Indexations
EMR Index Medicus
Index Copernicus
Index Pakistan
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CONTENTS

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INTERNATIONAL

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EDITORIAL

A CRITICAL REVIEW OF UNIVERSITY OF HEALTH SCIENCES INTEGRATED MODULAR CURRICULUM FOR MBBS

Tehseen Iqbal
RYK Medical College, Rahim Yar Khan, Pakistan

The University of Health Sciences Lahore has recently introduced an integrated modular curriculum (IMC) for its undergraduate MBBS program. There is no specific international obligation on Pakistan to follow an integrated modular curriculum for MBBS. Many medical schools in USA, UK, Australia and India have adopted and then left the integrated modular curriculum for undergraduate medical courses. Basic to clinical (vertical) integration is the problem of medical schools where basic medical sciences are taught by non-medical teachers. Pakistani medical colleges have physician teachers in all basic medical sciences. UHS curriculum has many flaws in designing, implementation and assessment. Without proper training of teachers, IMC is not suitable for Pakistan.

Keywords: Curriculum, Integrated, Modular, Medical Education, Undergraduate, UHS, Pakistan
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The University of Health Sciences Lahore has recently introduced an integrated modular curriculum (IMC) for its undergraduate medical (MBBS) program. These modules are designed to integrate the learning objectives, allowing students to develop a deeper understanding of the interconnectedness of different medical disciplines.[1]

There is no specific international obligation on Pakistan to follow an integrated modular curriculum for MBBS. Pakistani doctors who meet the licensing requirements of other countries can practice medicine internationally. The World Federation for Medical Education (WFME) has set global standards for medical education[2] which do not include the implementation of an integrated curriculum. Its standards do not offer a universal core curriculum and are not prescriptive.[3] Many medical schools in USA, UK, Australia and India have adopted and then left the IMC for undergraduate medical courses.

Basic to clinical (vertical) integration is the problem of medical schools where basic medical sciences are taught by non-medical teachers. Pakistani medical schools have physician-teachers in all basic medical sciences. During their lectures these physician-teachers refer to other medical subjects for horizontal as well as vertical integration.[4] Physiology teachers do a double integration. Firstly, they integrate basic sciences of Physics and Chemistry with basic medical sciences like Physiology and Biochemistry. Secondly, they integrate basic medical sciences with the clinical sciences like medicine, surgery etc. Although vertical integration is not a problem for basic medical science teachers in Pakistan, even then they need a proper training for implementation and assessment of IMC. There is no curricular development without teacher development.

When deciding about the type of curriculum for MBBS course, we should see the educational continuum of a doctor *in toto*. Before entering the MBBS course the students follow a subject-based curriculum in pre-medical classes. After MBBS, they will definitely pursue their postgraduation which is mostly subject-specific. What 'special' benefit can there be from following a zigzag course of study during MBBS. Medical field is not about patient dealing only; it includes Medical Teaching, Medical Administration, Medical Research, Medical Diagnostics, etc. Will IMC help them in their future careers? During MBBS the learning materials (books etc.) available are subject-specific. No book in the market is available which is theme-based which is the basis for IMC. In addition, the 1st year student has to purchase *all* books of MBBS course or the college has to purchase 5 times the number of books they now have in libraries.

UHS Integrated Modular Curriculum[1] is not, by definition, a 'Curriculum'. A curriculum is meant for a program or course of study, e.g., MBBS. UHS Integrated Modular Curriculum, is 'Syllabus' for First Year of MBBS. When we compare it with the curricula of other Medical Universities in Pakistan, King Edward Medical University is still following the previous curricula of UHS; FJMU is following curriculum of PMDC & HEC; the Rawalpindi Medical University's downloaded curriculum[5] is not 'Curriculum', that is just a timeline or a timetable for modules. Nishtar Medical University's (not yet published) Part-1 of the curriculum has a 'Subject-Based Modular Curriculum' which is a strange terminology for a modular curriculum. Khyber Medical College curriculum[6] is in the form of modules for each year of MBBS and is not a complete one-book curriculum. The downloaded curriculum (999 Pages) of Dow University of Health Sciences[7] is a curriculum in true sense; although it has a 'Semester System' of examination. It is clear that in Medical Colleges of Pakistan, there is no uniformity of curriculum, instruction, and assessment. For uniformity of curriculum, it would have been better to follow the '2022 Guidelines for Undergraduate Medical Education Curriculum (MBBS)' of Pakistan Medical Commission.[8]

UHS Curriculum does not mention that how many credit hours MBBS course has as is required internationally. This only mentions that the whole MBBS course is five-years duration, having 6,000 hours in total; 1,200 hours per year and 35 hours per week of study. The curriculum does not mention the subjects in the Curricular Framework mentioned at pages 52–54; but

inside the modules, wrongly mentioned is 'Curriculum of Individual Subjects', e.g., at page 60. This is a wrong terminology; the course contents of one subject for one examination are called 'syllabus.' UHS Curricular Framework is not complete, this only mentions the modules of the First Professional. The Curricular Framework of the whole MBBS course should have been completed first to decide which topics of any basic medical sciences are to be taught in which year of the MBBS course. UHS Curricular Framework does not mention teaching hours of each subject taught in a year or the total hours of a subject taught in five-years. To complete this Framework, senior teachers of each clinical specialty should identify the topics of each basic medical science subject they need to be taught with their subject topics. For example, teachers of Otorhinolaryngology and Ophthalmology will prefer Physiology of Special Senses to be taught in 4th Professional MBBS which is presently placed in 2nd Professional MBBS Framework. Likewise, teachers of Obstetrics and Gynaecology will prefer Physiology of Reproductive System to be taught in the 5th Professional MBBS. UHS Curricular Framework should also include 'Transcript' of the MBBS course.

Teachers from 4 to 5 Departments are involved in each module; coordination among different departments is difficult in our settings. As one subject is taught, in parts, in different years of the MBBS course, students will lose intra-subject integration which is important resulting in very superficial and patchy knowledge of each subject. List of Physiology Lab Practicals, given in the UHS Modular Curriculum is about half than in the traditional curriculum. There is no Physiology Lab work during Musculoskeletal Module. Interestingly, at Page 211, the assessment scheme shows one OSPE too with one Structured Viva station for 24 Marks! What will be assessed here? It is noteworthy that according to the 'Inspection Proforma for MBBS 5 Years Program'[9], issued by the UHS, many of the instruments of Physiology Lab become redundant.

For Physiology, the Foundation Module has lectures 40 and Practical 10 hours, Haematopoietic and Lymphatic Module has 20 lectures and 6 Practical hours; Musculoskeletal Module has 34 lectures and zero Practical hours; Cardiovascular Module has 75 lectures and 10 Practical hours; Respiratory Module has 45 lectures and 10 Practical hours; total being 250 hours in First Professional MBBS. According to the '2022 PMC Guidelines[8], time allocated to Physiology is 400 hours total (200 hours in 1st and 200 hours in 2nd Professional). If 250 hours are consumed in 1st Year, what will be the distribution of remaining 150 hours in other 4 Years? Guidelines of PMC (2022) at pages 29–32 may be followed with some modification.

The Assessment scheme at pages 210–212 shows that students have to pass a module 'as a whole'; not required to pass each subject separately. Previously, students used to study *topics* of a subject selectively; now students will study *subjects* selectively. If, for example, there are 10/100 MCQs from subject A, a student may skip subject A course altogether and aim for 55% from the remaining subjects, subject A being totally ignored!

Problems faced by medical colleges in Pakistan in implementing the modular curriculum are many. Some of them are: Present infrastructure, i.e., large classrooms, less tutorial rooms, possibly no or only one skills lab etc.; implementation of modular curriculum requires a different (larger) set of faculties and a different set of instruments. In some medical colleges, hospitals and college buildings are far away from each other making it difficult for faculty as well as students to shuttle between places. In case of failure a student will not know which part of the paper is to be prepared more for supplementary exam.

Inclusion of modern e-learning, Computerized Data Acquisition Systems, Virtual Dissection Table, Dissection, Telemedicine, Robotic Surgery, etc. should be considered. Without proper training of the teachers, IMC is not suitable for Pakistan.

