care are relatively new, but efforts are underway to test their efficacy in diabetic patients (Lorig *et al.*, 2010).

There have been limited researches comparing the use and effectiveness of Web-based interventions to non-Web-based interventions such as traditional face-to-face interactions and paper and pencil assessments. The introduction of the Internet into clinical practice as an information-sharing medium has brought about many opportunities for innovative interventions for individuals with chronic illnesses and their care providers. These interventions are often designed to address deficiencies in patient knowledge and chronic illness management skills. Improvements in these areas have been shown to lead to improve health outcomes (Wantland *et al.*, 2004).

Diabetes management education is a critical element of care for all people with diabetes and is necessary in order to improve patient outcomes (Funnell *et al.*, 2009). International Consensus Standards of Practice For Diabetes Education' focuses on the structure of diabetes education programs and the

only patient-centred outcome standards described are knowledge and clinical outcomes as glycemic control. However, in recent years diabetes education outcome measures are increasingly focusing on attitudes, self-care skills, lifestyle behaviours, psychological outcomes, quality of life and empowerment/self-efficacy and cost-effectiveness (Deakin *et al.*, 2005).

Patient education is one of the most important responsibilities of nurses. Educators emphasize on learning needs of the individual's (American Diabetes Association, 2007). Aiding patients in enhancing their diabetic-management requires consideration of their self-efficacy and motivation. Self-efficacy is a person's belief in his or her ability to overcome the difficulties inherent in a specific task, in a particular situation. Self efficacy influences the choices a person makes, the effort applied to a task and how long a person will persist when confronted with obstacles or failure (Wallace *et al.*, 2009; Al-Khawaldeha *et al.*, 2012).

Significance of the study:

Management of type 2 DM is challenging and often requires skillful integration of complex treatment regimen such as healthy diet, regular exercise, optimum weight control, self monitoring of blood glucose, and medication adjustment into the daily routine over long periods (Montague *et al.*, 2005). Diabetes educational strategies are of great importance because the adoption of healthy behaviors will produce optimum glycemic control for DM, which in turn will help minimize or prevent subsequent acute and long-term complications of the disease and they improve self efficacy and patients outcomes (Funnell *et al.*, 2004; Kennedy *et al.*, 2007; Al-Khawaldehaz *et al.*, 2012).

The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild *et al.*, 2004). An estimated 285 million people worldwide are affected by diabetes. With a further 7 million people developing diabetes each year, this number is expected to hit 438 million by 2030 (Canadian Diabetes Association, 2012).

In the Eastern Mediterranean Region, an estimated 22 million people have diabetes, out of a total adult population of 290 million. Studies conducted in different populations of the Region have reported high prevalence rates varying from 7% to 25% in the adult population. Approximately half of the countries have published incidence rates, the highest rates are reported in Egypt, Kuwait, Lebanon, Oman and Qatar. With current estimates indicating that the number of diabetes cases is set to double by 2025 (WHO, 2006).

Since 1980, the number of adults with diabetes worldwide has doubled. There will be an estimated 70% increase in the number of adults with diabetes in

the developing world and a 20% increased in the developed world between 2010 and 2030. (**Canadian Diabetes Association, 2012**).

Inadequate diabetic self-management remains a significant problem facing health care providers in all settings and populations. Based on the previous researches, it was noted that inadequate self-management poses a threat to satisfactory outcome. It was emphasized on the impact of adequate self-management on the patient's morbidity and mortality and on increasing the costs of medical treatment as cost of medication, cost of laboratory tests and cost in time and effort of the care providers in addition to the frustration for both the patients and the care providers. In contrast, other studies reported that the patients who had adequate self-management had better outcomes, live longer, enjoy a higher quality of life, and suffer fewer symptoms and complications.

Aim the study:

The aim of this study was to compare the efficiency of Web-Based education versus counseling on diabetic patients' outcomes through the following:

1. Assessment of patients' diabetic knowledge, level of self-efficacy, self-care activities and blood glucose level pre intervention.
2. Planning and implementation of educational intervention.
3. Evaluation and comparing the efficiency of Web-Based education versus counseling on diabetic patients' outcomes including knowledge, level of self-efficacy, self-care activities and blood glucose level post-intervention.

Research hypothesis:

It was hypothesized that:

- 1- Both web-Based education and counseling will improve diabetic patients' outcomes.
- 2- There will be a statistically significant difference between patients' who will be exposed to web-Based education versus counseling regarding diabetic patients' outcomes.

2.Subjects and Methods:

Research Design:

A quasi-experimental research design has been utilized in this study.

Research setting:

The study was conducted at the outpatient clinic for diabetes in Ain Shams University hospitals, Cairo, Egypt.

