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EDITORIAL

Simulation in Health Care

Rizwan Hashim

Simulation is usually perceived as the advancement in technology to create experiences that are focused towards clinical applications. This is not a holistic view of this sphere. In fact, it is the integration of knowledge and affective domains that are structured carefully to involve different techniques, related equipment's and courses. These are well structured activities that are designed for different levels of participants having diverse levels of expertise. Besides others, one reason for introduction of simulation in medical field is to groom physicians who are safe practitioners.[1] The history of clinical skill lab is traced back to the 17th century in France, where manikins using the foetal model and pelvis were used. These training were given to the paramedical staff namely midwives by Madame Du Coudray.[2] In modern times the basis for standardization of training in simulation medicine dates back to Resusci Anne who managed the training by providing manikins that were simple to use and were low cost. Later on Abrahamson developed the Sim-One simulator that was aimed to train the novice to learn the insertion of endotracheal tubes. This was a high fidelity simulator.[3,4] This development was followed in 1980 by the development of Comprehensive Anesthesia Simulation Environment (CASE) where the simulator was linked to a program.[5] From there on, Barrows introduced the concept of provision of an environment that was safe and helped the participant to practice where skills with simulated patients.[6] To this date more advanced technological equipments that have capabilities of running computer assisted programs, virtual reality and even tactile sensations modalities have been introduced.[7]

Recent advances in simulation

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There are a wide variety of simulators that are available for use; the choice mainly depends on the requirement of the training that it has to be used for. The common types that are extensively used are as follows:

Part-task trainer: This is commonly used for imparting training related to psychomotor and procedural skills. They are used for practice and achieving proficiency. It has both low fidelity such as venipuncture, male and female pelvic models; skin and tissue materials that can be used for practicing injections and suturing with various materials and needles, more over, it can also have high fidelity training equipment.[8]

Computer based systems: These have developed over the period from simple interfaces to advanced programs. They have been usually used for basic sciences with the provision that the participants can practice on them with their own pace. The ability to provide appropriate feedback helps the learner to reinforce what is right and correct what they have not attempted correctly.[9] Such interfaces are usually of low cost, whereas they have a drawback that they require a trained teacher to teach the participants how to use them.[10]

Virtual reality and haptic systems: In virtual reality the technology is used to generate images of objects and environment. These are visualized by the participants using high tech gears and goggles that are connected with various computer systems, the individual has the freedom to interact with those images and objects with the help of various gadgets that are provided with the setup. In haptic systems there is provision of adding the tactile and kinesthetic capability in the equipment. When the virtual reality and haptic systems are integrated they provide a much enriched experience to the learner. The presence of recordings and tracking systems in the devices helps to generate data that can be used for feedback as well as a record of practice sessions carried out by the participant.[11] These are usually applied for advanced training in complex skills like endoscopy and laparoscopy.[12]

Integrated simulator models: In these simulators

there is integration of the manikins with the computers and the outcomes are displayed on monitors. They are used to display common data regarding ECG, pulse oximetry and methodology like insertion of chest tube and passing of urinary catheter, the manikins also respond to the administration of various therapeutic agents. The various available models are both high and low fidelity simulators.[13]

Simulated environment: The simulation facilities are placed at a variety of settings according to the requirement of the learning outcomes for which they are planned to be used. These specific environments can range from simple teaching and learning rooms, where simulated environments are created to create patient wards, operating rooms, emergency rooms, intensive or coronary care units and sometimes domestic settings and road or ambulance environments.[14]

Tailored Training: In the clinical skill labs the training requirements can be designed according to the desires of the team or the individuals of the team. This includes both the beginners and the experts. In clinical skill labs the speed of the various training components of the events can be regulated and modified according to the requirements of trainee. In regular occurrences the physiological events following certain medical situations keep happening and for patient safety they have to be taken over by the treating physicians and the trainees do not get enough time for decision making and implementing their choices, this deficit can be easily managed in clinical skill labs on manikins and high fidelity equipment without compromising patient safety.[13,15]

In recent years medical curriculum has undergone major changes both internationally and nationally.[16] The introduction of outcome competency based curriculum has been adopted to develop required knowledge, skill and attitude. The same can be acquired through well designed activities in the clinical skill labs.[17] Amongst several issues, one of the many factors positively influencing the developments of clinical skill labs in both undergraduate and postgraduate teachings is the fact that, patients remain central to the teaching in medical education, but at the same time there is growing concern among the patients and their relatives that the medical students and practitioners need to be appropriately prepared before they start handling patients, they do not hesitate to voice their distress with the hospital management, for safe handling of the patients by the health care workers at all levels[18]. These growing issues can be attended by formal practice of a wide range of procedures and skills in simulated environments in the clinical skill labs both for the undergraduate and postgraduate trainees aiming at the better patient care and outcomes.

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ORIGINAL ARTICLE

Hepatitis C Virus Infection, A Risk Factor for Gallstone Disease: A Cross Sectional Survey

Samia Kausar[1], Fazilla Farid[2], Shamaila Burney[3], Kiran Fatima[4], Muhammad Farooq[5], Amina Shahzad[6]

ABSTRACT

Objective: To study the frequency of gallstone disease in patients with hepatitis C virus infection.

Study Design: Descriptive cross sectional study design.

Place and Duration of Study: From 1st March 2019 to, 31st December 2019 at Department of Medicine and Radiology of Pakistan Railway Hospital Rawalpindi

Materials and Methods: A total of 200 subjects were selected through non probability consecutive sampling from medical In-patient and Out-patient Department. They were screened for hepatitis C virus (HCV) infection by anti-HCV antibodies test by enzyme linked immunosorbant essay (ELISA). They were divided into two groups. HCV positive and HCV negative groups with 100 participants in each group. The study subjects were sent to Radiology Department for detection of gall stones by abdominal ultrasound. The data was obtained from each participant regarding demographic and clinical variables and analyzed using SPSS version21.

Results: HCV positive patients had significantly higher frequency of gallstones (12.5%) compared with HCV negative patients (4.5%). Higher percentage of males had gallstones in HCV positive group. Cirrhosis was present in 52% of HCV infected patients.

Conclusion: The HCV infection is associated with increased risk of gallstone disease.

Key Words: *Cirrhosis, Cholelithiasis, Gallstones, HCV.*

Introduction

Gallstone disease affects almost 20% of adults, two to three times more common in females with peak incidence at more than forty years of age. Majority patients are asymptomatic but more than 20% present with biliary symptoms and complications.[1] Cholelithiasis highly prevalent because of rising trend in obesity and modifiable life style factors. Factors increasing the risk are: infection, high body mass index, pregnancy, birth control pills, hereditary, diabetes, liver disease, rapid weight loss, smoking, lack of exercise, diet rich in red meat and hydrogenated fat.[2] Factors decreasing the risk are: Intake of fruits and vegetables.[3]

Worldwide HCV has infected almost 3% population,[4] transmitted mostly through unsterile medical equipment and injections. HCV infection is more prevalent in intravenous (IV) drug abuser and human immunodeficiency virus (HIV) positive patients.[5] Hepatitis C virus, a major cause of chronic liver disease increases the risk of gall stone formation,[6] and several explanations have been suggested for the possible link between the two. HCV impairs gallbladder motility and mucosal function that might contribute to gallstone formation.[7,8] It may be due to direct HCV infection of gallbladder as HCV ribonucleic acid (RNA) has been traced in gallbladder epithelium on autopsy.[9] HCV nonstructural protein leads to fatty liver by altering lipid metabolism thus promoting cholesterol lithogenesis.[10] Evidence in the current literature states that HCV infected patients develop gallstone even without cirrhosis and at a younger age.[6,11] They are more prone to have multiple gallstones and bile duct stones.[11,12]

So dissimilarity exists in clinical pattern of gallstone disease with or without HCV infection. The correlation between HCV infection and cholelithiasis should be explored further. The prevalence of HCV is high in Pakistan, almost 5%.[13] This will build up further if we do not put forward efforts on modes of transmission and virus behavior. There is added

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financial burden on hospitals due to gallstones and related complications. The beneficial effect of prevention of transmission and eradication of HCV infection by effective drugs lowers the risk and reduces associated morbidity and mortality. Community based epidemiological data from Pakistan is scarce on this association. Studying the link between gallstones formation and hepatitis C infection will create awareness about magnitude of problem in our population. So early recognition will accomplish better outcome.

Our study aimed to compare the frequency of gallstones in patients with hepatitis C virus infection to patients who are HCV negative.

Materials and Methods

A descriptive cross sectional study was carried out involving 200 patients. It was performed at Medicine and Radiology departments of Pakistan Railway Hospital Rawalpindi from March 2019 to December 2019. All patients were included from outdoor and in patients department of Hospital using consecutive non-probability sampling. They were split into two equal groups (100 each) depending on the presence of anti-HCV antibodies. Both HCV positive and negative individuals were referred for ultrasound abdomen for detection of gall stones. The patients who were HIV, Hepatitis B surface Antigen positive, having sickle cell disease, alcoholic liver disease, chronic liver disease other than HCV, thalassemia and malignancy were excluded. The Institutional Ethical Review Committee of Riphah University permitted to study. We took informed consent from all participants before enrollment.

A brief history and examination was done. Information about age, sex, parity, oral contraceptive use/ estrogen replacement therapy, heredity, diabetes, hypertension, hyperlipidemia and voluntary weight loss in last one year was recorded on structured performa. All participants were investigated for blood sugar, serum cholesterol levels and body mass index (BMI) was calculated. The HCV-positive group was further investigated for bilirubin, prothrombin time, serum albumin levels. Cirrhosis was diagnosed on aggregate of clinical, radiological (surface nodularity, coarse echo texture, enlarged portal vein splenomegaly and ascites[14]) and biochemical criteria. Child-pugh score was calculated for assessment of cirrhosis severity.

Ultrasound (US) was done by single trained radiologist. US machine with 5.0M HZ transducer was used. Gall stones were diagnosed when it showed dense reflection from gallstone along with posterior acoustic shadow. Moreover gallstone mobility was seen on repositioning of patient.[15] Anti–HCV antibodies were tested using ELISA method.

Data (parametric) was recorded on SPSS 21. Statistical variables like patient's age, gender and BMI were listed. Mean and frequencies were calculated. Independent sample t-test was used for group comparison.

Results

Out of 200 patients, 100 patients were HCV positive. The age range was 25 to 85 years. The mean age in HCV positive group was 54.90 ±8.93 and 56.14±9.23 in HCV negative group. The frequency of gall stones in HCV positive group was 12.5% and 4.5% in HCV negative group. Overall prevalence of gall stones in both groups was 17%.

Gender distribution with gallstones in HCV negative group was 79% female and 21% male while in HCV positive group 45% female and 55% male.

In HCV positive group with gallstones 48% did not have cirrhosis while 52% had cirrhosis. 15.38% patients belonged to child class A while 38.46% in child class B and 46.15% were in child class C.

Mean body mass index (BMI) was similar in both groups 27.31±0.6 and 26.99±0.5 in HCV negative and HCV positive group respectively. Mean fasting blood sugar and serum cholesterol were also comparable in both groups. There were 39% diabetics in HCV positive group and 35% diabetics in HCV negative groups. Almost 50% patients were found hypertensive in both groups. Regarding positive family history of gallstones it was present in 40% vs.35% in HCV negative group compared with HCV positive group.

Table I: Age and Gender of Study Population.
(Total Subjects=200)

Age (Years)	HCV-Negative Group		HCV-Positive Group		Total
	Male	Female	Male	Female	
25-45	14	12	14	09	50
46-65	14	22	13	21	69
66-86	12	26	18	25	82
Total	40	60	45	55	200

Table II: Frequency of Gallstones in Study Patients

Study Groups	Gallstones Present	Gallstones Absent	Total	P Value
HCV-Positive Group	25	75	100	0.001
Percentage of Total	12.5	37.5	50	
HCV-Negative Group	9	91	100	
Percentage of Total	4.5	45.5	50	
Total	34	166	200	
Percent	17%	83%	100	

Discussion

The results of our study showed that HCV infection was associated with high frequency of gallstones (12.5%) compared with patients without HCV infection (4.5%). Moreover, for patients with HCV infection and cirrhosis, the frequency of gallstones increased further with worsening of liver dysfunction.

Worldwide it has been observed that HCV is associated with increased prevalence of gallstones. Initially Chang et al. found gallstones were present in 11.7% of HCV positive patients without cirrhosis compared with 6% without infection.[12] Aljaky et al. from Egypt found prevalence of gall stone in HCV positive subjects 15.68% vs.9.9% in healthy individuals.[16] While studies from Pakistan showed increased prevalence with HCV infection 18.65% vs. 6.65% by Shah et al. and 22% vs. 8% by Haq et al. in healthy subjects.[11,17] As observed in above mentioned studies, HCV infection has been found to be risk factor for gallstones development even without cirrhosis. We verified as 48% HCV positive patient did not have cirrhosis. The results of our study are in agreement with previous studies.

It has been observed that in HCV infected patients prevalence of gall stone increased with age. Lee et al. concluded that hepatitis B and C virus led to greater frequency of gallstones in elderly.[18] In another study HCV infection increased the risk two to three fold in elderly when compared with HBV infection in the same age group. This difference was not observed in patients less than 60 years.[19] Hu et al. declared that males older than 55 years who were HCV infected had higher frequency of gallstones (7.8%) than HCV positive females (6.1%) of same age.[20] In our study we also observed greater prevalence of gallstones in patients more than 50 years of age in HCV infected patients. Our result was in contradiction to the observation made by Shah et al. from Pakistan. They found significantly higher prevalence of gallstones at or below 40 years of age in HCV positive subjects.[11]

In general population, females have three times higher prevalence of gallstones than males. In some studies cirrhosis was considered a risk factor for gallstones formation for men but not for women.[21] The reasoning for this gender specific dissimilarity is higher level of estrogen and progesterone in cirrhotic men which impair gall bladder emptying as in pregnant women. In Taiwan, study involving 1701 individuals HCV infection was related with gallstone formation specifically in male gender. Gallstone prevalence was not affected in females by HCV status.[22] While a study from United States showed that HCV infection significantly enhanced development of gallstones among males but not in females.[23] Similarly Shah et al. HCV positive males had higher prevalence of gall stones as compared to HCV positive female. The reason why male gender is more prone to gallstone formation with HCV infection is not known. It may be due different gender specific risk factors. However we also found higher frequency of gallstones in HCV positive males.

Cirrhosis augments the risk for gallstones. The risk of gallstones development is amplified two fold.[19] Conte et al. observed 618 patients with cirrhosis for 4 years and 22.8% of them developed gallstones.[24] Major mechanisms involved in gall stones development are chronic haemolysis due to hypersplenism, abnormal biliary lipids, low synthesis of bile salts and unconjugated bilirubin.[25] Moreover gall bladder hypo motility and structural changes in its wall accounts for impaired emptying.[25] HCV infection exaggerate gallstones risk in elderly patients with cirrhosis.[19]

Literature review showed that prevalence of gallstones increased with severity of cirrhosis (increased child pugh score). This was characteristically seen in HCV positive cirrhosis. Our study showed that there was increased prevalence of gallstones in child class C. While other studies disputed significant difference in gallstones

prevalence with child pugh score.[19]

The high frequency of gallstones in HCV infected patients suggests that HCV is significant causative link. As there is high prevalence of HCV in Pakistan there is need to work on prevention of transmission, early diagnosis and treatment of HCV infected subjects. Most people are unaware of infection, delayed diagnosis results in increased morbidity and mortality.

Limitation of our study was small a sample size and that the study results may not apply to general population. There is need for large scale epidemiological studies to confirm the conclusion.

Conclusion

The HCV infection is associated with increased risk of gallstone disease. The authors declare no conflict of interest. The authors alone are responsible for the content and writing of paper.

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ORIGINAL ARTICLE

Comparison of Oral Brush Cytology and Tissue Biopsy in Diagnosing Oral Lesions

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ABSTRACT

Objective: To assess the diagnostic efficacy of oral brush cytology in the diagnosis of potentially malignant and malignant lesions of the oral cavity.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: Outpatient Department of ENT and Maxillofacial Surgery, Pakistan Institute of Medical Sciences and Pathology Department of Pakistan Railway Hospital Rawalpindi from 1st March 2017 to 28th February 2018.

Materials and Methods: A total of 50 patients with oral lesions were enrolled through non-probability convenient sampling. All patients presented with oral potentially malignant lesions were included, while patients with age less than 10 years and with bleeding diathesis were excluded from the study. The oral lesions were first sampled by oral brush biopsy technique using a toothbrush and then later on by scalpel biopsy. Samples were then studied under microscope for diagnosis. The data was analyzed using SPSS software version 21.0. Sensitivity, specificity, positive predictive value and negative predictive values were calculated keeping histopathology as a gold standard. Pearson's Chi-Square Test was used for calculating p-value, where p-value of ≤ 0.05 was considered significant.

Results: In this study, the mean age of patients presented with non-malignant oral lesions was 59 ± 12 years, while those with oral cancers were 60 ± 12 years. Men were affected than women. Among 50 patients 39 were found to have oral cancers. The sensitivity of oral brush biopsy was 88%, specificity was 83.3%, positive predictive value was 97.6% and negative predictive value was 50%. The p-value was calculated as 0.001, which was significant.

Conclusion: Our study found that oral brush cytology is reliable and can be easily performed with less cost and discomfort to the patient. It can be used for screening of suspicious oral lesions. It is useful in those situations where a patient refuses to have a biopsy or where a patient with bleeding diathesis would be exposed to unnecessary surgical risks.

Key Words: *Brush Cytology, Dysplasia, Oral Squamous Cell Carcinoma, Potentially Malignant Disorders.*

Introduction

Oral lesions are a common presentation in our outpatient departments. Oral cancer involves cancer of the lips, tongue, floor of the mouth, cheeks, soft and hard palate and pharynx. It is the 6th most common cancer in the western world and second most common cancer in some parts of the subcontinent.[1] It is a global health problem with increasing incidence and mortality.[2] In 2013 oral cancer resulted in 135,000 deaths, which have increased from 84000 in 1990.[3]

Oral cancer has a multifactorial etiology. It can be caused by genetic reasons as well as environmental influences. Globally tobacco, alcohol and human papillomavirus are associated with oral cancers.[4] Studies have shown that the high incidence of oral cancer in the subcontinent is due to a strong association with tobacco chewing, use of gutka, pan, chaalia, naswar, hukka and cigarette smoking.[5]

In Pakistan, areas of khyber phuktun-khwa have a higher prevalence of oral cancers due to the frequent

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use of naswar and in Karachi due to use of paan and chaalia.[6] Karachi cancer registry shows that oral cancer is the second most common cancer in both men and women.[7] The Shaukat Khanum cancer registry in 2016 also shows oral cancer as the 8th most common cancer in Pakistan.[8] Men have twice the risk of oral cancer as compared to women and the risk increases after the age of 50 years.[9] However, some recent studies have shown an earlier incidence in the younger age group as well.[10]

Early detection of oral lesions has been the most effective approach to reduce morbidity and mortality, especially in the malignant ones.[11] It has been proven that benign oral lesions cannot be distinguished from cancers based on clinical examination alone and so when a suspicious oral lesion is encountered it should always be evaluated.[12]

Oral brush cytology utilizes a brush to obtain a complete trans-epithelial cytology specimen with cellular material from all three layers of the lesion i.e. basal, intermediate and superficial layers.[13] The technique is to make repetitive to and fro movements with the brush until there is punctate bleeding from the lamina propria of the lesion, thus ensuring that cells from all epithelial layers have been taken.[14] The yield of brush cytology can be further increased by using digital aids and other adjunctive techniques such as DNA analysis, Immunohistochemistry, molecular analysis and liquid based preparations. Brush cytology is indicated to aid in the diagnosis of an oral lesion which cannot be identified with clinical certainty or a probable benign lesion when a clinician wants to avoid unnecessary biopsy.[15]

Literature search reveals very limited local studies on efficacy of brush cytology. Our study intended to investigate this simple but useful technique. The objective of our study was to assess the efficacy of oral brush cytology in the diagnosis of potentially malignant and malignant lesions of the oral cavity.

Materials and Methods

This cross-sectional, analytical study was carried out at Outpatient Department of ENT and Maxillofacial Surgery, Pakistan Institute of Medical Sciences, and Pathology Department of Pakistan Railway Hospital from 1st March 2017 to 28th February 2018. A total of 50 patients were enrolled through Non-Probability Convenient Sampling. A written informed consent was taken from every patient. Approval for the study was taken from the Ethical Review committee of Riphah International University. Patients with oral lesions suspicious for malignancy, irrespective of the gender were included in the study. These lesions included leukoplakia, erythroplakia, actinic cheilosis and suspected oral carcinoma.

Before sample collection, patient's data were recorded on a pre-designed proforma. For oral brush cytology rinsing of the oral cavity was performed by every patient with ample water. The lesion was viewed with the aid of light. A toothbrush was disinfected in 0.2% of chlorhexidine gluconate mouth wash and was used to obtain a complete trans-epithelial biopsy with minimal discomfort. By using moderate pressure, the brush was repeatedly brushed in one direction over the entire lesion many times until pinpoint bleeding occurred, signaling entry into lamina propria. The material from the brush was smeared on two clean, dry glass slides. The smears were fixed with 95% isopropyl alcohol for staining with hematoxylin and eosin. Cytological smears were graded as follows:[16]

Class 0: Inadequate specimen, Class 1: Benign: No atypical cells identified, Class 2: Dysplastic: Cells exhibiting dysplasia, not sufficient for diagnosis of malignancy, Class 3: Cytology suggestive for malignant.