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ORIGINAL ARTICLE

ANTIOXIDANT EFFECT OF GHRELIN ON NICOTINE-INDUCED RENAL TISSUE DAMAGE IN MICE

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Background: Ghrelin is an orexiogenic peptide released from stomach. It protects multiple organs of the body by its antioxidant and anti-inflammatory properties. Ghrelin combats oxidative stress by increasing antioxidant enzymes and decreasing lipid peroxidation. This study was done to determine the nephroprotective effect of ghrelin against nicotine induced renal tissue damage in BALB/c mice by estimating tissue antioxidant enzyme levels, namely catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and lipid peroxidation marker malondialdehyde (MDA). **Methods:** This randomized control trial was conducted at Foundation University School of Health Sciences, Islamabad in collaboration with National Institute of Health, Islamabad. Total of 27 male mice from NIH were randomly allocated into 3 groups. Group I (control) received 1 ml/Kg body weight saline, Group II (nicotine only) received 2.5 mg/Kg of body weight nicotine daily and Group III (nicotine plus ghrelin) received nicotine 2.5 mg/Kg body weight each day along with ghrelin 10 µg/Kg on alternate days for 29 days via intraperitoneal administration. Renal tissue sampling was done on 30th day for estimation of tissue oxidative stress markers through ELISA. **Results:** Nicotine administration led to significantly increased levels of CAT (p<0.001), SOD (p<0.001), GR (p<0.001), and decreased MDA (p<0.001) levels in group II. Administration of ghrelin along with nicotine in Group III resulted in significant restoration (p<0.001) of antioxidant enzymes (CAT, SOD, GR) and lipid peroxidation (MDA) (p<0.001) levels. **Conclusion:** Ghrelin acted as a potent antioxidant in nicotine induced oxidative stress in renal tissue suggesting its potential nephroprotective role.

Keywords: Antioxidants, Ghrelin, Lipid peroxidation, Oxidative stress

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INTRODUCTION

Ghrelin is derived from the word 'ghre' depicting Proto-Indo-European origin meaning 'grow' based on its growth hormone releasing ability.[1] Earlier, ghrelin had only scarce known functions, including stimulation of appetite and growth hormone release. Different studies now reveal that it is a multifaceted hormone having multiple actions on different body systems.[2] It scavenges reactive oxygen by increasing the activity of various antioxidants thus helping the body to combat various diseases.[3,4] In the renal tissue ghrelin exerts its protective effect by reducing oxidative stress. Ghrelin protects kidneys from ischemic reperfusion injury by abolishing oxidative stress, decreasing inflammation and preventing apoptosis.[1,2]

Oxidative stress is altered balance between generation and degradation of reactive oxygen species in cells or decreased capacity to detoxify free radicals. Reactive Oxygen Species (ROS) are generated as a by-product of different reactions.[5] In healthy individuals production and oxidation of ROS is balanced by internal mechanism of body. In stress or diseased state, these internal mechanisms are exhausted, disrupting the natural balance leading to oxidative stress.[6,7]

Anti-oxidant are substances which ablate the damaging effects of free radicals. They are endogenous or exogenous substances. Endogenous enzymatic antioxidants include catalase, superoxide dismutase, glutathione reductase. Exogenous antioxidants include vitamin, C, E, zinc and drugs like acetylcysteine.[8] Oxidative stress has clinical implication in various disorders like cancer, diabetes, atherosclerosis, cardiovascular, hepatic and renal disorders.[6]

Nicotine, member of Solanaceae family and a natural alkaloid, has been shown to increase the production of reactive oxygen species, decrease the cell survival in various tissues including renal, ultimately causing chronic kidney disease.[9] Nicotine causes tremendous fall in antioxidant enzymes along with increased lipid peroxidation damaging renal tissue.[9–11]

Treatment with ghrelin increased CAT, SOD and GSH levels and decreased MDA levels thus nullifying oxidative damage.[12] Renal tissue damage resulting from nicotine induced oxidative stress has been well documented in literature; however, role of ghrelin in alleviating this damage has been scarcely studied.

The current study was planned to determine the antioxidant effect of ghrelin against nicotine induced oxidative stress by determination of antioxidant enzymes (CAT, SOD and GR) and lipid peroxidation (MDA) in renal tissue of Balb/c mice.

MATERIAL AND METHODS

This randomized control trial was conducted at Foundation University Medical College in collaboration with NIH Islamabad. Resource equation[13–16] (n=DF/k+1) was used to calculate sample size keeping

20% attrition whereas DF=Degree of freedom, *k*= number of groups, and *n*=number of animals per group. Total of 27 healthy male Balb/c mice were divided in three groups of 9 each and kept in separate cages in animal house. Room temperature was kept at 22 °C with 12/12 hours light/dark cycle. Intra peritoneal injections were given to all mice.[17]

Control group (I) received normal saline 1 ml/Kg body weight intraperitoneal (IP) daily.[9] Nicotine group (II) was given nicotine 2.5 mg/Kg bodyweight IP daily to cause oxidative stress in renal tissue.[9] Nicotine+ghrelin group (III) received ghrelin 10 µg/Kg (IP) every other day and nicotine 2.5 mg/Kg body weight (IP) consecutively for total duration of 29 days.[18] Dissection was done on 30th day and kidneys were removed to measure antioxidant enzymes (CAT, SOD, GR) and lipid peroxidation (MDA) in the renal tissue.

SPSS-21 was used for evaluation of mean and standard deviation of CAT, SOD, GR and MDA levels. Evaluation of significant difference among all groups was tested by ANOVA followed by Post hoc Tukey's test and $p \leq 0.05$ was considered statistically significant.

RESULTS

Comparison of control group (I) and nicotine group (II) revealed that nicotine administration resulted in significant fall in CAT, SOD, GR and rise in MDA in renal tissue ($p<0.001$) in group-II. In group-III, ghrelin administration with nicotine lead to restoration of CAT, SOD, GR and MDA levels in renal tissue ($p<0.001$) in comparison with group-II. (Table-1, 2).

Table-1: Comparison of renal tissue CAT, GR, SOD and MDA levels in study groups

Variable	Group I	Group II	Group III	*p*
Tissue CAT ng/ml	3.54	2.37	3.27	<0.001*
Tissue GR pg/ml	35.77	11.87	29.12	<0.001*
Tissue SOD ng/ml	1458.33	626.79	1350.56	<0.001*
Tissue MDA ng/ml	7.13	8.55	7.21	<0.001*

Mean was used to express the results, ANOVA was used to compare means, *Statistically significant

Table-2: Pair-wise comparison of renal tissue CAT, GR, SOD and MDA levels in study groups (*p*-values of Post Hoc Tukey's HSD test)

Variable	Group I with II	Group II with III	Group I with III
Tissue CAT (ng/ml)	<0.001*	<0.001*	0.45
Tissue GR (pg/ml)	<0.001*	<0.001*	0.07
Tissue SOD (ng/ml)	<0.001*	<0.001*	0.09
Tissue MDA (ng/ml)	<0.001*	<0.001*	0.94

*Statistically significant

DISCUSSION

Smoking affects multiple organs of the body by increasing free radical generation. Tobacco smoke leads to kidney dysfunction due to increased oxidative stress in kidneys.[19] In the current study nicotine induced oxidative stress in mice was ameliorated by ghrelin administration, depicting its antioxidant effect. We found that administration of nicotine lead to fall in antioxidant enzyme levels (CAT, GR and SOD) and increased lipid peroxidation (MDA) in nicotine group, and ghrelin administration with nicotine lead to decrease oxidative stress evident as increased levels of endogenous antioxidants (CAT, GR and SOD) and decreased lipid peroxidation marker (MDA) in ghrelin treated group. Ghrelin administration protected the renal tissue by nullifying oxidative stress.

Products of lipid peroxidation (MDA) are potent markers used for the assessment of oxidative stress and their increased levels signify oxidative damage.[20] We observed that nicotine induced oxidative damage resulted in membrane damage manifested by increased MDA levels. Administration of ghrelin along with nicotine markedly decreased lipid peroxidation as was evident by decreased MDA levels. Bademci *et al*[21] in their study done on hepatic tissue of Sprague-Dawely rats showed protective effect of ghrelin on reducing oxidative stress and lipid peroxidation. It was concluded that ghrelin administration resulted in marked reduction in MDA levels in hepatic tissue thus protecting liver from oxidative injury.[21] Akki *et al*[22] observed that administration of ghrelin resulted in marked reduction in lipid peroxidation marker (MDA) in testicular tissue. Findings of that study showed that ghrelin lowers MDA levels acting as an antioxidant.