Subjects:

Purposive sample of patients were included in the study. Patients for this study were adult and diagnosed with type 2 diabetes. Patients divided randomly into two equal groups (45 patients each) to constitute the web-based group and counseling group. Those who came first carried odd numbers constituted

the web-based group patients and even numbers assigned to counseling group. Inclusion criteria included that the patients should be able to read and write to understand the goals and procedure of the study, the web-based group had computers and an Internet access in their houses and ability to reach the web site by themselves or by one of their families.

The sample size was estimated with STATA 10 program. The estimated required sample size was 45 patients in each group, to achieve power of study 80%, power = 0-8000 and alpha=0.0500.

Study tools:

The following tools were used to collect data related to this study:

Tool I :Patient's assessment and clinical data sheet: The sheet was designed by the researchers to gather information related to age, sex, education of patients, work status, marital status and, also covered data related to blood sugar test, duration of illness.

Tool II: Diabetes Management Self-Efficacy Scale (DMSES): The scale is developed by van der Bijl, 1999 and also had acceptable reliability and validity. This tool was used to assess self-efficacy of diabetic patients pre- and post intervention. This tool composed of 20-item DMSES to know how the patient confident in doing certain activities with five responses scoring from 5-1, the high score was given for high self-efficacy and it was considered that > 60 % from total score was high self-efficacy.

Tool III: Diabetic patient's knowledge questionnaire sheet: It was used to assess patient's knowledge about diabetes mellitus and its management such as; definition, types, diet, medication, exercises, glucose monitoring, avoidance of complications such as; hyperglycemia, hypoglycemia and diabetic foot. It was written in Arabic language and developed by the researchers based on the related literature (; Lewis et al.,2007; Dewit, 2009; Morten, 2009; Ignatavicius &Workman, 2010 ; Nettina, 2010 ; Urden et al.,2010 ;;). It was composed of 20 questions. The Score was given for each correct answer and zero for incorrect answer. For each area of knowledge, the scores of the items were summed-up and the total score divided by the number of the items. These scores were converted into a percent score. The total nurses' knowledge was considered satisfactory if the percent score was 60% and more, and unsatisfactory if less than 60%.

Tool 4: Summary of Diabetes Self-Care Activities Scale (SDSCA): The scale is developed by **Toobert and Glasgow, 1994**, also had acceptable reliability and validity. The scale translated to Arabic by **Mason, 2005** according to WHO guidelines for translation .It contain 12 questions about diet, exercises, blood sugar test foot care and medication. The questions ask about Diabetes Self-Care Activities during the past 7 days,

so the scale graded from day one today seven and it was considered that, less than three days are inadequate, while more than three days are adequate.

• **Procedure:**

The current study was carried out on three phases, preparatory phase, implementation phase and evaluation phase.

Phase I: Preparatory phase:

- Human rights and ethical permission were obtained to conduct the study. Head of outpatient clinics gave permission to perform the study. Patients were fully informed of the study. The voluntary nature of participation was stressed as well as confidentiality. Consent was obtained from each patient.
- The researchers developed the counseling program for diabetic management intervention based on needs of patients and Booklet was developed illustrating diabetic management based on related literature (Daniels, *et al.*, 2007; Morten, 2009 ; Timby & Smith, 2010 ; Lewis *et al.*, 2011; potter *et al.*, 2011).
- Web site for diabetic management was determined and introduced to the Web-based group.

Phase 2: Implementation phase:

A pilot study was carried out by 5 patients to test the clarity, applicability, objectivity and feasibility of the tools to conduct the study. No Changes or modifications were done. The subjects included in the pilot study were included in the study.

Patients in the diabetic clinic who met the study criteria were included immediately after random assignment for counseling group and web-based group (net group). Patient's assessment data sheet was fulfilled as a baseline pre intervention data. Also Patient's assessment for diabetic management knowledge, diabetes self efficacy, diabetes self care activities and blood glucose measure were done pre intervention. Web site for diabetic management was determined and introduced to the Web-based group. Data collection and program implementation was carried out during the period from June 2011 to February 2012

Counseling was implemented according to DASIE technique. The counseling program was carried out using DASIE technique based on Richard Nelson Jonson 1997. The number of sessions was based on the patient's needs. Each patient was interviewed individually for 30 to 40 minutes counseling sessions were presented by the researcher according to DASIE technique (D: Develop the relationship and clarify problems; A: Assessment and restate problems in skills terms; S: State goals and plan interventions; I: Interview to develop life skills; E: Emphasize, take-away and end.)

Phase 3: Evaluation phase:

This phase aimed to evaluate the effect of web-based education versus counseling on Diabetic patients' Outcome through assessment of improving knowledge related to diabetic management, diabetes self efficacy, diabetes self care activities and improving in blood glucose measure after implementation and completion of both educational interventions.

