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EDITORIAL

Association of Hypothyroidism with Metabolic Syndrome

Aamir Ijaz

Metabolic Syndrome (MS) is diagnosed when three out of five cardiometabolic risk factors are present namely hyperglycaemia, low HDL-Cholesterol, high triglycerides, systolic hypertension and obesity.[1] Presence of metabolic syndrome increases the risk of cardiovascular diseases and type 2 diabetes mellitus (T2DM).[2] Other conditions have also been related to metabolic syndromes e.g. cancer, sleep apnea, polycystic ovary syndrome, thyroid disruptions and others.[3,4] There is a worldwide epidemic of MS, Pakistan and some other developing countries are no exception[4]. Hypothyroidism can be overt or sub-clinical. Subclinical-hypothyroidism (SCH) is defined when TSH values are more than 4.0 mIU/L but less than 10 mIU/L with normal thyroid hormones (fT4 and fT3).[5,6] The etiological factors for SCH and overt disease are the same with a difference of severity of the disease, so SHO is also called 'Mild Hypothyroidism' as by definition SCH is only a biochemical diagnosis and has nothing to do with the presence or absence of clinical features of thyroid disease. SHO has been shown to be much more common as compared to overt disease.[7] SCH becomes a dilemma for the physician regarding the question of treatment or waiting for the overt disease.[8,9] Amongst many concerned related to hypothyroidism, a propensity for dyslipidaemia is of great concern more so if the patients has other cardiovascular risk factors, too. Khan et al (2018) have recently shown that lipid parameters are adversely affected in hypothyroidism as a continuous function of increasing level of TSH. Lipid changes are found to be more subtle in the subclinical hypothyroid group than cases with overt hypothyroidism.[10] Most significant effect has been shown to be on LDL-cholesterol, non-HDL-cholesterol and urine albumin-creatinine ratio. In another recent study it has been shown that he association between MS and hypothyroidism depends on the presence of T2DM. The most important pathophysiological mechanism in T2DM is Insulin Resistance (IR), so it is difficult to ascertain the role of SCH in causation of MS in the presence of T2DM.[11]

Various components of metabolic syndrome i.e. high blood pressure, elevated triglycerides level, obesity, and IR have been shown to be closely related to subclinical hypothyroidism.[12,13] It has also been shown that even persons with TSH in the upper reference values (2·5–4·5 mu/l) were more obese, had higher triglycerides, and had an increased likeliness for the metabolic syndrome.[14] Slightly elevated serum TSH levels have also been shown to be associated with an increase in the occurrence of obesity.[15] Another puzzling finding about thyroid hormones and metabolic syndrome is from Wolffenbuttel et al (2017), who have shown that in men, lower FT4 is related to MS but in the highest free Triiodothyronine (FT3) and free thyroxine (FT4) quartiles, there is a 50–80% increased risk of having MS compared to the lowest quartile.[16] This has been confirmed in other recent studies showing MS developing in patient with high FT3 as well as higher FT3/FT4 ratio.[17,18] Insulin resistance is the major biochemical mechanism involved in the causation of MS as well as polycystic ovaries syndrome and non-alcoholic fatty liver disease.[19] Hypothyroidism is associated with elevated markers of insulin resistance such as homeostatic model of insulin resistance (HOMA-IR) in adults[20] and children.[21] Despite these known associations, the temporal relationships between subclinical hypothyroidism and assorted cardiovascular risk factors remain largely unexplored and studies are needed to find the chronology of development of components of MS with progression of hypothyroidism. Moreover, TSH should be taken as yardstick for decreasing thyroid function as it is a hormone of the mother gland and the anxiety of the mother gland (pituitary) cannot be compared with the concern of a small child (thyroid) who is totally oblivious of his health

Key Words: *Hypothyroidism, Metabolic Syndrome, Sub-clinical Hypothyroidism.*

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condition due to his shear ignorance.[22] TSH alone is a sufficient parameter for the early diagnosis and monitoring of hypothyroidism before one or more components of MS develop. In clinical practice, before starting treatment of dyslipidaemia, obesity or systolic hypertension, especially in a young patient, TSH estimation must be not be forgotten!

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ORIGINAL ARTICLE

Association of Hypothyroidism with Metabolic Syndrome

Fakhra Noureen[1], Muhammad Nadeem Akbar Khan[2], Abid Saeed khan[3], Shazia Qayyum[4], Aqsa Liaquat[5]

ABSTRACT

Objective: To determine the association between hypothyroidism and metabolic syndrome

Study Design: Observational cross sectional study

Place and Duration of Study: Data was collected from Medical unit and Department of Pathology, Pakistan Railways Hospital, Islamic International Medical College, Rawalpindi and Capital Hospital Islamabad from April 2017 to April 2018.

Materials and Methods: One hundred and fifty adult subjects participated in this study. Hundred hypothyroid subjects were recruited as cases on the basis of laboratory findings of raised serum thyroid stimulating hormone (TSH) levels and low serum free thyroxine (FT4) level. Newly diagnosed/ untreated cases of Hypothyroidism and adults of either sex were included. After an overnight fasting, participants were tested for various components of metabolic syndrome. Fifty euthyroid subjects were taken as controls. Data was analyzed by SPSS-21.

Results: Among 150 total recruited subjects, 25% hypothyroid cases and 10% euthyroid controls were diagnosed with metabolic syndrome. These results were statistically significant with p value 0.030. Mean serum triglycerides 183 ±26 and 153±26 mg/dl and mean fasting blood glucose 100 ±30 and 96 ±18 mg/dl respectively among hypothyroid and euthyroid patients were significant with p-value 0.001. Whereas, waist circumference, high density lipoprotein cholesterol and blood pressure measurement of hypothyroid and euthyroid individuals were not significant.

Conclusion: Hypothyroidism is associated with various components of metabolic syndrome.

Key Words: *Free Thyroxine, Fasting Blood Glucose, Hypothyroid, Metabolic Syndrome, Serum Triglycerides, Thyroid Stimulating Hormone*

Introduction

Thyroid dysfunction and metabolic syndrome are major encountered endocrine abnormalities in clinical practice.[1] High blood glucose level, raised serum triglyceride level, increased waist circumference, elevated blood pressure measurements and low high density lipoprotein cholesterol are common in these two entities.[2] Thyroid hormones regulate wide range of functions in body such as basal metabolic rate , protein synthesis, cardiac and gastrointestinal function, maturation of the central nervous system and maintain body mass index.[3] Hypothyroidism has prevalence of 3.8%–4.6% in Asian population as per data of different studies.[4] Definition of Metabolic syndrome given by American Heart association and the National Heart, Lung and Blood Institute (AHA/NHLBI) declared that clinical diagnosis of metabolic syndrome can be established if any three of the following factors are present, elevated triglyceride level (TG), elevated waist circumference, decreased HDL-cholesterol (HDL-C) level, elevated fasting plasma glucose and elevated blood pressure.[5] The estimated prevalence of metabolic syndrome in general population is between 17 and 25%.[3] Decreasing thyroid function is associated with occurrence of obesity and hence can potentially contribute to the development of MS.[6] As thyroid hormones decrease, components of metabolic syndrome get more prominent.[7] Study conducted in western population and India showed that thyroid

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hormones significantly affect each component of metabolic syndrome.[8, 9] Extensive literature review revealed that studies have been done regarding this topic in developed countries, however, reported clinical data is scarce in Pakistan. This knowledge gap was addressed by designing a study with an objective to find the relationship between hypothyroidism and metabolic syndrome.

Materials and Methods

It was an observational, cross-sectional study conducted at department of chemical pathology, Islamic International Medical College in collaboration with medical unit of Pakistan Railways Hospital, Rawalpindi and Medical unit of Capital Hospital Islamabad. The study extended over a period of 12 months from April 2017 to April 2018.

A total of 150 subjects were enrolled by non-probability convenient sampling after approval from Ethical Review Committee. Written informed consents were obtained from participants. With the help of medical specialist of Pakistan Railways and Capital hospital, serum TSH was done of clinically diagnosed patients who visited Medical OPD. Subjects who had raised serum TSH levels > 4.5 µIU/ml were further tested for serum fT4. Similarly hundred newly diagnosed patients of hypothyroidism on the basis of raised serum TSH and low serum Ft4 were taken as cases. Fifty age and gender matched controls with normal serum TSH level (0.4 – 4.5 µIU/ml) were enrolled.

Willing participants reported the next morning after an overnight 10-12 hours fast. 5ml blood was taken for analysis. The blood samples were centrifuged at 15000 rpm x g for about 15 minutes and serum was separated. Serum TSH and fT4 test was performed using the Vitros ECi Immunodiagnostic Systems. HDL-C was measured by enzymatic precipitation method on semi-automated chemistry analyzer micro-lab 300. Enzymatic end-point method (GPO-PAP) was used to determine the triglycerides level and Glucose concentrations were determined on fasting serum samples, using glucose oxidase method on semi-automated micro lab 300. Using the pubic crest and the umbilicus as land marks abdominal obesity was determined by measurement of the waist circumference in centimeters. Blood pressure was measured with the help of Mercury Sphygmomanometer. The average of two readings taken fifteen minutes apart was considered.

Data was entered and analyzed using Statistical Package for Social Sciences (SPSS) version 21. Simple descriptive statistics (frequencies, percentages) was computed for each categorical variable such as age and gender. Whereas mean and standard deviation was calculated for numerical (continuous) variables which included serum TSH, serum fT4, blood glucose fasting, serum HDL-C, serum triglycerides, waist circumference and blood pressure measurements. Independent sample t test was applied to compare the means of various components of metabolic syndrome. Chi square test was applied to determine the relationship between hypothyroid and different components of metabolic syndrome. p value of < 0.05 was considered statistically significant.

Results

Mean age (years) of total recruited subjects was 41.64±3 among them 33.3%were males and 66.7% were female. Mean age (years) of hypothyroid subjects was 42.48±11.56 having 26% male and 74%females. Among 50 euthyroid subjects age in years was 39.36±12.01 with 48% male and 52% female. Hypothyroid subjects who had metabolic syndrome were 25 in number with mean age of 46.50±8.97, and male to female ratio were 16.7 % and 83.3% respectively. Descriptive statistics of serum TSH in hypothyroid and euthyroid subjects was 32 ±28 and 3.2 ±0.6, which was statistically significant i.e (p 0.001). Whereas mean serum fT4 levels was 0.4 ±0.2 in hypothyroid subjects and 1.5 ±0.4 in euthyroid subjects and was statistically significant i.e (p 0.001). Mean serum TSH levels and fT4 levels were 35 ±28, 19 ±25 and 0.61 ±0.5, 0.8 ±0.5 in subjects having metabolic syndrome and subjects who did not present with metabolic syndrome respectively and was statistically significant (p-value 0.005) as shown in Table I.

Different variables of metabolic syndrome were compared among hypothyroid and euthyroid patients. Mean serum triglycerides mg/dl among hypothyroid and euthyroid patients were 183 ± 26 and 153 ± 26 respectively, the difference in two groups was significant (p value 0.001). Mean fasting blood glucose mg/dl levels were 100 ±30 and 96 ±18 in hypothyroid and euthyroid subjects and was statistically significant with p value 0.001. Independent sample t-test was used to compare

waist circumference, HDL and blood pressure measurements among hypothyroid and euthyroid subjects which was statistically not significant, as shown in Table II.

Chi-square test was applied to determine the association of hypothyroidism with metabolic syndrome as shown in Table III. Among 100 hypothyroid and 50 euthyroid subjects 25 (25%) and 05 (10%) individuals presented with metabolic syndrome respectively. The results were statistically significant with p value 0.030.

Table I : Descriptive Statistics of Serum TSH and Serum fT4 Levels in Metabolic Syndrome (MS) Patients

	MS present n= (30)	MS absent n= (120)	p value *
Serum TSH (0.4 –4.5U/ml)	35 ±28	19 ±25	0.005 *
Serum fT4 (0.78–2.19ng/dl)	0.61 ±0.5	0.8 ±0.5	0.005 *

*P<0.05 was taken as level of significant

Table II: Descriptive Statistics of Metabolic Syndrome Variables in Hypothyroid and Euthyroid Subjects

Metabolic syndrome Variables	Hypothyroid n= 100	Euthyroid n= 50	p value
Waist Circumference cms	91 ±12	86 ±9	0.07
HDL cholesterol mg/dl	40 ±7	44 ± 4	0.21
Serum Triglycerides mg/dl	183 ± 26	153 ± 26	0.001*
Blood Pressure systole mmHg	120 ± 12	118 ± 6	0.06
Blood Pressure diastole mmHg	79 ±8	78 ±7	0.16
Fasting Blood Glucose mg/dl	100 ±30	96 ±18	0.001*

*P<0.05 was taken as level of significant

Table III: Association of Hypothyroidism With Metabolic Syndrome

	Metabolic Syndrome		p-value
	present	absent	
Hypothyroid n=100	25 25%	75 75%	0.030*
Euthyroid n=50	5 10%	45 90%	

*P≤0.05 was taken as level of significant

Discussion

The study showed that mean age of hypothyroid and euthyroid participants were 42.48± 11.5 and 39± 12, respectively as compared to the patients of metabolic syndrome in which mean age was 46.5 ±8.9. This shows that prevalence of metabolic syndrome increases with age as decline in immunocompetence with age is accompanied by the increase in the incidence of autoimmune diseases. Similar findings are present in study by Ervin RB.[10]

In total of 150 participants, 30 subjects were diagnosed with metabolic syndrome in which 37.5% were male whereas 62.5 % were female which shows that in females MS is common. Study by Gurav et al reports similar findings.[11] Another study by Louai Razzouk et al demonstrated increase prevalence of the metabolic syndrome in older age and higher among females. This can be explained by increased caloric intake, dyslipidemia, and sedentary lifestyle, hormonal changes in women and obesity in older age.[12]

Mean serum TSH and fT4 in metabolic syndrome patients was 35 ±28, 19 ±25 and 0.61 ±0.5, 0.8 ±0.5 respectively which was statistically significant. TSH is more sensitive indicator of thyroid function. Even small changes in Thyroid hormone levels may cause a marked shift in TSH level these findings have shown a similar relationship with thyroid hormones by Shehzad et al.[13]

In our study it was found that 25(83%) hypothyroid subjects were diagnosed with MS and had high serum TSH levels as compared to the euthyroid subjects where 5 subjects were diagnosed with MS, who had TSH levels within normal reference range. The study conducted in Nepal by Gyawali et al showed significant difference.[14]

Fasting blood glucose levels were found higher in hypothyroid patients as compare to euthyroid (control) group. This finding is in agreement to previous study by Maratou at al.[15]

Serum triglyceride levels were found to be elevated; on the other hand the HDL-C levels were lower in hypothyroid patients. TH affects HDL-C through cholesterol ester transfer protein and hepatic lipase activity. These findings are similar with results of previous studies by Sanyal et al and Pearce et al.[16,17] In our study, it has been observed that high serum TSH levels are directly related with various components of metabolic syndrome. Study by Renehr et al has found increased serum TSH levels to be correlated

with obesity. This may be due to involvement of leptin protein from adipose tissues which also fluctuates with body adiposity.[18]

Extensive literature review has revealed that scanty work has been done in our setup to determine association between hypothyroidism and metabolic syndrome. Our study results showed that MS is highly prevalent among hypothyroid patients. There were 25 (83.3%) hypothyroid and 05 (16.7%) euthyroid patients (control group) who were diagnosed with metabolic syndrome. These results are in agreement with the previous study by Gyawali P[14] and Roos et al.[19] Another study conducted by Tehrani F on large cohort of 5786 subjects also found that hypothyroidism is associated with metabolic syndrome.[20]

Conclusions

Our study shows association of MS among hypothyroid patients in our setting in Rawalpindi and Islamabad, Pakistan. The coexistence of two diseases MS and thyroid dysfunction increases the chance of cardiovascular disease and diabetes. Therefore, diagnosed patients of hypothyroidism should be evaluated for all components of MS.

Limitations

The study design was Cross-Sectional, so it did not help to determine cause and effect relationship between low-normal thyroid function and MS. Sample size was small i.e. 100 hypothyroid patients and 50 euthyroid participants, a large scale clinical trials should be carried out. Due to limited resources subjects were recruited from two centers, a multicenter study should be carried out involving all main hospitals of the city.

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ORIGINAL ARTICLE

Chemical Cautery with 100% TCA Versus $AgNO_3$ For the Treatment of Xanthelasma Palpebrum: A Randomized Controlled Trial

Lubna Rani Faysal,[1] Farid Ur Rehman[2]

ABSTRACT

Objective: To compare the efficacy of chemical cautery with 100% trichloroacetic acid (TCA) versus silver nitrate ($AgNO_3$) for the treatment of xanthelasma palpebrum.

Study Design: Randomized controlled trial (RCT).

Place and Duration of Study: Department of Dermatology, Pakistan Railway Hospital, Rawalpindi from 1st June 2016 to 31st December 2017.

Materials and Methods: A total of 40 adult patients with xanthelasma palpebrum were enrolled and randomly divided into two equal groups. In group A patients, chemical cautery was done with TCA 100% while group B patients were treated with AgNO3. Results were recorded after single session of treatment at 02 weeks for improvement/ clearing of lesion and at 03 months for post inflammatory hypopigmentation /hyperpigmentation and recurrence.

Results: In group A, 19 patients (95%) out of 20 showed 75 – 100% clearing two weeks after the single treatment session in comparison to 04 (20%) patients in Group B. And only 01 patient (5%) in group A showed 50-75% clearing as compared to group B where 16 patients showed this response. None of the patient showed less than 50% clearing in group A while 01 patient (5%) in group B had this response. At the end of 03 months, 20% and 5% patients developed post-inflammatory hypopigmentation in group A and group B respectively. No recurrence of lesion was reported during this period.