For biopsy samples a local anaesthetic was injected at the site of oral lesion and a scalpel biopsy was taken. The biopsy specimen was kept in 10% formalin for fixation and sent for histopathology. Gross inspection of tissue was done and submitted for routine processing, slide preparation and then stained with Hematoxylin and Eosin for microscopy.

Based on the degree of dysplasia, architectural loss, invasion deep to the basement membrane and presence of atypical cells, these biopsy specimens were classified as benign, mild to moderate dysplasia, marked dysplasia or Carcinoma in Situ, well-differentiated squamous cell carcinoma, moderately differentiated squamous cell carcinoma and poorly differentiated squamous cell carcinoma. Olympus CX21 light microscope was used for examination of slides of both brush cytology and biopsy.

The data was entered and analyzed by using SPSS 21.0 (Statistical Package for Social Sciences).

Sensitivity, specificity, positive predictive value and negative predictive value with 95% confidence interval were calculated by 2 x 2 table, keeping histopathology as a gold standard. Pearson's Chi-Square Test was used for calculating p-value, where p-value of ≤ 0.05 was considered significant.

Results

The total numbers of cases were 50. The mean age of patients presented with oral cancer was 60. The age group most commonly affected was in 6th decade of life, with a male preponderance. The most common site of oral cancer was buccal mucosa as shown in Table I.

Table I: Table Showing Age, Gender, Site and Adverse Habits

Groups		Non-Malignant Group	Malignant Group
Age		59±12	60±12
Gender	Female	4 (44.4%)	16 (39%)
	Male	5 (55.5%)	25 (61%)
Site	Buccal	2 (22.2%)	15 (36.5%)
	Tongue	3 (33.3%)	12 (29%)
	Alveolus	1 (11.1%)	10 (24.3%)
	Lip	3 (33.3%)	2 (5%)
	Other	0	2 (5%)
Adverse Habits	Naswar	4 (44.4%)	25 (61%)
	Cigarette smoking	3 (33.3)	9 (22%)
	Paan	1 (11%)	3 (7%)
	Gutka	1 (11%)	3 (7%)

The brush cytology and biopsy results were classified into three classes, i-e Benign, dysplastic and malignant (Table II).

Table II: Table Showing the Classification of Oral Lesions on Brush Cytology and Biopsy

	Brush Cytology		Biopsy	
	Percentage	Frequency (n)/50	Percentage	Frequency (n)/50
Benign	20%	10	10%	5
Dysplastic	4%	2	8%	4
Malignant	76%	38	82%	41
Total	100%	50	100%	50

The analysis of the results of the study was done with the help of the 2x2 Table (Table III).

Table III: 2x2 Table Showing Brush Cytology Results Against the Tissue Biopsy

	Tissue Biopsy Positive	Tissue Biopsy Negative	Total
Brush Cytology Positive	(True Positive) 39	(False Positive) 1	40
Brush Cytology Negative	(False Negative) 5	(True Negative) 5	10
Total	44	6	50

In our study sensitivity and specificity, PPV and NPV were calculated. (Table IV). The true and false positives and negatives were based on the following:

- True positive: Samples that were positive on both biopsy and brush cytology.
- True negative: Samples that were negative on both biopsy and brush cytology.
- False positive: Samples those were negative on biopsy and positive on brush cytology.
- False negative: Samples those were positive on biopsy and negative on brush cytology.

Table IV: Statistical Values

Statistics	Value
Sensitivity TP/ (TP + FN)	88.6%
Specificity TN/ (TN + FP)	83%
PPV* TP/ (TP+FP)	97.5%
NPV TN/ (FN+TN)**	50%
Accuracy (TN + TP)/ (TN+TP+FN+FP)	88%

True Positive: TP, True negative: TN, False positive: FP, False Negative: FN. *PPV: Positive predictive value **NPV: Negative Predictive Value.

Pearson's Chi-Square Test was applied through SPSS version 21 and p-value was calculated as 0.001, which was significant.

Discussion

Our results showed that the mean age of patients presenting with malignant oral lesions was 60 years. The age group most commonly affected (30.7%) was 60-69 years. Majority of patients with oral malignancies were males accounting for 61% of the total patients while female patients were 39%. Previous studies also support this finding. Mehrotra et al[17] have documented that 58.9% of malignant oral lesions were males as compared to 41% in females. Naseem et al[18] have documented that 73.4% of cases with malignant oral lesions were males and 26.6% in females. The higher male incidence is attributed to the fact that males are more predisposed to the risk factors such as smoking, alcohol and smokeless tobacco like paan, gutka, naswar causing oral cancers.[19] The most common site of oral cancer in our study was buccal mucosa (37%) followed by the tongue (30%) and then alveolus (23%). This finding was consistent with other studies conducted in the South Asia region. Sharma et al[20] reported buccal mucosa as the most common site with involvement of 63.5%. The likely reason for buccal mucosa being

the most common site for oral cancers can be smokeless tobacco; naswar which is the most common addiction in our patients which is kept against the cheek. Secondly, cheek mucosa is also very thin and non-keratinized and hence more prone to irritants and carcinogens.

There was 88 % agreement among brush cytology and scalpel biopsy results, with a p-value of 0.001 which showed statistically significant agreement between two the tests. This show that the diagnostic accuracy of brush cytology in comparison with the scalpel biopsy was fair and hence brush cytology can be used as an adjunctive test for diagnosis of oral cancers.

In our study, 38 out of 50 patients were diagnosed as malignant, two cases were dysplasia and 10 cases were benign on cytology. When we compared the same cases on histopathology, we found that among 10 patients classified as benign on cytology, only 5 were benign while 5 were malignant (Table II). We found on biopsy that 41 cases were malignant as compared to 38 malignant cases on brush cytology. Hence true positive in our study were 39, true negative were 5, false negative results were 5 and false positive was 1 (Table III). This was consistent with other studies which showed that brush cytology had higher false negative cases than false positive cases.[21] The reason for the higher value of true positives in our study is firstly, being the inclusion of the cases which look malignant on visual examination and secondly the late presentation of oral malignancies in our setup. Reasons for the false negatives on brush cytology can be small sample size, wrong sampling technique, loss of malignant cells in toothbrush bristles and topographic error between the site of brush and scalpel biopsy. This false negative rate suggests that the suspicious oral lesions should undergo scalpel biopsy before they are labelled as benign on cytology.

In our study, the sensitivity was 88.6% and specificity was 83%, positive predictive value was 97.5% and the negative predictive value was 50 % (Table IV). These values are consistent with other studies. Trakroo et al[22] have found that the sensitivity and specificity of brush cytology in detecting dysplasia and oral squamous cell carcinoma were 84.37 % and 88.09 % respectively, and positive and negative predictive values were 93.10% and 76%, respectively. Moreover, when histopathology and brush cytology were compared, they showed good correlation with insignificant P values.[23] Mehrotra et al.[24] found in their study that when compared to scalpel biopsy, the statistical sensitivity of the brush cytology was greater than 76.8% ($P < .05$) while the statistical specificity was greater than 93.3% ($P < .05$). The limitations of this study were that this study was conducted at only one hospital. Strength of the study can be improved by conducting a multicentre study with a larger sample size. Secondly, most of the patients in this study were malignant and hence sensitivity and specificity of the brush cytology for benign lesions could not be ascertained beyond doubt.

Conclusion

Our study finds that oral brush cytology is reliable and can be easily performed with less cost and discomfort to the patient. It can be used for screening of suspicious oral lesions and may have applications in resource-constrained areas. It is useful in those situations where a patient refuses to have a biopsy or where a patient with bleeding diathesis exposes to unnecessary surgical risks.

Recommendation

Brush cytology can be used as a useful adjunct to scalpel biopsy in diagnosing oral lesions especially when the index of suspicion for malignancy is high.

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ORIGINAL ARTICLE

Effects of Ascorbic Acid on Aspartame Induced Nephrotoxicity: An Experimental Rat Model

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ABSTRACT

Objective: To assess the nephroprotective role of Ascorbic Acid against Aspartame induced nephrotoxicity in Albino Wistar rats.

Study Design: Quasi-experimental study.

Place and Duration of Study: Postgraduate research laboratory at ISRA University, Hyderabad from August 2018 until November 2018.

Materials and Methods: Thirty albino Wistar rats were divided into three groups: Group I (Control group), Group II (Aspartame only), and Group III (aspartame and ascorbic acid combination). Pre and post-experiment body weight, the biochemical analysis was done through ANOVA. While Fisher's exact test was used for histological analysis in SPSS version 22.

Results: Statistically significant difference in mean post-experimental body weight was observed in all three groups (P-value<0.05). Marked reduction in mean body weight was observed in group II (171.4±17.5) as compared to group III (191.80±15.1). A statistically significant difference in mean serum levels of serum biomarkers was also observed in all three groups (P-value<0.05). Marked elevation in serum levels of urea, creatinine, C-reactive protein while the decline in serum levels of glutathione peroxidase was seen in group II as compared to group III. Histological alterations (mean diameter of proximal and distal renal tubules) were also more pronounced in group II (110.3±7.4 and 185.98±5.9) respectively as compared with group III (89.59±6.1 and 95±6.8).

Conclusion: Aspartame consumption causes significant nephrotoxicity and disturbs normal renal functions. Ascorbic acid used as a potent antioxidant can limit and/or decrease the toxic effects caused by aspartame.

Key Words: *Ascorbic Acid, Aspartame, Biochemical, Histological, Nephrotoxicity.*

Introduction

Aspartame (APM) is amongst the most widely existing artificially sweetening compounds, consumed by over 100 million people globally.[1] It is one of the constituent ingredients present in diet carbonated cola beverages, tabletop sweeteners and a large number of pharmaceutical products (cough syrups, lozenges, multivitamins etc.).[2] It is commercially available in markets with different names like; Diet sweetener, Nutra sweet, Candril etc. Constituents of APM include aspartic acid and phenylalanine.[3] With the positive uses of aspartame, several negative effects make it one of the most controversial artificial sweeteners.[4] Each year, it claims numerous health problems like headaches, dizziness, nephrotoxicity etc.[5] Consumption of aspartame poses some serious effects on neuronal tissues and leads to neurodegenerative disorders.[6,7] It is confirmed as a multipotent carcinogenic agent.[1] Once consumed, APM metabolized in the gastrointestinal tract (GIT) into aspartic acid, phenylalanine and menthol.[8,9] This menthol then oxidized into cytotoxic formaldehyde and formic acid.[6] Formic acid is considered as the chief metabolite responsible for the detrimental effects of acute intoxication by menthol in humans and animal trials whereas, formaldehyde is a known potent carcinogen.[10] They are mainly responsible for oxidative stress at the cellular level that may impair renal functions.[9,11] Consumption of aspartame for longer duration leads to the production of reactive oxygen species (ROS) include free radicals that cause

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renal tissue injury.[6,11,12]

These free radicals attack the cell membranes causing peroxidation of fats.[13] Consequently, damage to the cellular components resulting in oxidative stress (OS).[7] The elevation of menthol level soon after administration of aspartame has demonstrated by studies on humans and animals.[8]

Ascorbic Acid (AA) is one of the most significant water-soluble antioxidants needed in the body for several processes.[14] It is an effective antioxidant that showed a protective role against ischemic conditions, toxicity and injurious effects induced by OS in animal models as well as human studies.[15] It reduces OS thus avoiding several damaging processes within cells and has the potential to reverse the negative or adverse effects of carcinogenic substances like APM.[16] It can affect the endothelial functions in a positive way and exert anti-inflammatory activities.[17] Due to the protective roles of AA, it is frequently used in the field of medicine.[14,17]

Histological alterations in liver architecture resulting from APM consumption is reported by some researchers.[12] Whereas, the hepatoprotective effect of AA is reported by different studies.[18,19] However, a very limited number of studies have demonstrated the role of AA against the nephrotoxicity by APM.[20]

To the best of our knowledge, no study has been conducted in Pakistan that has demonstrated the protective role of AA in APM induced nephrotoxicity. The current study, therefore, was designed to highlight the nephroprotective effects of AA against APM related nephrotoxicity. This will not only provide the baseline for future human studies but also help in designing community-based programs to educate masses to raise awareness related to the harmful effects of APM containing products on their kidneys. Moreover, any significant findings of the present study will also be helpful in providing guidelines for the stakeholders to include AA as an ingredient in APM containing products to prevent and reduce the morbidity and mortality rate.

The objective of the present research work was to assess the nephroprotective role (hematological, anti-oxidative and histological) of AA against APM induced nephrotoxicity in Albino Wistar rats.

Materials and Methods

Quasi-experimental study was conducted at the postgraduate research laboratory at ISRA University Hyderabad from August 2018 to November 2018. Thirty male and healthy Albino Wistar Rats, 8-12 weeks old, 150 to 250 grams were included in the present study through a non-random purposive sampling technique. The animals were handled according to the national research council guidelines for laboratory animal handling.[21] Ethical approval was sought from the ethical review committee of ISRA University.

After acclimatization period of 1 week, we randomly divided animals into three groups (n=10); group I (controls), group II (aspartame 200mg/kg/day orally)[22] and group III (aspartame 200mg/kg/day orally + ascorbic acid 100mg/kg/day orally).[23] Experimental drugs were crushed and mixed with a normal chow diet, which was fed to the animals for six weeks.

Blood samples for biochemical analysis (Serum Urea, Creatinine, C-reactive proteins (CRP) and serum glutathione peroxidase (GPX) were collected twice from each rat model (before and after APM induction) from retro-orbital plexus (before) and then through cardiac puncture (after) in the study to evaluate the renal changes. All tests were carried out by Roche/Hitachi diagnostic kit method on an automatic modular analyzer while GPX performed on the bioassay technology ELISA kit.

After six-week, all animals were sacrificed under anesthesia and kidneys of all groups were removed soon after sacrificing the animals. Kidneys were then washed with normal saline and gross abnormalities as well as morphological parameters like those that weight & size were recorded on electronic precision balance and measuring scale respectively.

Collected specimens (kidneys) from all groups were fixed in 10% formalin for histological analysis. Tissues were passed in ascending grades of ethyl alcohol (70%, 80%, 90% and 100%) then in xylene for clearing. The tissues were processed to prepare paraffin blocks by the paraffin embedding method. Four-micrometer sections were obtained using Rotary Microtome, 290 (by manual method), for slides preparation. All slides then stained with hematoxylin and eosin (H & E) to observe under a light microscope at 400 magnifications.

Data were analyzed by SPSS (Statistical packages for social sciences) version 22.0. ANOVA was applied to

compare the body weights and hematological findings. Whereas, Fisher Exact Test was used for histological findings. The significance level was set at P-value ≤ 0.05.

Results

Weight of all rats in groups I, II and III were observed prior to the experiment and were found to be 201.2±5.7 gm, 215.7±8.5 gm and 204.6±7.4 gm respectively. At the end of the experiment the rats have weighed again and statistically significant difference in mean post-experimental body weight was observed in all three groups (p-value <0.05) (Table I)

Table I: Mean Body Weight (In Grams) of Animal Groups (n=30)

Groups	Mean (SD ±)	F-Value	P-Value
Group I (Controls)	223.50 (15.7)	26.39	<0.05 (0.001)
Group II (Aspartame induced Group)	171.40 (17.5)		
Group III (Aspartame + Ascorbic acid Group)	191.80 (15.1)		

Pre experimental biochemical analysis of all three groups was performed in the present study. Pre-experimental mean level of serum urea, creatinine, CRP and GPX in group I was (19.05±2.61 mg/dl, 0.46±0.07 mg/dl, 0.117±0.05mg/L and 1.44±0.17 ng/ml respectively), in group II (19.72±2.52 mg/dl, 0.49±0.09 mg/dl, 0.117±0.07mg/L and 1.43±0.16 ng/ml respectively) while in group III these were found to be (19.33±2.51 mg/dl, 0.52±0.09 mg/dl, 0.12±0.08mg/L and 1.41±0.14 ng/ml respectively).

The mean ± SD of post-experimental biochemical analysis findings of all three-study groups of rats are shown in Table II. Aspartame induction resulted in a rise in serum levels of urea, creatinine and CRP whereas; significant decline serum level of GPX was observed in group II. There was a statistically significant difference (p<0.05) of mean levels of serum urea, creatinine, CRP and GPx in the post-experimental analysis of blood samples in group II comparison to the group I and III. (Table II)

Marked changes in the histology of mean Proximal Convoluted Tubules (PCT) and Distal Convoluted Tubules (DCT), as well as mean distance between visceral and parietal layers of Bowman's capsule (BC), observed in-group II in comparison with the group I and III. Changes were also observed in group III but less in comparison with group II. There was a statistically significant (p<0.05) difference in renal histology i.e. changes in the diameter of PCTs, DCTs and distance between visceral and parietal layers of Bowman's capsule (Table III).

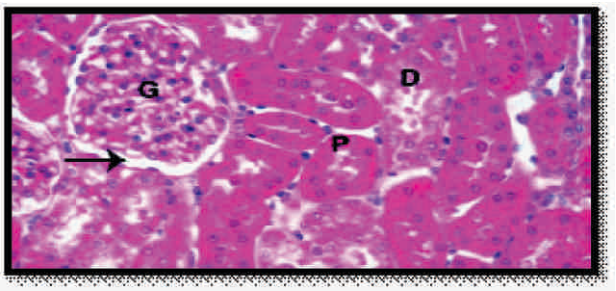
Table II: Mean Biochemical Analysis Findings of All Study Groups (n=30)

		Mean (SD±)	F-value	p-value
Serum Urea	Group I	21.12 (0.573)	2821.7	<0.05 (0.0001)
	Group II	39.83 (0.55)		
	Group III	26.71 (0.58)		
Serum Creatinine	Group I	0.48 (0.06)	176.09	<0.05 (0.0001)
	Group II	2.39 (0.28)		
	Group III	1.69 (0.266)		
C-Reactive protein	Group I	0.11 (0.0264)	375.5	<0.05 (0.0001)
	Group II	0.75 (0.061)		
	Group III	0.50 (0.062)		
Serum Glutathione peroxidase	Group I	1.51 (0.098)	264.9	<0.05 (0.0001)
	Group II	0.757 (0.079)		
	Group III	1.95 (0.160)		

Table III: Renal Histological Difference between the Study Groups (n=30)

Renal Changes		YES	NO	p-value*
Changes in diameter of the proximal convoluted tubule	Group I	0	10	<0.05 (0.001)
	Group II	8	2	
	Group III	3	7	
Changes in diameter of distal convoluted tubule lumen	Group I	1	9	<0.05 (0.018)
	Group II	7	3	
	Group III	3	7	
Changes in distance between visceral and parietal layers of the Bowman's capsule	Group I	0	10	<0.05 (0.003)
	Group II	7	3	
	Group III	6	4	

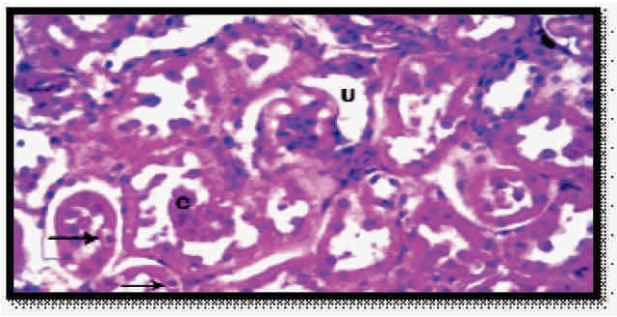
***Fisher Exact Test**



GROUP I

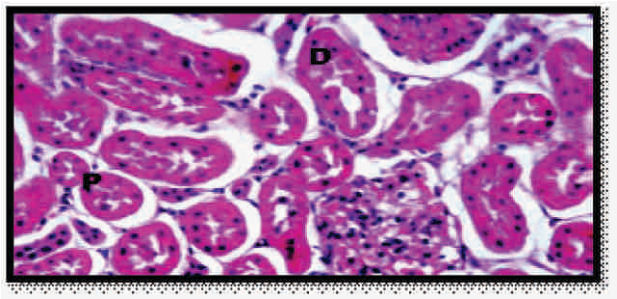
Normal Glomerular tuft (G) with urinary space (arrow)

and renal corpuscles seen in the renal cortex. Proximal convoluted tubules (P) and Distal convoluted tubules (D) lined by the cuboidal cells. Acidophilic cytoplasm and presence of apical brush border seen in PCT (P).
(H&E) X 400 (4µm)



GROUP II

Low cuboidal cells lining the renal tubules with a vacuolated cytoplasm (arrow) and pyknotic nuclei seen in this group. Shrinkage of renal corpuscle with widened urinary space (U). The dilated tubular lumens contain sloughing necrotic cells (C).
(H&E) X 400 (4µm)



GROUP III

Most of the glomeruli and tubules in the renal cortex seen. These are more or less similar to that of the control group. Brush borders in most of the proximal (P) and distal tubules (D) are preserved.
(H&E) X 400 (4µm)

Fig 1: Histological Slides of Renal Cortex

Discussion

APM is one of the most controversial artificial sweeteners used by people around the globe.[24] It is a Food and Drug Administration (FDA) approved sugar alternate but its use in different routine food and medicinal products is highly debated.[25] Although its consumption within normal and approved range is considered safe the findings of some experimental and epidemiological studies demonstrated its adverse effects like hyperglycemia, obesity, cardiovascular disease, metabolic syndrome, neurobehavioral disturbances, cancers etc.[8,9,11,13,26]

Several studies in recent years demonstrated the nephrotoxic effects of artificial sweeteners like APM and its close relationship with renal dysfunctions.[8,13,26] Similarly, studies also demonstrated that long-term consumption of APM could lead to an increase in the production of harmful free radicals that cause renal tissue damage.[11,13,27]

In the present study, we observed the nephroprotective effects of AA on the APM-induced nephrotoxicity on albino Wistar rats. We found that the majority of rat in-group II showed statistically significant ($P < 0.05$) loss in their body weights. Similar findings reported and supported by the studies conducted in the past few years.[8,12,26] This decrease in body weight may be attributed to the wasting of protein from the body secondary to the reduced availability of carbohydrates as a potential energy source. Another study found that consumption of APM triggers the secretion of a peptide in GIT (glucagon-like peptide) that may result in weight loss.[28] Furthermore, a statistically significant difference in mean weight-loss in-group III i.e. lesser decline weight loss) was observed compared with group II.