Data analysis:

Data entry, validation and analysis were done with the statistical package for social science version 13.0, the statistical tests used are number and percent distribution, mean and stander deviation .A value of $p < 0.05$ was considered to be statistically significant.

3. Results:

Table (1), shows that nearly half (48.9% , 51.1%) of counseling and web-based groups were less than 40 years respectively. Also male represent (51.1%) of counseling group and (43.2%) of web based group. Regarding level of education (53.3%) of counseling group read and write and (57.8%) of web based group had secondary education. Also more than two thirds of two groups (71.1 , 68.9%,) respectively had a work. As regard marital status it was found that more than half (51.1% , 55.6%) were married. Also mean of disease duration was (11.44±5.23, 11.29±5.85) respectively.

Table (2) shows satisfactory knowledge of diabetes in both counseling and web-based group. It illustrates that the minority of the two groups had satisfactory knowledge pre- intervention . However web based group had more knowledge about diabetes in all items except in diet, signs & symptoms of hypo- and hyper-glycemia and management of hyperglycemia than counseling group with no statistically significant differences between them regarding all items.

Table (3) shows the improvement of diabetes knowledge among the majority of two groups regarding general knowledge of diabetes. Conversely to pre intervention, the table shows that counseling group had more satisfactory knowledge than web-based group with no statistically significant difference in all items except for the management of hyperglycemia (p value 0.003).

Table (4) shows that the majority (91.1% & 84.4%) of counseling and web-based group had low level of self efficacy pre-intervention with no statistically significant difference between them. Also the mean score of total self efficacy pre intervention was (2.49±1.24 and 3.47±1.22) among two groups with highly statistically significant difference ($p > 0.000$). While post-intervention, the majority (88.9% & 86.7%) of two groups respectively had high level of self efficacy with mean score of total self efficacy (8.1±1.1 and 7.9±0.93) with no statistically significant difference between them ($p > 0.3$).

Table (1): Socio-demographic characteristics of counseling and web- based groups.

Parameters	(counseling group) Total=45		(web-based group) Total=45		Test	P value
	No	%	No	%		
Age/ years					X ²	
18-40	22	48.9	23	51.1	0.38	0.8
40-50	16	35.6	17	37.8		
above50	7	15.6	5	11.1		
Mean and standard deviation of age	39.24±9.61		37.11±9.69		T	0.2
					1.04	NS
Gender					X ²	
Male	23	51.1	19	43.2	0.56	0.45
female	22	48.9	25	56.8		
Education					X ²	
Read and write	24	53.3	12	26.7	9.4	0.009
Secondary	12	26.7	26	57.8		
Higher	9	20	7	15.6		
Work status					X ²	
Work	32	71.1	31	68.9	0.05	0.81
Not Work	13	28.9	14	31.1		
Marital status					X ²	
Single	8	17.8	6	13.3	0.36	0.8
Married	23	51.1	25	55.6		
Widow and divorced	14	31.1	14	31.1		
Duration of disease					X ²	
< 6	7	15.6	12	26.7	5.82	0.1
6-	15	33.3	12	26.7		
12-	21	46.7	14	31.3		
>18	2	4.4	7	15.6		
Mean and standard deviation of duration of disease	11.44±5.23		11.29±5.85		T	0.89
					0.13	NS

Table (2): Satisfactory knowledge difference between two groups pre –intervention.

Item	(counseling group) Total=45		(web-based group) Total=45		z	P-value
	No. (satisfactory)	%	No. (satisfactory)	%		
Basic knowledge:						
Def.	10	22.2	12	26.7	0.491	0.624
Types	13	28.9	15	33.3	0.455	0.649
Self care:						
Diet	11	24.4	10	22.2	0.249	0.803
Medication	9	20.0	11	24.4	0.507	0.612
Exercises	8	17.8	9	20.0	0.269	0.788
Glucose monitoring	7	15.6	8	17.8	0.283	0.777
Avoidance of complication						
Hypoglycemia:						
Def.	7	15.6	8	17.8	0.283	0.777
S&S	8	17.8	6	13.3	0.582	0.561
Management	9	20.0	10	22.2	0.258	0.796
Hyperglycemia:						
Def.	8	17.8	9	20.0	0.269	0.788
S&S	8	17.8	7	15.6	0.283	0.777
Management	10	22.2	8	17.8	0.527	0.598
Diabetic foot:						
Causes	9	20.0	10	22.2	0.258	0.796
Risk factor	6	13.3	7	15.6	0.300	0.764
Prevention	8	17.8	8	17.8	0.000	1.000

Table (3): Satisfactory knowledge difference between two groups post –intervention.