Conclusion: Chemical cautery for the treatment of Xanthelasma with 100% TCA gives better results than $AgNO_3$.

Key Words: *Chemical Cautery, Cryotherapy, Hyperlipidemia, Radiofrequency (RF), Trichloroacetic acid (TCA), Xanthelasma Palpebrum (XP).*

Introduction

Xanthelasma palpebrum (XP) is the commonest cutaneous xanthoma that develops around the eyes with the prevalence of roughly 1.1% in women and 0.3% in men.[1] Xanthomas are cholesterol-rich depositions that can appear anywhere in the body during various disease states. The term "xanthelasma" is derived from the Greek word xanthos (yellow) and elasma (beaten metal plate)[2] the age of onset ranges from 15 to 73 years, with a peak incidence between 30 and 50 years.[3]

Clinically Xanthelasma can be categorized into macular, flat plaques and papulonodular types. It is characterized by yellowish plaques occurring symmetrically distributed near the inner canthus of the eyelid, more often on the upper, rather than the lower lid. XP is composed of xanthoma cells or foam cells, histiocytes are laden with intracellular fat deposits, primarily located within the upper reticular dermis or in perivascular and periadnexal areas. Intrahistiocytic vacuoles contain esterified cholesterol.[4]

Xanthomas are usually associated with primary hyperlipidemias, especially types II and IV, having low high-density lipoprotein (HDL) levels, or secondary hyperlipidemias, such as hypothyroidism, diabetes mellitus. Drugs like glucocorticoids, cyclosporine, cimetidine, estrogens, some antihypertensive medications, retinoids, certain antiepileptic drugs, anabolic steroids and tamoxifen

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are associated with secondary hyperlipidemia.[5] About 60% of patients have associated hypercholesterolemia. XP has also been reported following erythroderma, inflammatory skin disorders, and allergic contact dermatitis despite normal lipid profiles. XP can occur in normolipidemic persons with low HDL levels.[6]

It is essentially a benign condition; treatment is totally of cosmetic importance. In patients with XP who have associated lipid disorders, plasma lipid levels including triglycerides, cholesterol, low density lipoprotein and HDL, and apolipoprotein B100 levels should be assessed. Medical management involves lifestyle modifications such as regular physical exercise and low-fat diet in addition to lipid-lowering drugs. Although important in the overall care of a patient with abnormal lipids, medical management has a limited role in the treatment of XP.

Several treatment modalities are used to treat XP but none of them produces satisfactory results. These include simple surgical excision, cryotherapy, chemical cauterization with trichloroacetic acid (TCA), radiofrequency (RF), and laser treatment.[7] All modalities have their own advantages and disadvantages. Simple excision with or without blepharoplasty and medial epicanthoplasty can be conducted in grades I and II lesions, whereas, in advanced cases, uncapping surgery, local flaps, and skin grafts can be carried out. The commonest method of surgery is full-thickness skin excision[8] there are many disadvantages associated with surgery. There is always need of systemic or local anesthesia. It is often followed by slight scarring, ectropion and dyspigmentation.[9] Radiofrequency (RF) is considered to be an easy, safe, inexpensive, and effective treatment but facility is not available in all clinical setups. This technique has minimal impact on the surrounding tissues, making it appropriate for delicate areas with temporary side effects include pain, pruritus, burning, swelling, and erythema.[10] Similarly advantages of lasers include better acceptance, avoidance of surgery, minimal tissue loss, and good functional and cosmetic results. Moreover, the procedure is easy to perform and gives fast results but the main disadvantages are high cost and unpredictable results sometimes. In addition, it is not possible to obtain a histopathological specimen.[11,12]

TCA is an affordable and versatile treatment modality, particularly in our setup. It is a short, simple, and inexpensive procedure. It has been observed that 100% TCA gives the best results in papulonodular lesions, 100% or 70% TCA give similar results in flat plaque xanthelasma, and 50% TCA is sufficient in macular lesions.[13] Hypopigmentation is the commonest side effect, followed by hyperpigmentation, irritation, and pain. Scarring, atrophy, and Koebner-like phenomenon are other rare side effects.

There is not sufficient data available in literature on use of AgNO3 in treatment of xanthelasma but there is evidence of its use to debride hyper granulation tissue and calloused rolled up edges in wounds and ulcerations. It is a caustic material that oxidizes organic matter, coagulates tissue resulting into tissue death.[14] The objective of this study was to compare the efficacy of chemical cautery with 100% trichloroacetic acid (TCA) versus silver nitrate ($AgNO_3$) for treatment of xanthelasma palpebrum in order to emphasize the importance and efficacy of this simple and cost effective treatment modality in the era of modern energy devices where Laser & radiofrequency have almost replaced such modalities.[10,15]

Materials and Methods

This randomized controlled trial was conducted on outdoor patients visiting department of Dermatology, Pakistan Railway Teaching hospital, which is an affiliate of Islamic International Medical College, Rawalpindi. A total of 40 patients with diagnosis of Xanthelasma palpebrum, fulfilling inclusion criteria (age > 30 years, patients irrespective their lipidemic status, and absence of hypersensitivity) were enrolled for the study. The trial began after the permission from the hospital ethical review board and all the ethical issues were addressed. The disease was diagnosed on the basis of history and clinical examination. Informed written consent was taken from patients after detailed explanation. The patients were divided in two equal groups (20 in each) by computer generated randomization list. Group allocation was done to group A and group B

Patients allocated to group-A were treated with 100% TCA and patients in group-B were treated with

AgNO3 (supersaturated sol). The chemical was applied to the lesion with help of wooden stick cotton swab; size of swab was customized manually to the size of lesion. Before application of chemical, area was cleaned with normal saline. Swab was dipped into the chemical and applied to the lesion with rotatory movement from outside to center of the lesion. The surrounding skin was protected by application of white soft paraffine around the lesion to prevent damage to the normal skin from accidental spillage of chemical. The endpoint for TCA application was frosting of lesion and for $AgNO_3$ was charring /blackening of the lesion. All patients were given Fusidic acid 2%, which had to be applied over the treated area twice daily for 01 week after the treatment.

All the patients were evaluated objectively in clinic for improvement of lesion after 02 weeks of treatment session. The results were scored on a 0–4point following scale:

0 - No improvement

1 - Moderate result (<25% improvement)

2 - Satisfactory result (25%–50% improvement)

3 - Good result (50%–75% improvement

4 - Excellent result (>75% improvement)

Follow up visits were done at 03months interval for post-inflammatory hyper/ hypo-pigmentation& recurrence of lesion.

Data was entered and analyzed by statistical package for social sciences (SPSS) version 21. Quantitative data was calculated in the form of mean and standard deviation. Qualitative data was calculated in the form of frequencies and percentages. Chi-square test was used to compare the efficacy in two groups. A probability (p) value of less than 0.05 was considered statistically significant.

Results

A total 40 patients with xanthelasma palpebrum were enrolled in the study, with 20 in each group, A & B respectively. The overall mean age of study population was 47 years with age range of 32 years to 68 years. There was no significant difference in the mean age of study population between two groups. There were total 13 males and 27 female patients. Patients in group-A were treated with TCA chemical cautery and patients in group-B were treated with $AgNO_3$.

In group-A, 19 (95%) patients showed excellent results (75%-100% improvement) at 02 weeks of treatment, and 01(5%) patient showed this response in group-B. A total of 04 (20%) patients showed good result i.e.50-75% improvement in group-A and 15 (75%) patients in group-B had this response. Only 01 (5%) patient had less than 50% improvement in group-B. (Table-I)

The percentage of patients with excellent improvement was significantly higher in group-A as compared to group-B, P-value <0.000. (Table-II)

At the end of 03months follow up period, 04 (20%) patients in group-A had post-inflammatory hypopigmentation while 01 (5%) patient had it in group-B. (Figure-I). No recurrence of lesion was reported during this time.

Table I: Comparsion of Efficiency of Treatment in Group A & Group B

Treatment Groups	Efficacy						Total (n)
	Less than 50%	%	50-75% response	%	75-100% response	%	
Group A	0	0%	1	5%	19	95%	20
Group B	1	5%	15	75%	4	20%	20
Total	1	5%	16	80%	23	58%	40

Table II: Chi- Squar Test Showing Significant Difference

	Value	Df	Asym.Sig. (2-sided)
Pearson Chi-Square	23.033	2	0.000
Likelihood Ratio	26.717	2	0.000
Linear-by-Linear Associate	20.975	1	0.000
N of valid Case	40		
A.2 cells (33.3%) have expected count less than 5			

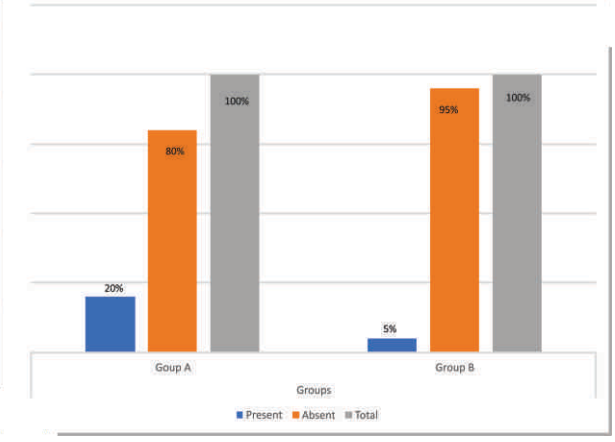

Fig 1: Comparison of Post Inflammatory Hypopigmentation In Both Groups

Discussion

The present study clearly shows the superior efficacy of TCA chemical cautery in treatment of xanthelasma palpebrum as compared to AgNO3. More than 90%

patients achieved excellent results at 02 week after a single treatment session with TCA 100% while the corresponding figure is very low, only 5% in group B.

In a retrospective review of 102 patients treated with TCA 95% for XP conducted by Cannon PS, et al. patient's outcome at 03 months and 12 months of treatment for recurrence/ persistence of the lesion showed high patient satisfaction. Overall, the success rate for TCA was 61% at a mean follow-up of 31.8 months[16] the mean number of TCA treatments was 1.68. Out of 44 reviewed in the clinic, there were 09 persistent lesions and 04 recurrences. Of 51 patients contacted by telephonic interview, 22 patients had experienced a recurrence, 09 patients had persistence of the lesion and 3 patients undergone surgical excision of the lesion since the last TCA treatment.

In our study, > 90% patients achieved excellent results with 100% TCA application and no persistence of lesion was recorded at 03 months follow up but to report for the recurrence, a longer follow up will be required.

The results of various prospective treatment trials with different strengths of TCA, 70% and 50% have shown its efficacy, satisfactory cosmetic result and acceptable recurrence rate.[13,17] But lowering the strength, increases average number of applications of chemical to achieve the desired result and hence the after effects of treatment like post-inflammatory hypo/ hyperpigmentation and scarring also get increased. Atrophy and a Koebner-like phenomenon has also been reported with repeated TCA applications.[18]

In our study only 04 patients reported with post inflammatory hypopigmentation at 03 months follow up, while no scarring, atrophy and koebnerization has been observed during follow up period. Hence higher concentration of TCA is more efficacious with better cosmetic results. It also prevents the repeated applications of TCA.

There is limited data available in literature on use of AgNO3 in treatment of xanthelasma but there is evidence of its use in treatment of XP.[14] In our study AgNO3 showed good results, 15 patients (75%) showed 50-75% clearing of lesion in single treatment while 4 (20%) had excellent results. It is relatively less efficacious than TCA and has comparatively less post-treatment pigmentary changes (5% in group-B vs 20% in group-A). But the requirement of repeated applications of $AgNO_3$ to achieve complete clearance of the lesion may lead to more post-treatment pigmentary changes and scarring compared to TCA100%. Hence further research with $AgNO_3$ in future will be required with longer follow up duration.

Conclusion

Chemical cautery with 100% TCA is more effective than AgNO3 and it is strongly recommended for treatment of Xanthelasma palpebrum.

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ORIGINAL ARTICLE

Effects of Aqueous and Methanolic Extracts of Cichorium Intybus Seeds on Gentamicin Induced Nephrotoxicity in Rats

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ABSTRACT

Objective: To evaluate the effects of Aqueous and Methanolic extracts of Cichorium Intybus seeds on Gentamicin induced nephrotoxicity in rats.

Study Design: Experimental Study

Place and Duration of Study: Study was conducted from 15 January to 31 March 2017 at National Institute of Health Sciences (NIH) in collaboration with Riphah Institute of Pharmaceutical Sciences (RIPS).

Materials and Methods: Forty healthy Sprague Dawly rats weighing 300-350gms were randomly divided in four groups with 10 rats each. Group A was the control group that received no medications. Group B was given Gentamicin 80mg/ kg body weight / day for 10 days intraperitoneally (IP). Group C was given Gentamicin 80mg/ kg body weight / day IP and Aqueous Extract of Cichorium Intybus seeds 500mg/kg body weight/ day through gavage tube for 10 days. Group D was given Gentamicin 80mg/ kg body weight / day IP and Methanolic Extract of Cichorium Intybus seeds 500mg/kg body weight / day through gavage tube for 10 days. Serum urea and creatinine were measured at day zero, 6, and 11. The values were compared within and between the groups.

Results: Elevated levels of serum urea and creatinine were found in group B indicating renal damage. Whereas administration of Aqueous and Methanolic extracts of Cichorium Intybus seeds reduced the elevated levels of serum urea and creatinine in group C & D. However more reduction was produced in group C.

Conclusion: Concomitant administration of Aqueous and Methanolic extracts of Cichorium Intybus seeds exerted nephroprotective effect on Gentamicin induced nephrotoxicity in rats. Aqueous extract produced greater protection as compared to Methanolic extract.

Key Words: *Cichorium Intybus Seeds, Gentamicin, Nephroprotective effect, Nephrotoxicity.*

Introduction

Kidneys play a vital role in the body performing important functions for example excretion of metabolic waste, drugs, homeostasis maintenance and extracellular environment regulation.[1] Direct or indirect exposure of drugs and different chemicals to kidneys results in nephrotoxicity. Many therapeutically used drugs may result in acute or chronic renal failure.[2] Across the globe approximately 19-25% cases of nephrotoxicity in critically ill patients results from nephrotoxic drugs.[1] Nephrotoxicity is indicated by the raised levels of serum urea and creatinine.[3] Drug groups which cause nephrotoxicity include immunosuppressant, anticancer drugs, radio contrast media and antibiotics.[4]

Aminoglycosides (AGs) is among one the widely prescribed antibiotic group throughout the world being cost effective.[4] Aminoglycosides are bactericidal antibiotics which irreversibly inhibit protein synthesis by binding to specific 30S-subunit ribosomal protein through interference of initiation of complex of peptide, breaking of polysome into monosomes and misreading of mRNA.[5] The major adverse effects caused by aminoglycosides are ototoxicity and nephrotoxicity, which limits their use. Aminoglycosides are excreted unchanged through glomerular filtration without metabolism.[7] During the process of concentration and reabsorption

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proximal tubular cells are mainly exposed to damage by the drugs. Free radicals generated due to oxidative stress causes mitochondrial damage in tubules resulting in cytotoxicity. Inflammation in glomerulus and tubules is induced by nephrotoxic drugs which ultimately result in fibroses of renal tissue.[8]

From ancient days medicinal plants have been widely used as a source of medication providing a cheap source of treatment for a majority of world's population. Herbal plants have got nephroprotective effect.[9] Several plants along with their extracts have got protective effect against nephrotoxic drugs due to their anti-oxidant, anti-inflammatory and diuretic properties.[10] Cichorium intybus , generally called chicory (Kasni) is vertical woody perennial plant distributed in Europe, Mediterranean region, Northern Asia and Pakistan.[11] All parts of Cichorium intybus is known to have antimicrobial, gastroprotective, hepatoprotective, anti-diabetic, antimalarial, anti-inflammatory and anti-oxidant effects due to the presence of phytocompounds like polyphenols , alkaloids, flavonoids and tannins.[12,13]

Previous studies have shown anti-oxidant effects of either Aqeous or methanolic extracts of Cichorium intybus roots, leaves and stem against gentamicin induced nephrotoxicity or hepatotoxicity in animal model.[10] Evaluation of both aqueous and methanolic extracts of Cichorium intybus seeds against gentamicin induced nephrotoxicity in literature is lacking. Present study was designed to evaluate the effect of both aqueous and methanolic extracts of Cichoriumintybus seeds against gentamicin induced nephrotoxicity in rat's model.

Materials and Methods

This experimental randomized control trial was conducted from 15th January 2017 to 31st March 2017 in the department of Pharmacology and Therapeutics, Islamic International Medical College, Rawalpindi in collaboration with the Riphah Institute of Pharmaceutical Sciences and Animal house at National Institute of Health (NIH), Islamabad. Forty healthy adult male Sprague Dawly rats were included through simple random sampling and the study was conducted after approval from Ethical Review Committee of Islamic International Medical College, Riphah International University (RIU), Islamabad.