In our study, we found that levels of GPx, CRP, urea and creatinine were disturbed significantly after the induction of APM in experimental groups. A statistically significant decline in GPx level in group II and elevation in levels of CRP, urea and creatinine in the same group noticed in comparison with the other two groups. This change in levels may occur due to the toxic consumption of APM resulting in renal dysfunction linked with the damage to glomerular epithelium and filtration resulting from APM metabolite. The findings of other studies are consistent with the current study.[9,11,13,29] These studies demonstrated the negative impact of APM on GPx and other markers of renal dysfunction. These studies also demonstrated that consumption of APM for a longer duration or in high doses causes injuries to the renal tissues leads to the depletion of GPx and eventually disturb the biochemical markers like CRP, serum urea and creatinine. The findings of our study are consistent with the findings these studies confirmed that consumption of APM linked closely with the renal dysfunction.

Moreover, we also observed the levels of serum GPx

that declined due to APM induction but remained sustained with AA i.e. in-group III. While levels of CRP, urea and creatinine found lower in AA treated group (group III) in comparison to group II. These differences in serum values of mentioned markers attributed to the anti-inflammatory and vaso-protective role of AA against the APM. These findings are consistent with the other experimental studies that reported the protective role of AA in the presence of nephrotoxic, hepatotoxic or other substances or drugs.[15,16,21]

Renal histological changes were also observed in this study. In comparison to the control group, in the APM alone group II there is complete to a partial loss of the brush-bordered lining of tubular epithelium and diameter of lumens detected. Remnants of the cell (exfoliated cells) were also present in the lumen of some tubules. We also noticed the increase in urinary space and atrophic glomeruli in some renal corpuscles etc. These findings are consistent with the findings of similar studies.[12,13,27,29]

We observed that AA is effective in decreasing the toxic effects of APM on renal tissues. In-group III, glomeruli appeared normal and showed no significant changes in comparison to group II. Slight edema of tubular cells, the mean diameter of PCTs and DCTs as well as the mean distance between visceral and parietal layers of Bowman's capsule, increased less in comparison with group II. Similar findings were observed by conducted in Egypt.[20]

This study is one of its kind, because to the best of knowledge no other studies are available demonstrating the protective effects of AA in APM induced nephrotoxicity. However, the present study had certain limitations. We had limited time and availability of funds due to which other laboratory tests of renal function (urine analysis, plasma levels of albumin, serum electrolytes) and oxidative stress (malondialdehyde, Catalase) could not be performed. Therefore, further research is recommended to assess the various protective effects of AA on various blood and urine parameters as well as on other organ systems. Moreover, further studies are also recommended to explore the effects of AA in combination with other anti-nephrotoxic agents such as L-Arginine and Resveratrol etc.

Conclusion

The current study concluded that aspartame causes significant hazardous effects on the body, consequently affecting the normal renal tissue and ultimately resulting in severe nephrotoxicity. Histological changes also endorsed these findings and showed the serious damage and alterations in normal renal histology.

On the other hand, ascorbic acid use showed promising results and highly significant protective effects on renal functions and its histological reparations, when given with APM.

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ORIGINAL ARTICLE

Correlation of Serum Uric Acid, Thyroid-Stimulating Hormone and Free-Thyroxine in Subclinical and Overt Hypothyroidism

Fakhra Noureen[1], Saddaf Ayub[2], Abid Saeed Khan[3]

ABSTRACT

Objective: To correlate the serum uric acid, Thyroid-stimulating hormone (TSH) and free-thyroxine (FT4) in overt and subclinical hypothyroidism.

Study Design: Cross sectional comparative.

Place and Duration of Study: Capital Development Hospital, Islamabad from January 2019 to June 2019.

Materials and Methods: Total 114 participants, recruited through convenient non-probability sampling technique, were sub-divided in three groups, comprising 38 participants each. Group I included patients of overt hypothyroidism. Group II had patients with subclinical hypothyroidism and Group III included healthy controls. Serum uric acid levels were measured for all participants. For data analysis, SPSS 21 was used. Statistical significance was estimated using one way ANOVA. *p* value of < 0.05 was considered significant. Correlation between numerical variables was determined by Pearson correlation coefficient.

Results: Mean serum uric acid level in overt hypothyroid patients (7.5±0.84 mg/dL) was significantly raised than the patients in the subclinical hypothyroid and control group (4.7±0.82, 4.6±1.09 mg/dL respectively) with *p* value <0.001. However, there was no significant difference of mean uric acid levels between subclinical hypothyroid group and the control group (*p* value =0.95).

A significantly positive relation was observed between serum uric acid and TSH in group I only (r = 0.53 and *p* < 0.001).

Conclusion: Uric acids levels are increased in hypothyroidism more profoundly in case of overt hypothyroidism, less so when subclinical hypothyroidism is present. In patients of overt hypothyroidism, serum TSH and uric acid levels should be monitored regularly to prevent renal complications in these patients.

Key Words: *Hypothyroidism, Thyroid Stimulating Hormone, Thyroxine, Uric Acid.*

Introduction

Clinically, the commonest endocrine disorder is hypothyroidism. It results from an insufficiency of the thyroid hormones, leading to generalized impairment of various metabolic processes. [1]

Prevalence of hypothyroidism is 3.8%–4.6% in Asian population.[2]

T3 and T4 are the two related hormones synthesized by the thyroid gland. These hormones serve an imperative role in cell differentiation, regulation of thermogenesis and metabolic homeostasis in adults.[3]

Numerous mechanistic links exist between thyroid hormones and the renal system. The kidneys cause local deiodination of T4 by enzyme T4-5 - deiodinase, resulting in production of T3, a more potent form biologically.[4] Conversely, thyroid hormones are crucial for the development of renal system and its physiology. They have effects, both pre renal as well as intrinsic renal, which in turn cause increased renal blood flow and glomerular filtration rate (GFR).[5]

Hypothyroid state causes hemodynamic changes, including a decline in both renal plasma flow and GFR, which may cause derangement of renal function.[6] Also, purine metabolism is affected by lower levels of T3 and T4, which may cause a modification in uric acid (UA) levels.[7]

There are numerous remaining gaps in knowledge with respect to the interactions between thyroid and kidney dysfunction.[6] Previous studies carried out to

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inquire the link between hypothyroidism and UA levels have shown contrary findings.[8,9] Therefore, the present study was designed to correlate the serum uric acid, Thyroid-stimulating hormone (TSH) and free-thyroxin (FT4) in overt and subclinical hypothyroidism.

Materials and Methods

It was a cross-sectional study conducted at Capital Development Hospital, Islamabad after getting approval from institutional Ethical Review Committee.

The study extended over 6 months from January 2019 to June 2019. A total of 114 participants were enrolled and were divided in three groups comprising of 38 participants each. Groups I included overt hypothyroid patients (TSH >4.5 µIU/ml and FT4 < 0.78ng/dL). Group II included subclinical hypothyroid subjects (TSH > 4.5 µIU/mL and normal FT4) and euthyroid individuals were labeled as Group III. Participants from both genders were enrolled through non-probability convenient sampling after taking informed consent. Known patients of diabetes, hypertension, renal and hepatic disorders, patients on treatment of hyperuricemia and pregnant women were excluded from this study. Age group < 18 years was also excluded as reference ranges of TSH and FT4 are universally different depending on age group. Serum TSH was done of clinically diagnosed patients who visited Medical OPD and who were admitted in wards. Subjects who had raised serum TSH levels > 4.5 µIU/mL were further tested for serum FT4. Participants who had TSH > 4.5 µIU/mL and FT4 less than 0.78 ng/dL were labeled as overt hypothyroid. Subjects having raised serum TSH but normal serum FT4 value were recruited as cases of subclinical hypothyroidism. Age and gender matched controls with normal serum TSH level (0.4 – 4.5 µIU/mL) were enrolled.

Venous blood sample was collected. Centrifugation of blood samples was done at 15000 rpm x g for about 15 minutes and serum was separated. Serum was stored at -70° C, until analysis of serum TSH, FT4 and UA was performed for all participants.

Serum TSH and FT4 test was performed using the VitrosECi Immunodiagnostic Systems using FDA approved kits of Johnson's and Johnson's. Serum UA was performed by uricase method on semi-automated chemistry analyzer micro-lab 300.

Data analysis was done by SPSS 21. Frequencies and percentages were computed for each categorical variable such as gender, whereas mean and standard deviation was estimated for numerical variables like age, serum TSH, serum FT4 and serum UA levels. To compare serum TSH, FT4 and uric acid levels in overt hypothyroidism, subclinical hypothyroidism and euthyroid groups, One way ANOVA was performed. Pearson correlation was calculated to find relationship of serum uric acid with serum TSH and FT4 in all the three groups.

Results

Mean age (years) of participants in group I, II and III were 47.5±9.92, 39.6 ±7.39 and 39.2±11.77 respectively. There were 21.1%, 44.7% and 52.6% males and 78.9%, 55.3% and 47.4% females in three groups respectively (Table I). The mean serum TSH, FT4 and UA levels in all three groups are summarized in Table II.

In group I, 58% patients had higher than normal serum uric acid levels for their gender. In group II, all the participants had normal serum uric acid levels while in group III, 8% participants had higher than normal serum uric acid level for their gender.

Among the three groups I, II and III, there was a statistically significant difference (p <0.001) in mean serum TSH levels (48.3±28.24, 23.5±33.11, 3.2±0.54 µIU/mL respectively), serum FT4 (0.42±0.20, 1.08±0.26, 1.54±0.40 ng/dL respectively) and serum UA (7.5±0.84, 4.7±0.82, 4.6±1.09 mg/dL respectively) as determined by one way ANOVA for each of these parameters(Table II).

Post HOC comparison using the Tukey test indicated that mean value of serum UA in overt hypothyroid patients (7.5±0.84 mg/dL) was raised significantly than the patients of the subclinical hypothyroidism and controls (4.7±0.82, 4.6±1.09 mg/dL) with p value <0.001. However, there was no significant difference in UA levels between the subclinical hypothyroid group and the control group (p value =0.95).

There was a significant positive relation between serum UA and TSH levels in group I (r =0.53 and p<0.001). No significant positive relation was found when TSH and FT4 were correlated with UA in each of the groups II and III. (Table III)

Discussion

This study indicates a worsening pattern for serum UA levels from euthyroid condition to overt

Table I: Descriptive Statistics of Age and Gender in Study Groups (N=114)

Variable	Group I (n=38)	Group II (n=38)	Group III (n=38)
Mean Age (years)	47.5±9.92	39.6±7.39	39.2±11.77
% Male Female	8 (21.1%) 30 (78.9%)	17(44.7%) 21(55.3%)	20 (52.6%) 18 (47.4%)

Table II: Comparison of Biochemical Parameters among Study Groups (N=114)

Parameters	Group I (n=38)	Group II n=38)	Group III (n=38)	*p* value (ANOVA)
TSH (µIU/mL)	48.33±28.24	23.53±33.11	3.21±0.54	0.000*
FT4 (ng/dL)	0.422±0.20	1.08±0.26	1.54±0.40	0.000*
Uric acid(mg/dL)	7.5±0.84	4.7±0.82	4.6±1.09	0.000*

*p<0.05 was taken as level of significance

Group I: Overt hypothyroid patients

Group II: Subclinical hypothyroid patients

Group III: Healthy adults

Table III: Correlation of Uric acid with serum TSH, FT4 in three Groups (N=114)

Variables	Uric acid					
	Group I (n=38)		Group II (n=38)		Group III (n=38)	
	r	*p* value	r	*p* value	r	*p* value
TSH	0.539*	0.000*	-0.250	0.130	0.117	0.486
FT4	-0.124	0.442	-0.119	0.476	0.151	0.364

r= correlation coefficient

p<0.05 was taken as level of significance

hypothyroidism. Previous studies investigating the relationship of overt hypothyroidism with hyperuricemia have shown contrary findings[8,9] Therefore, this study was designed to measure uric acid levels in hypothyroidism in our setup.

Our study demonstrated a significant increase in serum UA levels in overt hypothyroid patients when compared to the controls. This finding is in agreement with results of Marwah et al[8], Kaur et al[10], Karanikas[11], Khan et al[12] and Abebe et al.[13] The results of our study indicate a possible effect of thyroid abnormalities, mainly overt hypothyroidism, over purine nucleotide metabolism. This suggests that a reduction in renal blood flow and GFR in hypothyroidism may lead to hyperuricemia.[8] On the contrary, the results of an African study indicated that serum UA levels are reduced in both overt hypothyroidism and hyperthyroidism.[13] Similarly, a study conducted on a large cohort of 2359 patients diagnosed with thyroid dysfunction (hypothyroidism and hyperthyroidism) could not ascertain any positive relation between serum UA and TSH levels.[9] The disagreement between these findings and our study results can be explained through differences in sample size and study population of these studies.

The results of our study demonstrated that there was no significant difference in the mean serum UA levels between the subclinical hypothyroid group and the control group. This finding is in agreement with the results of Ye Y et al[14] who showed that the prevalence of hyperuricemia in euthyroid and subclinical hypothyroid groups had no significant difference (22.8 % vs 21.9% respectively). Similarly the work of K Ashizawa et al[15] demonstrated no significant association between hyperuricemia and subclinical hypothyroidism.

The possible reason that why the subclinical hypothyroid group did not show considerable change in serum uric acid levels when compared with euthypothyroid group can be explained by the gradual deterioration of thyroid function in subclinical hypothyroid patients , which might not be linked with metabolic complications at this stage of subclinical hypothyroidism.[16]

In our study, a significantly positive relation between serum UA and TSH in overt hypothyroid patients was observed. This finding is in agreement with the findings of Marwah et al[8] and Saini et al.[17] The reason for this rise in the levels of UA in case of hypothyroidism may either be an increased generation of uric acid due to hypothyroidism-associated myopathy or a deficient renal clearance resulting from reduced GFR.[17] This relationship may be of significance while evaluating patients having gout, because the excretion of uric acid in these patients might stem from a hypothyroid state. However our results are contrary to Arindam et al[18] who demonstrated no association between UA levels and TSH/FT4 in overt hypothyroidism.

Our study revealed no significant correlation of uric acid with either TSH or FT4 in the subclinical and euthyroid participants. This finding is supported by the results of Raber et al[9] which also did not establish any correlation of TFTs with uric acid in these groups. This might occur because the

derangement of thyroid function is less severe in patients of sub-clinical hypothyroidism as compared to overt hypothyroids.[16]

Limitations

The limitation of this study is that it was a uni-centred, cross sectional study, so it did not help to determine cause and effect relationship between hypothyroidism and uric acid.

Future Recommendations

Further multi-centred studies with larger sample size are recommended. Moreover other biochemical markers for renal functions (serum urea and creatinine along with muscle markers i.e serum aldolase and creatinine kinase) can be considered in relation to hypothyroidism when similar study is planned.

Conclusion

Uric acids levels are increased in hypothyroidism more profoundly in case of overt hypothyroidism, less so when subclinical hypothyroidism is present. A positive correlation of serum TSH and UA in overt hypothyroid patients calls for regular monitoring of serum UA levels in these patients to prevent renal complications.

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ORIGINAL ARTICLE

Anti-Hyperglycemic and Anti-Dyslipidemic Activities of Glycyrrhiza Glabra Root Extract In Diabetic Rats

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ABSTRACT

Objective: To compare the anti-hyperglycemic and anti hyperlipidemic activities of ethanolic extract of Glycyrrhiza glabra (licorice) roots with the standard drugs metformin and glimepiride in streptozotocin induced diabetic rats.

Study Design: Experimental study.

Place and Duration of Study: Animal House of Basic Medical Science Institute (BMSI). Jinnah post graduate medical center (JPMC), Karachi conducted from May 2018 till August 2018.

Materials and Methods: Total seven groups of Wistar albino rats comprising six rats in each were included. Study included negative control and positive control groups, to which 0.9% of sodium chloride was administered. Other five groups of streptozotocin induced diabetic rats were treated with metformin, glimepiride, rosuvastatin, ethanolic extract of Glycyrrhiza glabra (licorice) roots at a dose of 200 mg/kg and 400mg/kg, respectively. The treatment was given for 28 days followed by the laboratory estimation of fasting blood glucose (FBG), fasting serum insulin, Glycosylated Hemoglobin A1c (HbA1c), total lipid profile and serum amylase were evaluated.

Results: A significant decrease was observed in all the glycemic indices at both doses of Glycyrrhiza glabra (licorice) i.e. 200 mg/kg and 400mg/kg, but a more rampant decrease is observed at the dose of 400mg/kg. Similarly, both concentrations of extract showed significant decrease in all lipidemic indices that included HDL-C, VLDL-C, LDL-C, total cholesterol (TC), Triglycerides (TG) and the serum amylase levels.

Conclusion: This study concludes that the licorice herb has sufficient anti hyperglycemic and anti hyperlipidemic effects in diabetic rats without any aberration in pancreatic enzymes, hence it might be beneficial as additional dietary supplements for the effective management of diabetes mellitus along with standard drugs.

Key Words: *Diabetes Mellitus, Hyperglycemia, Hyperlipidemia, Licorice.*

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia along with weakened metabolism of carbohydrate and other essential energy yielding fuels, such as lipids and proteins."[1] Recently, the International Diabetes Federation (IDF) has reported that around 415 million people were having diabetes mellitus in 2015 and this figure is assumed to raise up to 642 million by 2040 throughout the world.[2] This alarming illness is mainly of two types i.e. type 1 which is caused by relative insulin deficiency while type 2 is mainly attributed to insulin resistance.[3] Along with other co-morbidities, dyslipidemia is also associated with poorly controlled diabetes mellitus which can lead to multiple micro and macro vasculopathies including coronary heart disease and stroke that explains early mortalities and morbidities in diabetic patients.[1]

The oral treatment regimen for diabetes mellitus is classified in to insulin sensitizers, insulin secretagogues and miscellaneous group.[4] The management and control of diabetes by these synthetic drugs without adverse effects is a great challenge because mostly all these oral anti hyper glycemic medications have various distressing complications with development of resistance on enduring exploitation.[5,6] Furthermore, conventionally used most oral hypoglycemic drugs have more side effects on which is the awful

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outcome of poorly controlled diabetes.[7] Herbal drugs remain the focus of attraction by the researchers not only in ancient times but still today a large number of World's population believes that herbal medications are sole remedies for a range of diseases.[8] There are about 45000 plants which possess different medicinal properties including anti hyperglycemic and anti-dyslipidemic activities. G.glabra is the root of Glycyrrhiza inflates (Fabaceae) which is commonly known as sweet wood or licorice. It is regularly used herb for culinary and ayurvedic purposes in South Asia since long time.[9,12] According to current literature, G.glabra is medically used for multiple purposes such an antioxidant, antidote for peptic ulcers and gastritis and prevention as well as treatment of common cold.[13] Furthermore, it has potent anti-tussive activity, muscle relaxing property, weight reduction potential, immune boosting action via increasing WBC counts and anti-diuretic and anti-inflammatory effects.[14] The naturally active constituents of G.glabra are glycyrrhizin, liquiritins, liquiritigenin, glycyrrhizin acids and flavones. Glycyrrhizin is the major saponin in licorice root and its metabolite glycyrrhetinic acid is main pharmacologically active form.[15] Together these flavonoids confirm significant anticancer, antioxidative, antimicrobial, and antiviral effects.[16] Moreover, licorice also reduces the liver damage significantly owing to its antioxidant and anti-inflammatory properties as indicated by Chen et al. in 2014.[17]

However, none of the studies mentioned above have aimed to find out the antidyslipidemic properties of G.glabra. Therefore, in our study we have used G.glabra on rats to find out its effects on the different lipidemic parameters. Moreover, we also aimed to find out the effect of different doses of G. glabra on the lipidemic parameters. Therefore, this study was conducted to compare the anti-hyperglycemic and anti hyperlipidemic activities of ethanolic extract of Glycyrrhiza glabra (licorice) roots with the standard drugs metformin and glimepiride in streptozotocin induced diabetic rats.