Our study revealed that ghrelin significantly increases antioxidant enzymes and decreases lipid peroxidation. Similar fact was reported in a study on male rats with normobaric hypoxia. It was observed that ghrelin at a dose of 80 µg/Kg/day for 2 days resulted in marked improvement in anti-oxidant defense in blood and brain of rats. Treatment with ghrelin resulted in fall in MDA levels and increased total antioxidant capacity in ghrelin treated group whereas no significant difference in levels of catalase, superoxide dismutase and glutathione peroxidase was found.[23] The difference in their results from our study is probably due to fact that kidney has higher levels of antioxidant enzyme system as compared to brain tissue. Brain tissue has decreased levels of superoxide dismutase and reduced activity of catalase which resulted in insignificant change in enzyme levels in brain tissue.[23,24] In our study due to higher levels of these anti-oxidant enzymes in kidneys, the levels of CAT, SOD and GR were increased significantly in renal tissue.

Our study depicted that ghrelin protects against oxidative damage caused by nicotine by increasing antioxidant enzyme levels (CAT, SOD, GR). Similar results were obtained in a study evaluating nephropective role of ghrelin in lipopolysachride induced renal damage. That study depicted marked improvement in renal function and oxidative stress in rats receiving ghrelin+lipopolysaccharide as compared to rats receiving lipopolysachharide only. The beneficial

effect of ghrelin on renal tissue was attributable to rise in levels of CAT, SOD and gluthion peroxidase and decrease in thiobarbituric acid reactive substances.[25] The findings of that study are in concordance with our study where ghrelin administration resulted in rise in anti-oxidant enzyme levels. The difference however lies in the point that in our project MDA was used instead of thiobarbituric acid reactive substances as lipid peroxidation marker.

The fall in lipid peroxidation marker and rise in antioxidant enzymes suggests significant role of ghrelin in alleviating renal oxidative stress caused by nicotine. Our study highlights the importance of ghrelin administration to prevent nicotine induced renal damage.

CONCLUSION

Ghrelin mitigates nicotine induced oxidative stress in renal tissue by increasing antioxidant enzymes (CAT, GR, SOD) and decreasing lipid peroxidation (MDA) thus acting as a potent renal antioxidant.

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HK: Acquisition and interpretation of data

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ORIGINAL ARTICLE

ASSOCIATION OF DIETARY INTAKE WITH SOCIO-DEMOGRAPHIC CHARACTERISTICS AMONG NURSES AT A TERTIARY CARE HOSPITAL IN KARACHI

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Background: Nurses' health status has great importance for their better and enhanced performance in hospital setup. Unhealthy diet may expose them to various diseases. The aim of this study was to identify dietary intake and its association with socio-demographic characteristics among nurses. **Methods:** The analytical cross-sectional study was conducted from 1st June to 31st July 2020 and included 337 nurses from Dr. Ruth Pfau Civil Hospital having minimum one-year working experience, using purposive sampling technique. The Food Frequency Questionnaire (33-items) was used to measure dietary intake in association with socio-demographics of nurses. Data was analysed using SPSS-23. **Results:** Majority (71.8%) of the study participants were female, 84.0% were married, and 49.2% were in 31–40 years old. Low fat dietary and dairy products were significantly associated with gender, marital status, age group, qualification, smoking, exercise, diabetes, and hypertension with p=0.02, 0.5, 0.03, <0.001, 0.03, <0.001, <0.01 and <0.01 respectively. Sea food and high-fat dairy products were significantly associated with exercise only (p=0.03). **Conclusion:** There was no significant association of fat and sweets intake with demographic characteristics except that low-fat diet and dairy products were significantly associated with all demographic variables. Sea food and high-fat dairy products had significant association with exercise.

Keywords: Association, Dietary intake, Nurses, Socio-demographics

Pak J Physiol 2023;19(2):6–9

INTRODUCTION

Nursing profession saves lives and nurses play critical role in the healthcare delivery system. Nurses help individuals to maintain the good quality of life.[1] The health status of health workers especially nurses has great importance because nurses must be fit and strong for their better and enhanced performance. The unhealthy diet, poor working conditions, shift duties and stress may lead to expose them to the hypertension. Hypertension was responsible for 1.5 million deaths every year among South-East Asian people till 2013.[2] Hypertension and cardiovascular disease are major health problems even in Pakistan and the incidence is increasing day by day.[3] The risk factors which are associated with hypertension are poor dietary intake, obesity, smoking, high cholesterol levels, deficient physical activity and diabetes.[3] Dietary habits contribute towards risk of developing obesity, cardiac diseases, and hypercholesterolemia etc. which in turn increase mortality.[4] A study conducted in Pakistan identified the association of age, gender, body mass index (BMI), use of tobacco, education, and marital status with hypertension among Pakistani adults.[5] Male gender, lower education, being married, adolescence, limited physical activity, and urban residence are factors associated with sedentary lifestyle and high-fat diet.[6] Higher intake of fruits and vegetables can prevent obesity and increased BMI, if high fat is not taken.[1–3,5,6] Nurses are facing hypertension as they are getting older and facing different demographic challenges. In addition hypertension affects the work productivity and quality of care through absenteeism and health conditions.[7] Poor dietary intake of nurses is linked with adverse health effects. There is association between poor dietary habits and psychological and cardio-metabolic problems in female nurses.[8]

Poor and unhealthy dietary trends among healthcare providers including nurses have resulted in higher prevalence of hypertension and other cardiovascular disorders.[9] There is a significant association between unhealthy diet intake of female nurses and their blood pressure.[10]

Limited studies are available on nurses' dietary intake and its association with socio-demographic characteristics. The aim of this study was to identify the association of dietary intake and its association with socio-demographics characteristics among nurses working at a tertiary care hospital in Karachi.

METHODOLOGY

This cross-sectional analytical study was conducted from 1st June to 31st July 2020 on both male and female nurses above the age of 20 years and with at least 1 year working experience at Dr. Ruth KM Pfau Hospital Karachi. Purposive sampling technique was used to select the participants. Nursing assistants, technicians and nurses having less than 1 year experience were excluded.

The sample size was calculated with Open Epi online software and the calculated size was 337 using

67.5% of nursing students had BMI less than 18 with significant level of 5% and 95% confidence interval.

A total of 337 nurses participated in this study with a 100% response rate. Ethical approval was taken from the Institutional Review Committee of Dow University of health Sciences vide (ION-MSN/2019/18/-67) and permission was obtained from Dr. Ruth KM Pfau Hospital Karachi. Written informed consent was taken from each participant. Information about socio-demographic characteristics including age, gender, marital status, qualification, experience, department, smoking status, BMI, exercise including more than 30 minutes daily walk, and history of conditions like diabetes and hypertension were recorded.

Data were collected using 33-item food frequency questionnaire (FFQ)[11] developed on the bases of widely employed Harvard FFQ. For all food items, nurses were asked how frequently they consume the food. These frequencies were standardized to number of times per day. BMI (Weight (Kg)/[Height (m)]^2 score <18.5 was taken as underweight, 18.5 to 24.9 as healthy, 25 to ≤30 as overweight, and >30 as obesity.

Data were analysed using SPSS-23. Mean±SD were calculated for all three sections of dietary intake. Frequencies and percentages were calculated for categorical variables. Mann-Whitney U test and Friedman test were applied to compare the median of different dietary sections with demographic variables, and $p \leq 0.05$ was considered as statistically significant.

RESULTS

Among 337 study participants (71.8%) were female and 84% were married. Majority (49.2%) of the participants were aged between 31 and 40 years and 56.1% were specialized nurses. The proportion of smokers, doing exercise, and having diabetes were 11.9%, 9.8% and 13.6% respectively. Nearly half of the study participants were normotensive (Table-1).

Fat and sweet intake was the only section showing no association with demographic characteristics and low-fat diet and dairy products section was significantly associated with gender, marital status, age group, qualification, smoking, exercise, diabetes and hypertension (p=0.02, 0.05, 0.03, <0.001, 0.03, <0.001, <0.01 and <0.01 respectively). Sea food and high-fat dairy products were significantly associated with exercise only (p=0.03). Daily median consumption of fat and sweets were insignificantly higher in male. Daily consumption of fat and sweets intake, low-fat diet and dairy products were higher in married nurses. For age group of 20–30 years daily consumption of fat and sweets were high as compared to other age groups, but the differences were not significant (p=0.08). Daily consumption in low-fat dietary and dairy products were higher in above 40 years of age.