Rats weighing between 300-350 grams with normal Serum urea and Creatinine levels were included in the study. Rats were first allowed to get acclimatized for one week in the NIH Animal house in 50-70% humidity at a room temperature of 24$\pm$2°C with a 12 hour light and dark cycle. [14] Rats were randomly divided into four groups of ten rats each (n=10). Group A was normal control, given diet and water ad libitum for ten days. Group B was administered Gentamicin at 80 mg/kg intraperitoneally once daily for ten days. Group C was administered Gentamicin 80mg/kg intraperitoneally along with Aqueous extract of Cichorium intybus seeds orally mixed in 2ml distal water through gavage tube at a dose of 500mg/kg once daily for ten days.[10] Group D was administered Gentamicin 80mg/kg body weight once daily intraperitoneally along with Methanolic extract 500mg/kg orally mixed in 2ml distilled water through gavage tube once daily for ten days.[15]

Dried seeds of Cichorium intybus were purchased and authenticated from herbarium department of National Agriculture and Research Centre (NARC) Islamabad. Aqueous extract of Cichoriumintybus seeds was prepared at RIPS, Islamabad by using fine homogenized powder of chicory seeds which were mixed with distilled water, the whole solution was boiled for 2 hours and after cooling was filtered through filter paper what man no 3. The aqueous extract was formed by using vacuum rotary evaporator and was frozen dried.[16] To obtain the methanolic extract, 1 kg of Cichorium Intybus seeds powder was soaked in 8 L of methanol (95%) for 24 h. The extract was filtered using what man paper No 3 and the filtrate was evaporated (78 °C) to dryness using a rotary evaporator; 25.64 g of dried methanolic extract were obtained giving an extraction yield of 2.56% (w/w based on the dried starting weight).[17] Sampling at day 0 and day 6 were done via lateral tail vein. Sampling at day 11th was done through cardiac puncture. Biochemical analysis of serum urea and serum creatinine was estimated through commercially available kits by Merk and auto analyzer Micro lab 300 on photometric system.

Statistical analysis of collected parametric data was done by using SPSS version 21 and Mean ± Standard Error of Mean was calculated. One way ANOVA and Post Hoc Tuckey tests were applied to compare the mean difference between control and rest of the groups and mean difference in between the groups.

p value of <0.05 was considered statistically significant.

Results

Mean initial Serum Urea for Group A (Normal Control Group) was 23.70 ± 1.521 mg/dl. Mean initial Serum Urea for Group B (Disease Control Group) was 24.00 ± 1.732 mg/dl. Mean initial Serum Urea for Group C (Aqueous extract Experimental group 1) was 25.56 ± 1.744 mg/dL. Mean initial Serum Urea for Group D (Methanolic extract Experimental group 2) was 22.10 ± 1.912 mg/dl. There was no significant difference in the serum urea levels among these groups on day 0 (p Value .589). Serum Urea on day 6 for Group A (Normal Control Group) was 24.70 ± 1.633 mg/dl, for Group B (Disease Control Group) was 83.30 ± 2.119 mg/dl, for Group C (Aqueous extract Experimental group 1) was 67.10 ± 1.703 mg/dl& for Group D (Methanolic extract Experimental group 2) was 84.00 ± 1.592 mg/dl. There was significant difference in the serum urea levels among these groups on day 6 (p Value .000). This suggests that nephrotoxicity was induced by Gentamicin.

Mean Serum Urea on day 11 for Group A (Normal Control Group) was 24.70 ± 5.165 mg/dl , for Group B (Disease Control Group) was 97.90 ±4.332 mg/dl, for Group C (Aqueous extract Experimental group 1) was 51.30 5.755 mg/dl& for Group D (Methanolic extract Experimental group 2) was 73.60mg/dl ±4.695. There was significant difference in the values of group C & D (p Value .000). This suggests that nephrotoxicity in Group C (Aqueous Extract Treated Group) was improved more than Group D (Methanolic Extract Treated Group).

Mean initial Serum Creatinine for Group A (Normal Control Group) was 0.750 ± 0.0654 mg/dl. Mean initial Serum Creatinine for Group B (Disease Control Group) was 0.740 ± 0.0562 mg/dl. Mean initial Serum Creatinine for Group C (Aqueous extract Experimental group 1) was 0.770 ± 0.0559 mg/dL. Mean initial Serum Creatinine for Group D (Methanolic extract Experimental group 2) was 0.770 ± 0.0667 mg/dL. There was no significant difference in the serum Creatinine levels among these groups on day 0. (p Value .980).

Mean Serum Creatinine on day 6 for Group A (Normal Control Group) was 0.750 ± 0.0619 mg/dL, for Group B (Disease Control Group) was 2.490 ± 0.0849 mg/dl, for Group C (Aqueous extract Experimental group 1) was 1.450 ± 0.0601 mg/dl& for Group D (Methanolic extract Experimental group 2) was 1.730 ± 0.0517 mg/dl. There was significant difference in the serum Creatinine levels among these groups on day 6 (p Value .000). This suggests that nephrotoxicity was induced by Gentamicin.

Mean Serum Creatinine on day 11 for Group A (Normal Control Group) was 0.750, ± 0.0619 mg/dl, for Group B (Disease Control Group) was 2.490, ± 0.0849 mg/dl, for Group C (Aqueous extract Experimental group 1) was 1.450 0.0601 mg/dl& for Group D (Methanolic extract Experimental group 2) was 1.730 ± 0.0517 mg/dl. There was significant difference in the values of group C & D (p Value .024). This suggests that nephrotoxicity in Group C (Aqueous Extract Treated Group) was improved more than Group D (Methanolic Extract Treated Group).

Table I: Comparison of Serum Urea and Creatinine Levels Among Groups By ANOVA at Day 0

		Sum of Squares	df	Mean Square	F	Sig.
Serum Urea	Between Groups	58.275	3	19.425	.648	.589
	Within Groups	1079.500	36	29.986		
	Total	1137.775	39			
Serum Creatinine	Between Groups	.007	3	.002	.060	.980
	Within Groups	1.351	36	.038		
	Total	1.358	39			

Table II: Comparison of Serum Urea and Creatinine Levels Among Groups By ANOVA at Day 06

		Sum of Squares	Df	Mean Square	F	Sig.
Serum Urea	Between Groups	23241.875	3	7747.292	246.141	.000
	Within Groups	1133.100	36	31.475		
	Total	24374.975	39			
Serum Creatinine	Between Groups	10.714	3	3.571	116.668	.000
	Within Groups	1.102	36	.031		
	Total	11.816	39			

Table III: Comparison of Serum Urea and Creatinine Levels Among Groups By ANOVA at Day 11

		Sum of Squares	Df	Mean Square	F	Sig.
Serum Urea	Between Groups	29290.875	3	9763.625	388.173	.000
	Within Groups	905.500	36	25.153		
	Total	30196.375	39			
Serum Creatinine	Between Groups	15.539	3	5.180	119.531	.000
	Within Groups	1.560	36	.043		
	Total	17.099	39			

Table IV: Multiple Comparison of Serum Urea and Creatinine levels Among all Groups By Post Hock Tuckey Test

Parameter	Groups	Mean difference	P Value
Serum Urea	Group A (control group) Vs Group B (Disease Control Group)	-73.200(*)	.000
	Group A (control group) Vs Group C (Experimental group 1)	-26.600(*)	.000
	Group A (control group) Vs Group D (Experimental group 2)	-48.900(*)	.000
	Group B (Disease Control Group) Vs Group C (Experimental group 1)	46.600(*)	.000
	Group B (Disease Control Group) Vs Group D (Experimental group 2)	24.300(*)	.000
	Group C (Experimental group 1) Vs Group D (Experimental group 2)	-22.300(*)	.000
Serum Creatinine	Group A (control group) Vs Group B (Disease Control Group)	-1.7400(*)	.000
	Group A (control group) Vs Group C (Experimental group 1)	-.7000(*)	.000
	Group A (control group) Vs Group D (Experimental group 2)	-.9800(*)	.000
	Group B (Disease Control Group) Vs Group C (Experimental group 1)	1.0400(*)	.000
	Group B (Disease Control Group) Vs Group D (Experimental group 2)	.7600(*)	.000
	Group C (Experimental group 1) Vs Group D (Experimental group 2)	-.2800(*)	.024

Discussion

Gentamicin is widely used to induce nephrotoxicity in animal models for research purposes and has been stated by numerous researchers for analyzing nephroprotective effects of natural plants. Key phenomenon elaborating the GM induced nephrotoxicity is damage by reactive oxygen species.[18] Review of literature showed that production of ROS lead to lipid peroxidation causing necrosis and denaturation of proteins. Various studies have concluded that the nephroprotective effects of natural plants are due to the presence of antioxidants.[19,20]

The current study was designed to investigate the protective effects of Aqueous and Methanolic extracts of Cichorium Intybus seeds in Gentamicin induced nephrotoxicity in rats via biochemical parameters. It was found that Gentamicin induced nephrotoxic changes were improved by both Aqueous and Methanolic extracts of Cichorium Intybus seeds but aqueous extract showed better nephroprotective effects against Gentamicin induced nephrotoxicity. In present study, GM induced nephrotoxicity is indicated by raised level of serum creatinine and urea as concluded previously by Nitha and Janardhanan.[21] The process glomerular filtration causes the accumulation of gentamycin in the proximal tubules of kidney. GM Produced ROS causes oxidative stress and tubular injury and is indicated by raised serum creatinine and urea.

Our study in accordance with findings of study by Tanweer khaliq who induced Nephrotoxicity in Albino rabbits with Gentamicin Intraperitoneally in a dose of 80 mg/kg and observed raised levels of biochemical markers.[10] Similarly our study is in correlation with another study performed by V. Chinnapa Reddy showing the nephrotoxic effects of Gentamicin in Rats which were indicated by raised levels of serum urea and creatinine.[16]

Current study is consistent with the study of Tanweer Khaliq who compared the nephroprotective effect of aqueous extract of Cichorium Intybus seeds against Gentamicin induced nephrotoxicity in which he used aqueous extract of Cichorium Intybus seeds in doses of 250 mg/kg administered via gavage tube. His study also evidenced the nephroprotection due to presumed antioxidant properties of Cichorium Intybus seeds.[10]

Review of literature shows that studies have been done on exploring nephroprotective effect of Cichorium intybus in combination with other herbal extracts and medical compounds like silymarin.[10] No individual study was done on aqueous and methanolic extract of Cichorium intybus seed indicating dosage use regarding submaximal, ceiling effect and toxicity. Current study shows the nephroprotective effect of both aqueous and methanolic extract of Cichorium intybus seeds. Further studies are needed to elaborate the mechanism of higher response of methanolic extract as compare to aqueous extract of Cichorium intybus seeds for reducing elevated renal markers. In addition administration through different routes and higher dosage can be tried to see the effect.

Conclusion

Aqueous and Methanolic extracts of Cichorium Intybus seeds exhibited protective effects against Gentamicin induced nephrotoxicity in rats. Aqueous extract Cichorium intybus seeds have exerted greater nephroprotective effect in comparison to Methanolic extract.

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ORIGINAL ARTICLE

Hepatoprotective Effect of Tamarixdioica Roots on Acetaminophen Induced Hepatotoxicity in Male Mice

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ABSTRACT

Objective: To study the hepatoprotective effect of Tamarixdioica at low and high doses on transaminases (Alanine aminotransferase and Aspartate aminotransferase) in acetaminophen induced hepatotoxicity in male mice.

Study Design: It was a Randomized Control Trial.

Place and Duration of Study: The study was conducted at department of Pharmacology, Islamic International Medical College, Rawalpindi from 1st April 2015 to 31st March 2016.

Materials and Methods: Forty Balb-c Albino male mice were randomly divided in four groups with 10 mice each. Group A was the control group and received no medications. In Group B (Disease Control Group) hepatotoxicity was induced by Acetaminophen 1000mg per kg body weight given daily for 4 weeks. Group C (Low Dose Experimental Group) was given Acetaminophen 1000 mg/kg/day orally in combination with Tamarixdioica (Aqueous Extract) that was given daily through gavage tube in a dose of 100mg/kg/day for 4 weeks. Group D (High Dose Experimental Group) followed the same protocol as Group C but the Tamarixdioica (Aqueous Extract) dose was increased to 200mg/kg/day for 4 weeks through gavage tube. ALT and AST were measured and compared in different groups to see the hepatoprotective effect of Tamirixdioica.

Results: Mean ALT in Group A, B and C were 35.50 2.24 U/L, 94.00 8.62 U/L and 50.90 4.56 U/L respectively. There was a significant difference among ALT values of Group A & B and among Group B & C (p Value .000). Mean AST in Group A, B and C were 27.00 3.11 U/L, 151.00 14.53 U/L and 66.90 7.77 U/L respectively. There was a significant difference among AST values of Group A & B and among Group B & C (p Value .000). This suggested that hepatotoxicity was induced by acetaminophen and hepatotoxicity was improved by Tamirixdioica. No significant difference was observed in ALT & AST values among Group C and D.

Conclusion: Acetaminophen induces hepatotoxicity at high dose. Concomitant treatment with aqueous extract of roots of Tamarixdioica prevents hepatotoxicity induced by Acetaminophen in mice. Extract of roots of Tamarixdioica showed improvement biochemically in both low and high doses, as compared to drug treated group in a dose independent manner.

Key Words*: Acetaminophen, Hepatoprotective effect, Hepatotoxicity, Tamarixdioica.*

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Introduction

According to available data, diseases of liver such as inflammatory disorders and hepatitis are considered to be the most prevailing diseases in world. Pakistan has sixth highest number of cases with liver diseases.[1] Viral Hepatitis makes a big proportion in chronic cases.[2] Drug induced hepatotoxicity is common in Acute settings. Pharmacological drugs account for 20-40% of all cases of fulminant hepatic failure. Approximately 75% of the idiosyncratic drug reactions result in liver failure or death.[3] Hepatic injury caused by drugs is the most common reason mentioned for withdrawal of an approved drug. About 2000 cases of acute liver failure occur annually in the United States, and drugs account for over 50% of them (39% are due to acetaminophen, 13% are idiosyncratic reactions due to other medications).

Drugs account for 2-5% of total cases of patients hospitalized with jaundice and approximately 10% of all cases of acute hepatitis.[4] Acetaminophen (Paracetamol, N-acetyl-p-aminophenol) is the most commonly used over-the counter analgesic and antipyretic drug. At therapeutic doses, it is believed to be safe, having analgesic and antipyretic effects. Paracetamol toxicity is the foremost cause of acute liver failure in the Western world.[5] Estimation of liver enzymes should be done to evaluate the liver function during the course of hepatic injury treatment.[6] The sensitive markers for the evaluation of liver function are serum ALT and AST as these are cytoplasmic in their location and found in circulation in case of cellular damage. ALT is the most sensitive marker indicating the liver injury.[7]

Herbal medicines represent one of the most important fields of traditional medicine throughout the world. WHO estimates that 80% of the world population relies on herbal medicine for primary health care? About 30% of the pharmaceutical preparations are still extracted from plant material. These are the source of good affordable effective drugs. The family Tamaricaceae consists of 60 different species, which are also commonly called salt cedars. Tamirix.dioica (Tamaricaceae), with the local name of Lai in Urdu Ghaz or khagal, is an evergreen shrub or small tree with reddish bark, vaginate leaves, and purple flowers. The tree is native to Pakistan, Afghanistan, Iran, India, Bangladesh, Bhutan, Kashmir, Nepal, and Myanmar.[8] Screening of phytochemicals constituents of Tamarixdioica showed positive results for the presence of flavonoids, alkaloids, phenols, steroids, glycosides, carbohydrates, terpenoids and tannins. In past studies has been carried out which concluded the antioxidant and hepatoprotective effects of Tamarixdioica by using methanolic extracts of its various phytochemical.[9] However studies on hepatoprotective effects of Tamarixdioica by using aqueous extracts of its roots is limited. Objective of the present study was to observe the hepatoprotective effects of Tamarixdioica (Aqueous Extract) at low and high doses against acetaminophen induced hepatotoxicity in male mice by evaluating ALT and AST levels as biochemical markers.

Materials and Methods

This randomized control trial was conducted from 1st April 2015 to 31st March 2016 in the department of Pharmacology, Islamic International Medical College, Rawalpindi in collaboration with Animal house at National Institute of Health (NIH), Islamabad Pakistan after getting approval from Ethical Review Committee of Riphah International University (RIU), Islamabad.

In this study 40 adult male Albino mice were used. Mice weighing between 30-50 grams with normal Serum Alanine aminotransferase and Aspartate aminotransferase levels were included while mice weighing less than 30 grams or mice with abnormal serum ALT and AST were excluded from the study. Mice were first allowed to get acclimatized for one week in the NIH Animal house in 50-70% humidity at a room temperature of $24\pm2^{\circ}C$ with a 12-hour light and dark cycle.[8]

Blood samples were taken randomly from 8 mice for estimating Serum ALT and AST levels at day 0. Forty healthy mice were then randomly divided into four groups of ten mice each (n=10). Group A was normal control, given diet and water adlibitum for four weeks. Group B (Disease Control Group) was administered Acetaminophen at 1000 mg per Kg body weight through gavage tube daily for 4 weeks. Group C (Low Dose Experimental Group) was administered Acetaminophen 1000 mg/Kg/day weight along with Aqueous extract of Tamarixdioica at a dose of 100mg/kg/day through gavage tube for four weeks.[7] Group D (High Dose Experimental Group) was administered Acetaminophen 1000 mg/Kg/day weight along with Aqueous extract of Tamarixdioica at a dose of 200mg/kg/day through gavage tube for four weeks.

Roots of Tamarixdioica were purchased and authenticated from the plant sciences department, Quaid-e-Azam University, Islamabad. Aqueous extract of Tamarixdioica root was prepared at RIPS, Islamabad by using fine homogenized powder of Tamarixdioica which were mixed with distilled water, the whole solution was boiled for 2 hours and after cooling was filtered through filter paper what man no 3. The aqueous extract was formed by using vacuum rotary evaporator and was frozen dried. Sampling at day 0 was done via lateral tail vein. Sampling after four weeks was done through cardiac puncture. Biochemical analysis of serum ALT and AST was estimated through commercially available kits

by Merk and auto analyzer Microlab 300 on photometric system.

Statistical analysis of data was done by using SPSS version 21 and Mean ± Standard Error of Mean was calculated. One-way ANOVA and Post hoc Tuckey tests were applied to compare the mean difference between control and rest of the groups and mean difference in between the groups. P value of <0.05 was considered statistically significant.

Results

Initial serum ALT for eight randomly selected mice at day 0 was 33.21 ± 1.02 U/L whereas mean initial serum AST for eight randomly selected mice were 26.12 ± 1.60 U/L. There was no significant difference in the serum ALT and AST among these mice on day 0 (p Value .980).