Materials and Methods

This was an experimental study conducted between May 2018 till August 2018 in Animal House of Basic Medical Science Institute (BMSI), Jinnah post graduate medical center (JPMC), Karachi. G.glabra root was obtained from the local market of Karachi. The plants were authenticated and identified from botany department of Karachi University and taxonomy number was obtained. (Taxonomic number of Glycyrrhiza glabra i.e. Licorice is: 17234) The study was approved from Ziauddin University.

The G.glabra roots were washed and dried separately in open air for 48 hours. The roots were then minced into powder using a mechanical grinder. The powder was then mixed and infused with absolute ethanol at a 1:10 ratio (100 gram in 1 L solvent) for 7 days in separate jars. The extract was filtered through a Whatman No 1 filter paper which was followed by rotory evaporation of fíltrate with the help of rotary evaporators so that the concentrated extract of herb was obtained which was free of ethanol. The crude extract was reconstituted in freshly prepared 2.5% dimethyl sulfoxide DMSO and kept in jar for evaluation of anti-hyperglycemic and anti-dyslipidemic properties in diabetic rats.

Forty-two adult male and female Wistar albino rats (aged 7-8 weeks, weighing 180–240 grams) were purchased from the animal house of Agha khan university hospital. However, it was made sure none of the rats purchased suffered from any other comorbidity that could have affected our results. All rats were kept in (25 ± 3 C, 12 h light/dark cycle) as well as the standard diet and clean tap water the rats were provided. Rats were divided in 7 groups: Group-I negative control non diabetic rats; treated with 0.9% sodium chloride (NaCl). Group II positive control diabetic rats; treated with 0.9% NaCl. Group-III diabetic rats and was treated with glimepiride at 0.1 mg/kg bw. Group-IV diabetic rats and was treated with metformin at 10 mg/kg bw. Group V diabetic rats; treated with rosuvastatin 10 mg/kg/day bw. Group- VI diabetic rats; treated with Ethanolic Extract of G.glabra at a dose of 200 mg/kg .Group- VII diabetic rats; treated with Ethanolic Extract of G.glabra at a dose of 400 mg/kg.

With the exception of negative control group, diabetes was induced to all animals by injecting the solution of Streptozotocin (STZ). The solution was made by dissolving dry powder of STZ in 0.1 M citrate buffer (pH 4.5) that was used after filtration.[17] It was injected as a single dose of 55 mg/kg via intra peritoneal route (i.p) to overnight fastening rats. On

3rd day 1ml blood was taken from tail for FBS from each rat. The rats whose blood glucose level was more than 250mg/dl were considered as diabetic.[18] Herbal extract and standard treatment were given to all the rats except positive control through metallic feeding syringe orally for a period of 28 days.

On 29th day, following overnight food deprivation, the rats were given an anesthesia that consisted of ether solution and were sacrificed as per Institutional Animal Ethics Committee (IAEC) guidelines. A blood sample of 10 ml was collected by cardiac puncture and was transferred into vacuum tubes which were then centrifuged at 3000 rpm for 10 minutes. After centrifugation, sera were separated for different biochemical assays.[19] Glycemic indices such as blood glucose level, serum insulin, Glycosylated Hemoglobin (HbA1c), serum amylase and lipid profile such as total cholesterol (TC), Triglycerides (TG), High density lipoprotein- Cholesterol (HDL-C), Very low density lipoproteins (VLDL- C), Low density lipoproteins - Cholesterol (LDL- C) were measured from serum samples by standard enzymatic methods using commercially available kits (Bartham, Trinder, Richmond and schettler) according to manufacturer advice respectively.

The data was analyzed using the software SPSS version 20.0. The glycemic índices and lipid levels were expressed as mean ± Standard Error of Mean (SEM) that were obtained by analysis of variance (ANOVA) test. The *P* values less than 0.05 were considered statistically significant for all treatment groups.

Results

Effects of Glycyrrhiza Glabra on Fasting Blood Glucose Levels

All the glycemic parameters showed a significant increase in the positive control group as compared to the negative control group at day 29 (Table I). A rampant decrease was observed in glimepiride group. The administration of licorice root extract at the dosage of 200mg/kg and 400mg/kg for 29 days to rats with hyperglycemia (induced by streptozotocin via intraperitoneal route of administration) a significant reduction in the blood glucose concentration, HbA1c and fasting serum insulin in comparison to the result obtained from the positive control group (mentioned in*).

Table I: Effect of Fasting Blood Sugar, Hba1c and Serum Concentration in Comparison With Negative and Positive Control

	FBG DAY 3	FBS DAY 29	HbA1C	Serum Insulin
Negative control	99.72 ± 3.44	103.94 ± 2.15	4.60 ± .44	3.34 ± .47
Positive control	492.16 ± 36.71*	613.33± 34.18*	14.78 ± 1.69*	7.76 ± .65*
Glimepiride	458.33 ± 7.36*	94.68 ± 3.05*	9.64 ± .294*	5.03 ± .16*
Metformin	494.16 ± 10.12*	116.63 ± 9.03*	9.29 ± .28*	5.38 ± .10*
GG 200 mg	447.00 ± 2.28*	285.70 ± 3.92*	14.78 ± 0.10	7.69 ± 0.18
GG 400 mg	451.16 ± 9.82*	259.79 ± 9.92*	14.69 ± 0.33	7.05 ± 0.15

GG: Glycyrrhiza Glabra
FBS: Fasting Blood Sugar
Glycosylated Hemoglobin
*shows P-value < 0.05 (highly significant)

Effects of Glycyrrhiza Glabra Root Onserum Lipid Concentration

Parameters associated with the lipid profile such as TC, TG, HDL- C, VLDL-C and LDL-C were considerably altered in positive control group in comparison with negative control (Table II). Rosuvastatin maximally normalized these values. Both doses of G. glabra also improved these parameters significantly as compared to positive control group (Table II).

Table II: Total Cholesterol TC, TG, HDL, VLDL, LDL, in Control and Diabetic Rats Treated With Glycyrrhiza Glabra

	TC	TG	HDL- C	VLDL	LDL
Negative Control	81.62 ± 1.78	39.43 ± 1.71	42.13 ± 1.59	7.79 ± 0.14	35.88 ± 1.17
Positive Control	264.88 ± 4.30*	211.78 ± 13.41*	32.79 ± 1.60*	54.54± 3.49*	124.45± .82*
Rosuvastatin	83.37 ± 1.80*	60.88 ± 0.96*	49.54 ± 0.50*	11.90± 0.51*	37.28± 0.60*
GG 200 mg	101.43 ± 2.76*	80.13 ± 1.07*	43.63 ± 1.41*	28.36 ± 1.40*	53.75± 0.77*
GG 400 mg	98.47 ± 1.26*	78.52 ± 1.47*	46.27 ± 0.65*	27.03 ± 1.63*	52.47± 1.33*

GG: Glycyrrhiza Glabra
TG: Triglycerides
TC: Total Cholesterol
HDL: High Density Lipoprotein
VLDL: Very Low-Density Lipoprotein
LDL: Low Density Lipoprotein
*shows p value is highly significant

Effects of Glycyrrhiza Glabra on Serum Amylase Levels

There was significant rise in serum amylase level in positive control group) when compared to negative control. G.glabra at both dosages reduced these values significantly (Table III).

Table III: Serum Amylase Level When Compared With Control Groups and G.Glabra Groups

GG: G.GLABRA

*shows p value is highly significant.

Groups	Serum Amylase(m.mol/L) ISD
Negative control	1142.83 ± 5.41
Positive control	1941.00 ± 139.41*
GG 200	1470.32±6.58*
GG 400	1487.21 ± 5.77*

Discussion

Management of diabetes by any means like with oral hypoglycemic drugs or by injecting drugs such as insulin or recently introduced exenatide[21] is a big challenge as nearly all of these drugs have a number of serious adverse effects and distressing complications. Moreover, these agents are generally used in combination to get maximum effects.[22] Finally, on enduring exploitation resistance develops gradually resulting in unsuccessful glucose control and appearance of fearsome complications.[22]

In this scenario the medicinal plants are great blessing as not only they have the potential to cure but also after diagnoses if received earlier herbal formulations derived from plants offer an innate approach for prevention of complications as well.[24] Hence for purpose of evaluating the anti-hyperglycemic properties of G.glabra we constructed a diabetic model of rats with streptozotocin.[25]

Firstly, it is to be noted that FBS values raised significantly at both doses of G. glabra i.e 200mg/kg and 400mg/kg as seen in table I and comparable with other studies done by Han S. *et al.*[26] The optimal blood glucose level must be below 140 mg/dl and this sugar level was achieved more or less only in standard groups. This finding can be attributed to the presence of non-hydrophilic flavonoids that showed alpha glucosidase inhibiting activity enzyme that hydrolyze polysaccharides into simpler form for better absorption of sugars from small intestine.[27] Other possible mechanism may be the activation of peroxisome proliferator-activated receptor-γ (PPAR-γ) as this receptor is responsible for the utilization of energy and homeostasis.[28] Our herb possesses both innate alpha-amylase inhibiting property along with natural PPAR-gamma inhibitory potential and thus modifies two different pathways of glucose metabolism which otherwise will be provided by two different classes of OHG agents.

Secondly, despite significant lowering of FBS after 29 days of treatment, G.glabra failed to improve HbA1c and fasting serum insulin levels when compared with positive control (p value 1.00). The underlying reason may be limited time duration of treatment i.e. 28 days which might be insufficient to produce obvious changes in HbA1c and insulin level.[1,9,29,30] As other studies conducted show that 12 month or greater time span yield in more positive result.[31] Thirdly, in our study the diabetic rats that were treated with G. glabra showed a considerable and significant improvement in all the parameters associated with dyslipidemia in comparison to the positive control group ($p<0.001$) just like the Rosuvastatin group. The anti dyslipidemic factor of G.glabra could be attributed to the presence of phytosterols and saponins in the G. glabra.[32,33,34,35] According to a study the phytosterol can replace the intestinal cholesterol which could lead to a decrease in the amount of cholestrol as it won't be absorbed properly from the intestine.[37] In one study, it was observed that when G. glabra roots when given as 5% and 10% diet for 4 weeks in hyper-cholesterolemic rats and it was observed that lipid levels were drastically reduced and excretion of cholesterol and bile acid seen in feces markedly increased.[35] In another study done by Shalaby *et al*. it was exhibited that G. glabra reduced the levels of TC and TG with no significant change observed in the levels of LDL, HDL and VLDL in male rats.[36] But these results are not consistent with our study as our results show a mark improvement in all parameters related to dyslipidemia which was also seen in Furukawa et al., 2017, Al-Rubeaan et al., 2017, King, 2012, Gaur et al., 2014.

According to the results of our study an increase in HDL and a decrease in TC is observed which can be owed to the increase stimulation of pre-β HDL-C and reverse cholesterol transport, as demonstrated in Rodriguez *et al's* study[38] or due to the suppression of hydroxyl methyl glutaryl-CoA synthase activity by Glycyrrhizin, the active component of G.glabra. The present study illustrated that research herb reduces the bad and improves the good cholesterol levels in streptozotocin induced diabetic rats which could be due to the saponin content of G.glabra root.

As pancreatitis is the common complication of few oral hypoglycemic drugs[39] and is usually encountered clinically as raised serum amylase levels.[17] Therefore,

we aimed to evaluate the effects of our herb on pancreatic enzymes and we found no aberration in this enzyme. The serum amylase levels were significantly increased in the diabetic rats in comparison to the rats in the positive control group. Furthermore the levels of serum amylase reduced back to an almost normal range after 28 days of treatment with G.glabra as seen in Table III.

This data is however scarce regarding the effects of this herb on pancreatic physiology but Xiaoying et al highlights the G.glabra's positive effect on the levels of serum alanine aminotransferase (ALT) and aspartate transaminase (AST) in a cadmium induced hepatotoxicity of animal model and its reversal effect on inflammatory changes of liver.[40]

Conclusion

With the evidence from this study we conclude that G.glabra has significant glucose lowering effects with a striking protective role against dyslipidemia. As an alternative, this herb can be timely utilized for the management of diabetes and associated dyslipidemia without any obvious irregularity of pancreatic functions.

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ORIGINAL ARTICLE

Comparative Study of Aescin and Atorvastatin on Lipid Profile of Albino Wistar Rats

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ABSTRACT

Objective: To observe the effects of Aescin and Atorvastatin on the lipid profile of Albino Wistar rats.

Study Design: Quasi-experimental study.

Place and Duration of Study: Postgraduate research laboratory at ISRA University, Hyderabad from 6th June 2018 to 7th October 2018.

Materials and Methods: Fifty albino Wistar rats were divided into five groups: Group A (Control), Group B (High-fat diet), Group C (Aescin + high-fat diet), Group D (Atorvastatin + high-fat diet), Group E (Aescin + Atorvastatin + high-fat diet). Pre and post-experimental body weight and biochemical analysis was done through ANOVA on SPSS version 22. The significance level was $p \leq 0.05$.

Results: Marked reduction in serum Total Cholesterol (71.36 ±10.1), Triglycerides (83 ±25.66), and Low-density lipoprotein-cholesterol (32 ± 3.76) while elevation in levels of High-density lipoprotein-cholesterol (45 ± 11.85) was observed in Group E as compared to Group B. Statistically significant difference in mean post-experimental body weight body was also observed between all study groups ($p \leq 0.05$).

Conclusion: Combination therapy of Aescin and Atorvastatin has significant protective effects on lipid profile when compared with individual therapy of either drug.

Key Words: *Aescin, Atorvastatin, Cholesterol, Hyperlipidemia, Triglycerides.*

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide, killing more people than any other disease annually.[1] In 2016, around 18 million people were reported to have died from CVDs, representing 31% of all deaths around the globe.[1] Ecological ethnographic studies have reported that South Asian people are comparatively at a higher risk of CVDs than other ethnicities.[2] Alarmingly, CVDs are responsible for more than 25% of deaths in this part of the world.[2] The estimates also show that one in every fifth middle-aged adult in Pakistan may be suffering from subclinical CVDs.[3] This rising toll of CVDs globally is related to the gross incidence of atherosclerotic diseases owing to a sedentary lifestyle and co-morbidities like; Diabetes, Hypertension, dyslipidemia, obesity, etc.

Dyslipidemia is a disorder of lipoprotein metabolism including lipoprotein overproduction or deficiency. It can be aggravated by increased level of Low-Density Lipoprotein-Cholesterol (LDL-C) and triglycerides (TAGs)) or a decrease in High-Density Lipoprotein-Cholesterol (HDL-C) in the blood.[4] The most common form of dyslipidemia is hyperlipidemia. Hyperlipidemias can be classified by specific genetic abnormalities, also termed as familial and alteration in plasma lipoprotein metabolism, which is acquired.[5]

Circulating LDL-C in the blood can invade the artery wall and lead to the development of fatty plaques in a process called atherosclerosis, which is also accompanied by primary endothelial injury.[6]

It has been observed that even 1% decrease in the concentration of plasma lipid levels by lipid-lowering therapies results in a 2% reduction in the prevalence of CVDs[7,8]

There are different classes of drugs that are used to treat hyperlipidemia, which include niacin, fibrates, and cholesterol binding drugs ezetimibe, omega 3 fatty acids and dietary supplements.[9]

Among these, statins are usually the first line lipid-lowering therapy, which primarily targets plasma LDL-C.[10] According to a study, patients who do not

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respond to statin treatment remain at a higher risk of developing CVDs.[11]

Atorvastatin is one of the most efficacious statins having major LDL-C lowering properties. It reduces the production of cholesterol through inhibiting 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA) in the liver.[12]

Similarly, another lipid-lowering agent, Aescin is an important ingredient taken out of Aesculus hippocastanum tree. It is very popular for being anti-inflammatory, anti-edematous and anti-oxidative. It also inhibits the pancreatic lipase in the gastrointestinal tract, preventing the absorption of lipids and increasing the excretion of fat content in feces thus decreasing the total cholesterol, very-low-density lipoprotein cholesterol (VLDL-C), LDL-C and TAGs and an increase in HDL-C levels in serum.[13]

After extensive literature review, it was found no study has been conducted in Pakistan that has demonstrated the comparative effects of aescin and atorvastatin on lipid profile of albino Wistar rats. The current study, therefore, was designed to highlight the potential protective effects of aescin and atorvastatin both individually and in combined form. This will not only provide the baseline for future human studies but also help in designing possible efficacious add-on therapies.

The objective of the current study was to observe the effects of Aescin and Atorvastatin on body weight and lipid profile of male Albino Wistar rats as well as to compare the difference of individual versus combination therapy in reduction of hyperlipidemia.

Materials and Methods

This quasi-experimental study was conducted at the Postgraduate center of ISRA University, Hyderabad from 6th June to 7th October 2018. Fifty healthy male albino Wistar rats of weight range of 175-300g were included using non-probability purposive sampling. All rats of female gender and with any sickness were excluded from the study. The study was approved by the Ethical Review Committee of ISRA University, Hyderabad. The rats were kept in a proper hygienic and well-ventilated environment. Room temperature of 25 ±2°C and day and night cycle per 12 hours was maintained. After an acclimatization period of ten days, all rats were equally divided into five different groups. Group A (Control) received standard chow diet and water ad libitum, Group B received a high-fat diet of 400mg/kg, Group C received Aescin 75 mg along with high-fat diet, Group D received Atorvastatin 80 mg along with high fat and Group E received Aescin 50mg + Atorvastatin 40mg along with high-fat diet.[14,15] Aescin was administered in the form of horse chestnut as its extract contains 70% Aescin.[15] Pre and post-experimental body weights of all experimental animals were recorded. All the rats were euthanized by placing them under the inverted glass jar with chloroform soaked cotton swabs. The rats were sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture through a syringe and then transferred to gel-tubes which were kept in a vertical position and then tubes were centrifuged at 5000 rpm for 5 min to separate serum which was used for biochemical analysis. The estimation of random lipid profile (Total cholesterol, LDL-C, TAGs, and HDL-C) was carried out by Roche diagnostic kit method on an automatic modular analyzer at Isra University Diagnostic Laboratory, Hyderabad.

The data was analyzed using SPSS (Statistical Package for Social Sciences) version 22. One-way analysis of variance (ANOVA) was applied to compare the means of various quantitative variables among groups A, B, and C, D, and E.Statistical significance was taken at $p \leq 0.05$.

Results

The Mean±SD post-experimental body weight in group A, B, C, D, and E was noted as 198+35.90, 284+19.71, 218+32.55, 251+55.01 and 202+48.46 grams respectively and a statistically significant difference was noted ($p<0.05$) among all the groups. A marked increase in body weight was observed in Group B. Aescin and Atorvastatin treated hyperlipidemic rats (groups C and D) revealed a decrease in body weight, with the Aescin group (Group C) showing better results. However, Aescin and Atorvastatin combination therapy group (Group E) showed the best results that reveal the combination therapy prevented the body weight gain significantly (Table I).

The post-experimental biochemical analysis (mean±SD) findings of all study groups are reported in Table II. A statistically significant difference ($p<0.05$) in mean levels of serum cholesterol, TAGs, HDL-C and LDL-C was observed among experimental groups. A significant increase in serum levels of

Table: I Mean Bodyweight (Grams) Levels Among Control and Experimental Groups

Groups	Mean(±SD)	P-value
Group A (Control)	**198(±35.90)**	**0.001***
Group B (Experimental control + High-fat diet)	**284(±19.71)**	
Group C (Aescin + High-fat diet)	**218(±32.55)**	
Group D (Atorvastatin + High-fat diet)	**251(±55.01)**	
Group E (Aescin + Atorvastatin + High fat Diet)	**202(±48.46)**	

Significant Findings (<0.05)

cholesterol, TAGs, and LDL-C while a decrease in serum levels of HDL-C was noted in the hyperlipidemic group (Group B). Aescin and Atorvastatin treated hyperlipidemic rats (groups C and D) revealed a decrease in levels of total cholesterol, TAGs, and LDL-C and an increase in HDL-C levels, with Aescin group (Group C) showing comparatively better results. However, Aescin and Atorvastatin combination therapy group (Group E) showed significant results with near-normal levels of all lipid profile parameters.

Table: II Mean Levels of Lipid Profile Parameters among Control and Experimental Groups

	Groups	Mean± SD	p-value
Serum Cholesterol (mg/dl)	A	78 (±16.7)	0.01
	B	158 (±30.91)	
	C	87 (±25.90)	
	D	96.83 (±27.79)	
	E	71.36 (±10.1)	
Serum Triglycerides (mg/dl)	A	81(±21.20)	0.02
	B	130(±26.42)	
	C	92.6(±36.88)	
	D	110(±28.79)	
	E	83(±25.66)	
Serum HDL-C (mg/dl)	A	40(±9.91)	0.01
	B	27(±10.32)	
	C	42(±12.81)	
	D	30(±9.67)	
	E	45(±11.85)	
Serum LDL-C (mg/dl)	A	38(±6.45)	0.01
	B	88(±8.82)	
	C	37(±2.30)	
	D	50(±5.75)	
	E	32(±3.76)	

Significant Findings (<0.05)

Discussion

The present study is based on comparing the individual and combined effects of Aescin and Atorvastatin respectively. There are few studies that have been conducted on Aescin and its role as a lipid-lowering agent but literature is scarce in terms of finding a research article on combination therapy of Aescin and Atorvastatin.[16] The present study showed that both aescin and atorvastatin have lipid-lowering effects, however, combination therapy of both the drugs is a more potent and efficacious lipid-lowering regimen.