Item	(counseling group) Total=45		(web-based group) Total=45		z	P-value
	No. (Satisfactory).	%	No. (satisfactory)	%		
Basic knowledge:						
Def.	40	88.9	38	84.4	0.620	0.535
Types	44	97.8	39	86.7	1.968	0.049
Self care:						
Diet	43	95.6	40	88.9	1.181	0.238
Medication	40	88.9	37	82.2	0.900	0.368
Exercises	42	93.3	40	88.9	0.741	0.459
Glucose monitoring	43	95.6	39	86.7	1.482	0.138
Avoidance of complication						
Hypoglycemia:						
Def.	39	86.7	38	84.4	0.300	0.764
S&S	40	88.9	39	86.7	0.322	0.748
Management	41	91.1	37	82.2	1.240	0.215
Hyperglycemia:						
Def.	42	93.3	39	86.7	1.054	0.292
S&S	40	88.9	36	80.0	1.163	0.245
Management	44	97.8	35	77.8	2.896	0.004
Diabetic foot:						
Causes	41	91.1	38	84.4	0.965	0.334
Risk factor	40	88.9	37	82.2	0.900	0.368
Prevention	42	93.3	39	86.7	1.054	0.292

Table (4): Self- efficacy difference between two groups pre and post intervention

Parameters	(counseling group) Total=45		(web-based group) Total=45		Test	P value
	No	%	No	%		
Pre intervention						
Low level of self efficacy	41	91.1	38	84.4	X ² 0.93	0.3
High level of self efficacy	4	8.9	7	15.6		
Mean score of total self efficacy pre intervention	2.49±1.24		3.47±1.22		T -3.77	0.000
Post intervention						
Low level of self efficacy	5	11.1	6	13.3	X ² 0.1	0.7
High level of self efficacy	40	88.9	39	86.7		
Mean score of total self efficacy	8.1±1.1		7.9±0.93		T 0.96	0.3

Table (5): Self care activities difference between two groups pre-intervention

Parameters	(Counseling group) Total=45		(web-based group) Total=45		T-Test	P value
	No	%	No	%		
1-Diet						
0-3 days (inadequate)	41	91.1	42	93.3		
>3-7 days (adequate)	4	8.9	3	6.7		
Mean score of diet	2.12±0.66		2±0.59		0.87	0.3
2-Exercise						
0-3 days (inadequate)	43	95.6	42	93.3		
>3-7 days (adequate)	2	4.4	3	6.7		
Mean score of Exercise	0.84±0.68		0.94±0.86		-0.61	0.5
3-Blood sugar test						
0-3 days (inadequate)	44	97.8	44	97.8		
>3-7 days (adequate)	1	2.2	1	2.2		
Mean score of Blood sugar test	0.34±0.58		0.47±0.67		-1.004	0.3
4- Foot care						
0-3 days (inadequate)	42	93.3	42	93.3		
>3-7 days (adequate)	3	6.7	3	6.7		
Mean score of foot care	0.27±0.88		0.48±0.96		-1.08	0.2
4- Medication						
7 days (adequate)	45	100	45	100	No test available	

Table (6): Self care activities difference between two groups post- intervention

Parameters	(counseling group) Total=45		(web-based group) Total=45		T Test	P value
	No	%	No	%		
1- Diet						
0-3 days (inadequate)	7	15.6	12	26.7		
>3-7 days (adequate)	38	84.4	33	73.3		
Mean score of diet	5.52±1.15		4.88±1.26		2.5	0.01
2- Exercise						
0-3 days (inadequate)	22	48.9	17	37.8		
>3-7 days (adequate)	23	51.1	28	62.2		
Mean score of Exercise	4.17±1.44		3.84±0.76		1.36	0.1
3- Blood sugar test						
0-3 days (inadequate)	2	4.4	22	48.9		
>3-7 days (adequate)	43	95.6	23	51.1		
Mean score of Blood sugar test	5.36±1.25		3.44±0.46		9.63	0.000
4-Foot care						
0-3 days (inadequate)	7	15.6	25	55.6		
>3-7 days(adequate)	38	84.4	20	44.4		
Mean score of foot care	5.04±1.51		3.44±0.50		9.72	0.004
4- Medication					No test available	
7 days (adequate)	45	100	45	100		

Table (7): Fasting Blood sugar difference between two groups pre- and post- intervention

Parameters	(counseling group) Total=45		(web-based group) Total=45		X ²	P value
	No	%	No	%		
Fasting Bl. Sugar test pre-intervention						
Normal	12	26.7	7	15.6	1.6	0.19 NS
Abnormal	33	73.3	38	84.4	6	
Mean score of blood sugar test	163.22±41.4		185±31.75			
Fasting Bl. Sugar test post-intervention						
Normal	39	86.7	34	75.6	1.8	0.17 NS
Abnormal	6	13.3	11	24.4	1	
Mean score of fasting blood sugar test	128.9±133.5		116±13.2			

Table (8): Relation between mean of fasting blood sugar and self efficacy level pre- and post-intervention.