Mean Serum ALT after four weeks for Group A (Normal Control Group) was 35.50 ± 2.24 U/L, for Group B (Disease Control Group) was 94.00 ± 8.62 U/L, for Group C (Low dose Experimental group) was 50.90 4.56 U/L & for Group D (High dose Experimental group) was 49.90 ±4.99 U/L. There was a significant difference in the values of Group A & B (p Value .000). This suggests that hepatotoxicity was induced by acetaminophen in group B. There was also significant difference in the values of Group B & C and in values of group B & D (p Value .000). This suggests that Hepatotoxicity in Group C & D was improved. More than Group D (Methanolic Extract Treated Group). There was no significant difference in the serum ALT levels among Group C & D Suggesting that both low and high dose of Tamarixdioica extract have an equal hepatoprotective effect. (P Value .999).

Mean Serum AST after four weeks for Group A (Normal Control Group) was 27.00 ± 3.11 U/L, for Group B (Disease Control Group) was 151.00 ± 14.53 U/L, for Group C (Low dose Experimental group) was 66.90 7.77U/L & for Group D (High dose Experimental group) was 53.00 ± 10.08 U/L. There was significant difference in the values of group A & B (p Value .000). This suggests that hepatotoxicity was induced by acetaminophen in group B. There was also significant difference in the values of group B & C and in values of group B & D (p Value .000). This suggests that Hepatotoxicity in Group C & D was improved. There was no significant difference in the serum AST levels among group C & D (p Value .748) Signifying that Aqueous extract of Tamarixdioica both in low and high dose have same efficacy in protecting liver against acetaminophen induced hepatic injury.

Table I: ANOVA of ALT and AST in all Groups after Four Weeks

		Sum of Squares	df	Mean Square	F	Sig.
ALT	Between Groups	19175.475	3	6391.825	20.421	.000
	Within Groups	11268.300	36	313.008		
	Total	30443.775	39			
AST	Between Groups	86285.075	3	28761.692	30.031	.000
	Within Groups	34478.900	36	957.747		
	Total	120763.975	39			

Table II: Multiple Comparison of Serum ALT and AST Among all Groups by Post Hock Tuckey Test

Parameter	Groups	Mean difference	P Value
Serum ALT	Group A (control group) Vs Group B (Disease Control Group)	-58.500(*)	.000
	Group A (control group) Vs Group C (Low Dose Experimental Group)	-15.400	.227
	Group A (control group) Vs Group D (High Dose Experimental Group)	-14.400	.281
	Group B (Disease Control Group) Vs Group C (Low Dose Experimental Group)	43.100(*)	.000
	Group B (Disease Control Group) Vs Group D (High Dose Experimental Group)	44.100(*)	.000
	Group C (Low Dose Experimental Group) Vs Group D (High Dose Experimental Group)	1.000	.999
Serum AST	Group A (control group) Vs Group B (Disease Control Group)	-124.000(*)	.000
	Group A (control group) Vs Group C (Low Dose Experimental Group)	-39.900(*)	.032
	Group A (control group) Vs Group D (High Dose Experimental Group)	-26.000(*)	.255
	Group B (Disease Control Group) Vs Group C (Low Dose Experimental Group)	84.100(*)	.000
	Group B (Disease Control Group) Vs Group D (High Dose Expe rimental Group)	98.000(*)	.000
	Group C (Low Dose Experimental Group) Vs Group D (High Dose Experimental Group)	13.900	.748

Discussion

Acetaminophen is the commonest analgesic and anti-pyretic used worldwide. Hepatotoxicity is produced by Acetaminophen and is deduced by the raised serum levels of ALT and AST. The current study investigates the protective effects of Tamarixdioica (Aqueous Extract) at low and high doses in Acetaminophen induced hepatotoxicity in male mice via biochemical parameters. We find that Acetaminophen induced hepatotoxic changes can be improved by both high and low doses of aqueous extracts of Tamarixdioica. In present study in comparison with the normal control Group A (which received normal standard diet) Acetaminophen induced hepatotoxicity is observed in Group B, Group C and Group D with resultant increase in levels of biochemical markers i.e. serum ALT and AST.

The present work is in accordance with findings of Boyd and Mitchell who reported hepatotoxicity in rodents when treated with higher doses of Acetaminophen.[11] whereas the rats were not very sensitive to the hepatotoxicity, both mice and hamsters proved to be more sensitive. Following this initial report, few cases of acetaminophen overdose were reported. Boyer and Rouff described the main clinical symptoms as development of nausea and vomiting, 2–3 h of ingestion followed by abdominal pain in the right upper quadrant. Liver dysfunction occurred within 24 h and reached a maximum approximately 3–4 days after ingestion.[12] The current study is also consistent with Prescott (Ihab Talat Abdel-Raheem; 2009) in which hepatotoxicity of Acetaminophen was evaluated by increased alanine aminotransferase (ALT) and aspartate aminotransferase levels along with mild hyperbilirubinemia, and increased prothrombin time.[13]

In our study when results of Group C (Low Dose Experimental Group) and Group D (High Dose Experimental Group) were compared regarding ALT and AST no significant difference was observed (p Value > 0.05). These results indicate that hepatoprotective role of Tamarixdioica remain almost same when given in low or high Dose and it is not dose dependent. Similar findings were observed by Krishnaiah and his colleagues in which they found the hepatoprotective role of Tamarixdioica in both low and high doses. They have attributed this hepatoprotective property of Tamarixdioica to presence of flavonoids and tannins present in abundance in Tamarixdioica.[6]

Our study is also in accordance with study carried by Abouzid S and his colleagues which showed a marked reduction in tissue glutathione level in rats. The hydro-alcoholic extract of Tamarix (100 mg/kg body weight) ameliorated the adverse effects of carbon tetrachloride on Hepatocytes and returned the altered levels of biochemical markers near to the normal levels. We have compared results of Tamarixdioica to acetaminophen and not CCl4.[14] However same mechanism has probably produced hepatoprotective effect in our study also.

Previously studies have been done on exploring hepatoprotective effect of Tamarixdioica in combination with medical and other herbal compounds and extracts. No dose dependent study was done individually on aqueous extract of Tamarixdioica roots extract which guides us about the submaximal, ceiling effect and toxicity. Our study confirms the hepatoprotective effect of aqueous extract of Tamarixdioica roots both in low and high dose. Further studies are needed to determine dosage for submaximal and ceiling effect of Tamarixdioica roots extract. In addition a different route of administration can be tried to see the same effect.

Conclusion

Aqueous extract of Tamarixdioica roots have significant hepatoprotective effect on Acetaminophen induced hepatotoxicity both in low and high doses.

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ORIGINAL ARTICLE

Anemia in Elderly Hospitalized Patients: Frequency and Association with Comorbidities

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ABSTRACT

Objective: To determine the type and severity of anemia in elderly hospitalized patients and their association with comorbidities.

Study Design: Descriptive study.

Place and Duration of Study: Medical Department of Pakistan Railways Hospital from October 2015 to September 2016.

Materials and Methods: One hundred elderly patients admitted in the Medical Ward of Pakistan Railways Hospital were selected by non- probability convenient sampling. We investigated the frequency of anemia in these patients and its relationship to common comorbidities in this age group such as hypertension, diabetes, heart disease and chronic kidney disease etc. Anemia was defined according to the World Health Organization (WHO) criteria: Hemoglobin <13 g/dL in males and <12 g/dL in females. The patients were classified according to the severity of anemia as well as morphological type of anemia based on hemoglobin level, blood cell indices and peripheral film Three grades of anemia were differentiated as per WHO criteria: mild (hemoglobin between 11g/dl and lower limit of normal), moderate (hemoglobin between 8 g/dl and 10.9 g/dl), and severe (hemoglobin <8g/dl). Microcytic anemia was defined as mean corpuscular volume (MCV) below 77 fl, normocytic as MCV between 77 fl and 96 fl, and macrocytic by an MCV above 96 fl. Data analysis was done on the basis of symptomatology and routine laboratory parameters done on hospital admission.

Results: Out of the 100 patients studied, 63% were found to be anemic. The frequency of mild, moderate and severe anemia was 57%, 30% & 12% respectively. Normocytic normochromic anemia was the predominant morphological type seen in 61.9 % patients. Nearly half (49%) of the anemic patients had 3 or more comorbidities as compared to 21% non- anemic patients and the correlation was statistically significant at 0.01 level (p value 0.009). Chronic kidney disease was thrice as common in anemic patients and had a significant association with anemia.

Conclusion: Anemia is a frequent occurrence in hospitalized geriatric patients and is directly related to the number of comorbidities. Early diagnosis and management of anemia can have a significant impact on the overall disease outcome in elderly patients and should be part of a comprehensive geriatric assessment.

Key Words: *Anemia, Elderly, Geriatric, Hospitalized, Co morbidities*.

Introduction

The geriatric population is expected to show an alarming rise by the end of 21st century posing fresh challenges to the health care provider and putting additional constraints on meager health resources. Elderly are a neglected population in Pakistan health care system and geriatric medicine is still not practiced as a distinct specialty. With advancing age, the frequency of chronic diseases and psychological ailments increases. Anemia is a frequently encountered problem in geriatric practice world over and has rightly been called "an emerging problem for the 21st century."[1]

The mean prevalence of anemia in elderly ranges from 12% in the community to 40% in hospitalized patients.[2,3] Anemia in older patients is often mild and asymptomatic but it is associated with many adverse outcomes such as cognitive decline, dementia, depression, functional deterioration and frequent incidence of falls.[3,5] It is also an independent

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predictor of mortality as concluded by Leiden 85 study.[6] There has been a growing interest in the recent few years to unfold the association between anemia and various comorbidities found in this age group. There is now sufficient evidence to suggest that anemia has a negative impact on the outcome of diseases like diabetes and hypertension.[7,8] A study conducted by Nathavitharana et al has suggested that presence of anemia in hospitalized elderly patients is associated with more frequent readmissions, longer hospital stay, and adverse outcomes in terms of morbidity and mortality.[9] More recently, Abrahamsen et al concluded that more elderly patients with explained anemia died or were readmitted after one year of acute hospitalization as compared to their non-anemic counterparts or those with unexplained anemia.[10] This highlights the significance of early diagnosis and treatment of correctable causes of anemia such as nutritional anemia in this vulnerable age group.

Despite such conclusive evidence, there is a dearth of information regarding prevalence of anemia in our geriatric population and its clinical impact. Present study was conducted to determine the type and severity of anemia in elderly hospitalized patients and their association with comorbidities.

Materials and Methods

This descriptive study was conducted in the Medical Department of Pakistan Railways Hospital from October 2015 to September 2016. A total of 100 hospitalized elderly patients (65 years and above), both male and female were selected by non-consecutive convenient sampling. Sixty-five years age was used as a cutoff point which is widely accepted and utilized in previous studies. The study was initiated after the approval of study proposal by the Institutional Ethical Committee. Informed consent from all participants was obtained. Patients with malignancy, critical illness/those requiring ICU admission, unstable congestive cardiac failure, decompensated liver disease, congenital hemoglobinopathies, history of recent surgery or blood transfusion and those on anti-anemic treatment were excluded. Data analysis was done on the basis of detailed history, medical records, and routine laboratory parameters done on hospital admission. Complete blood counts were generated through Sysmex XP-100 hematology analyzer with routine quality control. Peripheral blood films were examined by consultant hematologist after staining with Leishman's stain. Urinalysis, fasting blood sugar, renal function tests (serum urea and creatinine), electrolytes (serum sodium and potassium), liver function tests (serum bilirubin, alanine transaminase, alkaline phosphatase), and electrocardiogram (ECG) were performed as part of admission protocol. We used the same reference ranges for various tests as established by our laboratory.

Anemia was defined according to the World Health Organization (WHO) criteria: Hemoglobin <13 g/dL in males and <12 g/dL in females.[11] The patients were classified according to the severity of anemia as well as morphological type of anemia based on hemoglobin level, blood cell indices and peripheral film . Three grades of anemia were differentiated as per WHO criteria: mild (hemoglobin between 11g/dl and lower limit of normal), moderate (hemoglobin between 8 g/dl and 10.9 g/dl), and severe (hemoglobin <8g/dl). [11] Based on data from literature and reference values for hemoglobin level, hematocrit, total red blood cell count, blood cell indices and peripheral smear, anemia was classified as microcytic (<77 fl), normocytic (77–96 fl), or macrocytic (>96 fl). [12]

Data was analyzed using IBM SPSS 21. Categorical data was calculated as frequencies and percentages. Comparison of study variables was performed by Chi-square test. P-value of less than 0.05 was considered significant at 95% confidence interval.

Results

A total of 100 hospitalized patients > 65 years of age were included after meeting inclusion & exclusion criteria. Of these, 53 were male and 47 were female patients. According to WHO classification, 63 (63%) patients were found to be anemic. Females showed a higher incidence of anemia (70.2% vs. 56.6%) but the gender difference was not statistically significant. Amongst 65-69 years age group, 60.4% (29/48) patients, in 70-79 years, 60.5% (23/38) and in patients 80 years above, frequency of anemia was 78.5% (11/14). No statistically significant association was found between patient's age & hemoglobin levels although the prevalence of anemia increased with rising age. There was no association between age and severity of anemia. In majority of the

patients (36/63 or 57%) anemia was mild. Figure 1 shows frequency of mild, moderate and severe anemia in our study.

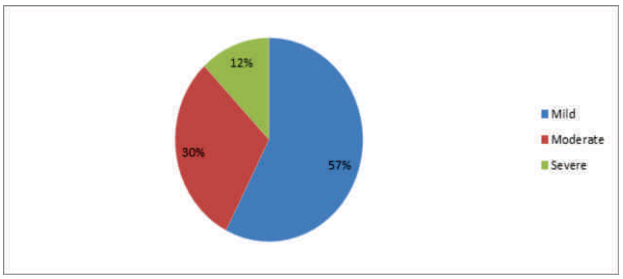

Fig 1: Frequency of Anemia According to Severity

Normocytic normochromic anemia was the dominant morphological pattern seen in 61.9% (39/63) patients. Figure 2 shows distribution of anemia according to morphological pattern.

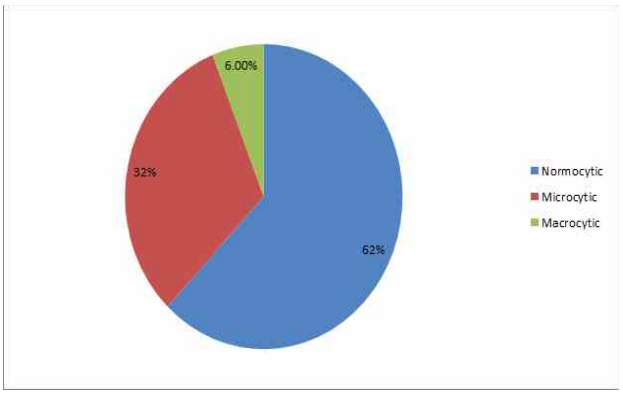

Fig 2: Distribution of Anemia According to Morphology

All patients were evaluated for comorbidities common for this age group. These included diabetes, hypertension, chronic kidney disease, heart disease, stroke, obstructive airway disease, rheumatological disorders, chronic liver disease & infections. Hypertension, diabetes, heart disease and chronic kidney disease were the most common diseases seen in this study. All these conditions were more frequently seen in anemic rather than non-anemic patients (table I). The association between anemia and chronic kidney disease was also statistically significant. (p value < 0.01) When comparing anemic and non- anemic patients in terms of number of comorbidities in a single patient, anemic patients had a higher number of comorbidities. Nearly half (31/63 or 49%) of the anemic patients had 3 or more comorbidities as compared to 21% (8/37) non-anemic patients and the correlation was statistically significant at 0.01 level (p value 0.009).

Table I: Common Comorbidities in Non-anemic and Anemic Patients

Comorbidity	Non-anemic patients (%)	Anemic patients (%)
Hypertension	29.7	42.2
Diabetes	35.1	44.4
Heart disease	32.4	34.9
CKD	10.8	42.8
Infections	37.8	28.5
COPD	18.9	15.8
CLD	16.2	11.0
Arthritis	10.8	9.5

Discussion

The results of our study confirm a high prevalence (63%) of anemia in our geriatric hospitalized patients. Majority of the patients had a mild normocytic normochromic anemia. Presence of anemia in a single patient was significantly correlated with number of comorbidities mainly diabetes, hypertension, heart disease and chronic kidney disease. Frequency of anemia in the present study was higher than the frequency reported in community-dwelling adults, but is in agreement with the data reported from nursing home residents and hospitalized patients.[2,3] Bach et al. while analyzing data from a large European university hospital cohort reported frequency of anemia as 21.1 %. [13] Another observational cohort study found the prevalence of anemia 48%. [14] The highest frequency of anemia in geriatric patients was determined by Giesel et al in a retrospective hospital based study in which 63.3% of their elderly patients were found to be anemic.[15] Results of our study therefore, are in accordance with Geisel et al.

Literature review suggests that anemia is significantly correlated with advanced age and male sex.[13] The percentage rises with age and is reported as high as 50% in individuals 80 years and above.[16,17] Results of our study also show that anemia increases with advancing age as 78.8% of our patients >80 years of age were anemic. However, female patients in our study were found to be more frequently anemic than their male counterparts (70.2% vs. 56.6%). A simple explanation of this is the fact that women in developing parts of the world are inherently at a greater risk of anemia due to socioeconomic, cultural and biological factors with low body iron stores resulting from early marriages, multiple pregnancies etc.