Diploma holder nurses consumed fat and sweet intake, low-fat dietary, and dairy products more compared to nurses with other qualifications. The daily consumption of fat and sweet were higher in nurses who were smoking, were not doing exercise, and having diabetes. Consumption of low-fat and dairy products were higher in nurses who were not smoking, were doing exercise, and having diabetes (p=0.03, <0.001, and 0.01 respectively). Consumption of fat and sweet were higher in pre-hypertensive nurses, however hypertensive nurses consumed low-fat. Approximately same median score were found for sea food and high-fat dairy products. (Table-2).

Table-1: Descriptive statistics of baseline characteristics (n=337)

Characteristics	Number (%)
Gender	
Male	95 (28.2)
Female	242 (71.8)
Marital Status	
Married	283 (84.0)
Unmarried	54 (16.0)
Age group	
20 to 30 years	37 (11.0)
31 to 40 years	166 (49.2)
>40 years	134 (39.8)
Qualification	
General Nursing	19 (5.6)
Specialization	189 (56.1)
BSN	97 (28.8)
MSN	32 (9.5)
Experience	
1 to 5 years	63 (18.7)
6 to 10 years	96 (28.5)
11 to 15 years	83 (24.6)
>16 years	95 (28.2)
Departments	
Ward	180 (53.4)
HDU	21 (6.3)
ICU	67 (19.9)
Emergency	51 (15.1)
Administration	18 (5.3)
Use of Smokeless tobacco	
Past	33 (9.8)
Current	53 (15.7)
Never	251 (74.5)
Smoking status	
Smoker	40 (1.9)
Non-Smoker	297 (88.1)
Exercise Status	
Yes	33 (9.8)
No	304 (90.2)
BMI	
Normal weight	21 (6.3)
Overweight	50 (14.8)
Obese	266 (78.9)
Diabetes status	
Diabetics	46 (13.6)
Non-Diabetics	291 (86.3)
Hypertension	
Normotensive	170 (50.5)
Pre-hypertensive	117 (34.7)
Hypertensive	50 (14.8)

Table-2: Association between demographic characteristics and dietary intake

Characteristics	Fat and sweet intake		Low-fat dietary and dairy products		Sea food and high-fat dairy products	
	Median (IQR)	*p*	Median (IQR)	*p*	Median (IQR)	*p*
Gender						
Male	5.21 (1.94)	0.35^	1.95 (0.97)	0.02*^	1.38 (0.68)	0.49^
Female	4.90 (2.11)		2.19 (0.95)		1.33 (0.75)	
Marital Status						
Married	5.02 (2.18)	0.72^	2.20 (1.00)	0.05*^	1.35 (0.71)	0.55^
Unmarried	4.88 (1.73)		1.95 (0.72)		1.35 (0.80)	
Age (Years)						
20–30	5.37 (2.52)	0.08~	2.06 (1.43)	0.03*~	1.30 (0.77)	0.61~
31–40	5.06 (2.12)		2.05 (0.81)		1.33 (0.76)	
>40	4.70 (1.98)		2.28 (0.91)		1.42 (0.71)	
Qualification						
General Nursing	5.67 (2.60)	0.42~	2.78 (2.28)	<0.001**~	1.45 (0.92)	0.25~
Specialization	5.02 (1.92)		2.16 (0.91)		1.38 (0.77)	
BSN	4.84 (2.06)		1.95 (0.71)		1.24 (0.64)	
MSN	5.17 (2.40)		2.39 (2.01)		1.37 (1.22)	
Smoking						
Yes	5.26 (1.91)	0.23^	1.89 (0.90)	0.03*^	1.28 (0.65)	0.38^
No	4.92 (2.11)		2.16 (0.97)		1.35 (0.75)	
Exercise						
Yes	4.63 (2.78)	0.44^	2.85 (2.90)	<0.001**^	1.52 (1.65)	0.03*^
No	5.05 (2.09)		2.08 (0.88)		1.33 (0.68)	
Diabetes						
No Disease	4.89 (2.21)	0.41^	2.06 (0.92)	0.01*^	1.35 (0.72)	0.18^
Diabetes	5.47 (1.81)		2.40 (0.76)		1.35 (0.77)	
Hypertension						
Normotensive	4.86 (2.19)	0.08~	2.10 (1.05)	0.01*~	1.31 (0.90)	0.95~
Prehypertensive	5.27 (1.66)		2.06 (0.81)		1.38 (0.56)	
Hypertensive	4.65 (2.43)		2.35 (1.30)		1.36 (1.00)	

DISCUSSION

In this cross-sectional study, three dietary categories were identified among nurses of Karachi, including fat and sweet, low-fat dietary and dairy products and sea food and high-fat dairy products.

The fat and sweet intake is the only one that has no significant association with demographic characteristics, whereas the low-fat diet and dairy products intake has a significant correlation with gender, marital status, age group, qualification, smoking, exercise, diabetes, and hypertension. Consumption of seafood, high-fat diet, and dairy products are linked to increased physical activity. This intake was found to be linked to male gender, smoking, and lower socioeconomic status.[12]

Daily median consumption of fat was insignificantly higher in males. Similar results have been reported that consumption of sweet snacks, sports drinks, fried foods, energy drinks, soft drinks and vegetables was significantly more in male compared to female participants.[9] An earlier study conducted in Australia observed higher prevalence of hypertension and consumption of fat and sweets, low-fat diet among single women and married men.[13] Daily consumption of fat and sweet intake, low-fat dietary and dairy products were higher in married nurses. Similar results were reported in past studies[11,14,15].

For age group 20–30 years daily consumption of fat and sweet were higher compared to other age groups while daily consumption of low-fat dietary and dairy products were higher in >40 years of age. Similar results were noted in prior research[16]. In contrast, other studies showed that fat and sweet consumption was higher in older age participants.[13,17]

An increased intake of fat, especially saturated fat, is a main driver of obesity and increased incidence of cardiometabolic disease.[18] In our study, the daily consumption of fat and sweets was higher in nurses who were smoking, were not doing exercise, or had diabetes. Consumption of low fat and dairy products were higher in nurses who were non-smoker, were doing exercise, or having diabetes. In a past study[15], smoking was established as a contributing factor of hypertension and cardiac disorders. On the other hand, no significant association prevailed between smoking, dietary intake, and hypertension in an earlier study[19]. A previous study did not establish any significant association of smoking and hypertension.[20] However, hypertension was found significantly associated with dietary intake and diabetes mellitus.[19] Similarly, the risk of getting hypertension was found quite increased among participants with diabetes mellitus.[5,15] Exercise reduce the risk of becoming hypertensive and getting cardiac diseases.[1,2,5,19,21]

High consumption of organ meat is practiced by our subjects. In a previous study, parallel findings that increase risk of hypertension was found among the participants with high consumption of meat products.[22] According to Gharibzahedi[22], the nurses also eat less Naan Roti/Chappati, which should be raised to lower the risk of heart problems. Our subjects consumed cooked vegetables in huge amounts. Repeatedly heated vegetables[23] and over cooking[24] is a risk factor for heart problems. Margarine was also used in minor volumes which is good to secure cardiac health. Consumption of margarine is associated with cardiovascular ailments.[24] Our study participants used cream in ordinary amounts which is healthful indication. Nestel *et al*[25] reported their study subjects to be taking more cream and potatos, and developing hypertension and other cardiac diseases. Our subjects also consumed more potatoes in their daily food intake.

CONCLUSION

Mostly participants consumed two categories of diet including high-fat with sweet products and low-fat dietary and dairy products. The consumption of fat and sweet were higher in males compared to females. Higher consumption of fat and sweets was observed in hypertensive participant.

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FR: Concept, literature search, manuscript writing and data collection
SN: Study and questionnaire design, analysis of manuscript
KH: Data collection and data entry, manuscript writing and drafting
AA: Data analysis, data interpretation, analysis of manuscript
TA: Critically analysis of manuscript and final approval

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ORIGINAL ARTICLE

EFFECT OF *CUSCUTA REFLEXA* EXTRACT ON GASTROINTESTINAL MOTILITY

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Background: Motility disorders of the gut are one of the major challenges to the medical profession. The stem of *Cuscuta reflexa* had been used by traditional practitioners for the treatment of gastrointestinal and bilious disorders. The objective of the current study was to determine the effects of an extract of *C. reflexa* on carbachol-induced contractions of isolated rabbit ileum. **Methods:** The study was conducted from January to July 2020 at Department of Pharmacology, Muhammad College of Medicine, Peshawar after approval from the Ethical Committee of the College. The sample size was calculated using the 'Degree of Freedom in ANOVA' formula. The rabbit's ileum tissues were dissected and divided into two main groups, and each was further divided into three subgroups. Each subgroup consisted of six isolated rabbit's ileum tissues. After equilibration and stabilization of tissues in an organ bath containing Tyrode's solution, the effect of atropine and *C. reflexa* extract on carbachol-induced contractions in isolated rabbit's ileum was recorded. Results were analysed statistically, and $p \leq 0.05$ was considered significant. **Results:** Ethanolic extract of *C. reflexa* of different concentrations significantly decreased the amplitude of carbachol-induced contractions of rabbit ileum ($p<0.05$). The most powerful response of ethanolic extract of *C. reflexa* was observed at a concentration of 0.4 mg/ml. **Conclusion:** The ethanol extract of *C. reflexa* produces antimuscarinic effects on smooth muscles of the rabbit ileum.