Zhang et al. observed in their experiment that bodyweight of albino rats decreased when Aescin was used in high-fat diet groups. These effects were due to their enzyme inhibition and antioxidant activity. These results are consistent with the present study.[17] In our study, we found Aescin to be effective in improving the lipid profile of Wistar rats. The findings of our study are consistent with the study of Sood S et al. which concluded that Aescin derived from hippocastanum plants is effective in preventing the rise of total cholesterol level.[16]

Lella M et al. and Prasad A et al. reported about combined therapy of Atorvastatin and cholesterol binding drug (Ezetimibe) the studies are consistent with our study that Atorvastatin shows better results in combination therapy but in our study, we used Aescin instead of ezetimibe.[18,19] In this study, we observed that Aescin has significant protective effects on lipid profile of albino rats. However, these protective effects were more pronounced when Aescin was used at a comparatively lower dose in combination with Atorvastatin than Aescin alone.

Avci G et al. conducted a similar study on Aescin and high fed diet rat models, according to their findings, total cholesterol and TAGs didn't show any significant decrease in experimental groups.[20] This particular finding is inconsistent with our study. This difference could be due to the short duration of their study (2 weeks) as compared to the duration of this study being 5 weeks. However, the results are consistent with the present study in terms of HDL-C and LDL-C levels as in both studies HDL levels have increased and LDL-C levels decreased with treatment of Aescin both on low and high doses respectively.[20]

Sood S et al. reported in a very similar study on Aescin and its effects on hypercholesteremia as a lipid-lowering agent, their results in terms of HDL-C and LDL-C are similar to the results of the present study as in both HDL-C levels are being increased and LDL-C levels are decreasing.[16] Chatley P et al. conducted an experiment in which he evaluated that the low dose of Atorvastatin (5mg/day) and Finofibrats (160mg/day) in combination therapy was equally effective as compared to high dose of

Atorvastatin (10-40mg) and fenofibrate (160mg-200mg) when given individually.[21] These findings were consistent with the present study. However, they also observed that the combination therapy not only decrease the lipid profile but cause side effects related to high dose. However, the side effects were not observed in the present study, but can be recommended for further studies to strengthen the present study.

With strengths, our study had certain limitations. We could not see the effects of the drugs on other parameters such as high-fat diet-induced cardiovascular toxicity and oxidative stress due to monetary limitations and time constraints. Therefore, further work should be carried out to see the effects of these drugs on other organ systems as well as to compare the side effects of statins and Aescin. Aescin can be used as an add on therapy to conventional treatment of hyperlipidemia. However, this can be made available by conducting maximum experimental and clinical trial to further prove its significance.

Conclusion

This study concludes that both Aescin and Atorvastatin are efficacious in lowering lipid levels. However, Aescin showed significant results as compared to Atorvastatin, whereas combination therapy is most effective in reducing hyperlipidemia.

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ORIGINAL ARTICLE

Awareness and Perceptions Regarding thalassemia Major and Premarital Screening in General Public of Cantonment areas of Rawalpindi

Iffat Noor[1], Rukshana Roshan[2], Rehama Gilani[3]

ABSTRACT

Objective: To assess awareness and perception of general public regarding thalassemia and premarital screening and determine the association of various socio demographic variables with awareness of public.

Study Design: This was a descriptive cross-sectional study.

Place and Duration of Study: The study was conducted at Rawalpindi cantonment areas from 1st June 2017 to 20th December 2017.

Materials and Methods: This study was conducted among 200 people in 20 to 60 years age group, residing in different settings of Rawalpindi cantonment over a period of six months. Data was collected from respondents selected through multistage probability sampling by pretested and validated questionnaire.

Results: Statistical analysis showed 48.5% of participants had adequate knowledge about thalassemia and premarital screening, while 39.8% perceived its importance and 39.2% perceived that legislation will be beneficial for prevention of thalassemia in our country.

Conclusion: Based on study results it is concluded that majority of participants had adequate knowledge regarding thalassemia and premarital screening. Education is highlighted as significant factors towards their perception regarding importance of mandatory premarital screening legislation in Pakistan.

Key Words: *Awareness, Genetic, Pre-Marital Screening, Thalassemia.*

Introduction

Thalassemia is a hereditary blood disorder, which along with medical complications having psychological, social and financial impact at the patient, their family and whole society. It is categorized into alpha or beta thalassemia depending on absence of globin chain. Its occurrence is high in a broad belt which extends from Mediterranean basin through to Middle East, South East Asia, and Indian subcontinent. Globally 15 million patients have clinically apparent disorder.[1] In Pakistan, frequency of β-thalassemia gene is 5-8% and is present in all ethnic groups. It is estimated that approximately 9 million β-thalassemia carrier are here leading to more than 5000 births of transfusion-dependent thalassemia (TDT) each year in Pakistan.[2] Presently there are estimated 100,000 cases of thalassemia in Pakistan, which making up for almost 5% of world cases. This situation is serious and alarming in our country as thalassemia can be a serious threat in coming years in absence of appropriate genetic counseling and proper screening. Consanguinity is the main factor to high prevalence in Pakistan.[3]

Thalassemia diagnosis is clinical along with laboratory assistance in the form of blood complete examination, Hb electrophoresis and confirmation by genetic analysis if obligatory. The treatment approach varies according to socioeconomic condition of parents and the country. The best treatment choice is bone marrow transplant that is available in our country but its high cost is a major hurdle. The other treatment options are supportive like repeated blood transfusion along with iron chelation therapy, splenectomy and Hb F augmentation. This lifelong transfusion therapy with iron chelation therapy puts a huge financial burden.[4]

Thalassemia is a major public health problem, but it is preventable by adopting various preventive strategies and methods. Incidence of thalassemia major has been reduced in many countries through

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effective preventive programs. According to Pakistan Bureau of Statistics, per capita annual income during 2013 was $1380 as, comparison to the estimated 6000 dollar per year cost of appropriate management of a thalassemia case.[5] Thalassemia screening preceding marriage is actually a more easier and cost-effective tool. Government of Pakistan is now planning for the implementation of a law for pre-marital screening as a mandatory procedure to curtail the burden of this disease. The aim of this study was to determine the awareness of general population about thalassemia and their perception regarding the pre-marital screening law for thalassemia, as no such survey has been conducted among the general population.

Materials and Methods

This descriptive cross sectional study was conducted in areas of Rawalpindi cantonment during six months from 1st June 2017 to 30th December 2017. Study population comprised of general public residing in the sampled areas. People of both genders between age group of 20-60 years, willing to participate, were included in the study. The ethical approval was taken from Institution Review Board (IRB). Sample size was calculated based on the prevalence of knowledge about thalassemia in Pakistan.[6] Multistage probability sampling technique was used. At first stage of sampling, ward and areas were selected. Rawalpindi cantonment consists of twelve wards, each having 12-14 residential areas. Through simple random lottery mechanism, ward number eight was selected, which has twelve residential areas. From this ward every third area was selected by using simple random lottery again. Fifty participants were selected from each area by convenience sampling method to achieve a total sample size of 200 respondents. The data collection instrument used was a self-designed, self-administered questionnaire developed with the help of previous studies comprising of both open and close-ended questions. Data analysis was done by using version 22 of SPSS. In descriptive analysis mean and standard deviation of age and years of education was calculated. Frequency and percentage of all categorical variables like gender, marital status, consanguinity of parents, consanguinity of couples, family history of thalassemia and knowledge (adequate, inadequate) were determined. Chi square test was applied to examine possible association between socio demographic characteristics and awareness of participants p- value < 0.05 was taken as significant.

Results

The number of sampled participants completed the questionnaire were 200 with a response rate of 99.5%. The sociodemographic characteristics are given in table I. A significant number of study participants 140 (73.3%) claimed having awareness about thalassemia. Among them 115(61.5%) could correctly identify thalassemia as hereditary diseases. 140(76.1%) participants correctly identified pallor as most apparent sign of thalassemia. 157(87.2%) respondents correctly identified thalassemia can lead to decreased blood formation. 114 (62.3%) were not aware regarding availability of thalassemia screening test, 64(35.2%) identified television as the major source of information, 147(86.0%) correctly identified blood transfusion as main treatment of thalassemia, 119(66.9%) participants knew about the importance of premarital screening who identified it as a blood test of a couple before marriag74(39.8%) of participants strongly agreed that premarital screening is a reliable preventive measure for thalassemia71(35.5%) participants strongly agreed, that legislation is important 105 (51.6%) considered that involvement of religious scholars will be one of the beneficial preventive strategies for thalassemia, adequate awareness was observed about thalassemia and premarital

Table I: Demographics Characteristics of Participants

Serial	Variables		N (%)
1.	**Gender**		
	a.	Male	105(52.5%)
	b.	Female	95(47.5%)
2.	**Marital Status**		
	a.	married	139(70.9%)
	b.	unmarried	57(29.1%)
3.	**Consanguinity of Parents**		
	a.	Yes	117(62.2%)
	b.	No	71(37.8%)
4.	**Consanguinity of Couples**		
	a.	Yes	85(43.8%)
	b.	No	69(35.6%)
	c.	Not applicable	39(20.1%)
5.	**History of thalassemia in family**		
	a.	Yes	35(18.9%)
	b.	No	135(73.0%)
	c.	Do not know	15(8.1%)

screening in 103(51.5%) participants. Chi square test showed that educated participants were more likely to have adequate knowledge while consanguineous marriages had association with inadequate knowledge.

Table II: Association between Demographic Characteristics and Awareness of Respondent about Thalassemia (N=200)

Demographic characteristics	Awareness Score		Chi square (df)	P-value
	Adequate	Inadequate		
Age				
Mean and below mean	52(46.4%)	60(53.6%)	0.533(1)	0.457
Above mean	42(51.9%)	39(48.1%)		
Gender				
Male	50(47.6%)	55(52.4%)	0.69(1)	0.793
Female	47(49.5%)	48(50.5%)		
Marital status				
Married	64(46.0%)	75(54.0%)	1.649(1)	0.199
Unmarried	32(56.1%)	25(43.9%)		
Educational status				
Educated	89(66.9%)	44(33.1%)	40.86(1)	0.001
Uneducated	6(12.8%)	41(87.2%)		
Consanguinity of couple				
Yes	30(35.3%)	55(64.7%)	11.48(2)	0.003
No	38(55.1%)	31(44.9%)		
Not applicable	26(65.0%)	14(35.0%)		
Consanguinity of Parents				
Yes	56(47.9%)	61(52.1%)	0.143(1)	0.706
No	36(50.7%)	35(49.3%)		
Family history of Thalassemia				
Yes	17(48.6%)	18(51.4%)	1.654(2)	0.437
No	67(49.6%)	68(50.4%)		

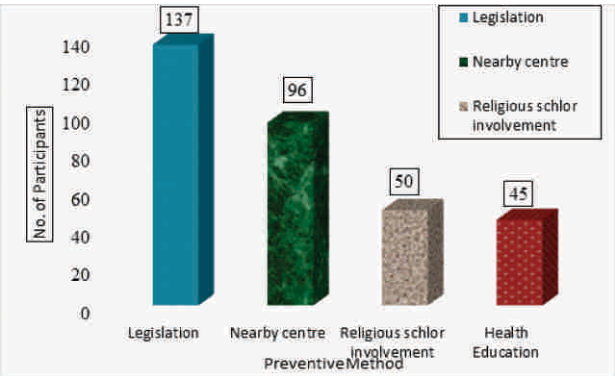

Fig 1: Opinion of Participants about Most Appropriate Preventive Method for Thalassemia

Discussion

In this study, participants with different sociodemographic background were approached and their awareness and perceptions were assessed. Only education was found to be associated with better awareness while no association was found among other variables (age, gender and marital status). This is in accordance with a study conducted in Kolkata.[6] Another study in Iran[7] showed females reflected better knowledge of thalassemia, and in Bahrain[8] university students and professionals who were married had better knowledge of thalassemia. Adequate awareness about thalassemia and premarital screening was observed in 48.5% of participants in our study which is higher than a study conducted in Karachi University where 78% students did not have adequate awareness about the disease and its consequences.[9] Kolkata[6] and Sri Lanka[7] study, conducted in general population showed 57.94% respondents had better knowledge about thalassemia. The study conducted at rural Bengal by Mittak et al[8] and at Lahore by Ishaq et al[9] showed 22.27% and 44.6% participants respectively were aware that thalassemia is a genetic disorder. Our study revealed that about 61.05% had adequate knowledge about the inherited nature of the disease; this is similar to Kolkata[6] study. It was also observed in Kolkata study[6] that two third of the study population were aware about blood transfusion as the essential treatment. The reason is the untiring efforts of many local and international thalassemia societies working at grassroot level in the communities. Another reason is that regular blood transfusion creates much economic strain on families that people near to them also become aware of this difficult treatment. Premarital screening is playing a vital role in reducing burden of disease in countries where it is mandatory by law. Studies conducted in Saudi Arabia[10,11,12] Oman[13] and Palestine[14] showed that majority of respondents were aware of premarital screening program but survey conducted at Quetta[15] showed only 35.2% respondents had awareness about premarital screening. The limitation of this study is the short duration of the project and it does not give insight about the disease among the rural population.

Conclusion

This study concluded that although our local population has adequate knowledge regarding thalassemia and premarital screening, it is imperative that government should implement the law in letter and spirit in order to reduce the burden of disease.

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ORIGINAL ARTICLE

Clinical Patterns of Aluminum Phosphide Poisoning and Its Association with Different Age Group & Gender

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ABSTRACT

Objective: To determine common clinical patterns of Aluminum Phosphide poisoning in patients received at tertiary level and its association with different age groups and gender.

Study Design: Descriptive cross sectional study design.

Place and Duration of Study: This study was conducted in Forensic Medicine Department and Medicine Department, Khyber Medical College, Peshawar from April 2017 to March 2018.

Materials and Methods: A total of 264 patients presenting and admitted with Aluminum Phosphide poisoning were recruited in this study through consecutive non-probability sampling and clinical features were recorded. A pre-designed performa was used to extract the data. SPSS 25.0 was used to measure mean±S.D for numerical variables and frequency with percentages for categorical variables. Association was made via Chi-Square; p-value of ≤0.05 was taken as significant.

Results: Sample of 264 patients had a mean age of 32.07±11.03 years, in which 64.4% were males & 35.6% were females. In the study, 45.1% had arrhythmias, 60.2% presented with ECG changes, 30.3% had shortness of breath and 80.3% with vomiting. The study recorded P-value of ≤ 0.05 for gender and age groups with all clinical features except shortness of breath, showing a significant association.

Conclusion: It was concluded that vomiting is the most common clinical feature in Aluminum Phosphide poisoning, in which males of younger age are more prone to this poisoning. A significant association was recorded for age groups and gender with all clinical features except shortness of breath.

Key Words: *Aluminum(MeSH),Arrhythmias (MeSH), Clinical Patterns (MeSH), Poisoning (MeSH),Shortness of Breath (MeSH), Vomiting (MeSH).*

Introduction

Aluminum phosphide is one of the most emerging poisons worldwide with no antidote available for the poison.[1]In recent past, this poison is nowbeing used as product of preservation in agriculture industry, with beneficial sidesthe same poisonhas also contributed a lot in suicidal and homicidal deaths.[2] Since 1940's this poison is being in the industry for its uses because of cheap, long lasting and effective fumigant characteristics and the gaseous form has easy with quick penetration producing early results.[2] A tablet of brownish dark color 3gm each having 20mm diameter and thickness measured as 5mm is being packed in pen structured plastic/tin with air tight feature keeping it fresh till not opened.[3]

The tablet after exposure to moisture releases phosphine gas which has some toxic effects. This toxin release has its effects on cardiovascular, respiratory, gastrointestinal and uro-genital systems.[4] The poisoning presents with nausea with vomiting, pain in the abdomen, shock which is unresponsive to the traditional treatment, ECG changes including arrhythmias and respiratory effects like edema and dyspnea.[5] Rare clinical manifestations like sensorium, tubular necrosis (acute form) and complications like pericarditis, cardiac failures can also be appreciated.[5] The active form of the compound is mostly fresh and has high mortality due to metabolic acidosis.In contrast the inactive and granular form leads to less severe effects than active form and has low death rates.[6] Dosage, severity, duration, failure to response are few criteria which determine the mortality of this poison, and in addition, hypomagnesaemia also contribute to be a major mortality factor.[7]Severity of

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hypotension, metabolic acidosis and severe vomiting are few other factors that determines the increase indeath ratio of this poison.[8] In clinical suspicion, history holds main pillars for detection and diagnosis of this poison, while in labs exhalation of phosphine detected by positive silver nitrate paper test is taken gold standard. This biochemical examination is the second mean used for confirmation.[2] Early detection holds an important landmark in saving life of such patients which are further treated with early gastric lavage followed by vasopressors and supportive care. Magnesium sulphate intravenously may act as treatment of choice however no antidote is available.[9] In a research, Gupta, et al.[10] studied 30 positive cases of this poison to find out the clinical profile, most of the poison was orally taken and was leading towards cardio-pulmonary shock in extreme poisoning cases. In literature acute renal failure and myocarditis were being reported as end organ damage after 12 hours.[9,10] Due to high mortality of this poison it is being regarded as "Killer with high mortality rate" in human beings.[9] Metabolic acidosis post vomiting is being reported to be as commonest cause leading to death in cases with this poison.

This study will enlighten the clinical patterns after Aluminum Phosphide poisoning, which would help all practitioners in general and medical clinicianswith forensic specialist in specific to identify the poisoning and to start further workup neededto decrease morbidity or mortality related to this poison. The objective of the study was to determine common clinical pattern of Aluminum Phosphide poisoning in patients received at Khyber Medical College, Peshawarand its association with different age groups and gender.

Materials and Methods

This cross-sectional study was conducted in the in the Department of Forensic Medicine and Medicine, Khyber Medical College, Peshawar from April 2017 to March 2018. A total of 264 patients based on consecutive non-probability sampling and presenting with AluminumPhosphide poisoning were recruited in the study. Ethical & Review board approval was taken from Institutional Ethical & Review board. All the patients of both gender and age ranging from 14 to 60 years presenting to the Emergency department of Khyber Teaching Hospital and being diagnosed as Aluminum Phosphide poisoning (vide Toxicology Laboratory of Forensic Medicine Department KMC) and then admitted in Medicine Department were included in this study. Patients with co-morbidities like history of asthma, COPD, known cardiac diseases, and history of thyrotoxicosis were excluded from the study. After taking informed written consent a self-administered pre-designed performa was used to extract the data including clinical features. To analyze the parametric data SPSS v25.0 was used and descriptive statistics were applied on numerical and categorical variables. Association was made vide Chi-Square; p-value less than ≤0.05 was taken significant

Results

The study was conducted on 264 patients presenting with Aluminum Phosphate poisoning. The mean age of the sample was 32.07±11.03 years. On grouping the sample in different age groups, it was observed that 101 (38.3%) of patients were in the age group of 14-25 years, 69 (26.1%) were in the age group of >25 to 35 years, 52 (19.7%) patients were in the age group of >35 to 45 years and 42 (15.9%) were in the age group of >45 to 60 years.

While distributing the patients with regards to gender, it was observed that in our study 170(64.4%) of the sample were male and 94(35.6%) were female gender. Out of 264 patients presenting with Aluminum Phosphate poisoning, 119(45.1%) had arrhythmias, 159(60.2%) had ECG changes, 80 (30.3%) had shortness of breath and 212 (80.3%)had vomiting.

Table I: Association of Clinical Features with Different Age Groups

Age Group	Clinical Patterns							
	Arrhythmia	Sign*	ECG Changes	Sign*	Shortness of Breath	Sign*	Vomiting	Sign*
14 to 25 years	25	< 0.001	38	< 0.001	38	0.098	77	< 0.001
> 25 to 35 years	14		41		14		69	
>35 to 45 years	38		38		14		38	
> 45 to 60 years	42		42		14		28	

*Chi-Square test was used for significance.

Table II: Association of Clinical Patterns with Gender

Gender	Clinical Patterns							
	Arrhythmia	Sign*	ECG Changes	Sign*	Shortness of Breath	Sign*	Vomiting	Sign*
Male	92	< 0.001	132	< 0.001	53	0.678	145	0.006
Female	27		27		27		67	

*Chi-Square test was used for significance.

The clinical features with regards to different age groups and gender were stratified and association was measured using chi square test taking p value of ≤ 0.05 as significant. All clinical features like arrhythmias, ECG changes and vomiting showed a significant relationship with age group having a P-value of < 0.001 for all while shortness of breath had insignificant relationship with a P-value of 0.098. In the same way, when gender was compared with clinical features, all features except showed a significant result with a P-value of < 0.001 while again shortness of breath had an insignificant association with P-value of 0.678.

Discussion

This cross-sectional study showed male predominance in Aluminumphosphide poisoning cases with a frequency of 64.4%. The study also revealed that mostly young adults with a mean of 32.07 ± 11.03 years are reported with this poisoning. A significant relationship was recorded between age group and gender with all clinical features (p-value = <0.001) except shortness of breath.