Group	Self efficacy	Mean of fasting blood sugar level	N Total=45	%	Std. Deviation	F Test	P value	
Pre intervention (counseling group)	Low	162.68	41	91.1	41.53	8.03	0.006	
	High	168.7500	4	8.9	45.89			
	(web-based group)	Low	183.07	38	84.4			34.05
		High	197.14	7	15.6			6.98
Post intervention (counseling group)	Low	114.0	5	11.1	11.40	0.41	0.52	
	High	130.77	40	88.9	141.71			
	(web-based group)	Low	114.16	6	13.3			10.20621
		High	116.28	39	86.7			13.70353

Table (9): Relation between mean of blood sugar and mean of self care activity pre- and post-intervention.

Group	Fasting Bl.Sugar test level	Mean of self care activity	N Total=45	%	Std. Deviation	F Test	P value
Pre intervention (counseling group)	Normal	1.33	12	26.7	0.25	0.33	0.56 NS
	abnormal	1.07	33	73.3	0.30		
(web-based group)	Normal	1.1	7	15.6	0.30		
	abnormal	1.2	38	84.4	0.39		
Post intervention (counseling group)	Normal	5.10	39	86.7	0.71	54.1	0.000 HS
	abnormal	5.25	6	13.3	0.48		
(web-based group)	Normal	4.11	34	75.6	0.64		
	abnormal	4.04	11	24.4	0.62		

Table (10): Relation between level of self-efficacy level and mean of self care activity pre- and post-intervention.

Group	Self efficacy	Mean of self care activity	Total=45N	%	Std. Deviation	F Test	P value
Pre intervention (counseling group)	Low	1.13	41	91.1	0.31	0.33	0.56 NS
	High	1.22	4	8.9	0.33		
(web-based group)	Low	1.18	38	84.4	0.35		
	High	1.18	7	15.6	0.49		
Post intervention (counseling group)	Low	4.78	5	11.1	0.73	54.16	0.000 HS
	High	5.17	40	88.9	0.67		
(web-based group)	Low	3.75	6	13.3	0.75		
	High	4.15	39	86.7	0.60		

Table (5) illustrates inadequate level of self care activities pre intervention in two groups. The majority of two groups had inadequate self care activities (0-3 days from 7 days for all items of self care activities) with no statistically significant difference between them except regarding medication, all of both groups had adequate self care activities.

Table (6) illustrates the improvement of level of self care activities post intervention among two groups. Regarding diet the table illustrated that (84.4%) of counseling group had adequate diet self care (the days >3-7 days) compared to (73.3%) of web based group with mean score (5.52±1.15) and (4.88±1.26) among two groups respectively with statistically significant difference between two groups (p value= 0.01). Regarding exercise, it was found that (51.1%) of counseling group had adequate exercise self care compared to (62.2%) of web based group with mean score (4.17±1.44) and (3.84±0.76) among two group respectively with no statistically significant difference between two

groups (p value= 0.1). Concerning blood sugar test, it was found that (95.6%) of counseling group had adequate blood sugar test compared to (51.1%) of web based group with mean score (5.36±1.25) and (3.44±0.46) among two groups respectively with highly statistically significant difference between two groups (p value 0.000). Also, this table shows that (84.4%) of counseling group had adequate foot care compared to (44.4%) of web based group with mean score (5.04±1.51) and (3.44±0.50) among two groups with statistically significant difference (p value=0.004). This table also clarifies that improvement in self care activities in counseling group than web-based group post intervention in all items of self care activities except in exercises.

Table (7) clarifies that (26.7%) of counseling group compared to (15.6%) of web-based group their fasting blood sugar test were normal pre-intervention with no statistically significant differences whereas the mean of fasting blood sugar test were (163.22±41.4) and (185±31.75) between two groups respectively. While, post-

intervention it was found that the majority of both groups achieved glycemic control, whereas (86.7%) of counseling group compared to (75.6%) of web-based group their sugar test were normal with no statistically significant differences whereas the mean of blood sugar test were (128.9±133.5) and (116±13.2) between two groups respectively. This table also clarifies that improvement in blood sugar in counseling group than web-based group post intervention.

Table (8), shows that the minority (8.9% & 15.6%) of two groups respectively had high self efficacy and high mean of blood sugar level pre-intervention with statistically significant relation between blood sugar and self efficacy (p value 0.006). While post intervention, it was found that the majority (88.9% & 86.7) of two groups respectively had high self efficacy and low mean of sugar level with no statistically significant relation between blood sugar and self efficacy (p value = 0.52).