Worldwide the dominant morphological pattern of

anemia reported is normocytic normochromic with majority of the elderly population having only a mild anemia.[13,17] More than half (57%) of our study population had mild anemia, however an equally large number of patients had moderate (30%) or severe (12%) anemia. It may be argued that this was due to the fact that ours was a hospital based study, however in comparison to previous hospital based studies these figures were still alarmingly high.[13,15] Type and severity of anemia can be a useful predictor of in-hospital and post discharge mortality. In a most recent study, Riva et al [18] concluded that even mild anemia was a significant predictor of hospital re-admission in older patients and the 3 month mortality risk was directly proportional to the severity of anemia. The study results further showed that normocytic normochromic anemia was associated with worst prognosis irrespective of severity. This is understandable, as prevalence of serious illnesses like diabetes, hypertension, heart disease etc. all increase with age resulting in anemia of chronic disease which is the major cause of normocytic normochromic anemia. Predominant morphological pattern of anemia in our study was also normocytic normochromic (61.9%) which is in keeping with previous studies.[13,17] However, in contrast to Western studies, frequency of microcytic anemia in our study population was quite high (31.7%) with almost every third patient having microcytic hypochromic anemia. This is a significant finding keeping in view that the most common cause of microcytic anemia is iron deficiency which is a readily correctable cause of anemia. Vitamin B12 and folate deficiency are also correctable causes of anemia and are a common cause of macrocytic anemia in developing countries like Pakistan.[19] In the present study, 6.3% of our patients had macrocytic anemia. In a previous study, we have reported frequency of vitamin B12 and folate deficiency as 10% and 7% respectively.[20] Prevalence of nutritional anemia in the developing world is high. The National Health and Nutrition Examination Survey (NHANES III), also reports that one-third of the elderly anemic patients have nutritional anemia mainly iron deficiency.[16] Anemia in elderly is often complicated by the fact that majority of these patients have nutritional anemia in the background of anemia of chronic disease. It may not always be possible to treat the underlying disorder however, correction of coexisting nutritional anemia such as iron deficiency can significantly influence the outcome in many diseases such as chronic kidney disease and heart failure.[21,22]

One of the most significant aspects of our study was that it highlights the association between anemia and presence of comorbidities in a patient. In a recently published study, Migone et al have concluded that anemia is independently associated with higher number of comorbidities in a single patient.[14] This can have important therapeutic implications as untreated anemia is known to negatively affect outcomes in terms of primary illness such as diabetes,[7] hypertension,[8] heart failure,[22] chronic obstructive pulmonary disease,[23] etc.[14,24] While the prevalence of all these diseases was overall high in our study population, the frequency of most of these disorders was much higher in anemic patients. Chronic kidney disease was thrice as common in anemic patients and had a significant association with anemia. Correction of anemia in chronic kidney disease is an integral part of management of patients with renal disease.[21] The association between anemia and chronic kidney disease is well known and renal disease should be excluded in every elderly individual with unexplained anemia. In terms of number of comorbidities in a single patient, more than half of our anemic patients had at least 3 or higher number of comorbidities as compared to their non-anemic counterparts (49% vs. 21%). The correlation between presence of anemia and number of comorbidities per single patient was statistically significant (p value < 0.01).

The present study was not aimed to determine the etiology of anemia therefore the data is based only on previously diagnosed coexisting diseases in our patients which may be considered as a limitation of the study. It may be argued that primary illness of an individual patient can act as confounder, affecting presence or severity of anemia. However, we applied stringent exclusion criteria and an attempt was made to include only stable patients admitted in general medical ward with diseases common in this age group. Furthermore, the exact causal link between anemia and different comorbidities needs to be evaluated.

The disease burden in Pakistani geriatric population

is very high with diabetes, hypertension and arthritis as the most common illnesses.[25] Anemia often remains under diagnosed because of vague signs and symptoms which are generally attributed to co existing illnesses or simply old age. Given the clear association between even mild anemia and morbidity in this age group, findings of our study may have clinical significance in the management of elderly patients with anemia. More studies to explore various aspects of clinical impact of anemia are needed to further evaluate this important aspect of geriatric health.

Conclusion

The present study has shown that every second hospitalized elderly patient in our set ups is likely to be anemic with every third patient having moderate to severe anemia. It has further shown that frequency of common diseases such as diabetes and hypertension is higher in patients with anemia and that presence of anemia is directly related to number of comorbidities in a single patient. We conclude that physicians should have a high index of suspicion and low threshold for treating anemia in geriatric patients as this can significantly impact the disease outcome.

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ORIGINAL ARTICLE

The Protective Effect of Olive Oil on Arsenic Induced Histological Changes in the Liver of Albino Rats

Hira Waqas Cheema[1], Shabanba Ali [2]

ABSTRACT

Objective: To study the protective effect of olive oil against histological changes induced by arsenic in liver of albino rats.

Study Design: Randomized control trail.

Place and Duration of Study: The research was carried out from 1st to 30th November 2017 at National institute of Health, Islamabad.

Materials and Methods: Forty five male adult albino rats were placed in three cages having 15 rats each. Distilled water was given to rats of control group I for 30 days. The rats of group II were given 40mg per kg per day of Sodium Arsenite dissolved in drinking water for 30 days. Rats in group III, in addition to sodium Arsenite received olive oil, 0.2ml per day for 30 days along with Sodium Arsenite. Dissection was done after 30 days and liver was dissected out for histological changes.

Results: The use of olive oil improved the gross and microscopic changes induced by Arsenic in liver lobes(right lateral and left lateral) of Albino rats of group III as compared to group II rats, which received only arsenic**.** Among microscopic parameters, sinusoidal dilation, pyknosis and necrosis was markedly reduced by use of olive oil in group III rats whereas hemorrhage was absent in group III.

Conclusion: Olive oil protects histological changes caused by arsenic in liver of Albino rats which include sinusoidal dilation, congestion, pyknosis, necrosis and haemorrhage.

Key Words: *EVOO (extra virgin olive oil), Sodium arsenite, Oleuropein.*

Introduction

Liver, the largest organ in the body, usually weighs about 1.5 kg. It is an organ of metabolism and production of energy; its other main functions include: storage of iron, trace elements, vitamins and bile production. The weight of human liver is 2 to 3%, whereas rat liver is 5% of total body weight.[1] Various metals have acute and chronic effects on liver; Arsenic is one of them which also produce toxic effects on liver. Arsenic, a "protoplasmic poison" interferes with mitosis, cell respiration, enzymes due to its effect on sulfhydryl group of cells.[2] It can also exert its toxic effects by generating "reactive oxygen species (ROS)" and "reactive nitrogen species (RNS)" leading to necrosis, oxidative damage to proteins, lipids and DNA in cells.[1,3,4] Several acute and chronic hepatic effects have been associated with arsenic poisoning.[5] Various antioxidants are available which can reduce the effect of arsenic on liver and various organs, olive oil is one of them which can be used to avoid disastrous effects of arsenic on liver. Olive oil contains about 70% Oleic acid and phenolic compounds that provide health benefits. Olive oil itself has a greater antioxidant capacity than most other seed oils.[6]

Arsenic affects most of the organs involved in absorption, accumulation and excretion,[7] Long-term exposure to inorganic arsenic can cause dysfunction of endocrine system[8,9] nervous system,[10] and reproductive system,[11] and may also cause loss of body weight.[4] Exposure to arsenic also causes, liver fibrosis, metabolic disorder such as diabetes,[12,13] chronic lung disease, gangrene of toes,[9] cancer of internal sites and skin.[14] It has been found to have toxic effects on gonadal tissues of laboratory animals as well.[11]

Various antioxidants have been used to ameliorate

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toxic effects of arsenic on various organs. Vitamin E, Ca and Olive oil is used to improve Arsenic induced histological changes in ovary.[15,6] Antioxidants like L ascorbic acid, biochanin A, menthe piperita and aloe Vera has been used to improve toxic effects of arsenic on liver of rats.[16,19] Oleuropein, one of the component of olive oil is found to be effective antioxidant in literature, therefore olive oil may be used in our study to ameliorate arsenic induced hepatotoxic effects.[20] The present study was designed to study the protective effect of olive oil against histological changes induced by arsenic in liver of albino rats.

Materials and Methods

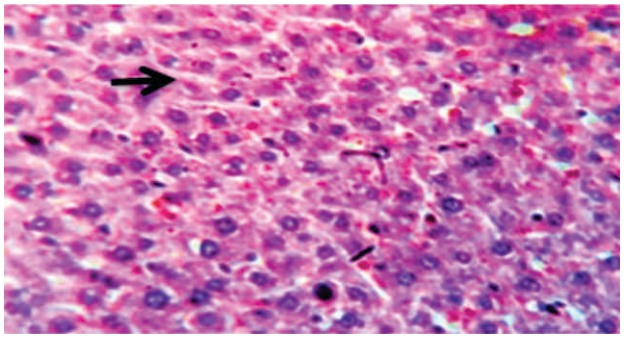
The experiment was carried on the basis of randomized control trial under supervision of animal house at NIH Islamabad from 1st to 30th November 2017. Forty five male albino rats, weighing 250 to 300gm were kept in three cages with a number of 15 rats per cage. The simple random sampling technique was used. The research was approved by Ethical Review Committee. A controlled standard living environment suitable to their class with adjusted diet was given. A well ventilated room with cycles of 12 hours light and 12 hours dark were maintained under 20 to 26 ^{0}C. The rats were adult of age 2 to 4 months and those with any known pathology and female rats were excluded. Animals were grouped accordingly as mentioned below.

Group I Control group n = 15	Control group rats consumed distilled water as drinking water for 4 weeks. These rats were dissected after 4 weeks.
Group II Experimental group n= 15	Rats consumed a solution of arsenic (40mg/kg) as sodium arsenite for 4 weeks They were also dissected and observed after 4 weeks to observe any change in histology of liver lobes.The longitudinal sections were taken from left lateral and right laterateral lobes.
Group III Experimental group n = 15	Rats consumed a solution of arsenic (40mg/kg) as sodium arsenite along with olive oil 0.2ml/day for 4 weeks. After 4 weeks these rats were analyzed to obsereve hepatotoxic effects of arsenic which were prevented due to use of olive oil.

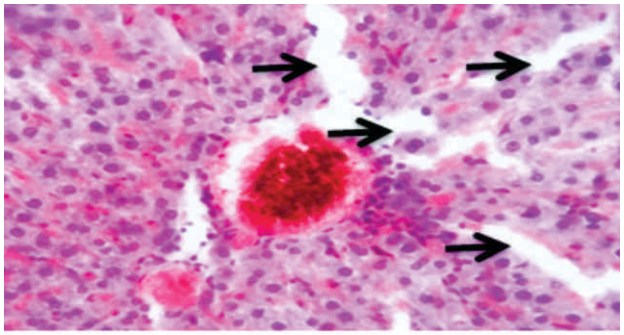
After accomplishment of 4 weeks duration of experiment, rats were anesthetized with chloroform and dissected. After fixation and embedding, eosin and hematoxylin stains were used for histological sections. The slides were examined in detail under X10 and X40 power of light microscope. The microscopic qualitative parameters were observed which include Sinusoidal dilation, Congestion, Pyknosis, Necrosis and haemorrhage

Results

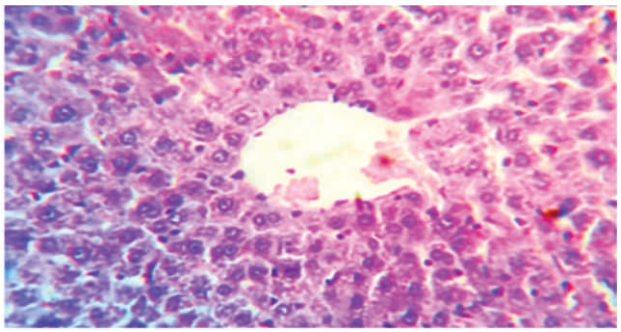
In control group I, experimental animals showed normal sinusoids while in group II and group III sinusoidal dilation was present in 100% of experimental animals. The use of olive oil in group III has significantly reduced the severity and amount of sinusoidal dilation caused by arsenic Table 1 and Figure 1).



Group I



Group II



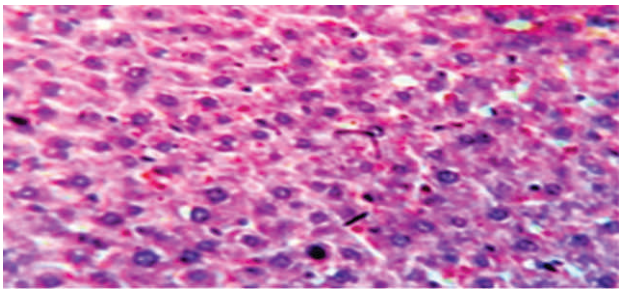
Group III (mild sinusoidal dilation)

Fig 1: Group Wise Distribution of Sinusoidal Dilation of Hepatic Lobule Showing Normal Sinusoid in Group I L7B1, Severe Sinusoidal Dilation in Group II L4D and Mild Sinusoidal Dilation in Group III L6 A.(H and E,X 40) (Indicated by Arrows in Group 1 and Group II of Figure 1)

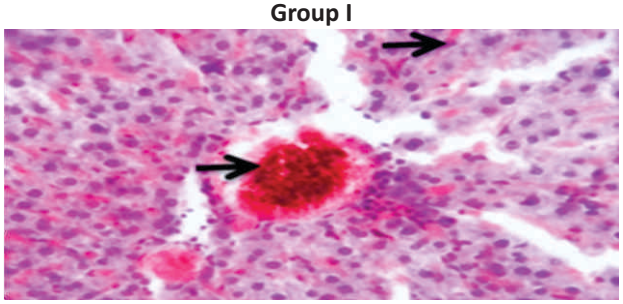
Table I :Group Wise Distribution of Sinusoidal Dilation in Hepatic Lobule Among Control and Experiental Groups of Albino Rats

Groups	Yes N(%)	No N(%)	Total	P value
Group I	0(0%)	15(100%)	15	0.000*
Group II	15(100%)	0(0%)	15	
Group III	15(100%)	0(0%)	15	
Total	30	15	45	

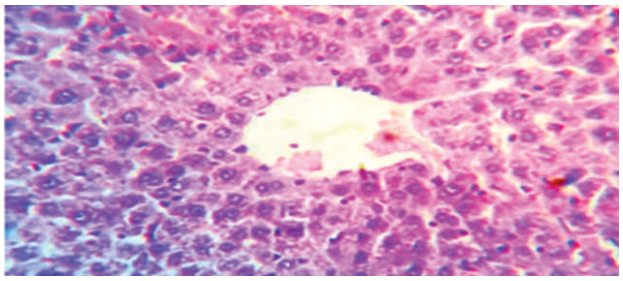
The control group showed no congestion in sinusoids and central vein. 100 % of experimental animals in group II showed congestion mainly in central vein and also in sinusoids,whereas 46.7% of rats in group III showed congestion but 53.3% showed no congestion.In this way olive oil has decreased the number of rats in group III showing congestion. (figure 2)



Group I



Group II Congestion in Central Vein and Sinusoids



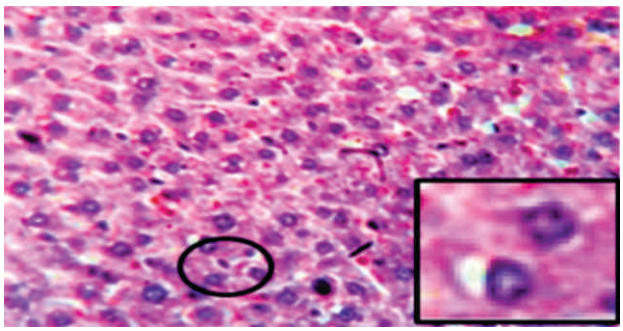
Group III

Fig 2: Group Wise Distribution of Congestion of Sinusoids and Central Vein Showing Normal Sinusoids and Central Vein in Group I L7D, Congestion in Central Vein and Sinusoid in Group II L4B, Whereas no Congestion in Group III L3C. (H and E, X 40). (Indicated by Arrow Heads in Group II Figure 2)

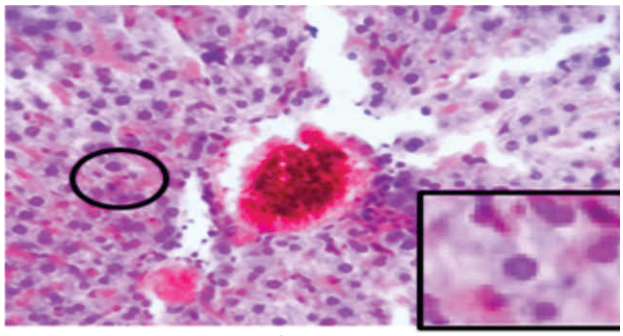
Table II: Group Wise Distribution of Congestion of Sinusoids and Central Vein Among Control and Experimental Groups

Groups	Yes N(%)	No N(%)	Total	P value
Group I	0(0%)	15(100%)	15	0.000*
Group II	15(100%)	0(0%)	15	
Group III	7(46.7%)	8(53.3%)	15	
Total	30	15	45	

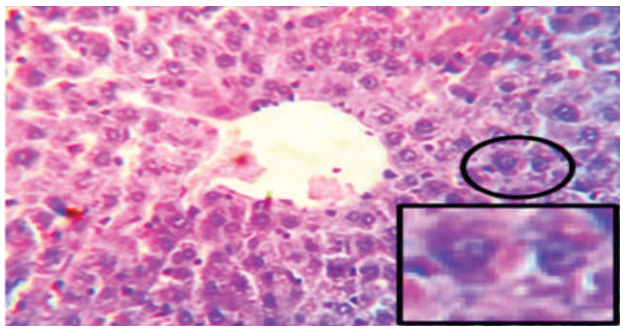
There was normal size of nucleus of hepatocytes in the control group I, pyknosis was present in 100 % of experimental animals of group II,Olive oil in group III has significantly reduced the number of rats showing pyknosis to 60%.(Table 4) (figure 3).