This poison is extremely dangerous in its early stage, the ingestion of which is mostly suicidal and very much uncommon being accidental and almost rarely it is used as homicidal. The absence of specific treatment particularly the antidote has made this poison a killer and has a high mortality. With time this poison has gained a lot of importance for its preservative properties in agricultural sector and is now easily available.[11]

Khodabandeh, et al.[12] suggests figures with a male–female ratio of 55:45 and mean age of 26 ± 8 years which is slightly different from what we extracted however in both studies young age group and males as gender was more prone to poisoning.

The effects of this poison on cardiovascular system, particularly heart in the form of arrhythmias and ECG changes can be compared with many studies, a study conducted at India in 1991 showed cardiac arrhythmias and disturbance in about 38.2 % of patients which is quite near to the results extracted in this study.[13] In another study conducted at Iran about 80% of patients ingesting Aluminum Phosphide showed different cardiac signs on ECG which is quite different from results of this study.[14]Cardiac toxicity of this poison comprises of circulatory failure like hypotension, heart congestion, edematous myocardial fibres due to edema, vacoulation of cardiac myocytes, necrosis having infiltrates of neutrophil and eosinophil are mostly found, detected and appreciated in autopsy. Significant increase of ventricular dimension mostly left leading to hypokinesia, akinesia and reduction of ejection fraction which in turn causes severe form of hypotension, and disrupts systemic venous pressure while pulmonary artery wedge pressure remain normal, ECG shows particular abnormalities which can be differentiated easily.[15,20]

Corrosive lesions of gastrointestinal tract particularly stomach and esophagus leads to hematemasis after vomiting, epigastric pain, erosion at both duodenum and esophagus, strictures (which in turn lead to dysphagia) and fistulas formation.[21-22]This apparent and visual form of dysphagia may appear with in 3 to 4 days after ingestion but it also may take upto 2 weeks.[23]Farzaneh E, et al.[14] in a study conducted at Iran also showed Nausea/Vomiting as a major clinical presentation of Almuninium Phosphide poisoning along with other gastrointestinal features which is in similarity with the this study.

Shortness of breath demonstrated in this study can also be correlated with a study by Chugh SN, et al.[24] which shows 40% patients showing respiratory clinical feature like tachypnea, dyspnea, crepitations and rhoonchi. All such patterns tends to appear with in or after four to forty eight hour of ingesting this poison causing arterial pressure to be reduced, O_2 saturation decreased without a possible increase in pulmonary artery wedge pressure and clearly suggesting the non- cardiogenic effect. These findings obviously states that this feature is produced by this poison.[25] The non-cardiogenic feature may also lead to adult respiratory distress with non-specified edema that would be protein rich and hemorrhagic.[8]

It is assumed that the region of study has a lot of Aluminum phosphide poisoning cases but due to lack of support for ordinary people and insufficient awareness, maximum aren't reported to concerned authorities. This compelled the authors to have a smaller sample size. To validate the study further and incorporate more convincing results in literature, it is suggested that a study plan involving different regions and a big sample will prove much fruitful.

Conclusion

It is concluded that vomiting is the most common clinical feature in Aluminum Phosphide poisoning, in which males of younger age are more prone to this poisoning. A significant association was recorded for age groups and gender with all clinical features except shortness of breath.

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ORIGINAL ARTICLE

Incidence of Aphthous Ulcers in All Forms of Tobacco Users, Mixed Habits and Non-Users

Rabia Masood, Hadia Malik, Laiba Gul, Zarmeena Imtiaz, Ume Hani Sajjad

ABSTRACT

Objective: The aim of this study was to test the association between recurrent aphthous ulcers and different forms of tobacco habits.

Study Design: Hospital based cross-sectional study.

Place and Duration of Study: The study population consisted of patients attending the Out Patient Department of Islamic International Dental Hospital Islamabad. A hospital based study is carried out for 2 successive months (July-August) 2018.

Materials and Methods: Study was conducted on 500 patients to assess the presence of aphthous ulcers. Questionnaire based data was collected along with the clinical examination. Questionnaire included both quantitative and qualitative variables. Quantitative variables; Age, Frequency of addictive habits, Duration of addiction, Size of ulcer, No. of lesions, Duration of ulcer and Qualitative variables; All types of Addictive habits (smoking and smokeless tobacco), Medical history, Frequency of recurrence of ulcers, Site of ulceration, Type of aphthous ulcers. Statistical analysis was carried out using SPSS software version 23 and chi-squared test was applied.

Results: Out of 500 subjects, 33 (6.6%) participants presented with aphthous ulcers. 78 subjects had addictive habits of smoking tobacco. Among them, Cigarette Smokers were 75 (15%), Hookah Smokers were 2 (0.4%) and 1 was a Bidi Smoker (0.2%). 23 subjects had addictive habits of using smokeless tobacco. Among which, Paan Chewers were 7 (1.4%), Gutka Chewers were 3 (0.6%) and 13 were Naswar Chewers (2.6%).

Conclusion: Although no significant association has been found between aphthous ulcers and smoking habits but ulcers were found to be lower in patients who are smokers as compared to the non-smokers.

Key Words: *Stomatitis, Aphthous Ulcer, Tobacco Smoking, Smokeless.*

Aphthous ulcer is a common condition, also known as “canker sores” or “aphthous stomatitis”. The term aphthae is derived from Greek word “Aphthi” which means “to set on fire” or “to inflame”.[1,2] It is characterized by the repeated formation of benign and non-contagious ulceration of the oral mucous membrane.[3] The ulcers present as lesions having yellow ulcerated base surrounded by erythematous halos and covered by fibrino-purulent membrane.[4,5] [6]Morbidity of Recurrent Aphthous Ulcer (RAS) is quite high affecting quality of life of patients in a way that they are painful and recurrent mucosal lesions causing discomfort while eating, drinking and speaking.

There are three clinical variations of aphthous stomatitis;

- Minor aphthous ulceration
- Major aphthous ulceration
- Herpetiform aphthous ulceration

Exact etiology of RAS is unknown but the condition is associated with multiple factors including autoimmunity, genetic predispositions, hematologic abnormalities (anemia), HIV, hormonal fluctuations, arthritis, stress/anxiety, nutritional deficiencies, trauma, drugs, food hypersensitivity, smoking cessation and allergies.[1,3,5,7]

RAS is associated with human leukocyte antigen (HLA) and immune-dysregulation. Lymphocytes are the predominant cells in pathogenesis of RAS with a variation in CD4:CD8 ratio during pre-ulceration, ulceration, and healing stage. [4,5] Tobacco reduces immunity and T cell response to various antigens so that the association appears to be biologically plausible. [8]

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The management of patients with RAS comprises application of topical analgesics, corticosteroids, antibiotics and anti-inflammatory agents that only provide symptomatic relief.[13]

There are different types of tobacco being used in Pakistan which includes *smoking tobacco* i.e. cigarettes, cigar, pipe, hookah, shisha and bidi and *smokeless tobacco* include paan, gutka, naswar, oral snuff, snuss (moist snuff), khaini (tobacco and lime) and lozenges.

Although studies have failed to find the exact etiology of Recurrent Aphthous Stomatitis but tobacco use is the one most debatable and confused anticipated factor as tobacco usage is associated with various oral pathologies such as Oral squamous cell carcinoma, periodontitis, gingivitis, tobacco pouch keratosis, oral sub mucous fibrosis and nicotine stomatitis etc., so tobacco usage should logically lead to occurrence of Recurrent Aphthous Stomatitis. However, in contrast to this a number of studies have shown negative correlation between RAS and tobacco usage and positive therapeutic effects of smoking. [3] Tobacco usage causes thickening (keratinization) of oral mucosa which renders the mucosa less susceptible to ulceration. [4 5] Smokers quitting with nicotine chewing gums have less chances to develop ulcers than those without nicotine replacement therapy. [9]

Previous studies have suggested negative association between tobacco usage and RAU but most of those studies assessed relationship between RAS and tobacco by using methods that were based on interviews, questionnaire, or on self-reporting of smoking status.[3,10,11]

However, the studies that were previously carried out did not evaluate occurrence of aphthous ulcers in different forms of tobacco users, mixed habits and non-users. In our study we wanted to evaluate the strength of association between occurrence of aphthous ulcer and tobacco usage and incidence of aphthous ulcer among different types of tobacco users in our population and comparing them with non-users because no such study has been done in our community.

The objective of this study was to assess the association between recurrent aphthous ulcers and different forms of tobacco habits.

Materials and Methods

Hospital based cross-sectional study design was used to assess the incidence of aphthous ulcers in tobacco users, non-users and those with mixed habits.

The study population consisted of patients attending the Out Patient Department of Islamic International Dental Hospital Islamabad. A hospital based study was carried out for 2 successive months (July-August) 2018. Study was conducted on 500 patients who visited OPD of dental hospital for seeking dental treatment. All subjects were interviewed and a structured questionnaire was developed to record their details. The questionnaire contained four main sections (addictive habits/tobacco usage history, aphthous ulcer related medical history, ulcer characteristics and demographics). The *Addictive habits section* had two domains; Smoking tobacco domain comprised of six tobacco usage habits (smoking cigarettes, cigar, hookah, pipe, shisha, bidi) and Smokeless tobacco domain also had six habits (paan, ghutka, naswar, snuff, lozenges, other habits). *Medical history* associated with the occurrence of aphthous ulcers included Anemia, HIV, Hormonal fluctuations, GI disorders, Arthritis, Stress/anxiety, Allergies and genetic predisposition. *Ulcer characteristics* comprised size of ulcer, number of lesions, site of ulceration, frequency of recurrence and duration of ulcers.

Informed Consent was taken from all the participants before conducting the study. The participants were asked whether they had oral ulcers (aphthous ulcers) present in their mouth after describing aphthosis to them as recurrent painful ulcers. Additional information about ulcers like duration, location, size, recurrence, and no. of ulcers was noted. Moreover, risk factors that might be related to condition were inquired (stress, hormonal factors, GD disorders, allergies).

Participants were classified into 3 groups and the selection criteria for the groups are given below:

Control Group:

Inclusion criteria included male and female of 15 years and above, subjecting without any ulcers and without any addictive habits.

Exclusion criteria included patients under 15 years, subjecting with ulcers and with addictive habits

Smokers group:

Inclusion criteria included male and female patients of 15 years and above, subjecting with smoking habits (Cigarette, cigar, pipe, hookah, shisha, bidi) and with/without ulcers.

Exclusion criteria included patients under 15 year

subjecting without any smoking habits.

Non-smokers group:

Inclusion criteria included male and female patients of 15 years and above, Subjecting without any smoking habits, with smokeless tobacco habits (paan, gutka, naswar, snuff, lozenges) and with/without ulcers.

Exclusion criteria included patients less than 15 years, subjecting with smoking habits and without any smokeless tobacco habits.

To assess the presence of aphthous ulcers, oral mucosal examination and questionnaire were completed for 500 patients reporting to the OPD over a 2-month interval by four examiners. History of addictive habits was taken and tobacco usage was measured on the basis of type of tobacco used, frequency of consumption per day and the duration for which the individual maintained this frequency. To avoid confounding, patients with known history of systemic diseases and other conditions that might influence occurrence of aphthous ulcer were also recorded. And finally on the basis of ulcer characteristics, aphthous ulcerations were categorized into minor, major and herpetiform ulcers.

Both quantitative and qualitative variables were part of this study.

Quantitative variables; age, frequency of addictive habits, duration of addiction, size of ulcer, no. of lesions, and duration of ulcer.

Qualitative variables; All types of Addictive habits (smoking and smokeless tobacco), Medical history, Frequency of recurrence of ulcers, Site of ulceration and types of aphthous ulcers.

Statistical analysis was carried out using SPSS software version 23. Frequency and percentages of different variables were calculated using SPSS and formulated in tables 1, 2, and 3.

Results

All 500 subjects were asked about their medical histories. Out of 500, only 5 subjects (1%) were anemic. 10 subjects (2%) had hormonal disorders related with puberty, menstrual cycle and pregnancy, 83 subjects (16.6%) had GI disorders related to acidity, 7 subjects (1.4%) had arthritis, 74 subjects (14.8) experienced stress related ulcerations during exams or social issues. 61 subjects (12.2%) were allergic to dust, pollen and medications, and 5 subjects (1%) presented with family history of recurrent ulcers.

Table I: Self-Reported Medical History of Patients

	Anemia	Hormonal	HIV	GI disorders	Arthritis	Stress	Allergies	Genetics
Frequency	5	10	0	83	7	74	61	5
Total	500	500	500	500	500	500	500	500
Percentage	1	2	0	16.6	1.4	14.8	12.2	1

Out of 500 subjects, 78 subjects had addictive habits of smoking tobacco. Among those 78, *Cigarette Smokers* were 75 (15%), *Hookah Smokers* were 2 (0.4%) and 1 was a *Bidi Smoker* (0.2%). (Graph 1). From 500 subjects, 23 subjects had addictive habits of using smokeless tobacco. And of those 23, *Paan Chewers* were 7 (1.4%), *Gutka Chewers* were 3 (0.6%) and 13 were *Naswar Chewers* (2.6%). (Graph 2). From a group of 101 subjects that presented with addictive habits of either smoking or smokeless tobacco 46 were addicted for more than a period of 7 years. Table-II illustrates distribution of duration of addiction among addicts:

Table II: Duration of Tobacco Addiction

Duration of Addiction	Frequency (percentages)
Less than 2 years	8 (1.6%)
2-5 years	22(4.4%)
5-7 years	25 (5%)
7-10 years	12 (2.4%)
More than 10 years	34 (6.8%)
Total	101 (20.2%)

33 (6.6%) participants presented with aphthous ulcers. Pertaining to the ulcer characteristics given in **Table III**, 2 patients presented with Major Aphthous Ulceration and 31 patients presented with Minor Aphthous Ulcerations. None of the patients presented with Herpetiform Aphthous Ulcerations during the period of sample collection. And out of these 33 subjects who presented with aphthous ulcers, 5 were cigarette smokers while remaining 28 had no history of any addictive habits (smoking or smokeless tobacco).

Presence of aphthous ulcers was correlated with self-reported medical conditions; 5 out of 33 subjects (15.1%) were allergic, 9 (27.2%) had GI disorders, 3 (9%) had hormonal disorders, 12 (36.4%) had stress-related ulcers, 4 (12.1%) had genetic association and 6 (18.2%) subjects presented without any significant medical history. Occurrence of RAU is affected by a number of other variables, with no statistically significant influence of tobacco usage.

Table III: Ulcer Characteristics

Ulcer Characteristics	Variables	Frequency (percentages)
Duration	7-14 days	28 (5.6%)
	2-6 weeks	2 (0.4%)
	5-7 days	3 (0.6%)
	Total	**33 (6.6%)**
No. of Lesions	1-5	30 (6%)
	1-10	2 (0.4%)
	10-100	1 (0.2%)
	Total	**33 (6.6%)**
Frequency of Recurrence	Non recurrent	4 (0.8%)
	Recur frequently	25 (5%)
	Recur rarely	4 (0.8%)
	Total	**33 (6.6%)**
Site of Ulceration	Non keratinized	25 (5%)
	Keratinized	8 (1.6%)
	Total	**33 (6.6%)**
Size of Individual Lesion	1-3mm	21 (4.2%)
	3-10mm	10 (2%)
	3cm	2 (0.4%)
	Total	**33 (6.6%)**
TOTAL		500

Incidence of RAU in tobacco users and non-users was statistically analyzed by using Chi-squared test. Cigarette smoking was considered to represent tobacco usage as significant number of tobacco users were cigarette smokers as compared with negligible amount of other tobacco variables. Cigarette smoking was compared with presence of RAU and type of ulcers if present. Statistical analysis of our study showed no significant association between the presence of aphthous ulcers and cigarette smoking (p value = 0.98) and between cigarette smoking and type of aphthous ulcers (p value = 0.72) as shown in the **Table IV and Table V.**

Table IV: Relationship of Cigarette Smoking with Aphthous Ulcer

Type of Ulcer	Cigarette smoking	
	Yes	**No**
Minor aphthous ulcers	5	26
Major aphthous ulcers	0	2

TABLE V: Incidence of Aphthous Ulcer among Smokers and Non-Smokers

		Cigarette smoking	
Ulcer	**Yes**	5	28
	No	70	397

Discussion

Aphthous ulcers are recurrent and painful condition of oral mucosa, etiology of which is still unknown.[3] There are certain risk factors that are associated with occurrence of RAU including immune reaction, genetic factors, hormonal factors, stress, infections, GI disorders etc. No randomized control trial have shown any treatment, that could help in preventing or curing RAU.[4]

RELATIONSHIP BETWEEN TOBACCO HABITS AND RAU: An inverse relationship is observed between RAU frequency and smoking habits according to previous studies held.[3,5,9] The observations previously made by Tony Axell and Vingent Henricsson also presents that there is a negative association between tobacco habits and RAU. According to them, surface structures like leukoedema and keratin prevent the penetration of antigenic substances into the oral epithelium.[9] Shapiro et al. found that there is a negative relation between RAU and smoking. They pointed that genetic, familial, psychological and environmental factors are important considerations in the formation of recurrent aphthous ulceration. They suggest that meaningful data can be obtained by multidisciplinary longitudinal studies. According to Banoczy and Sallay there is a negative association between keratinization of oral mucosa and aphthae.[12] The findings of the case control study given by PA Atkin, X Xu, and MH Thornhill indicate that patients with RAU have low levels of smoking than in matched controls, and they support that there is a negative correlation between minor RAU and smoking.[4] The negative correlation of smokeless tobacco with recurrent aphthous stomatitis is also given in a study by Grady et al.[3] The case control study given by Shamaz Mohamed and Chandrashekar Janakiram found the statistical association between the RAU and usage of tobacco smoking. The association that exists between smoking and aphthous mouth ulcers is negative. The non- tobacco users tend to have 55% more chance of occurrence of RAU than tobacco users.[1]

However, study carried out by Slebioda Z and Dorocka, showed there is no significant association found between smoking tobacco habits and occurrence of Recurrent Aphthous ulcers.[13]

Protective Effect of Smoking

Epidemiological studies suggest a protective effect of smoking. These studies show that mouth ulcers are more common in nonsmokers than in smokers.[4,14,15] The reason that might be associated with this protective effect of tobacco use could be increased keratinization of oral mucous membrane [3] or some substances present in cigarette smoke absorbed

causing decrease in frequency of RAU. Case studies suggest that the nicotine chewing gums are helpful for the nonsmokers who have mouth ulcers.[16] Most of the population, on cessation of smoking appear to develop RAU for the first time or any previous RAU that existed, has exacerbated.[4,17] This might possibly be due to increased keratinization of oral mucosa, antibacterial effect of tobacco smoke[17,18] or smoking cessation have effects on immune system like stress generated due to withdrawal.[17]

Comparison with Literature: Most of these previous studies assessed relationship between RAU and tobacco by using methods that were based on interviews, questionnaires, or on self-reporting of smoking status.[3,10,11]

Our study also used the same method as special questionnaire was designed according to which significantly smaller population of RAU patients were smokers (15%) as compared to control group who were nonsmokers (84.4%) in a sample of 500 patients. Most of the incidences of RAU were found among sample population who were non tobacco users. Some daily tobacco habits were found in patients among which smoking was most common habit especially cigarette smoking while some in rest of the sample were addicted to other forms of tobacco (smoking and smokeless) and no ulcers were found among them. In contrast to other studies [1 3 5 15] our study showed no significant association between presence of ulcers and cigarette smoking and no association between cigarette smoking and type of ulcers.

Limitations and Future Recommendations

The factors that might have affected our results could be that these lesion are not fixed long standing lesions, that can be evaluated at any time by the physician , but are short lived that may not be present at the time of examination[15] statistical evaluation of RAU might have been affected by this fact. In addition, the methods of assessing smoking status could be inaccurate as smokers may hide their smoking status or underestimate their level of smoking. Our study was unable to show incidence of aphthous ulcers between different genders and the medical conditions that might affect the occurrence of aphthous ulcers in our community, so in future we would suggest that further studies be carried out on these aspects.

Conclusion

Incidence of RAU in tobacco users and non-users has been assessed and statistically analyzed showing that occurrence of ulcers is lower in patients who are smokers as compared to non-smokers. However, no significant association has been found between ulcer occurrence and smoking habits. These findings substantiate with the previous similar studies and can serve as a base for further research in future.

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ORIGINAL ARTICLE

Belief System as Determinant of Treatment Outcome in Low Back Pain Patients

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ABSTRACT

Objective: To determine resilience, and health belief as significant predictors of treatment outcomes in low back pain patients.

Study Design: Quantitative cross sectional survey research design.

Place and Duration of Study: Data was collected from rehabilitation centers of Lahore between March, 2018 to October, 2018.

Materials and Methods: The subjects (n=300) were recruited after screening them through a detailed clinical inventory on the basis of low back pain as acquired in the course of life happening and not as an outcome of some accidental or infection-induced events. Standardized scales were used to collect the data such as Health Locus of Control Belief Scale, Resilience Scale, and Treatment Outcome Efficacy Scale. Data collected was analysed through SPSS 23.00.

Results: There were 330 respondents who filled the questionnaires but thirty respondents evaluated in first phase during pilot study were not included in the final data set. Among 300 finally recruited subjects after screening for low back pain, results of Pearson product moment correlation analysis exposed significant relationship in study variables. Further it was established through regression analyses that resilience and health beliefs sustain as significant positive predictors of treatment outcome efficacy while significant gender differences in health beliefs were observed.