Table (9), shows that the minority (26.7% & 15.6%) of two groups respectively had normal blood sugar level and low mean of self care activity pre-intervention with no statistically significant relation between mean of blood sugar and mean of self care activity (p value = 0.5). Meanwhile post intervention, it was found that the majority (86.7% & 75.6%) respectively of two groups had normal blood sugar level and high mean of self care activity with a highly statistically significant relation between mean of blood sugar and mean of self care activity (p value= 0.000).

Table (10) shows that the minority (8.9% & 15.6%) of two groups respectively had high self efficacy level and low mean of self care activity pre-intervention with no statistically significant relation between level of self efficacy and mean of self care activity (p value = 0.5). Meanwhile post intervention, it was found that the majority of two groups (88.9% & 86.7%) respectively had high self efficacy level and high mean of self care activity with highly statistically significant relation between level of self efficacy and mean of self care activity (p value= 0.000).

4. Discussion

This quasi-experimental study evaluated the effect of counseling versus web-based education on diabetic patients' outcome. The benefits of web-based education include its easy access, without limitations in time and place, for those who have Internet access. The Internet is already utilized as a source of health-related information, especially by patients with chronic illness and diabetes. The long-term management of diabetes as a chronic disease

is very important. Patients with diabetes need to change their lifestyles and, equally importantly, adhere to the regimen over their lifetime that known as self efficacy. They often perform well after receiving education (*Eigenmann & Colagiuri R, 2007; Song et al., 2009*).

The results of the present study revealed that nearly half of counseling and web-based groups were less than 40 years. Also nearly half were male and married. More than half of counseling group read and write and more than half of web-based group had secondary education. Also more than two thirds of both groups work. Also mean of disease duration was (11.44±5.23, 11.29±5.85) respectively. While *Karakurt and Kas,ıkçı, 2012* found that 67% of patients with type 2 diabetes were women, 39% were in the age group of 50–59, 88% were married, 61% were primary school graduates, 67% were housewives. Also they found that duration of diabetes was 1–5 years.

The results of the present study revealed that no statistically significant differences between counseling and web-based groups regarding sociodemographic characteristics. This was supported by *Tjam et al., 2006* who found no statistically significant differences between two groups when studied Physiological Outcomes of an Internet Disease Management Program vs. In-person Counseling.

As regard knowledge of diabetes and its management pre-intervention, the present findings illustrated that there was lack of knowledge among two groups, also knowledge was similar in the two groups with no statistically significant differences between them. This was congruent with *Upadhyay et al., 2008* who found that knowledge and practice of the diabetic patients had low knowledge so the researchers suggested the educational intervention to improve knowledge and self care activities of diabetes and its management.

After the diabetic management educational intervention, the current results showed improvement of knowledge of two groups. Also, the results showed that counseling group had more satisfactory knowledge than web-based group with no statistically significant difference. This was supported by *Malathy et al., 2011* who found that counseling improved knowledge of diabetes and its management and *Krishna and Boren, 2008* who found that web-based group improved knowledge of diabetes and its management.

Concerning knowledge related to self care, the present findings illustrated that there was lack of knowledge among two groups, also knowledge was similar in the two groups with no statistically significant differences between them pre

intervention. This was in the same line with *Murphy et al., 2011* who revealed that all participants had lack of knowledge about medication and diet to manage their diabetes effectively regardless of the time since diagnosis.

While post intervention, the current results showed improvement of knowledge related to self care of two groups with no statistically significant differences between them. This was in the same line of *Rodrigues et al., 2009* examined the knowledge and attitudes of patients with DM participating in a self-care educational program and found that participants obtained a good score in diabetes and self-care knowledge. Also, *Kim and Song 2008* who found that web-based group improved self care knowledge of diabetes as medication and foot care and *Rurike et al., 2010* who found that counseling improved self care knowledge of diabetes.

As regard self efficacy, the present finding showed that the majority of counseling and web-based group had low level of self efficacy pre-intervention with no statistically significant differences between them. This was congruent with *Glasgow et al., 2010* who found that self efficacy of the diabetic patients was low so the researcher suggested the educational intervention to improve knowledge and self efficacy of diabetes and its management.

While post-intervention, the majority of two groups respectively had high level of self efficacy with no statistically significant differences between them. The present study was consistent with *Robertson et al., 2007 and Wangberg 2008* who found that web-based education improved the self-efficacy of diabetic patients. Also, *Pansila 2008* found that counseling improved the self-efficacy of diabetic patients. Also, other study by *Lee et al., 2009* revealed that the patients who received education reported better self-care practices including healthier lifestyles and higher self-efficacy and controlled their blood glucose better than those who did not received. While *Angeles et al., 2011* found that web- based education was more effective than other methods regarding patients' satisfaction and self efficacy.