Group I



Group II



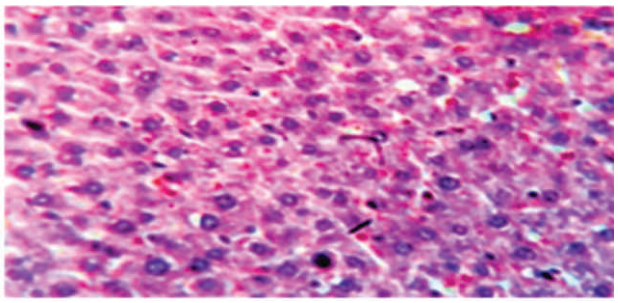
Group III

Fig 3: Group Wise Distribution of Pyknosis of Hepatocytes Among Control and Experimental Groups Shows Normal Nuclear Size in Group IL7A, Presence of Pyknosis in GroupII L4D and Absence of Pyknosis in Group III L3 B(H and E,X 40) .(Indicated by Circles in Figure 3)

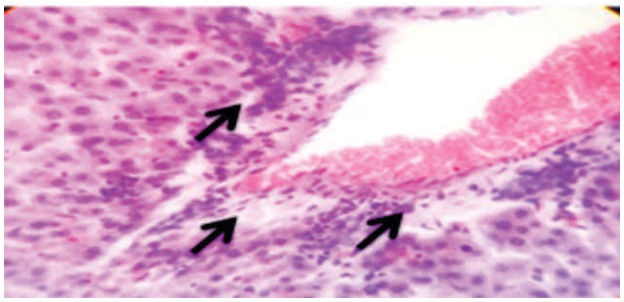
Table III: Group Wise Distribution of Pyknosis of Hepatocytes among Control and Experimental Groups of Albino Rats

Groups	Yes N (%)	No N (%)	Total	P value
Group I	0(0%)	15(100%)	15	0.000*
Group II	15(100%)	0(0%)	15	
Group III	9(60%)	6(40%)	15	
Total	24(53.3%)	21(46.7%)	45	

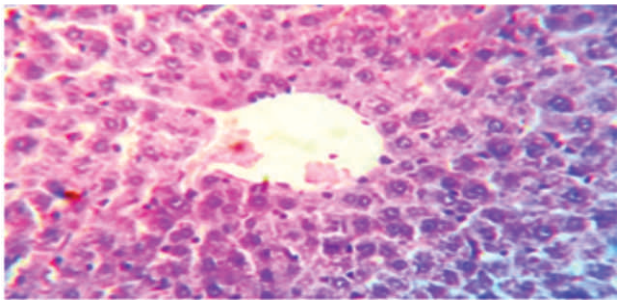
The normal hepatic parenchyma was observed in group I while 100% of experimental rats in group II showed necrosis in hepatic parenchyma, whereas use of olive oil along with arsenic in group III has significantly reduced the necrosis to 53.3% of rats in group III



Group I

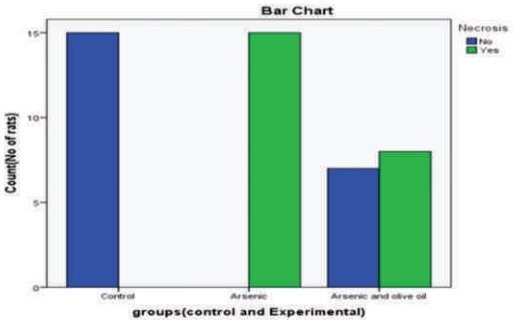


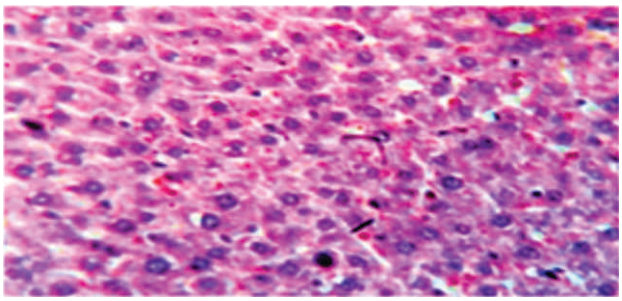
Group II (periportal necrosis)



Group III

Fig 4: Group Wise Distribution of Necrosis of Hepatic Parenchymma among Rat Groups Shows Normal Hepatic Parenchyma in Group IL7A2, Presence of Coagulative Necrosis in Periportal Areas in Group II L5B, and Absence of Necrosis in Group III L3A(H and E,X 40). (Indicated by Arrow Head in Group II Figure 4)





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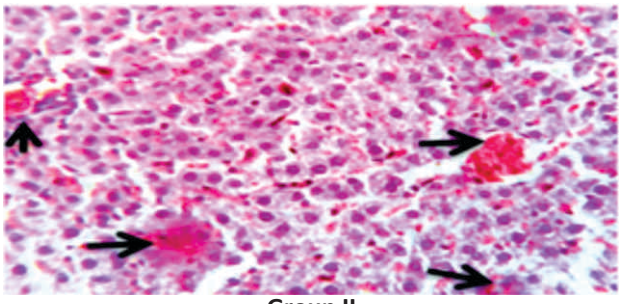
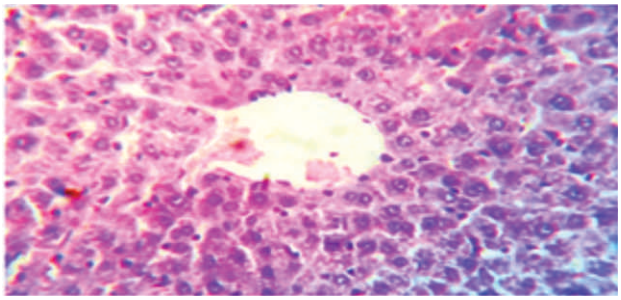


Group II



Group III

Fig 5: Bar Chart Showing Distribution of Necrosis of Hepatic Parenchyma among Rat Groups

In group I, hepatic parenchyma appeared to be normal, 46% of rats in group II showed hemorrhage. In group III, olive oil has significantly improved hemorrhage as it was absent in all rats. (Table IV)(Figure 6)

In group I, hepatic parenchyma appeared to be normal, 46% of rats in group II showed hemorrhage group III, olive oil has significantly improved hemorrhage as it was absent in all rats.(Table

IV)(figure6)

Figure 6 Group wise distribution of haemorrhage in hepatic parenchyma among groups

shows normal parenchyma in group I L7 C,haemorrhage indicated by arrow in group II L4D whereas no haemorrhage in group III L6A.(H and E, X 40).

Table IV: Group Wise Distribution of Haemorrhage in Hepatic parenchyma among among Control and Experimental Groups of Albino Rats

Groups	Yes N(%)	No N(%)	Total	P value
Group I	0(0%)	15(100%)	15	**0.000***
Group II	7(46.7%)	8(53.3%)	15	
Group III	0(0%)	15(100%)	15	
Total	7(15.6%)	38(84.4%)	45	

*P≤0.05

Discussion

The sinusoidal dilation was observed in groups I,II and III. In control group I, experimental animals showed normal sinusoids while in group II and group III sinusoidal dilation was present in 100% of experimental animals. The use of olive oil in group III has significantly reduced the sinusoidal dilation as compared to dilation in group III caused by arsenic. In one study carried out by Kharroub et al, sodium arsenite was given at 1mg and 10mg for 45 and 90 days respectively which showed that sinusoidal dilation is dose and duration dependent. The dilation was greater at 10mg of arsenic for 90 days as compared to 1mg at 45 days.[21] In another study conducted by Sohini Singh,4 to 10mg of arsenic was given for 30 days to rats which also caused sinusoidal dilation of liver.[16] In group III, there was slight sinusoidal dilation which is due to hepatoprotective effect of olive oil given simultaneously with arsenic to this group. Farag in his study showed natural antioxidant effect to prevent sinusoidal dilation and congestion caused by oxidative damage to liver.[22] Congestion of sinusoids and central vein is caused due to inflammation of liver. All rats of group II showed congestion mainly in central vein and also in sinusoids due to effect of arsenic.46.7% of rats of group III showed congestion but 53.3% showed no congestion. [23] In the study conducted by Oyagbemi et al, arsenic was given to three different groups at different doses for 4 weeks, they showed congestion in hepatic vessels both in central vein and sinusoids.[24] The use of olive oil in group III has significantly reduced congestion to only 46.7% of rats. In a study by Azab, when olive leave extract was given along with carbendazim, then congestion in central vein and sinusoids was remarkably decreased as compared to the group which was given only carbendazim.[25]

There was normal size of nucleus of hepatocytes in the control group I, pyknosis was present in 100 % of experimental animals of group II, Olive oil in group III has significantly reduced the number of rats showing pyknosis to 60%. In a study conducted by Somia Bashir of India, arsenic was given to three groups in three different doses for acute period of 24 hours and pyknosis was found only in group which was given higher dose. This study support that Pyknosis is early sign of necrosis.[26]

Necrosis occurs as a result of inflammation of liver caused by arsenic and it occurs after sinusoidal dilation and congestion in which degeneration of hepatocytes takes place due to ischemia. In the recent study, there is absence of necrosis in experimental animals of group I while 100% of experimental rats in group II showed necrosis whereas 53.3% of rats in group III showed necrosis. In this way olive oil has significantly decreased necrosis. In group II, there was periportal necrosis which is zone I as well as zone III which is pericentral, least oxygenated. In group III treated by both arsenic and olive oil, there is only pericentral necrosis.[27] In one of the study carried out by Sujata Das, sodium arsenite was also given in dose of 40mg per kg in drinking water to mice for 30 days and he found periportal necrosis similar to recent study.[28] The use of olive oil in group III has improved necrosis in our study. Metin Ogun, used oleuropein in dose of 30mg per kg along with 5mg per kg of sodium arsenite for only 15 days and he found that group which was given only arsenic ,6 mice showed necrosis but the oleuropein group showed necrosis in only 1 mouse.

The haemorrhage occurs late in process of inflammation due to damage of endothelial lining of sinusoids leading to extravasation of blood into parenchyma. The livers of group I showed normal parenchyma but in group II, 46% of rats only show hemorrhage as arsenic was given to them for 30 days. In group III, hepatic parenchyma was normal with no hemorrhage.[29] Daqian Yang in his study gave As_2O_3 (arsenic trioxide) intraperitoneally in a dose of

3mg per kg for 2 weeks to rats and he found hemorrhage in liver histological examination. When olive oil was given to group III along with arsenic, due to its antioxidant effect it prevents the liver of rat to develop hemorrhage. In support of ameliorating effect of olive oil to improve hemorrhage of liver of our study, olive leave extract was used to improve hemorrhage in liver caused by carbendazim in a study done by Azab of Libya.[25]

Conclusion

Olive oil protects histological changes caused by arsenic in liver of albina rats, which include sinusoidal dilation, congestion, pyknosis, necrosis and haemorrhage.

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ORIGINAL ARTICLE

Comparison of Postprandial Insulin Levels in Withania Coagulans and Liraglutide Treated Diabetic Rats

Abdul Samad,[1] Shazia Ali,[2] Noman Sadiq[3]

ABSTRACT

Objective: To investigate and compare the antidiabetic effect of withania coagulans and Liraglutide on serum postprandial insulin levels in streptozotocin induced diabetes in rats.

Study Design: Randomized Control Trial.

Place and Duration of Study: The study was conducted from 1st April 2016 to 31st March 2017 at Islamic International Medical College in collaboration with National Institute of Health Islamabad.

Materials and Methods: Total 40 male Sprague dawley rats were randomly divided into two groups; Group A (n=10) and Experimental Group (n=30). Group A was given normal diet for 5 days whereas experimental group was given normal diet plus streptozotocin (30mg/kg/day) intraperitoneally for 5 days and diabetes was confirmed in experimental group by fasting blood glucose (mg/dl). Experimental group was further divided into B (Diabetic control), C (Withania coagulans treated) and D (Liraglutide treated). First sampling group A and B was done after 5 days. Group C were given Withania coagulans and Group D were given Liraglutide along with normal diet for 30 days. Second sampling (fasting blood glucose, postprandial glucose and insulin level) was done from group C and D.

Results: Fasting blood glucose of group C (98 mg/dl ± 1.80) was significantly reduced than group D (102 mg/dl ± 2.04). Postprandial glucose and insulin levels of Group D (163 mg/dl ± 3.95 and 6.06 µU/ml ±0.17) were significantly decreased and increased as compared to Group C PPG (183 mg/dl ± 6.30) and insulin level (5.54 µU/ml ± 0.23).

Conclusion: Withania Coagulans significantly improves postprandial insulin levels as compared to liraglutide.

Key Words: *Diabetes, Insulin, Liraglutide, Withania Coagulans.*

Introduction

Diabetes mellitus is a disorder of carbohydrate metabolism in which the body sugar is unable to be oxidized due to lack or improper functioning of pancreatic hormone mainly insulin.[1,2] Type 2 diabetes is a medical condition characterized by hyperglycemia in which glucose is not metabolized due to insulin resistance and pancreatic beta cells can not compensate this insulin resistance.[3,4] Studies have shown that in type 2 diabetes pancreas fails to produce sufficient quantity of insulin in response to glucose stimulation which leads to chronic postprandial and fasting hyperglycemia which causes serious complications leading to excess morbidity and mortality.[5,6]

A number of pharmaceutical preparations are available for treating type 2 diabetes which targets insulin release from pancreas like sulphonylurea. These formulations are found to have a number of clinical limitations, the most serious long term limitation being the eventual need for insulin replacement therapy. These drugs which stimulate insulin secretion from pancreas are also associated with a common side effect-hypoglycemia. A new drug was discovered called Liraglutide (GLP-1 mime tics) which causes insulin secretion from pancreas only in response to hyperglycemia to avoid the risk of producing hypoglycemia and have enjoyed a great deal of success in treating type 2 diabetes.[7,8] Basic mechanism of action of liraglutide is through enhancement of insulin secretion from pancreas and

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can also be taken in combination with insulin sensitizer like metformin.[9,10]

The main drawback of this drug is that these formulations are not available in oral form so it has to be administered parentally. Continuous subcutaneous or intravenous administration of this drug is another challenge faced by patients of type 2 diabetes who take liraglutide.[11] Due to adverse effects of hypoglycemic drugs and exogenous insulin, use of medicinal plant has become a common practice as these are reported to have very less side effects and are easily accessible and affordable. In this category of medicinal plants, Withania Coagulans (paneer doda) belongs to a family Solanaceae is a famous herb known long for its antidiabetic property. This plant is cultivated in India, Pakistan, Iran and Afghanistan.[12,14]

Different compounds of Withania Coagulans have been studied for their different medicinal properties including phenolic compounds, fruit berry extract, seeds, flowers and alcoholic extract. Aqueous extract of Withania Coagulans (aqWC) dried fruits have specially been studied for its antidiabetic property because of presence of active compound called Withanolides in it. Several studies have proved that aqueous extract of Withania Coagulans has shown significant improvement in blood glucose levels and its effects were compared with biguanides and sulphonylureas.[15,16] Studies have also revealed that Withania coagulans treated rats exhibits increase level of serum insulin and results were compared with glibenclamide(sulphonylurea).[17,18] None of the previous studies has attempted to elucidate the level of postprandial insulin level and its comparison with GLP-1 analogues. So the aim of this study was to investigate the effect of aqueous extract of Withania Coagulans (aqWC) and Liraglutide on postprandial insulin, fasting and postprandial blood glucose levels, along with their comparison.

Materials and Methods

This Randomized Control Trial was carried out at Physiology department and Multidisciplinary research laboratory, Islamic International Medical College, Rawalpindi in collaboration with NIH animal house after approval from Ethics Review Committee.

Rats weighing between 200-300 grams were included in the study. Rats were first allowed to get acclimatized for one week in the NIH Animal house in 50-70% humidity at a room temperature of 24$\pm$2°C with a 12 hour light and dark cycle.

A total 40 male Sprague dawley rats were taken and randomly divided into two groups; Group A (n=10) and Experimental Group (n=30). Group A was given normal diet for 5 days whereas experimental group was given normal diet plus streptozotocin (30mg/kg/day) intraperitoneally for 5 days. After 5 days, diabetes was confirmed in experimental group by measuring and comparing Fasting blood glucose levels (mg/dl) with group A. Experimental group was then further divided into three groups i.e. B (Diabetic control), C (Withania coagulans treated) and D (Liraglutide treated). First sampling of the experiment was done after 5 days from group A and B which included postprandial glucose (mg/dl), serum insulin (µU/ml). Group C rats were given normal diet along with aqueous extract of Withania coagulans (1000mg/kg/day) orally mixed in drinking water for 30 days. Group D rats were given normal diet along with Liraglutide (Victoza pen) drug (0.3mg/kg/day) subcutaneously for 30 days. After 30 days of treatment, second sampling of the experiment was done from group C and D which included fasting blood glucose (mg/dl), postprandial glucose (mg/dl), and serum postprandial insulin (µU/ml).

The dried fruits of Withania Coagulans were identified and authenticated (# IIMC 03) by herbarium section of National Agriculture Research Center (NARC) Islamabad. Aqueous extract was prepared in Multi-disciplinary research labortatory IIMC at a dose 250mg/ml and was used for experimental work. Liraglutide belongs to GLP-1 mimetics was administered subcutaneously at a dose of 0.3mg/kg body weight for 30 days to diabetic rats.

Sample for blood glucose was collected from rat tail vein with 1 ml syringe. Blood glucose levels were assessed using the EASY GLUCO Ultra plus Auto Coding meter by Isotech Co.Ltd., Korea. Through Intracardiac sampling 2 mL of blood was withdrawn .The blood was saved in labeled gel tubes and kept in a laboratory ice box at 2-8 $^{\circ}$C until they were analyzed for insulin estimation (ng/mL) which was done using Sandwich-ELISA method. Statistical analysis was done applying the SPSS 21. Results were documented as mean $\pm$ SEM. Comparisons among the two groups was analyzed using the independent

sample t-test and correlation among the variables was done using Pearson's correlation coefficient. P value of <0.05 was considered significant for both analyses.