Keywords: *Cuscuta reflexa*, ethanol extract, antimuscarinic effect, intestinal motility

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INTRODUCTION

The gastrointestinal tract (GIT) is responsible for intake, digestion, assimilation and absorption of food, and excretion of waste products. Absorption, secretion and peristaltic activity are under the control of the enteric nervous system (ENS), central nervous system (CNS), and gastrointestinal hormones. Endogenous ligands, various neurotransmitters and hormonal substances play important role in regulation of normal motility of gut.[1] ENS is the only part of the autonomic nervous system (ANS) which can function independently. Peristalsis of the gastrointestinal tract is a necessary physiological function. Contraction of the circular muscle in GIT, is due to the influx of calcium into smooth muscle cells through voltage-sensitive calcium channels and calcium release from intracellular stores.[2] Motor neurons receive input from excitatory and inhibitory interneurons.

Acetylcholine (Ach) is an excitatory neurotransmitter of the motor neurons. The cholinergic receptors are classified as muscarinic receptors (subclasses M_1, M_2, M_3, M_4, M_5) and nicotinic receptors (subclasses N_N, N_M). Muscarinic receptors are found in all organs, tissues, and cell types. M_3 receptors are present in smooth muscles of GIT, bronchi and detrusor muscles of the bladder.[3] Functions of muscarinic receptors are mediated by interaction with G-proteins induced changes in the function of membrane-bound effector molecules.

Carbachol is a muscarinic agonist that causes concentration-dependent increase in amplitude of contractions by acting on muscarinic receptors (M_3).[4] Activation of M_3 receptors produces an inositol triphosphate (IP_3) mediated release of intracellular calcium and release of diacylglycerol (DAG) which activates protein kinase-C causing contraction of smooth muscles, and its effects are antagonized by atropine.[5]

Gastrointestinal motility disorders result in abnormal intestinal contractions.[6] These disorders may be primary or secondary to pathological diseases including irritable bowel syndrome, inflammatory bowel disease, infective diarrhoea, neoplastic diseases, Ogilvie syndrome etc. These disorders may cause severe colicky pain, gastroesophageal reflux disease, recurrent vomiting, abdominal distention and constipation.[7]

Herbal remedies have played a significant role in treating and preventing a variety of diseases, and 25 to 50% of all medicines currently available are derived from plants.[8] There is a lot of scientific information on phytoconstituents and therapeutic uses of plants. Medicinal plants have formed the basis for traditional medicinal systems for thousands of years, with the first records dating from about 2,600 BC in Mesopotamia. Isolation of active principles from herbs and plants began in early 1800s.[9]

The plant *Cuscuta reflexa* is a perennial herb of Convolvulaceae family, commonly known as '*Amarbel,* امربیل' or '*Akaashbel,* اکاش بیل'. This plant contains flavonoids, glycosides, alkaloids, cuscutin, cuscutalin, amarvel, amarbelin, betasterol, stigmasterol, kaempferol, dulcitol, qurecetin, astragallin, myrecetin, benzopyrones, glucopyranosides, Violaxanthin, lutein, lycopene, carotene, α-cryptoxanthin, and bergenin.[10] The plant is

used to treat the headache, paralysis, diphtheria, and fever. Extract are used externally to relieve itches and internally for relief of liver disorder, fruits are used to treat cough, and paste of the plant is applied to promote healing of tongue ulcers.[11] The aim of the current study was to determine the anti-muscarinic effect of *C. reflexa* ethanol extract on isolated tissues of rabbit jejunum and compare it with anti-muscarinic effects of atropine on isolated rabbit ileum tissues.

MATERIAL AND METHODS

Animal experimental *in vitro* study was designed to achieve our goal. The study was carried out at Department of Pharmacology, Muhammad College of Medicine, Peshawar after ethical approval. The sample size was calculated using the 'Degree of Freedom in ANOVA' formula. A random sampling technique was used. Isolated pieces of rabbit ileum were isolated. The isolated tissue samples were divided into two main groups, and each was further sub-divided into three subgroups. Each subgroup consisted of six isolated tissues, making the total as 36 tissues. The stems of *C. reflexa* were collected from a garden in Wah Cantt, Pakistan. A specimen was deposited in the Department of Plant Sciences, Quaid-i-Azam University, Islamabad, for identification. The sample was dried under shade at a cool dry place, cleaned off and coarsely grounded. A semi-solid mass of dark brown colour ethanolic extract of the powdered plant material was obtained. At the time of the experiment solution was freshly prepared by dissolving extract in distilled water.

Rabbits of either sex weighing 1–1.5 Kg were purchased from the local market. They were deprived of food but not water 18 hours before the experiment. The rabbit was sacrificed and a length of ileum proximal to Peyer's patch was removed and placed in a dish containing Tyrode's solution at 37 °C. Thirty minutes were allowed for equilibration before the addition of drugs. During this period preparation was washed with fresh Tyrode's solution every ten minutes. The tissue was stabilized with sub maximum concentration of acetylcholine (8 µg) at 5 minutes intervals with washing in between until constant responses were recorded. Isotonic contractions were recorded on graph paper by Harvard kymograph. Responses were measured in mm and concentration-response curves were plotted.

Increasing concentrations of carbachol, as selected from preliminary experiments were added in organ bath in cumulative way. Responses were recorded and measured in mm displacement. Carbachol induced contractions were recorded for one minute. After this, three doubling concentrations of plant extract (0.1 mg/ml, 0.2 mg/ml and 0.4 mg/ml) and atropine (0.002 µM, 0.005 µM and 0.011 µM) were added in organ bath in cumulative way, following concentrations and time cycle, as suggested by preliminary experiments.

Data were analysed on SPSS-20 and described as Mean±SEM. ANOVA was applied to determine the significant differences between means. Post hoc Scheffe test was applied where applicable, and $p \leq 0.05$ was considered significant.

RESULTS

Three increasing concentrations of carbachol 0.22 µM, 0.44 µM, and 0.88 µM produced a response of 15.75±2.83 mm, 22.33±3.42 mm and 29.66±4.27 mm, respectively. The effect of the same concentrations of carbachol after the addition of three increasing concentrations of *C. reflexa* showed that 0.1 mg/ml of *C. reflexa* extract decreased these responses to 11±2.38, 14.66±2.65 and 20.83±4.87 mm. Decrease in response was significant with *p*-values 0.02, 0.004 and 0.006, respectively. *C. reflexa* extract 0.2 mg/ml decreased these responses to 8.83±2.06, 10.58±2.19, and 13.25±2.33 mm. The decrease in response was significant with *p*-values 0.02, 0.001 and 0.001, respectively. *C. reflexa* extract 0.4 mg/ml further decreased these responses to 5.91±1.74, 7.16±1.74, and 8.66±1.67 mm. The decrease in response was significant with *p*-values 0.002, 0.001 and 0.001, respectively.

Figure-1 shows the effect of carbachol in the absence and presence of three increasing concentrations of *C. reflexa* extract in one of the experiments.

Table-1 gives results of 6 experiments with Mean±SEM, mean decrease in response, % decrease in response as compared to control (100%). These values were used to construct a dose-response curve. The extract caused a rightward shift of the concentration response curve of carbachol (Figure-2).