Concerning self care activities pre intervention, the present study illustrated that inadequate level of self care activities among two groups except for medication. This finding was congruent with *Xu et al., 2010* who stated that the participants were more likely to carry out self-management in relation to taking medication, but were less likely to carry out self-management with their diet, exercise, self monitoring of blood glucose (SMBG), and foot care. The behavior

patterns may indicate that it is easier for individuals with diabetes to follow medication regimens than to change their lifestyle.

Research among people with type 2 diabetes has suggested that the most frequent negative aspects of self-management are negative physical reactions, time constraints, lack of financial resources and performing tasks that they do not like (*Nagelkerk et al., 2006 ; Pun et al., 2009*).

While post intervention, the present finding illustrated the improvement of level of self care activities post intervention among two groups which was greater among counseling group with statistically significant difference between two groups. This were in congruence with a study evaluated minimal and moderate support versions of an Internet-based diabetes self-management program, compared to an enhanced usual care condition by *Glasgow et al., 2010* who revealed that the Internet-based intervention produced significantly greater improvements than the enhanced usual care condition on behavioral outcomes.

In several studies performed with patients with type 2 diabetes, it was found that the education given to patients had a positive effect on their self-care activities and their knowledge about their disease (*Cosar, 2003; Ko and Gu, 2004; Huang et al., 2005; Gallegos et al., 2006 Karakurt and Kas,ıkçı, 2012*). In this research, the increase in the average scores of the post education scale indicate that self-care activities of patients have changed positively and the education given to patients have been beneficial. Also this may be due to willingness of patients included in the research to participate regularly in the given education may provide an increase in their self care points.

As regard diet and exercise self care activities pre intervention, the results illustrated that the minority of two groups had adequate diet and exercise self care activities with no statistically significant difference them. The findings of the current study were in accordance with *Oftedal et al., 2011* who stated that less than half of the respondents stated that they adhered to their diet 6–7 days per week. On the other hand, less than 10% reported exercising every day

While diet self care activities post intervention, the results of the present study illustrated the improvement of diet self care activities post intervention among two groups which was greater among counseling group with statistically significant difference between two groups The present study was in consistent with *Wangberg 2008* and *Rurike et al., 2010* who found that counseling and web-based education

improved diet self care activities of diabetic patients respectively.

Regarding exercise self care activities post intervention, it was found that nearly half of counseling group had adequate exercise self care compared to near two third of web-based group with no statistically significant difference. The present study was in consistent with *Robertson et al., 2007* and *Malathy et al., 2011* who found that counseling and web-based education improved exercise self care activities of diabetic patients respectively.

Concerning blood sugar test, the present study clarified that low percent of two groups their sugar test were normal in pre intervention with no statistically significant differences. This was supported by *Tjam et al., 2006* who found no statistically significant differences between two groups when studied Physiological Outcomes of an Internet Disease Management Program versus In-person Counseling regarding blood glucose level pre intervention with low percent of two groups their sugar test were normal.

While, post intervention, it was found the majority of counseling group had adequate blood sugar test compared to about half of web based group with no statistically significant difference. This was supported by *Angeles et al., 2011* who found the improvement in blood glucose control post intervention and web-based education was more effective than other methods in glucose control. Also, *Lea et al., 2007* found that web-based education increased the patient's ability to trace blood glucose level. While *Tjam et al., 2006* found no statistically significant differences between two groups in blood glucose control post intervention.

Studies involving smaller samples have used technological interventions, such as cellular phone-based and computer feedback-based methods, successfully for glycemic control (*McMahon et al., 2005; Kim, 2007; Kim & Song, 2008*). Also, the present study were consistent with *Lu et al., 2011* who assess whether self-monitoring of quantitative urine glucose or blood glucose is effective, convenient and safe for glycaemic control in non-insulin treated type 2 diabetes and stated that all patients experienced significant reductions in HbA1c and fasting plasma glucose. Self monitoring of urine glucose and self-monitoring of blood glucose shared comparable efficacy in glycemic control.

Concerning medication self care activities, the present study clarified that all of the study subjects in both groups had adequate self care activities pre and post intervention. This was contradicted with

Kim et al., 2006 who found inadequate medication self care activities pre-intervention and web-based education for diabetic patients improved medication self care activities post-intervention. Also the present findings were in accordance with *Toumas, et al., 2009* who found that during comparing the effectiveness of small-group training in correct inhaler technique with self-directed Internet-based training, there was a significant improvement in the number of participants demonstrating correct technique in both groups post intervention, with no significant statistically difference between the groups.

Regarding foot self care activities pre intervention, the results illustrated that the minority of two groups had adequate foot self care activities with no statistically significant difference them. While post intervention, the results showed that the majority of counseling group had adequate foot care compared to less than half of web-based group with statistically significant difference between them. The findings of the current study were in the same line with *Kim et al., 2006* who found inadequate foot self care activities pre-intervention and web-based education for diabetic patients improved foot self care activities post-intervention.