Results

Fasting Blood Glucose (FBG) mg/dl

Fasting blood glucose (mg/dl) levels in group B rats (131 ± 3.05mg/dl) were significantly higher (P<0.05) than group A rats (80 ± 3.19mg/dl). While on comparison FBG levels in group C rats (98 ± 1.80mg/dl) and group D rats (102 ± 2.04) were significantly lower (P<0.05) than group B rats (131 ± 3.05mg/dl). Comparison of Mean ± SEM of FBG mg/dl levels in all four Groups (A, B, C, D) are shown in Table I.

Postprandial Glucose (PPG) mg/dl

Comparison of Mean ± SEM of PPG (mg/dl) in all four Groups (A, B, C, D) is displayed in Table I. Group B rats showed increased PPG (330± 15.95mg/dl) levels which were significantly higher (P<0.05) than Group A rats (143 ±5.34mg/dl). Significantly lower (P<0.05) PPG levels of group C rats (183± 6.30mg/dl) and Group D rats (163 ±3.95 mg/dl) were observed on comparison with Group B (330± 15.95mg/dl) rats.

Serum Insulin Levels (µU/ml)

Serum insulin levels of Group B were observed to be (3.5 ± 0.19 µU/ml) which were significantly reduced (P<0.05) as compared to the group A (5.9 ± 0.19 µU/ml) rats. While serum insulin levels of group C rats (5.54 ± 0.23 µU/ml) and Group D (6.06 ± 0.17 µU/ml) rats were significantly raised (P<0.05) as compared to the Group B (3.5 ± 0.19 µU/ml) rats. Comparison of Mean ± SEM of Serum Insulin (µU/ml) levels in all four Groups (A, B, C, D) is shown in Table I.

Table I: Comparison of Mean ± SEM of Fasting Blood Glucose (FBG) mg/dl, Postprandial glucose (PPG) mg/dl and Serum Insulin (µU/ml) levels in all four Groups (A,B,C,D):

Parameters	Group A (Control)	Group B (Diabetic)	Group C (WC treated)	Group D (Liraglutide treated)
FBG (mg/dl)	80 ±3.19	131 ± 3.05*[a]	98 ± 1.80*[b]	102 ± 2.04*[c]
PPG (mg/dl)	143 ±5.34	330± 15.95*[a]	183± 6.30*[b]	163 ±3.95*[c]
Serum Insulin	5.9 ± 0.19	3.5 ± 0.19*[a]	5.54 ± 0.23*[b]	6.06 ± 0.17*[c]

Withania coagulans **(**WC)

*=P < 0.05 is considered statistically significant.

*[a]= Group A vs B

*[b]= Group B vs C

*[c] =Group B vs D

Discussion

Type 2 diabetes is a major health problem with serious complications results in substantial health-care costs. Treatment of type 2 diabetes includes parenteral therapy (Insulin and GLP-1 mimetics) and oral hypoglycemic drug which included sulphonylurea, metformin and thiazolidiendiones. In the present study antidiabetic effect of withania coagulans and liraglutide on fasting, postprandial blood glucose and serum postprandial insulin level was evaluated along with their comparison.

Jaiswal et al have concluded that hypoglycemic potential of withania coagulans fruit may be due to activation of insulin gene expression through CREB (Calcium Responsive Element Binding protein) responsible for exocytosis of stored insulin from pancreatic beta cells.[13] Current study also seconds this fact concluded by by jaiswal et al as postparandial insulin level was raised after use of withania coagulans extract.

Fasting and postprandial glucose levels in the current study were also similar to the results of hamatha et al ho gave aqueous extract of withania coagulans for 7 days instead of 30 days as were in present study.[12] Their study revealed the antidiabetic and antihyperlipidemic effect of aqueous extract of Withania coagulans although serum postprandial insulin levels were not explored.

Results of the present study were also similar to results obtained by Shukla et al who concluded that along with blood glucose and insulin level, treatment of aqueous extract of Withania coagulans (500mg/kg/wt) has showed marked effect on increasing carbohydrate metabolizing enzymes i.e. glucokinase and phosphofructokinase.[14]

Serum insulin level were significantly increased in the results of present study which were inconsistent with the results of experiment done by Bharti SK et al who investigated the effect of aqueous extract of withania coagulans in poloxomer-induced diabetic rats. The study mentioned that administration of aqueous extract of withania coagulans (200mg /kg body wt) to diabetic rats for 5 weeks causes significant decrease in serum fasting insulin level and insulin resistance (measured by HOMA-IR) on comparison with those in diabetic rats.[15] This dissimilarity between the results of two studies could be due to the reason that in present study

postprandial insulin level was measured whereas Bharti SK et al estimated fasting serum insulin level.

Results of the present study were also similar to the Study conducted by Davies et al on type 2 diabetic patients who evaluated the efficacy of liraglutide on serum insulin levels.[19]

Upadhyay and vandata concluded that powder of Withania coagulans (10 g) when taken orally by diabetic patients showed marked improvement in fasting and postprandial glucose level which was similar to the results in present study in which aqueous extract of Withania coagulans (1000mg/kg) were administered to the diabetic rats.[20]

Another human study carried out by Alam A et al showed that administration of 150 ml of water containing 10 seeds of Withania coagulans twice a day showed reduction in serum level of fasting and postprandial glucose which were similar to the results of present study carried out in streptozotocin induced diabetic rats.[18] In current study because of cost and availability issue immunohistochemistry of pancreas tissue was not done. Further studies need to be conducted on aqueous extract of Withania Caogulans dried fruits on microscopic morphological features of intestinal L cells which release glucagon like Peptide-1.

Conclusion

Withania coagulans reduces fasting and postprandial blood glucose levels while increasing serum insulin levels. This can be used as a better treatment option for type 2 diabetes because of its oral intake and lack of adverse effects usually caused by liraglutide.

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ORIGINAL ARTICLE

Root Canal Configuration of the Mesio-buccal Root of Maxillary First Permanent Molars in Local Population

Mansoor Khan[1], Rana Muhammad Ahmad Khan[2], Muhammad Qasim Javed[3], Alia Ahmed[4]

ABSTRACT

Objective: The purpose of this in vitro study was to investigate variations in the root canal morphology of mesiobuccal root of maxillary first permanent molars in local population of Rawalpindi and Islamabad.

Study Design: Descriptive cross sectional study.

Place and Duration of Study: The study was conducted from 1st January to 30th June 2017 at Islamic International Dental Hospital (IIDH), Riphah International University, Pakistan.

Materials and Methods: Mesiobuccal root of eighty two maxillary first permanent molar teeth collected from Islamabad and Rawalpindi were analyzed using the Clearing Technique. Convenience sampling technique was used. Data was analyzed by using SPSS version 24. Descriptive statistics were used. Frequency and percentages were calculated for types of canal configuration.

Results: The frequency of a single canal orifices in the mesiobuccal root of maxillary first molar was 48.7% (type I, III, V and others), that with two canal orifices was 50% (type II, IV, VI and others), whereas with three canal orifices was 1.23% .

Conclusion: According to the results of this study, a second canal is common occurrence in local population of Rawalpindi and Islamabad. The distribution of canal configurations of our population differs from other populations suggesting ethnic/racial divergence.

Key Words: *Canal Configuration, Maxillary First Molar, Second Mesiobuccal Canal.*

Introduction

The Principal aim of carrying out endodontic treatment is to shape and clean all the pulpal spaces, thoroughly.[1,2,3] Root canal morphology of the teeth is often very complex and extremely variable.[4,5] False presupposition regarding root canal morphology of tooth leads to inaccurate diagnosis, inappropriate debridement, ledge formation and instrument separation during endodontic treatment.[6,7] Subsequently, it results in an inadequate root canal treatment and insufficient apical seal.[1,8] All these factors can decrease the prognosis of the endodontic treatment.[9]

Morphologically, Maxillary permanent first molar is one of the tooth with most variations.[10,14] Current literature highlights that the most common variation in permanent maxillary first molars is the presence of second canal in the mesio-buccal root [MB2].[1,3]The frequency of second mesio-buccal canal in Caucasian population was found to lie between 28-62%.[15,16] The frequency of the second canal is reported to be higher in the Asiatic population.[17,20] Weine et al reported presence of two root canals in mesio-buccal root of maxillary first molar teeth in 54% cases of the Japanese sub-population.[13] Factors contributing to the variation of morphology are ethnic/racial background,[20] age[21,23] and gender[24,26] of the population.

There is a paucity of research in this area in Pakistani population, which is limited to one study that was carried out in Turner dental school, Manchester. The study found that the prevalence of MB2, in 33 extracted teeth collected from Punjab dental hospital Lahore, were 53%.[18] Considering this the objective of the study was to determine the root

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canal configuration of the mesio-buccal root of permanent maxillary first molars by utilizing clearing technique[10] on extracted teeth collected from dental hospitals in Rawalpindi and Islamabad. This knowledge will contribute to the sparse statistics in Pakistan and will be valuable in the clinical training of Pakistani dental students.

Materials and Methods

Current descriptive cross-sectional study was carried out at Islamic International Dental Hospital, Riphah International University from 1st January to 30th June 2017. Convenience sampling technique was used. 150 Extracted permanent maxillary first molars from residents of Rawalpindi and Islamabad, Pakistan were collected. The study was approved by the ethical review board of IIDH. Teeth with fully formed roots, closed apex, intact floor of pulp chamber and intact external morphology apical to the cement-enamel junction were included in the study. On the other hand, teeth exhibiting root resorption, root fracture, calcified canals, root caries apical to cemento-enamel junction, or root canal treated were excluded. Extracted permanent maxillary first molar teeth were collected from four dental hospitals based in Rawalpindi and Islamabad by the investigators over a period of the six months and examined with naked eye. Out of 150 teeth, adherent soft tissue along with the fragments of bone and calculus were removed from the 82 teeth[27] that were included in the study, by manual scaling before storage in 10% formalin prior to transport to the study site.

On completion of specimen collection, the laboratory process was initiated where each tooth was prepared by creating an endodontic access cavity with diamond fissure burs (Mani, Tochigi, Japan) by holding tooth in hand. After locating orifice using an endodontic explorer, teeth were put in 5.25% sodium hypochlorite (PD, Switzerland) for two days to dissolve the remnants and debris of pulp. Thorough rinsing of all the teeth was performed with running water for four hours with the aim of cleaning the debris from the root canals. Subsequently, demineralization of teeth was done by using 5% nitric acid for 3 days (Merck, Darmstadt, Germany) at room temperature (25-30 ^{0}C). The solution of nitric acid solution was changed daily. At the end of the demineralization stage, the specimens were again rinsed for 4 hours in running water. Before the next step of dehydration of the tooth, the root canals were injected with India ink (BDH, UK), for canal elaboration. The dehydration procedure was comprised of sequential rinses of ethyl alcohol (Merck, Darmstadt, Germany), beginning with eighty percent solution for the duration of night, succeeded by ninety percent solution for sixty minutes and then hundred percent solutions for sixty minutes. Methyl salicylate (Merck, Darmstadt, Germany) was utilized for making the teeth transparent. The teeth were dehydrated for two hours in methyl salicylate. Examination was carried out using a magnifying glass at 5 x magnifications by holding teeth in forceps. A standardized data collection proforma was used for recording the details of canal configuration. Three of the authors first identify canal configurations individually. For 20% of the canal configurations difference of observation was noted, upon initial examination by individual operators. Then these disputed teeth were configured by mutual consensus of the three operators. All the collected data was analyzed using Statistical Package for Social Sciences (SPSS version 24). Descriptive statistics were used. Frequency and percentages were calculated for types of canal configuration.

Results

On the basis of inclusion criteria, 82 first maxillary permanent molar teeth were included in the study. Isthmuses (complete or partial) were observed in 37 (45.1%) of Mesio-buccal roots in the present study. 78 (95.1%) of teeth fell into Vertucci classification (Table-I and Figure-2), whereas, 4 teeth (4.9%) either fell into other classification or remain unclassified (Figure-1). Among them one tooth had 3 canals in mesio-buccal root all joining at apical third (Gulabivala type I (3-1)) (Figure- 1c). The remaining three teeth (3.69%) had one mesio-buccal canal with a pinhole at apical third (Figure-1a and 1b).

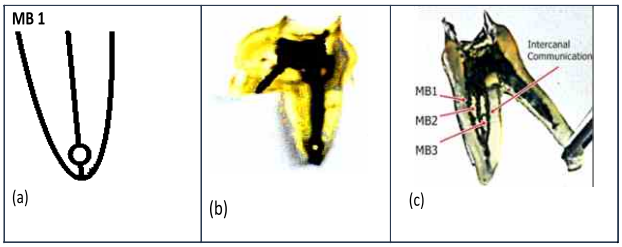

Fig 1: Configuration of Canals other than Vertucci's Classification

Table I: Configuration of The Mesiobuccal Root of Permanent Maxillary First Molars According to Vertucci Classification[2]

	TYPE ACCORDING TO VERTUCCI'S CLASSIFICATION					
	I	II	III	IV	V	VI
Number (n)	32	18	1	18	6	3
Percentage (%)	39	21.95	1.23	21.95	7.3	3.6

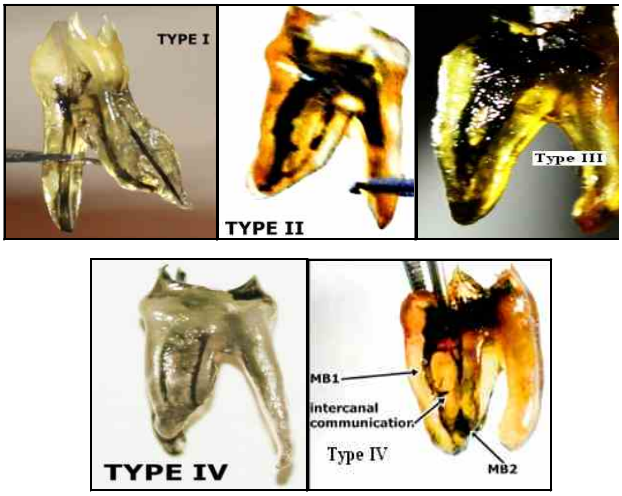

Fig 2: Canal Morphology of Transparent Teeth According to Vertucci's Classification[2]

Discussion

Procedural errors that occur during the course of endodontic treatment of permanent molars teeth highlight the importance of better understanding of morphology of root canal systems. Failure of endodontic treatment of first maxillary permanent molar is most likely because of missed second mesio-buccal canal.[8,28] Weine and colleagues[13] has emphasized the importance of a MB2 in the mesiobuccal root of maxillary first molars. Therefore, the purpose of current study was to investigate the variations in the root canal morphology of mesiobuccal root of Maxillary first molar teeth. The findings of the current research differ from those noted in other populations. The difference is particularly noted in relation to the presence of Type III, V and VI root canal configurations as noted in Table II. The results of the present study are in line with the results of research by Shahriaretal[29] on Iranian population. Shahriaretal[29] reported two canals in the mesioobuccal roots in 58.4% and one canal in 37.96% of cases. This correlation between the results of two studies may be attributed to the geographic region and ethnicity as suggested by Sert and Bayirli[24] Sert and Bayirli argued that gender and ethnic origin must be given due consideration while carrying out the preoperative evaluation for root canal therapy.[24]

In Caucasians, Type I canal configuration was reported to be as high as 72%[15] which is much higher than the frequency in Iranian[29] and Pakistani[18] population. The two populations in which Type III canal configuration were present other than this study were Australian[30] and Turkish.[31] In most of the studies Type V and Type VI canal configuration were not present except Wastietal,[18] Pienda,[21] PT Robyn[30] and Shahriaretal.[29]

Table II: Root canal configuration of the mesial root of maxillary first permanent molar teeth based on Vertucci's classification. (*Studies carried out in USA)

		Percentages (%) for types I-VI based on Vertucci's classification(%)						
Investigators	Teeth (N)	I	II	III	IV	V	VI	Other than Vertucci's
*Pomeranz&Fishelberg[15]	71	72	17	0	11.0	0	0	0
*Pineda & Kuttler[16]	262	39.3	12.2	0	35.7	12.7	0	0
*Vertucci [2]	100	45.0	37.0	0	18.0	0	0	0
PT Robyn[30](Australia)	216	26.4	19.9	27.3	1.9	12.0	0	0
Shahriar et al[29](Iran)	137	37.96	24.08	0	24.08	9.5	4.38	0
Wasti et al[18](Pakistani)	30	33.3	23.3	0	23.3	13.3	6.8	0
Alavi et al[19](Thai)	52	32.7	17.3	1.9	44.2	1.9	0	1.9
This study(Pakistani)	82	39.0	21.95	1.23	21.95	7.3	3.6	4.9

The highest incidence of Type I canal configuration was 72%[15] in American population. It ranged between 5.9-72%, whereas in current study it was 39% which is very close to study carried out on Iranian population by Shahriaretal[29] and previous study on Pakistani population by Wastietal,[18] 37.9% and 33.3%, respectively. Highest incidence of Type II canal configuration was also in American population 37% as reported by Seidbergetal.[11] In the present study, it was 21.95% which was again consistent with the results of Iranian study[29] and previous study done on Pakistani population[18] that was 24.08% and 23.3%, respectively. Type III canal configuration was found in few populations.[19,30,31,32] Its highest incidence was in Australian population[30] at 27.3% whereas in Pakistani population it was 1.23%. Highest incidence of Type IV was 59.9%,[33] in the current study it was found in 21.95% that is close to results of shahriaretal[29] and wastietal[18] which are 24.08% and 23.3% respectively. Type VI canal configuration in the

mesiobuccal root of permanent maxillary first molars, beside this study (7.3%), was only present in two studies Shahriar et al[29] 4.38% and Wastietal[18] 6.8% which are again very consistent with our results as close proximity of the ethnic/ racial origin and same geographical location. In the current study, Type I (3-1) Gulabivala canal configuration and was found to be in 1.23% of teeth that was previously reported only in Thai population[19] in 1.9% of the samples, Moreover, loops/pinholes were found in 3.67% of teeth at the apical end whereas the their incidence was reported as high as 66.7% in the Italian population[34]. Isthmuses (complete or partial) were frequently observed 37 of teeth (45.1% of the MB roots) in our study, which are reported to be as high as 100% in some previous studies.[35,37] The frequency of MB2 found in the current study is 50% which is in agreement with the previous study conducted by Wastietal[18] at 53%. In American population frequency of MB2 is in the range of 28-33% [11,15] while in European population it is 78-80%[33,34,38] Low incidence of MB2 in American studies may be due to their racial variation from our population. Thus, it is important to be aware of such variations to achieve a successful endodontic treatment.[39] In spite of the morphological knowledge of MB root of Maxillary first molars, in clinics, location of MB2 canal is almost always challenging.[40,41] Sometimes MB2 can be difficult to locate and treat, because it may share an orifice with MB1 canal.[25,34] So proper protocol must be followed while carrying out the endodontic treatment. Moreover, clinician should have adequate knowledge of canal morphology, schedule adequate clinical time and look for the MB2 by removing mesial dentinal protuberance overlying canal orifice under optimum magnification.[25,42] Additionally, the variation and inconsistency of incidence of MB2 among different studies can be result of insufficient sample size in most of the studies, methods used for the study (in vivo/in vitro), method of canal localization (roentgenographic examination, root sectioning, root clearing or computed tomography), age of the patient when tooth got extracted, ethnicity, geographic location and operators variability.