Three increasing concentrations of carbachol 0.22 µM, 0.44 µM and 0.88 µM produced a response of 11.83±1.62 mm, 23.33±4.50 mm and 32.83±4.61 mm, respectively. Atropine 0.002 µM decreased these responses to 8.83±1.25 mm, 12.66±1.56 mm and 20.5±1.28 mm, respectively. Decrease in response was significant (*p*-values 0.11, 0.04 and 0.03, respectively). Atropine 0.005 µM decreased these responses to 7.83±1.30 mm, 11±1.24 mm and 17±1.44 mm, respectively. The decrease in response was significant (*p*-values 0.01, 0.05, and 0.02 respectively). Atropine 0.011 µM decreased these responses to 5.33±0.76 mm, 8.16±1.25 mm and 13.33±1.52 mm, respectively. The decrease in response was significant (*p* 0.004, 0.02 and 0.01, respectively). Figure-3 shows the effect of carbachol in the absence and presence of three increasing concentrations of atropine in one experiment.

Table-2 gives results of six experiments with Mean±SEM, mean decrease in response, % decrease in response as compared to control (100%) and *p*-values. These values were used to construct a dose-response curve. Atropine caused a rightward shift in the concentration response curve of carbachol (Figure-4).

Table-1: Effect of *C. reflexa* extract on carbachol induced contractions of rabbit ileum, (2-tailed paired samples *t*-test)

	Carbachol (control)		Carbachol & *C. reflexa* extract (0.1 mg/ml)				Carbachol & *C. reflexa* extract (0.2 mg/ml)			Carbachol & *C. reflexa* extract (0.4 mg/ml)		
Concentration	0.22	0.44	0.88	0.22	0.44	0.88	0.22	0.44	0.88	0.22	0.44	0.88
Log concentration	-0.66	-0.35	-0.05	-	-	-	-	-	-	-	-	-
Responses (Height of Contractions)												
1 mm	18	22	26	14	16	24	8	10	13	6	8	10
2 mm	7.5	11	20	5	10	13	4.5	6	8	4.5	6	7
3 mm	15	17	24	5	7	9	3	4	9.5	2	3	7
4 mm	14	28	36	13	20	23	13.5	13.5	14	6	7	8
5 mm	12	21	24	9	11	14	8	11	11	3	4	4
6 mm	28	35	48	20	24	42	16	19	24	14	15	16
Response (mm) Mean±SEM	15.75± 2.83	22.33± 3.42	29.66± 4.27	11± 2.38	14.66± 2.65	20.83± 4.87	8.83± 2.06	10.58± 2.19	13.25± 2.33	5.91± 1.74	7.16± 1.74	8.66± 1.67
Decrease in response (mm) Mean±SEM	-	-	-	4.75± 2.38	7.67± 2.65	8.83± 4.87	6.92± 2.06	11.75± 2.33	16.41± 2.33	9.84± 1.74	15.17± 1,74	21± 3.03
% decrease in response	-	-	-	30.16	29.05	29.78	43.94	48.79	55.33	62.48	65.35	70.81
p	-	-	-	0.02	0.004	0.006	0.02	0.001	0.001	0.002	0.001	0.001

Table-2: Effect of atropine on carbachol induced contractions of rabbit ileum, (2-tailed paired samples *t*-test)

	Carbachol (control)		Carbachol & atropine (0.002 µM)				Carbachol & atropine (0.005 µM)			Carbachol & atropine (0.011 µM)		
Concentration	0.22	0.44	0.88	0.22	0.44	0.88	0.22	0.44	0.88	0.22	0.44	0.88
Log concentration	-0.66	-0.35	-0.05	-	-	-	-	-	-	-	-	-
Responses (Height of cotractions)												
1 mm	9	15	25	9	9	23	7	8	22	5	7	19
2 mm	9	13	20	4	8	16	3	9	13	3	4	9
3 mm	8	20	32	9	12	17	10	13	17	4	8	16
4 mm	18	34	45	12	17	22	9	11	19	7	10	11
5 mm	15	40	48	7	13	23	6	9	13	5	7	11
6 mm	12	18	27	12	17	22	12	16	18	8	13	14
Mean response±SEM (mm)	11.83 ±1.62	23.33 ±4.50	32.83 ±4.61	8.83 ±1.25	12.66 ±1.56	20.5 ±1.28	7.83 ±1.30	11 ±1.24	17 ±1.44	5.33 ±0.76	8.16 ±1.25	13.33 ±1.52
Mean decrease in response ±SEM (mm)	-	-	-	3 ±1.54	10.67 ±3.92	12.33 ±4.12	4 ±1.91	12.33 ±4.81	15.83 ±5.03	6.5 ±1.31	15.17 ±4.46	19.5 ±5.24
% decrease in response	-	-	-	25.36	45.74	37.56	33.82	52.86	48.22	54.95	65.03	59.4
p	-	-	-	0.11	0.04	0.03	0.01	0.05	0.02	0.004	0.02	0.01

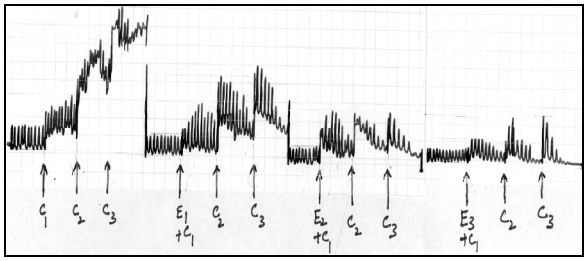
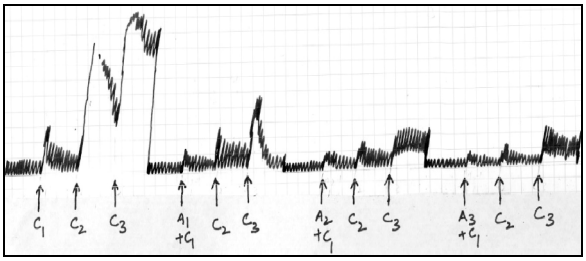


Figure-1: Effect of *C. reflexa* extract on carbachol induced contractions of rabbit ileum (n=6)

C1=Carbachol: 0.22 µM, C2=Carbachol: 0.44 µM, C3=Carbachol: 0.88 µM, E1=*C. reflexa* extract: 0.1 mg/ml, E2=*C. reflexa* extract: 0.2 mg/ml, E3=*C. reflexa* extract: 0.4 mg/ml



Figure=3: Effect of atropine on carbachol induced contractions of rabbit ileum

C1= Carbachol: 0.22 µM, C2= Carbachol: 0.44 µM, C3= Carbachol: 0.88 µM, A1= Atropine 0.002 µM, A2= Atropine: 0.005 µM, A3= Atropine:0.011 µM

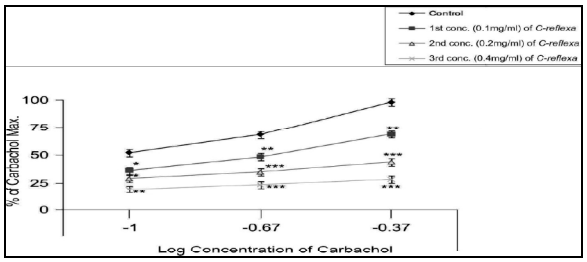


Figure-2: Response curves for carbachol in absence and presence of *C. reflexa* extract on rabbit ileum

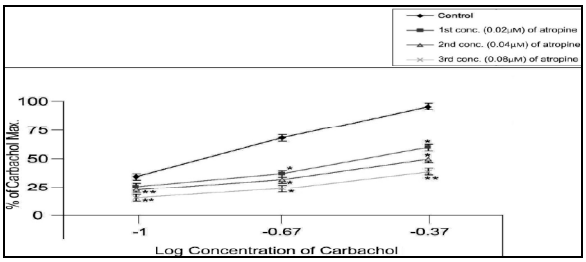


Figure-4: Response curves for carbachol in absence and presence of atropine on rabbit's ileum

DISCUSSION

Peristalsis of gastrointestinal tract is an essential physiological function. Contraction of the circular muscle in GIT is due to influx of calcium into smooth muscle cells through voltage-sensitive calcium channels and calcium release from intracellular stores. In experiments on isolated tissues as well as in studies on intact animals, GIT muscle contraction is mainly because of inward movement of calcium (blocked by L-type calcium channel blockers). Depolarization of muscle causes opening of calcium channels. Calcium present inside cell is involved in depolarization of smooth muscles of gastrointestinal tract by bringing the muscle cells close to threshold for calcium influx.[12] Motility disorders describe a variety of conditions in which the gut has lost its ability to coordinate muscular activity because of endogenous and exogenous causes.[13]