The present results showed that the majority of two groups had low self efficacy and high mean of blood sugar level pre intervention with statistically significant relation between self efficacy and mean of blood sugar level. Meanwhile post intervention, it was found that the majority of two groups had high self efficacy and low mean of sugar level with no statistically significant difference. Our findings were in the same line with *Nakahara et al., 2006* who stated that self-efficacy has been shown to have a consistent relationship with glycemic control. Also *Chih et al., 2010* added that patients with higher self-efficacy have a higher probability of reaching target glucose control.

The present results found that the majority of two groups had abnormal blood sugar level and low mean of self care activity pre intervention with no statistically significant relation between self care activity and mean of blood sugar level. Meanwhile post intervention, it was found that the majority of two groups had normal blood sugar level and high mean of self care activity with highly statistically significant difference.

The results of the present study was congruent with *Compeán et al., 2010* who stated that to achieve adequate glycemic control, patients should maintain a correct balance between different elements of a comprehensive treatment, such as diet, exercise, medication, glucose monitoring and permanent education. Also the researchers added

that better self-care corresponded to lower HbA1c levels which mean better glycemic control. Also this finding was in the same line with *Sousa et al., 2005* and *Sigh and Press, 2008* who reported that better self-care predicted better glycemic control (low HbA1c).

Our finding revealed that the majority of two groups had low self efficacy level and low mean of self care activity pre intervention with no statistically significant relation between self efficacy level and mean of self care activity pre intervention. Meanwhile post intervention, it was found that the majority of two groups had high self efficacy level and high mean of self care activity with highly statistically significant relation between them.. This findings was congruent with *Wang and Shiu, 2004* found that patients with greater self-efficacy were better able to manage their diabetes self-care.

Furthermore, *Wu et al., 2007* proposed using the self-efficacy model as a framework for understanding compliance with self-care. Nurses can discuss self-efficacy with patients with type 2 diabetes to promote improvement in their behavior and health outcomes (*Shi et al., 2010*). Self efficacy is strongly related to healthy eating and calories expended in physical activity (*King et al., 2010*) and is also a better predictor of other aspects of self-care besides diet and exercise (*Johnston-Brooks et al., 2002*).

Also *Sharoni and Wu, 2012* showed that there was a positive relationship between self-efficacy and self care behavior which was statistically significant .Health education strategy is very important and the nursing profession needs to review it periodically. The concept of self-efficacy should be included in nursing interventions with particular focus on healthy eating, physical exercise, monitoring of blood glucose concentrations and risk reduction behavior.

The present results found that the majority of two groups had abnormal blood sugar level and low mean of self care activity in pre intervention with no statistically significant relation between self care activity level and blood sugar level pre intervention. Meanwhile post intervention, it was found that the majority of two groups had normal blood sugar level and high mean of self care activity with highly statistically significant relation.

The present study in accordance with *Compeán et al., 2010* who stated that to achieve adequate glycemic control, patients should maintain a correct balance between different elements of a comprehensive treatment, such as diet, exercise, medication, glucose monitoring and permanent education. Also he added that Better self-care corresponded to lower HbA1c levels

which mean better glycemic control. Also this finding is in line with other studies *Sousa et al., 2005* and *Sigh and Press, 2008* who reported that better self-care predicted better glycemic control (low HbA1c).

5. Conclusion:

It was concluded that, The majority of counseling and web-based group had unsatisfactory knowledge, low level of self efficacy, inadequate self care activities and abnormal glucose level with no statistically significant difference between them pre-intervention. While, post-intervention, The majority of counseling and web-based group had satisfactory knowledge, high level of self efficacy, adequate self care activities and normal glucose level with. Also, Counseling group had more satisfactory knowledge, high level of self efficacy, adequate self care activities and normal blood glucose level than web-based group with no statistically significant difference in all items except for self care activities

Recommendations:

As results of the current research, the following suggestions are proposed:

1. TRY TO INCREASE THE PUBLIC HEALTH IMPACT OF INTERNET-BASED PROGRAMS WHICH CAN SUPPORT DIABETES MONITORING AND SELF-CARE.
2. DEVELOP WEB SITES WHICH ALLOW PATIENTS ONLINE INTERACTION WITH HEALTH CARE PROVIDER TO ENCOURAGE WEB BASED LEARNING USE.
3. FURTHER RESEARCH INTO THE FULL USE OF THE AVAILABLE TECHNOLOGY IS IMPERATIVE FOR IMPROVING THE QUALITY OF NURSING INTERVENTION.

Limitation of the study:

Inadequate researches which performed to compare Efficiency of Web-Based Education versus Counseling on Diabetic Patients' Outcomes.

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