The limitations of the current research include convenient sampling technique and the lack of demographic information particularly regarding the age, gender and the ethnicity of the individuals from which specimens were collected. Additionally, the sample was collected form Rawalpindi and Islamabad; hence, cautious approach should be taken while generalizing the results of the study. Thus, future multicenter studies are recommended across Pakistan. This will provide the better understanding of the correlation between the race/ethnicity and morphological variations in the maxillary first permanent molar teeth.

Conclusion

In the light of the findings of the current study, the frequency of MB2 in the mesio buccal root of first maxillary permanent molar teeth in the local residents of Rawalpindi and Islamabad is high. Moreover, the frequency of pattern of root canal system, in the maxillary first permanent molars in local population of Rawalpindi and Islamabad, differs from other populations but is very close to north-west Iranian population. The multi-ethnic nature of today's urban populations means that dentists treat an increasing number of patients of different and mixed racial/ethnic origin.

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ORIGINAL ARTICLE

Learning in Clinical Skill Lab: The Users' Perspective

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ABSTRACT

Objective: To explore the factors that enhance and hamper learning of medical nursing students using clinical skill lab.

Study Design: Basic qualitative study.

Place and Duration of Study: This study was carried out from 03 April to 29 September 2017, in Clinical skill lab of Fazaia Medical College, Islamabad affiliated hospital.

Materials and Methods: One to one semi structured interviews was conducted and qualitative data was collected. Literature search was done extensively before formulating the interview questionnaire. This was followed by expert validation. Audio recording was done of all the interviews, the same were transcribed, coded and analyzed both manually and by importing it into Pro 11 version of NVivo; this was followed by thematic analysis.

Results: Twenty participants, 6 males and 14 females participated in the study, with minimum of 6 to 9 months training in CSL. All the participants confirmed that the training in the CSL increased their motivation and confidence, a large majority agreed that their teachers/facilitators demonstrated the skills in a professional manner, the facilitators were vigilant during the teaching sessions and observed the trainees while they practiced, most of the trainees acknowledged that they had completed their training utilizing all the equipment. The participants appreciated the learning in CSL, the knowledge, skills, experiences and confidence, they acquired. However they mentioned the shortage of space, time given and equipment provided for their training.

Conclusion: All the CSL trainees strongly endorsed this method of hands on training, they narrated that inspirations, motivation, training and guidance provided by the instructors enhanced their learning in CSL, while shortage of time for practice, space in CSL and manikins hampered their learning.

Key Words: *Clinical Skill Lab, Confidence, Manikins, Motivation.*

Introduction

In Health sciences didactic teaching and training had been traditional. This is intended to acquire as much knowledge as possible. Many medical institutes in South Asia are still following the traditional teaching. Regional medical institutions commonly adopt demonstrations, various types of lecture presentations to help the health care students to learn and understand ranging domains of knowledge, skills and attitudes.[1,2]

However various medical careers are adopting new teaching and learning methodologies, These new teaching modalities have also been adopted and the change has been initiated for better learning and contributing towards patient care.[3] The health care policy makers have overwhelmingly advocated the incorporation of quality initiatives in learning and teaching that would ensure all aspects of patient safety.[4]

They stress the need to acquire adequate level of skill expertise and understanding of its application before trainees manage the real patients. The policy managers have also emphasized and promoted the use of simulation based training to address the issues of patient safety.[5] The students of this era are trained from their school days using innovative teaching methodologies and technologies.

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Therefore, once they enter the health care careers here too adoption of upgraded teaching methodologies and utilization of latest technologies is the way forward to bridge the gap of learning the new procedures and complex skills that need to be learnt by the health care providers for better patient outcome.[6] The development of CSL is one of the many initiatives that have been taken by the health care trainers to address the issues on innovative technologies for learning and patient safety.[7,8]

The development of CSL is one of the many initiatives that have been taken by the trainers. Hence, to address the patient concerns, effective methodologies for teaching that provide appropriate equipment and environment for learning are genuine and compelling reasons for the training institutes to establish Clinical Skill labs (CSL). However, despite provision of some CSL, there is dearth of literature in the local context that had explored the factors that hamper or enhance the learning of students using CSL.[9]

Materials and Methods

This basic qualitative study was conducted in a CSL of hospital affiliated with Fazaia Medical College, Islamabad from 03 April to 29 September, 2017. This center provides the training to nursing (male and female) students. The nursing course total duration is 4 years. The nursing students were trained for 6 to 9 months in CSL. They learn various techniques and procedures during their training in CSL. At the end of the course the trainees are evaluated before they are allowed to transfer their skills to real patients.

Twenty medical nursing students; 6 male and 14 female, consented to participate in the study. The CSL trainees were informed regarding their voluntary participation. All trainees gave their informed written consent, the undertaking and assurance was given to participants regarding securities of information, anonymity and confidentiality. The trainees were also informed that there will be no reward or payment given to them for their participation. The permission from Institutional Review Board (IRB) of Fazaia Medical College was taken. Only those nursing students were included in the study who completed minimum of 6 months training in the CSL.

All participants of the study had one to one semi structured interview with the researcher. Extensive literature search was done before formulating the questionnaire, the main areas of interest that were pertinent to this research were identified and the questions were framed accordingly. The areas were explored in further details to find the answer to the study research question. To enhance the research quality and give sequence to the questions, before the real interviews were carried out, the validation of questionnaire was done, using the questions that had to be used. Pilot interviews were conducted with 3 nursing students. The research question and the tools were aligned, followed by deductive coding and thematic analysis. Triangulation was done by taking field notes, member checking and interviewee comparisons. The analysis of qualitative data was done by using Nvivo Pro 11 software.

Results

The total number of participants who volunteered for the study was 20, (6 males and 14 females). All the participants had undergone a training of minimum 6 to 9 months in the CSL. The training comprised of using plastic models, low and high fidelity equipment/manikins/devices. Along their training in CSL, the participants also worked in the wards as per schedule of their duties. All the participants confirmed that the training in the CSL increased their motivation for learning clinical subjects and procedures, *P4:* "Each individual performs on manikins and our interest increases". "We get the inspiration from our instructor." P5: "This experience motivated me in doing my work in a better way," they also expressed that this training also improved their confidence, P2: "I gained confidence to perform my skills ....." P3: "I felt confident while attending the patients." P4: "CSL builds confidence in us." They commonly reflected positively regarding the experience and expertise they gained through the training in the CSL. They also indicated that CSL training was important to them, when they performed various clinical skills on real patients in the wards or the outpatient department. *P6:* "CSL training is much important to treat patients." P14: "We are taught many clinical skills in CSL." P16: "CSL provided a great opportunity to learn many skills and procedures."

The bulk of the participants approved that their teachers/facilitators demonstrated the skills during their teaching sessions and that the trainees

developed the desired level of expertise. *P3:* "Our instructor took keen interest to make us learn." P5: "We practiced on manikins many times." Popularly the opinions of the nursing student matched that the facilitators were vigilant during their teaching sessions, they observed the trainees while they practiced. *P3:* "The instructor asked us to conduct presentation and practice on manikins." P6: "My instructor in CSL taught me the basics of nursing and helped us to work and practice on manikins…" P9: "I practically performed many procedures in the CSL." Masses of the trainees indicated that they had completed the training utilizing all the equipment

Table I: The Responses to the Open Ended Question Codes, Sub Themes, and Themes of Text Analysis by Nvivo Pro 11

S. No	Theme	Subtheme	Codes	Representative quote (P = Participant no.)
Feelings of the participants				
1	Good Experience & high Motivation	Experience	The experience was good and I felt happy and excited	"My experience using Clinical skill lab was that I was so excited"(P1); "My experience was good while learning in Clinical skill lab"(P2); "In using Clinical skill lab my experience was very good"(P4);
		Motivation	Intrinsic interest to work, interest to work in CSL	The clinical skill lab increased my motivation"(P2); "I get motivation from the Clinical skill lab"(P7);
Factors affecting their learning: Hampering and enhancing their learning				
2	Scarcity of infrastructure, equipment, quality of training and trainers	Infrastructure and equipment issues	Shortage of: time, manikins, space, and trained staff	Manikins are less in quantity "(P1); "there is shortage of manikins "(P2); "Manikins present in our skill lab are not sufficient for the practice --"(P5); "there are less dummies to practice--"(P6); "there should be more manikins"(P5); "there should be more space for learning and teaching"(P6); learning stations should be increased-(P9);
		Administrative issues	The proper time management required along with more duration to work and more space	there should be time management for practice for each trainee"(P5); "specialized lectures should be arranged"(P6); "practice time should be increased for the trainees"(P9); "there were also space problems using clinical skill lab"(P12); "the duration of our training is most important problem"(P13); "there is no proper time management for clinical skill lab training"(P15); "provide us more space"(P12); "give us one place and proper duration--"(P14); "we need to spend more time in clinical skill lab and in practice"(P20);
		Issues of trainers	Dedicated and Qualified trainers required	"in clinical skill lab there should be specific teachers"(P1); the instructors should be available to teach accordingly to the strength of trainees"(P4); "without a trainer our teacher it is not possible to have any skill"(P16); "shortage of teacher"(P17);
		Practical procedures	Passing of I/V cannula, endotracheal, nasogastric, learning CPR	"I have learnt how to put an intravenous cannula "(P12); "I have learnt many procedure like passing naso-gastric tube"(P13); "We are taught stitching, intramuscular injections, passing catheter"(P14); "I have learnt cardio pulmonary resuscitation , doing ECG, passing naso-gastric tube"(P17);

that was arranged for their learning. *P13:* "I learnt many procedures that were necessary for a medical nursing student." P14: "Our teacher taught us many clinical skills on manikins." A few of them acknowledged that they were fearful that something might go wrong in the CSL that could damage the equipment. Predominantly the trainees replied that they were not fearful and were convinced that they were working in an environment where no harm would occur to any person or equipment while they gained the required experience and practice. *P6:* "The training helped to work and practice with manikins without hesitation," It was commonly expressed by the trainees that they feared they could have hurt the real patients had they not learnt and practiced the procedures in the CSL. Moreover, many of the students expressed their confidence that they could transfer the skills to the real patients that they had learnt in the CSL, P12*:* "It is impossible to do efficient work in wards without CSL training,"

Mostly, the trainees appreciated the training for various procedures like cardiopulmonary resuscitation, how to administer various treatments, manage patients, the basic nursing skills, passing of cannula in the vein, passing catheter in urethra, passing the nasogastric tube, endotracheal tube, to take blood pressure and temperature. *P6:*"We learnt many procedures like passing of NG tube, CPR….," P12: "I learned passing of endotracheal tube and how to give enema," P16: "CSL provided great opportunities to learn skills and procedures,"

All the trainees also expressed their feelings of gaining confidence with the training of CSL and that their work in the wards became more efficient. They felt motivated and excited while getting the training courses and practicing procedures in clinical skill lab. However, majority also expressed their concern regarding the shortage of: space and time for training and the numbers of manikins. *P18* "We could not practice on manikins due to shortage of time," P19 "….. There was shortage of time, less space and less manikins. The summary of codes, sub themes and themes of text analysis are presented in Table I.

Discussion

Until recently, clinical skills were taught using real patients. Due to many concerns like trainees and patients safety, distress to the patients where real patients were used to practice various skill and procedures, concept of developing core competencies during training of health care professionals, technology enabled teaching devices and manikins, increasing number of students being inducted in each session, now the health professional training institution are gradually moving towards adding the Clinical Skill Lab on their premises. However medical literature is deficient in documenting the factors that enhance or hamper the learning of students using CSL. This study explores these factors.

Due to ever changing demands of society and deeper understanding of various disease mechanisms, educators have been encountering many educational challenges and are involved in the dynamic process to reform the curriculum and teaching methodologies.[10]

Nevertheless the efforts are underway, even in developing countries to bridge the gaps in teaching and learning by providing opportunities for training and practice in safe environment.[11] This is to keep both trainees and the patients away from harm[12], this is where CSL setup provides the concepts of safe environment for practice and learning.

This study reflects the responses of those who use the CSL for their training. The responses received from the participants in this study revealed that they were happy and excited to attend the sessions of the clinical skill lab. They learnt many procedures like suturing and resuscitation. This was achieved, as a wide range of manikins and equipment were available. The same observations were noted in a study where clinical skill labs provided learning opportunities with provision of various equipment that ranged from low to high fidelity.[13,14]

The nursing students also expressed their feelings that they practiced in a safe environment in the CSL and they understood that their practice will not harm anyone and it's safe to make mistakes. The same is also reported in literature that CSL are venues where training practice of skills and its applications are carried out in environment that is safe.[11]

The CSL are established to ensure that students are provided with the required and adequate exposure to the clinical cases in a standardized manner.[15]

All the participants in this study confirmed that their training in the clinical skill lab enhanced their

motivation for learning the procedures, similar findings were supported by other local studies where clinical skill lab was used for integrating basic sciences subjects and the participants felt motivated using CSL.[15] The students using the CSL in this study also expressed that they felt more confident by using the CSL equipment ,the literature has quoted similar benefits of CSL where students using CSL gained confidence while using simulation.[16]

Most of the students expressed their confidence that they shall be able to transfer their learnt skills to real patients. This is an important attribute of learning in CSL. Various students have reported similar findings where simulation training helped in transferring of skills for clinical application.[17] This study noted that the training in the CSL was important to the students as they understood that they would perform the same procedure on real patients, other studies also narrated that simulated settings were more effective for training and long term preservation of the skills.[13,18]

The majority of participants in this study agreed that their facilitators demonstrated the skills during their teaching session; this was aimed to make them understand the various steps that were required to carry out different procedures. It was also observed by the participants that the trainers/facilitators were vigilant during their teaching sessions, they observed the training and that the trainees learnt in an organized manner. The literature has also identified similar findings that skills are strengthened through time and consolidated through demonstration of skills.[19]

The students also shared their views that in CSL the facilitators demonstrated them various procedure and skills. The trainers supervised and observed the trainees while they practiced. Similar finding have been reported in studies in Malaysia where supervisors reflected regarding the trainees development of various competencies during their supervisory sessions.[2,15]

Majority of the students expressed their confidence that they could transfer the learnt skills to the real patients. Research supports this claim of the students that use of simulators was received optimistically both by the educators and students, as it enhanced the transferability of the skills.[20]

It is well understood that there will be no replacement for physician and teachers in medical education but technological advancements will provide the facilitators many platforms for designing more creative and interesting training sessions that have better learning outcomes and also improve quality care for patients.[21]

Educational technologies are taking hold and can provide the experiences never imagined before.[22] As medical educators, we need to encourage our students to practice and enhance their level of expertise in skills in venues that are both safe for students and patient.

The study also emphasized that most nursing students perceived that they needed institutional support for CSL equipment maintenance and enhancement both in numbers and fidelity, they required dedicated staff for teaching, demonstrations and managing the CSL facilities. They also expressed that the duration of sessions needed to be enhanced to provide more opportunities for practice and learning. Our findings are in contrast with literature in a study carried out in Saudi Arabia where most students had the opinion that adequate staff, institutional support and learning sessions of desired durations were provided.[23,24] Overall the trainees expressed their feeling of satisfaction, excitement and happiness regarding their training in CSL.

Conclusion

All the trainees in the CSL strongly endorsed this method of hands on training. The trainees identified the factors that enhanced their learning, these were: training, guidance, inspiration and motivation for learning provided by the trainers, the variety of manikins models and equipment provided for learning, the personal and equipment safety and safe environment arranged for practice. The factors that hampered their learning were: shortage of space in CSL, the allocated time for training and the relatively less number of manikins available for practice.

Recommendations from the study

It is suggested that while designing the course content for training in CSL for nursing students their representatives need to be involved to augment the factors that enhance their learning and eradicate the factors that hamper their learning.

Future Study

For determining the core factors that enhance or hamper the learning of the nursing students using CSL, future studies can be designed to capture the perspective of the users to take preventive and corrective actions for optimizing the learning of CSL users.

Limitation

Future studies require the increase in sample size of the participants who receive their training in CSL and belong to various institutes.

Conflict of Interest

No funding was received from anywhere for this project. There is no conflict of interest of any author.

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