Traditional medicinal plants had been a source of medicinal treatments for thousands of years, and medicines derived from plants play an important role in primary health care. *C. reflexa* plant is traditionally used as a carminative, to control vomiting, in bilious disorders, flatulence and stomachache.[14] Intestinal motility is controlled by multiple physiological mediators, mainly acetylcholine, histamine, serotonin, bradykinins, prostaglandins, substance-P, and cholecystokinin which achieve their contractile effects through an increase in cytosolic calcium.[15] Antagonists of all the above-mentioned mediators inhibit responses by their respective agonists but calcium channel blockers will inhibit responses of all agonists.[16]

In our study, carbachol was selected as a muscarinic agonist because it is resistant to hydrolysis by cholinesterase enzyme hence cumulative dose effect could be recorded.[17] In our experiment, both atropine and *C. reflexa* extract reduced the amplitude of contractions, i.e., *C. reflexa* extract significantly decreased the magnitude of spontaneous contractions in a dose-dependent manner. Our finding is related to findings of Prasad *et al*[18], who showed that a freshly prepared aqueous solution of crude extract of *C. reflexa* possesses antispasmodic activity on guinea pig and rabbit isolated ileum against acetylcholine. However, according to Kayath *et al*[19], the extract of *C. reflexa* showed a cholinomimetic effect on rabbit ileum, frog rectus and heart muscles which were blocked by atropine on ileum and heart and by pancuronium on rectus abdominis muscle of frog. This contradiction may be because in the present study, the effects of freshly prepared solution of extract were studied while earlier Kayath *et al*[19] used 5–6 days older solution. This fact is supported by the study of Paudel *et al*[20] who observed that 4–5 days old solution of crude extract of *C. reflexa* produced marked contractions of isolated ileum of guinea pig and rabbit in a concentration which had earlier exhibited relaxant and spasmolytic effects. Given these findings, freshly prepared *C. reflexa* extract has a relaxant effect on intestinal smooth muscles of rabbit.

CONCLUSION

The freshly prepared *C. reflexa* extract has an inhibitory effect on the amplitude of carbachol-induced contractions of isolated rabbit ileum through antimuscarinic activity. It may be a valuable antispasmodic therapy. Further studies are recommended to explore the exact mechanism of action and to rule out the involvement of other receptors.

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ORIGINAL ARTICLE

VITAMIN D SCREENING BEFORE FERTILITY TREATMENT PLANS: PILOT STUDY IN PCOS AND NON-PCOS INFERTILE WOMEN

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Background: Polycystic ovarian syndrome (PCOS) and hypovitaminosis D are two most common endocrine disorders in young women leading to many adverse metabolic consequences. Objective of this study was to compare Vitamin D (VD) levels, Body Mass Index (BMI), lipid profile, hormonal parameters, and oocytes in PCOS and non-PCOS infertile females and to explore any association of VD levels with these parameters. **Methods:** This cross-sectional study was conducted from July 2019 to June 2020 after ethical approval at Aga Khan University in collaboration with Australian Concept of Infertility Medical Centre (ACIMC). It was conducted on 88 infertile females with age range 25–45 years recruited for Intracytoplasmic Sperm Injection (ICSI). Subjects were divided into two groups; PCOS (n=37) and non-PCOS (n=51) based on diagnostic criteria of PCOS. Serum VD was analyzed using ELISA. Statistical analysis was performed on SPSS-20. Mann-Whitney U-test and Spearman's rank correlation were applied. **Results:** The mean BMI was significantly higher among PCOS as compared to non-PCOS women (p<0.001). There were statistically significant differences in total cholesterol, triglycerides, Low Density Lipoprotein (LDL-C), High Density Lipoprotein (HDL-C), and Very Low Density Lipoprotein (VLDL) among groups (p<0.05). There was significant correlation of vitamin D with maturity of oocytes (r=0.836, p<0.0001). **Conclusion:** The findings indicated that PCOs women were obese, had abnormal lipid profile with low VD levels. Low levels of VD were associated with poor maturity of oocytes which is required for successful conception.

Keywords: Polycystic Ovary Syndrome, PCOS, Vitamin D, deficiency, oocyte, body mass index, BMI, lipid profile

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. It is classically characterized by the presence of oligo-anovulation, ovarian polycystic morphology, and hyperandrogenism.[1] Prevalence rates of PCOS are estimated to be around 4–18% worldwide and are particularly high for South Asian women, especially in Pakistani women, i.e., around 52%.[2] Classification methodologies currently being employed to serve as diagnostic criteria for PCOS include the National Institute of Health criteria, Androgen Excess and PCOS Society criteria, and the Rotterdam criteria being the most comprehensive one allowing diagnosis of non-classical phenotypical presentation of PCOS. Recently, serum anti-Müllerian hormone levels have also been suggested as a diagnostic marker for PCOS.[3]

The PCOS, being a multifactorial and heterogeneous syndrome, has a complex aetiology and its pathophysiological pathways have been under extensive research in the past decade. Vitamin D deficiency (VDD) has been identified as one of the potential risks factors.[4] In the Pakistani population, where VD status displays a staggering 53.5% VDD, it is imperative to investigate its potential association with PCOS, VDD, though very common in the general population worldwide, is even more prevalent in PCOS patients, seen in approximately 67–85%.[5] The existing literature hints towards a possible pathophysiological association involving VD metabolites affecting oocyte competence and embryo fertilization rates.[6] Research into the therapeutic effects of VD supplementation for PCOS patients has further substantiated this association.[7]

Regarding the Pakistani population, only limited work has been done till now to establish an association between VDD and PCOS, with little to no insight into the possible pathophysiological mechanisms responsible for this association. The objective of this study was to compare VD levels and relate VD levels with lipid profile, hormonal parameters and maturity of oocytes as well as Body Mass Index and in in PCOS/non-PCOS infertile women.

METHODOLOGY

This cross-sectional study was conducted from July 2019 to June 2020 after ethical approval at Aga Khan University in collaboration with Australian Concept of Infertility Medical Centre (ACIMC).

A data collection form was filled at the time of recruitment to record clinical data such as age, height, weight, calculation of BMI, blood pressure, smoking habits, hormonal treatment, and clinical history.

A total of 88 infertile women recruited from all ethnic backgrounds for Intracytoplasmic Sperm

Injection (ICSI) with age 25–45 years after obtaining written and informed consent. Subjects were further stratified into two groups; PCOS (n=37) and non-PCOS (n=51) based on diagnosis. Women on VD therapy, calcium supplementation (for the last 6 months) or thyroxin replacement therapy were excluded. Women with diabetes, hypertension, and serious general health status were also excluded. The serum VD was analysed using Enzyme-linked immunosorbent assay kit (Cat# ab213966). The analytical sensitivity of the assay was 1.98 ng/ml, and detection range was 0.5–1010 ng/ml.

Statistical analysis was performed on SPSS-20. Mann-Whitney U-test and Spearman's rank correlation were used for comparison of results, and $p<0.05$ was considered statistically significant.

RESULTS

A total of 88 infertile women were included in the study and were stratified further into two groups: PCOS 37 (42.1%) and non-PCOS 51 (57.9%).

The mean age of women in PCOS group was slightly higher (12.197±1.471) as compared to females in non-PCOS group (12.102±1.692), but it was not significant. The mean BMI was significantly higher among PCOS women as compared to non-PCOS women (28.966±5.227 *vs* 29.33±5.712, $p<0.001$).

Significantly low VD levels were observed in PCOS group (13.35±4.757) as compared to non-PCOS group (20.798±7.763). Number of oocytes were more in PCOS group (11.297±1.17) as compared to non-PCOS group (8.74±1.79). However, number of mature oocytes were less in PCOS group (4.0±1.17) as compared to non-PCOS group (7.78±2.4). The mean BP (Systolic and Diastolic) of women in PCOS group was slightly lower (121.243±4.734 and 75.081±3.647 respectively) as compared to non-PCOS groups (121.451±5.394 and 75.98±4.324 respectively), but it was not significant. The Follicular Stimulation Hormone (FSH), Luteinizing Hormone (LH), Anti-Müllerian Hormone (AMH), Prolactin and other parameters of women in PCOS and non-PCOS groups are shown in Table-1.

Table-2 illustrates a significant positive association of vitamin D levels with maturity of oocytes. There was a significant negative correlation of VD with all lipid parameters except HDL.

Table-3 describes correlation of VD levels with number of total oocytes (correlation coefficient, r= -0.0356 and r=0.841 in PCOS and non-PCOS) which was significant.