Conclusion: Health belief and resilience are significant predictors of treatment outcome efficacy in lower back pain patients**.** Enhancing health beliefs and resilience may improve treatment outcome efficacy in patients with lower back pain. This research is expedient among health care practitioners for dealing with the people with low back pain with more insightful understanding of psychological dimensions.

Key Words: *Health Beliefs, Low Back Pain Patients, Resilience, Treatment Outcome Efficacy.*

Introduction

In Pakistan due to poor awareness about dietry intake, inappropriate postures adoption and due to sedentary life styles, the reported clinical evidence for low back pain patients is on rise.[1] This grave phenomenon invokes with it the colossal loss for all in form of lower productivity, impaired daily life functioning, restraints mobility and lost work days, causing immense income loss. Enigmatic semblance of lower back pain has been found to produce quite diverse effects in different repondents.[2] In fact; chronic low back pain poses enigmatic challenges to medical practitioners due to its non-specific nature. Few practitioners have prophecies it to be the future's greatest medical disaster due to its diffused triggers and specifies. Consequently Biopsychosocial approach is relied on to find solution to such intricate condition. Biopsychosocial model somehow implicates to elucidate its origin, maintenance, assessment and management. Here an individual's thoughts, cognitions, emotions and behaviours gain primal significance. In spite of many technologically advanced treatment strategies in lower back pain modalities, this has been observed that individual's volunteer involvement in learning such behaviours that would lead to control and manage the chronic pains is pivotally important.[3] Multidimensional approaches in managing lower back pain have also been emphasized by health psychologists and medical practitioners. Numerous empirical studies have substantiated that internal health locus of

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control beliefs are significantly associated with intact life patterns and better physical and psychosocial health.[4] Health locus of control beliefs have been enumerated as the degree to which sufferers of lower back pain rely on parameters that they rely on in order to control their distress.[5] Health belief locus of control somehow helps in developing the self-sufficiency phenomenon. A person with internal locus of control beliefs assumes that his or her health is controlled by internal factors rather than by chance, luck, environmental triggers, or social happenings. The pain experiences in lower back pain patients have been presented as multifaceted, involving sensory, affective, and cognitive experiences which ultimately impair one's health and psychological well-being. Few researches have highlighted that cognitions associated with chronic lower back pain are catastrophizing and may lead the sufferer into debilitating state.[6]

Resilience has been a phenomenon originating in studies wherein children were examined for standing intact in their physical, psychological and emotional health at the wake of various environmentally posed challenges. Some children manifested effective growth and surmounted ordeals better than others. This lead to generalize the construct of resilience onto other ages and to individuals facing encounters of differing nature. Not all people who survive well at the wake of physical challenges also survive better in emotionally vulnerable situations. Resilience is the knack or ability to maintain positive levels of functioning in spite of calamity or adversity. This is in fact one of the several strengths that can assist people in leading positive life.[7] Likewise the demeanour of exhibiting resilience and growth varies a lot across individuals. Resilience in pain helps in adapting to the phenomenon of pain thus here it is reflected in individuals possessed sustainable attribute of effective coping in response to ardent stressors such as pain.[8] Some research studies have demonstrated that resilience moderates the relationship between pain severity and treatment outcome efficacy while others have shown insignificant associations.[9] Smith and Zautra have enumerated that resilience may entail such resources as self-control, optimism, determination in life, and pain management cum acceptance. Such dispositional aspects help him or her in managing the pain much more efficaciously.[10] Hence in the light of above literature, this empirical study ventures to examine whether health locus of control beliefs and resilience contribute in predicting treatment outcome efficacy. Extensive literature review herein helps in identifying the gaps and after reviewing literature and reported clinical data, this was realized that very few studies have addressed systematically the psychological dimensions as significant predictors of pain management in lower back pain patients. The objective of the current study was to determine resilience and health belief as significant predictors of treatment outcomes in low back pain patients.

Materials and Methods

It was a cross sectional survey research design based study, executed in rehabilitation centres of Lahore. The study lasted for eight months from March, 2018 to Oct, 2018.

A total of 330 participants both men and women equally distributed across gender were recruited. The age range of the respondents was 35 to 45 years. Respondents were selected after fulfilment of all ethic's consideration and guidelines. Formal permission was obtained from all concerned authorities and informed consent was sought from all participants after clarifying them the nature of the study and after ensuring them confidentiality. Only willing participants volunteering for research were included. This was also affirmed that participants had right to withdraw with their will, at any stage of the study.

All the respondents were screened on primary clinical inventory that filtered such respondents that had some prior accident or medical reason for back pain or who reported less than two months duration from its onset. Those reporting mild to moderate lower back pain were also excluded. Respondents reporting undiagnosed, diffused reasons for chronic low back pain and having no other comorbid physiological disease or conditions were taken. This stringent recruitment criterion bargained longer time for data collection but this was pertinent to be done to rule out the cases with intricate and complex features and consequences of pain. Furthermore, only literate patients with at least matriculation education were taken so that they could read and understand all the scales.

Resilience Scale[11]; Multidimensional Health Locus of Control Scale[12]; Treatment Outcome Scale[13] were the major measures used as tools for effective data collection. First of all, a pilot study was undertaken in order to ascertain the reliability estimates of the scales. This was found profoundly sound when administered on thirty respondents. After screening all the scales for any possible ambiguity and after ruling out the administration feasibility constraints, the target data was collected. Pilot study data was kept separate from ultimate data set.

Firstly, consent form was filled by the respondents. After that demographic information sheet and tools related to resilience, beliefs and treatment efficacy were given to the respondents.

Resilience was measured through the Brief Resilience Sales (BRS)[11] that was developed by Smith et al. This consists of total six items. Out of these six, three are scored reverse while other three are scored forwardly. The participants were made to respond on options spanning from strongly disagree to strongly agree. Western empirical evidences support that reliability, validity and internal consistency of the items of the scale is quite high. In one of his validation analysis study on Brief Resilience Scale, convergent validity and discriminant predictive validity were also established by Smith et al.[11] as quite sound. Second scale was Health Locus of Control Belief Scale (HLCBS).[12] This scale happens to be the multidimensional reflecting the extent to which individuals believe their health is controlled by various sources. This is one of the most efficient measures of health-related beliefs for more than a quarter of a century. It has 18 items and is very efficient in tapping and understanding health behaviour's. Third scale comprised of items pertaining treatment-efficacy. Treatment Outcome Efficacy Scale[13] was a ten items questionnaire. It tends to assess the confidence of people with treatment in post treatment settings with any type of chronic pain. Each item is rated on a 7 point scale from 0 = not at all confident to 6 = completely confident.

The parametric data was analysed with the help of SPSS version23.00. Regression analyses were used in addition to Pearson Product Moment Correlation and Independent Sample t-test.

Results

Cronbach's alpha reliabilities mean and standard deviation values were computed and grouped in table I. The values of Cronbach's alpha of Brief Resilience Scale, Health Locus of Control Belief and Treatment Outcome Efficiency Scale in this research were 0.82, 0.85 & 0.83 respectively. Demographics analysed through descriptive in SPSS divulged that mean age of the participants was 42.13 years while this was 37.23 for females and 41.22 for males. It also revealed that 55% belonged to lower middle class, 30% belonged to middle class, 10% belonged to lower income group and 5% belonged to higher income group. 73% reported that they had it from six years, 21% reported this to be from more than two to five years' time, and 6% maintained that they had it from past four months to two years. 48% reported partial impairment in their daily life functioning due to lower back pain while 52% maintained debilitating severe impairments in their daily life tasks performance due to lower back pain.

Table I: Reliability and Descriptive Analysis of the Scales *(N=300)*

Scales	M	SD	.
Brief Resilience	4.70	3.48	.82
Health Locus of Control Belief	3.47	2.22	.85
Treatment Outcome Efficacy	3.03	1.98	.83

Note. M=Mean; SD=Standard Deviation;
α = Cronbach's alpha

Table II: Correlation among Demographic Variables and Study Variables (N=300)

Study Variables	1	2	3
1.Resilence		.63	.85**
2.Health Locus of Control			.58*
3. Treatment Outcome Efficacy			

Note. *.p < .05; **.p< .01; ***.p< .001; M= Mean;
SD= Standard Deviation

Discussion

This empirical study has main aim to determine the health locus of control beliefs and resilience as predictors of treatment outcome efficacy in low back pain patients. Moreover, gender differences in resilience, health belief locus of control and treatment outcome efficacy in lower back pain patients were also investigated. The primary

Table III: Multiple Regression Used to indicate the Predictors of Treatment Outcome Efficacy (N=300)

	Treatment Outcome Efficacy		
	B	*CI*	
Variables		*LL*	*UL*
Constant	32.552	18.755	46.349
Resilience	.34	.39	.073
Health Locus of Control	.183	.034	.350
R^2	.119**		
F	5.18**		
ΔR^2	.096		

Note. *p<.05; **p<.01; ***p<.001; B = Unstandardized Co efficient; ΔR^2= R Square change; CI=Confidence Interval

Table IV: Gender Differences on Health Locus of Control Beliefs, Resilience, and Treatment Outcome Efficacy (N=300)

	Male (n=150)		Females (n=150)				95 % CI		
Variables	M	SD	M	SD	t	p	LL	UL	Cohen's d
Treatment Outcome Efficacy	31.92	9.95	28.72	7.69	1.60	.005	-.760	7.16	0.40
Resilience	66.82	7.21	65.87	13.76	.559	.28	-4.99	8.89	0.13
Health Locus of Control	72.65	9.84	66.45	10.08	2.78	.95	1.76	10.63	0.10

Note. * p < .05; M= Mean; SD= Standard Deviation; CI=Confidence Interval; LL= Lower Limit; UL= Upper Limit.

hypothesis of this research investigated the relationship among health locus of control beliefs, resilience and treatment outcome efficacy in patients with lower back pain. The findings from inferential analysis indicated that health locus of control and resilience was significantly correlated with treatment outcome efficacy in lower back pain patients. However, resilience and health locus of control were not significantly correlated with each other. These findings are in alignment to empirical findings of Smith et.al.[14] Similarly, Turner and Dworkin et al. reported that beliefs regarding pain played an important role in coping back pain problems among patients.[15] The current study has extended preceding researches in which ample support has been catered to Social Cognitive Theory. According to this theory the expectations and self-efficacious beliefs gained through health belief locus of control are likely to improve treatment outcome efficacy.

Offering support for expectations within Social Cognitive Theory that pain related self-efficacy predicts treatment benefit.[16] Pain control beliefs extended in this regard are amply appreciable as they offer a whole new domain of understanding this debilitating phenomenon with dynamic control over this. Resilience was found insignificantly associated with health locus of control. This is somehow in contradiction with findings divulged by some other researchers[17] that reveal that those who report more resilience also tend to have more internal locus of control and acknowledge significant link between their health and lifestyle. Such people dynamically get involved in such activities through which their health can improve and they can reduce the pain.[17] This is justified along these lines that resilience somehow helps us in coping with lower back pain and it helps us in attaining relative adjustment but somehow this is not directly associated with health beliefs. One potential explanation for our unexpected finding is that patients with lower resilience also somehow develop adaptation to pain due to presence of health beliefs and other psychosocial dimensions such as self-efficacy and proactive health behaviours. This is one of the reasons that lower back pain patients' treatment efficacy shows significant association but resilience does not. In another supporting investigation, this was found that resilience was not markedly associated with pain-related disability over time.[18]

The regression analysis reveals that health locus of control establish as significant predictor of treatment outcome efficacy in lower back pain patients. Previous research in accordance to this has shown that the stronger the belief in one's personal control, the better the outcome wills be.[19] The justification to this finding is also catered by our general attitude and belief patterns related to pain. People form beliefs about the pain that they encounter. These pain-specific beliefs either enable or disable them in their functional aspects of life. Since beliefs are potent predictors of health care utilization, people going through lower back pain timely utilize resources and benefit more, showing better treatment outcome efficacy.

Results of Independent sample t test showed that there were significant gender differences among patients in treatment outcome efficacy. Furthermore, it was noted that male have greater resilience and health beliefs as compared to women Demographic data also revealed that females

diagnosed chronic back pain problems in their earlier age of onset while males were reported in their later age domains. There are numerous past researches highlighting that differences in lower back pain between males and females exist; as reported by other epidemiological surveys [20-23] on general pain that showed greater frequency and intensity of pain for women. Indeed, according to the research by Barros, Cesar, Carandina and Torre the prevalence of pain related diseases in Brazil is higher for women.[24]

There are fewer limitations of this research and some suggestion aligned with them. This study was conducted on a small scale sample so, it is suggested that in future a large sample from various rehabilitation centres should be included. Likewise, the research design was cross sectional that might have limited scope of the data; longitudinal research design may benefit more in yielding convincing findings. Research includes only quantitative results if it includes qualitative results it would affirm better. Present study focused on limited number of predictors of treatment outcome efficacy. A step forward for research would be to develop more complex model to predict treatment outcome efficacy by entailing both physical psychological and dispositional factors. Further studies should investigate whether physical therapists beliefs during a patient-health care provider relationship predict patients' beliefs and clinical outcomes. If so, strategies to improve physical therapists decision-making should be considered in primary health care.

This research is useful among patients with low back pain. It applies to all those people such as adults, aged, injured actors models, caretakers, and physiotherapists etc. who either suffer from low back pain or get involved in dealing with lower back pain patients. These findings implicate the role of enhancing health belief and resilience at the wake of ordeals in patients with lower back pain in order to improve their treatment outcome efficacy. Results of this research are also helpful for future researchers in order to design more effective strategies and programs for adopting multidisciplinary/ multidimensional approach in treating lower back pain patients.

Conclusion

Health locus of control and resilience to confront debilitating lower back pain are found to be significant predictors of treatment outcome efficacy.

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ORIGINAL ARTICLE

Histological Effects of Caffeinated Energy Drink Consumption and Its Withdrawal on Kidneys of Experimental Rats

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ABSTRACT

Objective: The present research was carried out to observe withdrawal effects of energy drinks, whether these histomorphological changes are reversible or not.

Study Design: Laboratory based experimental study.

Place and Duration of Study: The research was carried out from 1st July to 30th August 2019 at national institute of health Islamabad.

Materials and Methods: Total thirty adult male albino rats were divided into 3 groups by simple random sampling, with ten rats in each group. Group I was control group, while energy drink group II received 3.57ml/kg body weight red bull corresponding to one can of energy drink (250ml) in humans orally for eight weeks. Rats in withdrawal group III received energy drink for first four weeks followed by normal diet and water for last four weeks. After eight weeks, rats were sacrificed and their right kidneys were removed. Slides were prepared using hematoxylin eosin and Periodic Acid Schiff Stain, results were analyzed by SPSS.

Results: The results showed that use of energy drink for 8 weeks resulted in increase in weight of kidneys along with histological alterations in renal cortex of rat kidneys. Grade 4 (severe) congestion, hemorrhage, loss of brush border and necrosis was observed in energy drink group II. Withdrawal of energy drink in group III resulted in weight of kidneys near to control group along with significant reduction in congestion, hemorrhage, loss of brush border and necrosis grades from grade 4 to grade 3 and 2 with $P \leq 0.05$.

Conclusion: Caffeinated energy drinks are having damaging effects on kidneys of albino rats and these histological changes caused by caffeinated energy drinks in this duration of study and in low doses corresponding to one can of energy drink (250ml) in humans are reversible.

Key Words: *Caffeinated, Energy Drink, Histological, Kidneys, Withdrawal.*

Introduction

Energy drinks (EDs) were first introduced in UK in 1929 as hospital drink; in 1980 they were promoted for replenishing lost energy. They are now available in > 140 countries as part of a multi- billion dollar business.[1] In 1960s they appeared in Asia and Europe.[2] There are diverse types of energy drinks available in Pakistan. First and most trendy caffeinated energy drink to be introduced is red bull.

Target of these caffeinated drinks market is mainly youth. In Pakistan majority of energy drink users are youngsters with age group of 13-35 years. EDs claim to provide burst of energy by using a combination of caffeine (chief active ingredient), guarana, yerba mate, taurine, glucose, fructose and glucuronolactone.[3] Caffeinated energy drinks (EDs) contain higher levels of caffeine along with other ingredients that are not commonly found in sodas and juices, marketed as providing mental and physical stimulation especially in youth. They differ from soft or sport drinks due to their unique composition.[4]

Caffeine present in these drinks is up to 500 mg per 20 oz. (600 mL) serving that is 15 times the amount of caffeine present in a 12-ounce (360 mL) serving of cola. Beverages that contains guarana actual levels of caffeine are much higher than levels mentioned on the label.[5] Youth using EDs is unaware of these high

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levels of caffeine and its adverse effects that is alarming.

The kidneys are the organs that filter waste products from the blood. Kidneys are predominantly vulnerable to ischemic and toxic damage. Many studies have depicted adverse health effects of EDs, one of them is hastened progression of renal micro vascular impairment and chronic kidney disease. [6] Literature illustrates that exposure of rats to energy drink leads to kidney damage causing renal vascular congestion, hemorrhage of interstitial tissue, focal atrophy and degeneration of lining epithelium of Proximal and Distal convoluted tubule.[7] There is limited data available regarding withdrawal effects of these drinks, therefore present study was conducted to observe nature of renal damage and to observe changes after withdrawal of these drinks whether these changes are reversible or not.[1]

Materials and Methods

This laboratory based experimental study was conducted from 1st July to 30th August 2019 by mutual collaboration with national institute of health (NIH) and Islamic international medical college (anatomy department) after approval from ethical review committee (ERC).

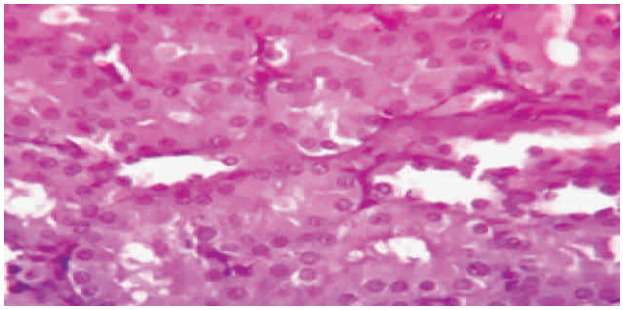
Thirty adult male healthy albinos Sprague Dawley rats (*n*=10/group) weighing 250±10 gm were used in experiment. They were housed at animal house of NIH Chak Shahzad Islamabad and were acclimatized to laboratory surroundings with free food and water access under natural dark and light rhythms prior to commencement of study. Female rats and rats with any disease or pathology were excluded. Total thirty animals were divided into three groups each group having ten rats selected by simple random sampling and treated in this way:

Control Group I (*n*=10)	Received normal diet and water for eight weeks.
Energy Drink Group II (*n*=10)	Received 3.57ml/kg red bull orally for eight weeks.
Withdrawal Group III (*n*=10)	Received 3.57ml/kg red bull orally for first four weeks followed by routine diet in next four weeks.

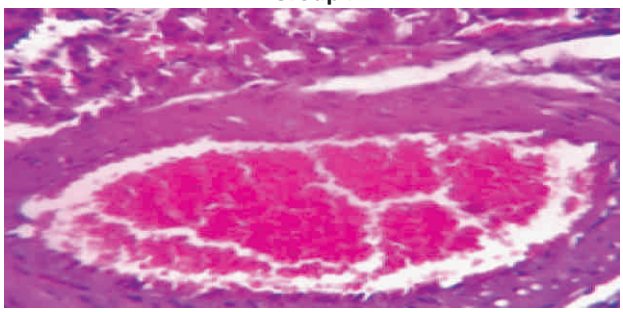
After 8 weeks of completion of experimental study rats were dissected and kidneys were removed. After fixation and embedding transverse sections of 5 µm thickness were obtained. Staining was done with Hematoxylin and eosin and Periodic Acid-Schiff stain. The slides were examined under light microscope in X10, X40 Power. Parameters observed were weight of kidneys, congestion, hemorrhage, loss of brush border, and necrosis. Using SPSS version 21 non parametric data was analyzed by means of chi square test. A *p* value of equal or less than 0.05 was considered as significant value.

Results

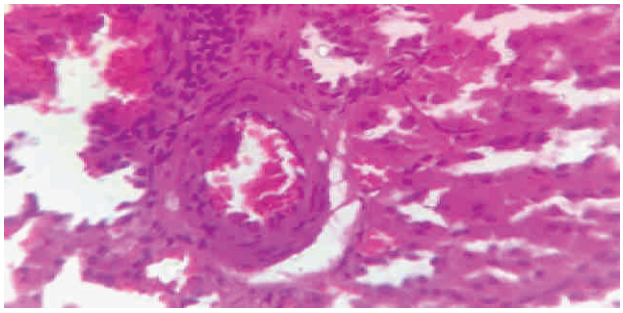
Renal cortical parenchyma appeared to be normal in 100% rats in control group I, while 25% rats showed minimal congestion (grade 1). In ED group severe congestion (grade 4) was noted in 62.5% of rats. In withdrawal group 62.5% lesions were moderate (grade 3) showing reversal of histological alterations caused by EDs (Fig: 1, Table I).



Group I



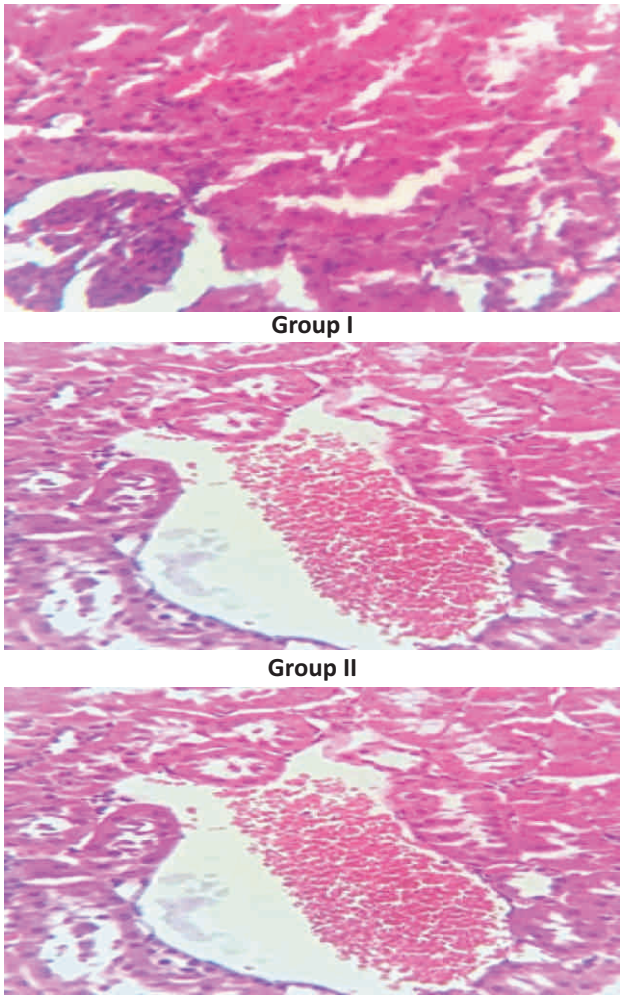
Group II



Group III

Fig 1: Distribution of Renal Cortical Parenchymal Congestion Among Different Groups, Group I K1R5 Showing No Congestion, Group II K1R2 Showing Severe Congestion And Arrows Indicating Moderate Congestion n Group III K1R7 (H&E, X400).

In 100% rats of control group I no hemorrhage was observed in renal cortex while in ED group severe hemorrhage (grade 4) was noted in 75% of rats. In withdrawal group 75% lesions were mild (grade 2) showing significant reduction in hemorrhage grade when compared to ED group (Fig: 2 and Table I).



Group I

Group II

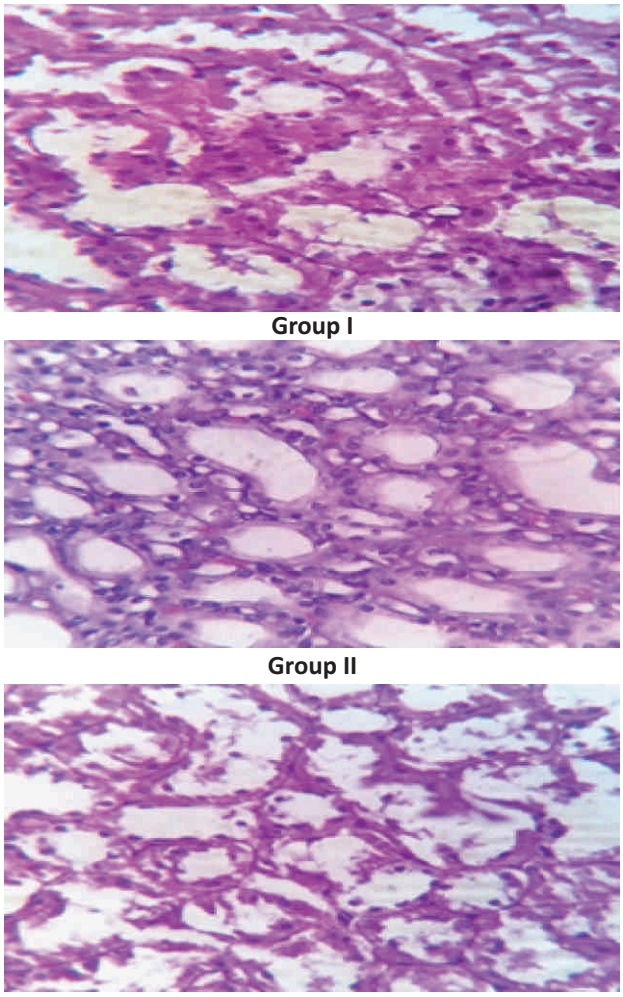
Group III

Fig 2: Distribution of Hemorrhage In Renal Cortical Parenchyma of Different Groups, Group I K1R2 Showing No Hemorrhage, Group II K1R8 Showing Severe Hemorrhage and Arrows Indicating Mild Hemorrhage In Group III K1R9 (H&E, X400).

Table I: Distribution of Grades of Congestion and Hemorrhage In Control and Experimental Groups of Albino Rats By Chi-Square Test

Grades	Congestion			Hemorrhage		
Grade 0	100%*	0%	0%	100%	0%	0%
Grade 1	0%	0%	0%	0%	0%	12.5%
Grade 2	0%	0%	25%	0%	12.5%	75% *
Grade 3	0%	37.5%	62.5%*	0%	12.5%	12.5%
Grade 4	0%	62.5%*	12.5%	0%	75%*	0%
***p* value**	0.000*			0.000*		

Renal cortex appeared to be normal in 100% rats in control group I. In ED group severe (grade 4) loss of brush border was observed in PCT of 87.5% rats. In withdrawal group mild loss of brush border in 50% of rats was seen showing significant reversal of histological alteration after withdrawal of ED. (Fig: 3, Table II)



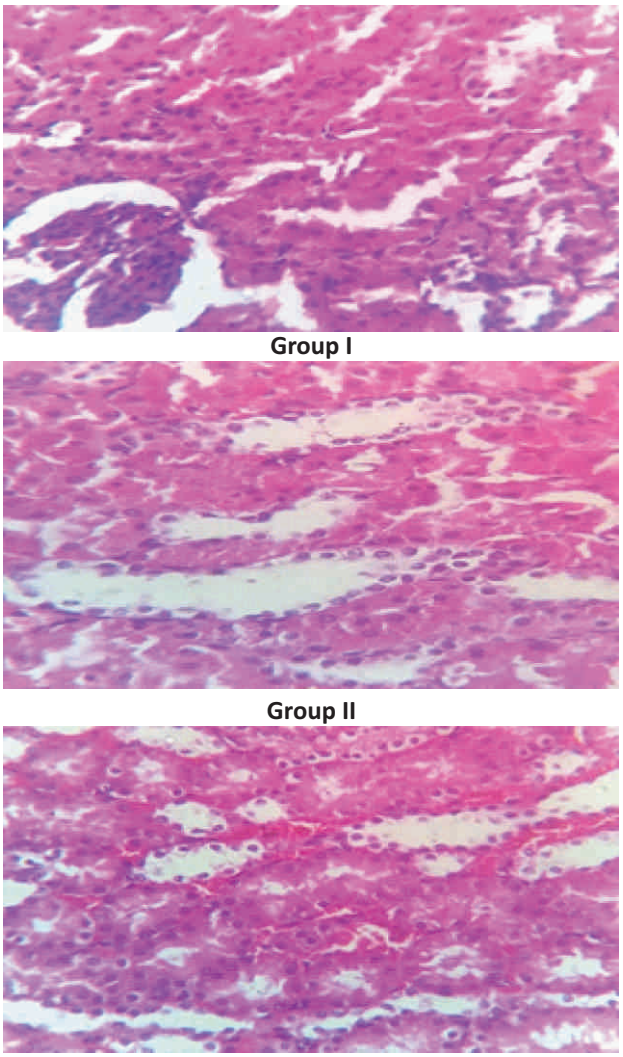
Group I

Group II

Group III

Fig 3: Distribution of Loss of Brush Border In PCT Of Different Groups, Group I K1R5 Showing Prominent Apical Brush Border, Group II K1R9 Showing Severe Loss of Brush Border In PCT And Arrows Indicating Mild Loss of Brush Border In Group III K1R1 (PAS, X400).

In control group 100% rats showed normal renal cortical architecture. In ED group severe (grade 4) necrosis was observed in 100% of rats. In group 3 moderate (grade 3) necrosis was seen in 75.5% rats showing significant reduction in grades of necrosis when compared to energy drink group. (Fig: 4 and Table II)



Group I

Group II

Group III

Fig 4: Distribution of Necrosis In Renal Tubules of Different Groups, Group I K1R3 Showing No Necrosis, Group II K1R5 Showing Severe Tubular Necrosis and Arrows Indicating Moderate Necrosis In Group III K1R7 (H&E, X400).

Table II: Distribution of Grades of Loss of Brush Border and Necrosis in Control and Experimental Groups of Albino Rats By Chi-Square Test

Grades	Loss of brush border			Necrosis		
Grade 0	100%*	0%	0%	100%	0%	0%
Grade 1	0%	0%	12.5%	0%	0%	12.5%
Grade 2	0%	0%	50%*	0%	0%	12.5%
Grade 3	0%	12.5%	37.5%	0%	0%	75%*
Grade 4	0%	87.5%*	0%	0%	100%*	0%
***p* value**	0.000*			0.000*		

p value ≤ 0.05

Discussion

This study investigated the reversal of renal histomorphological features after withdrawal of ED in kidneys of male rats. In this study we observed no congestion with normal renal cortical parenchyma in control group. In ED group severe congestion in renal cortical parenchyma was observed. In withdrawal group, moderate congestion was seen. Impairment of venous outflow due to inflammatory mediators results in a localized increase in blood to different areas of kidney, which is demonstrated histologically as congestion.[8] Taiwo et al documented congestion after administration of energy drink in different doses to rabbits.[9] He documented that changes observed were reversible in 28 days duration of study.[9] Similarly results has been supported by many other studies.[10,11,12]

In our study severe hemorrhage was observed in ED group. In rats of group III mild hemorrhages was seen. Hemorrhage observed was due to the effect of inflammation leading to expansion of blood vessels, as result vessels rupture and blood flows out. A previous study showed similar findings after administration of ED 1ml/animal/day orally for 4 weeks.[13] Comparable findings were observed in liver of rats after administration of high dose of EDs.[14] Akande also proved that damaged caused by ED was reversible in 28 days duration.[15]

The present study showed severe loss of brush border of PCT in ED group, while in withdrawal group mild loss of brush border was observed that showed reversal of histological changes caused by red bull after withdrawal of ED. This might be explained by vulnerability of cellular membranes to toxins, leading to decrease cellular production of ATP and accumulation of reactive oxygen species causing cell damage and detachment of epithelial cell from basement membrane. Similar results were shown in albino rabbits by Salih, results were ascribed to high level of caffeine in EDs.[16]

Most common cause of acute kidney injury is acute tubular necrosis that causes death of tubular epithelial cells leading to renal failure. In group III moderate necrosis was seen which was significantly reverted after withdrawal of ED.[17]Coagulative pattern of necrosis was observed in various experimental groups which is more common in toxic or ischemic injury.[18]The observed necrosis may be due to carbon dioxide present in energy drinks that damages membranes of mitochondria, change in ATP production, hypoxia and cell deah.[13]In another

study after administration of red bull, severe necrosis was observed in seminiferous tubules of male rats.[19]

In this study we observed the severity of renal injury in relation to time duration for which EDs were used. In ED group significant difference was observed in weight of kidneys, congestion, hemorrhage, loss of brush border and necrosis. This indicates that EDs leads to renal damage that is duration or time dependent, since more damage was observed in ED group when compared to group III, it showed reversal of these histological features after withdrawal of ED. Our findings are consistent with the results of study that proved that effects of caffeine on cell survival are highly time and dose dependent; in low doses it increases cell survival and at higher doses it increases super oxide production.[20,21] Also the study proved that chronic caffeine intake has age dependent effects on brain.[22]

Conclusion

In conclusion caffeinated energy drinks are having damaging effects on kidneys of rats, besides that with low doses, corresponding to one can of ED (250ml in humans) and with this duration of study histomorphological changes caused by caffeinated EDs are reversible.

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CASE REPORT

Maternal Depression as a Predictor of Intellectual Disability: Bio Psychosocial Model Speaks up

Rabia Khadim, Ayesha Jabeen

ABSTRACT

With an upsurge interest in holistic view of physical and psychological disorders, bio psychosocial model has gained much attention. Maternal depression is considered to manifold risks and vulnerabilities and early child developmental problems, including impaired cognitive, social and academic functioning. The present case study will highlight the intricacies linked with maternal depression in the infancy and early childhood of a 12 years old child, studying in a special education institute of city Lahore. The history file revealed that low socioeconomic status and parental conflicts induced depression in the mother of the child, who showed carelessness to the extent of making child vulnerable of experiencing intellectual disability.

Key Words: *Intellectual Disability, Maternal Depression, Maltreatment.*

Introduction

Parents are the primary source of survival and development of children throughout their whole lives, but early childhood development puts a strong impact and determines the future of children. One of the disruptions in the healthy development of children is maternal depression that is a broad term for a spectrum of depressive conditions affecting women during pregnancy and up to one year postpartum.[1] Studies have repeatedly revealed that maternal depression is associated with less optimal and insecure parenting. Maternal mental health is more emphasized in postnatal period as compared to prenatal period as the postpartum depression (PPD) is associated with weak emotional involvement, neglect, lack of attention, and hostility towards the child.[2] Warmness that is the charm of mother-child affiliation lacks in postpartum depression and is replaced by hostility towards child. There are pertinent associations between maternal depression and multiple disruptions in child including early child adjustment, developmental abnormalities, problematic parenting, family conflicts, parental negligence and other family difficulties.[3]

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The impact of maternal depression is not limited to mother only but its exposure is found to have multiple negative impacts on child's normal development in infancy and early development, including developmental delays, impaired cognitive, emotional and behavioral functioning.[4] Infants of postpartum depressive mother have been reported to show patterns of dys-regulated attention and arousal.[5]

The most solemn negative effects of postpartum depression are on the child's cognitive development, including language, IQ, and Piaget's object concept task. However, these effects are completely mingled and contextual influences. The studies on child behavior indorse the sound effects of postpartum depression on children's behavior, antisocial behaviors, and psychiatric disorders at home and at school.[6]

Among these cognitive impairments in children, one is Intellectual Disability (ID). Intellectual disability is a developmental disorder describing the condition in which individual's intellectual functioning level and adaptive skills are significantly below the average of his chronological age. As a result of ID, individual's practical, social and conceptual functioning is disturbed.[7]

Family conflicts can cause maternal depression in postpartum period that is the significant predictors of child's emotional and behavioral behavior problems along with Intellectual Disability (ID). The aim of the current study was to emphasize the importance of family conflicts, mental health of primary caregivers, specifically mothers who can have permanent negative impacts in child's development.

Case Study

The present case study is about a 12 years old child who was referred by his teacher with the presenting complaints of speech difficulties, academics and behavioral problems of being hyper, aggressive and hitting others. The in-depth clinical interview with the father of child revealed that child's early developmental years made him vulnerable towards intellectual disability. Apparently child's socio-economic status was below-average. There were inter-parental conflicts and relationship difficulties. The mother of child also had poor mental health that made her vulnerable towards maternal depression. The child had a healthy normal birth and there was no sign of abnormality. He appropriately achieved the entire developmental milestone at 2 years except speech and toilet training. According to father, mother had no affiliation with her child and she used to physically abuse him. She had careless and neglected attitude towards child. In case of illness, mother used to pick those medicines for child that induced dizziness and sleepy effects as she used to give child extra doses of cough syrup. After the child got accidently burned, she misused the doctor's prescription for child which had the abnormal effect on child's development. The frequency of doses was unknown to father but he was suspicious about mother regarding child's condition. Neglecting parenting and conflicts caused divorce of parents and child remained with his father. Afterwards, child stopped speaking and showed inappropriate attention, aggression and hyperactivity as teasing and hitting others.

The child started formal education at 4 years of age but had various unsuccessful experiences at normal schooling because of his speech and behavioral problems. So, at 7 years of age, he was admitted to a special education school where he was suspected with Intellectual Disability (ID-moderate), affecting conceptual, social and practical domains of child. The developmental assessment tool, Portage Guide to Early Education (PGEE) revealed the 9-10 years of overall discrepancy between chronological and functional age of child. He had 2-3 words speech, lack of socialization and cognitive impairment. Bender Gestalt Test (BGT) was administered to find the developmental maturation of child that was up to 3 years.

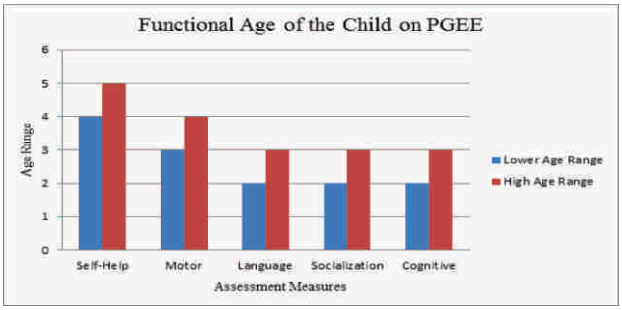

Fig. 1: This Figure is Showing the Functional Level of the Child on Different Areas of PGEE

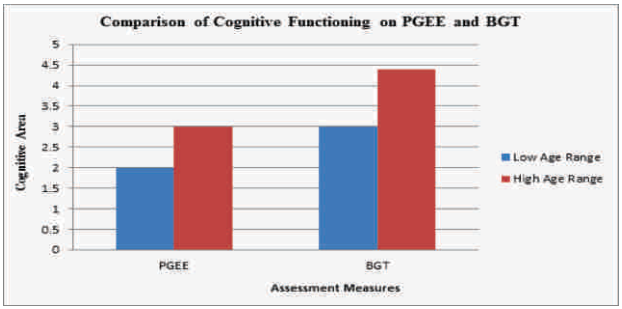

Fig. 2: This Figure is Showing the Comparison of Cognitive Functioning On PGEE and BGT

Table I: Conceptual, Social and Practical Domain of Child according to his Severity Level

Conceptual Domain	Social Domain	Practical Domain
The child's conceptual skills were markedly behind those of his peers.	The child had poor social Skills as his speech was not clear and developed to 2-3 words.	The child's function in practical domain regarding self-help but he needed support in complex daily living tasks in comparisons to his peers
The child had no basic concept of reading and writing as he could draw only lines in imitation and didn't have the concepts of alphabets.	He didn't interact with his peers and had poor participation in social activities. He used to give no social expression as smiley and greetings.	He couldn't buy something for him.
He had no understanding of time and money management.	He had no friends and didn't perceive or interpret the social cues accurately.	He couldn't perform practical tasks as simple nutritious food preparation, health benefits, managing social expectations, money management and responsibilities.

Discussion

Family provides the grounds for the upbringing of children where parents are the primary caregivers. Parental factors contribute towards the bio-psycho and social developmental processes of children that have long lasting impact on future life. There are multiple factors in family processes that can influence the normal growth patterns of children including parental conflicts, poor mental health, negligence, lack of support and parental divorce. This case study brought all such factors into light that can have adverse effect on normal children throughout their whole life and make it worse for them.

As in child's case, his parent's maltreatment made life full of negative challenges effecting his cognitions, emotions and behaviors. Parental neglection, conflicts, financial worries and relationship difficulties can put negative impact of one of the spouse as mother's mental health (maternal depression) was affected badly in current case. Maternal mental illness is associated with child's cognitive, social and academic impairment.[4,8] Maternal depression is associated with multiple adverse outcomes for child. Due to mental suffering of mother during postpartum depression (PPD), her hostile behavior towards child put into danger that lead towards overdose of medication. Studies have revealed through the reports of overdose with promethazine (phenergan) can originate significant variations into the nervous system including dizziness, restlessness, agitation, and confusion.[6] These adverse effects put the psychology of child into danger that come out as hyperactivity, aggressiveness and hitting behavior. Along with these behavioral issues, the overdose of promethazine plays a significant role towards the likelihood of Intellectual Disability (ID). As a result, the child's intellectual and adaptive functioning was disrupted.

Studies have revealed that the social factors of parental conflicts, divorce and destructive home environment are those social factors that can lead towards maladjustment of children affecting their biological, psychological and social functioning. Similarly, a disruptive home environment also has the profound effect on the wellbeing of children and interferes with the healthy development.[3]

The key goal of this study was to understand all the parental factors including maternal depression which effect the healthy growth of children.

Table II: Case Formulation of Child According to Bio-Psycho-Social Factors

Factors	Remote	Present	Future Recommendations
Bio	Maternal depression	Speech problem	Speech therapist
	Overdose of medication		
Psycho	-	Hyperactivity	Behavior modification
		Aggressiveness	
		Hitting behavior	
Social	Parental conflict	Destructive home environment	Family counseling
	Parental divorce	Lack of supervision	

The intervention plan was developed according to the needs of child in which goals were taught in one setting. Because of single parent, father couldn't give proper attention to child. Family processes are accountable for child's development and intervention as both parents have their own specific roles to play.

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- Sampling technique
- Mention about permission of ethical review board and other ethical issues addressed.
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