Figure-1 shows a significant positive correlation of both groups with maturity of oocytes (r=0.836, $p<0.001$). Figure-2a and b illustrate correlation of VD with number of oocytes in PCOS and non-PCOS, the correlation of both groups with maturity of oocytes is represented in Figure-3a and b.

Table-1: Descriptive statistics of the subjects (n=88)

Parameters	PCOS (n=37)	Non-PCOS (n=51)	*p*
Age	12.20±1.47	12.10±1.69	0.784
BMI	28.97±5.23	29.33±5.71	0.761
Systolic BP (mmHg)	121.24±4.73	121.45±5.39	0.852
Diastolic BP (mmHg)	75.08±3.65	75.98±4.32	0.307
FSH (mIU/mL)	12.22±17.54	6.47±2.69	0.023
LH (mIU/mL)	8.07±10.27	4.73±2.22	0.026
AMH (pmol/L)	2.60±2.82	2.14±1.48	0.324
Prolactin (ng/mL)	14.96±14.68	13.65±9.02	0.605
Estradiol (pg/mL)	4897.57±2587.97	5363.12±3600.73	0.504
No. of Oocytes/patients	11.30±1.17	8.75±1.80	0.000
No. of Mature Oocytes	4.03±1.66	7.78±2.42	0.000
Vitamin D (ng/ml)	13.35±4.76	20.80±7.76	0.000
Total Cholesterol (mg/dL)	223.76±17.98	176.20±15.02	0.000
Triglycerides (mg/dL)	210.38±40.94	133.71±25.02	0.000
HDL (mg/dL)	32.38±4.58	48.94±5.98	0.000
LDL (mg/dL)	138.32±10.11	114.12±16.45	0.000
VLDL (mg/dL)	46.00±7.87	23.82±4.76	0.000

HDL=High Density Lipoproteins, LDL=Low Density Lipoproteins, VLDL=Very low-Density Lipoproteins

Table-2: Correlation of study variables with Vitamin-D in the subjects

Variables	*r*	*p*
No of oocytes/patient	0.052	0.628
Oocyte maturity	0.836	0.000*
Age	-0.017	0.873
BMI	0.062	0.569
Systolic Blood Pressure	-0.016	0.883
Diastolic Blood Pressure	0.001	0.993
Follicle Stimulating Hormone	-0.064	0.553
Luteinizing Hormone	-0.139	0.198
Anti-Müllerian Hormone	-0.058	0.589
Prolactin	-0.013	0.905
Estradiol	0.160	0.137
Total Cholesterol	-0.427	0.000*
Triglycerides	-0.428	0.000*
HDL	0.359	0.001*
LDL	-0.377	0.000*
VLDL	-0.410	0.000*

*Significant at $p<0.01$

Table-3: Correlation of VD with study parameters among PCOS and non-PCOS groups

	PCOS (n=37)		Non-PCOS (n=51)	
Parameters	*r*	*p*	*r*	*p*
No. of oocytes/patient	-0.356	0.031	0.841	0.000
Oocyte maturity	0.784	0.000	0.792	0.000
Age	-0.152	0.368	-0.001	0.993
BMI	0.096	0.571	0.036	0.804
Systolic BP	-0.156	0.355	0.019	0.894
Diastolic BP	-0.048	0.778	-0.066	0.644
FSH	0.148	0.381	-0.027	0.852
LH	-0.002	0.990	-0.122	0.395
AMH	-0.046	0.785	0.023	0.873
Prolactin	0.179	0.290	-0.097	0.499
Estradiol	-0.169	0.318	0.242	0.086
Leptin	0.192	0.256	-0.083	0.563
CBC	0.249	0.137	-0.046	0.748
Total Cholesterol	-0.019	0.910	-0.072	0.615
Triglycerides	0.131	0.441	-0.291	0.038
HDL	0.099	0.559	-0.167	0.243
LDL	-0.020	0.908	-0.112	0.435
VLDL	-0.031	0.855	0.087	0.544

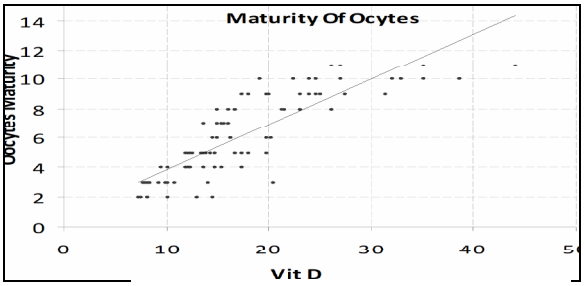

Figure-1: Correlation of VD Levels with mature oocytes in the study population

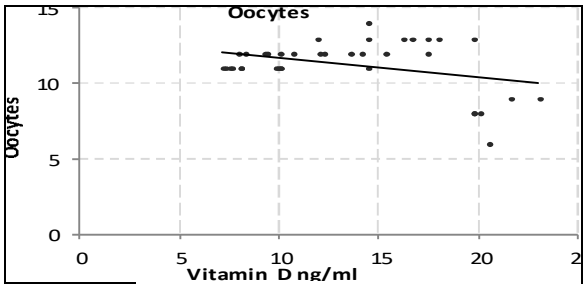

Figure-2a: Correlation of VD Levels with number of oocytes in PCOS group

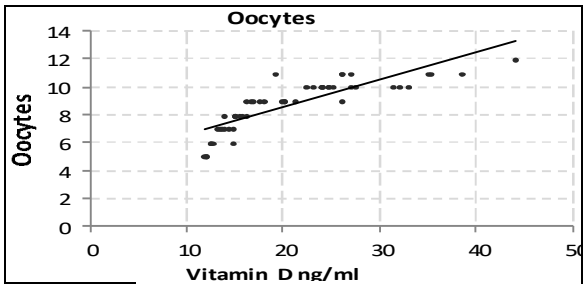

Figure-2b: Correlation of VD Levels with number of oocytes in non-PCOS group

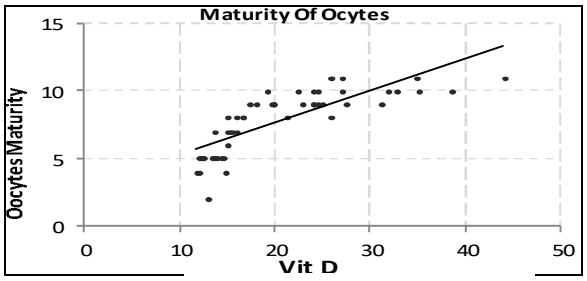

Figure-3a: Correlation of VD Levels with maturity of oocytes in PCOS group

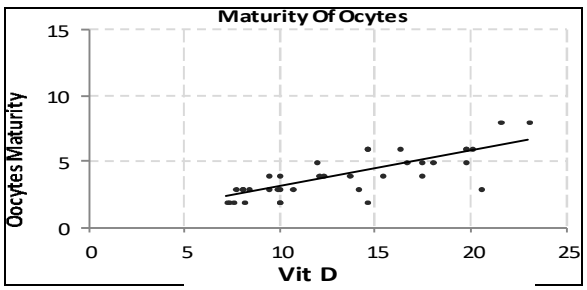

Figure-3b: Correlation of VD Levels with maturity of oocytes in non-PCOS group

DISCUSSION

Our study findings of high BMI in PCOS women corroborates with some other studies[4,8]. A study in North India found a maximum number of obese women to have hirsutism and oligo ovulation and less commonly, polycystic ovaries as well. A higher BMI did not impact the LH/FSH ratio; it was comparable between PCOS women with a high BMI and those with a normal BMI.[9] Compared to PCOS-lean women, PCOS-obese women had significantly higher systolic BP, total cholesterol, LDL, and triglycerides.[10] One possible reason for relation between high BMI and PCOS is that obesity is a well-known risk factor for PCOS. PCOS is associated with higher androgen levels coupled with a reduced androgen clearance. Conversely, PCOS can also be a risk factor for obesity and higher BMI.

Women with PCOS have a higher upper-body or android obesity when compared with control women of similar BMI, the reason being that hyperandrogenism is associated with a smaller size of femoral adipocytes causing a shift of fat distribution to the upper body.[11] This android obesity then contributes to insulin resistance, hyperinsulinemia, increased risk of cardiovascular diseases and diabetes in PCOS women. Hyperandrogenism or high testosterone levels also impacts fat distribution in such a way that visceral fat increases, further impairing insulin sensitivity.[12] It is for all these reasons that weight reduction can lead to better fertility outcomes due to decreased anti-Müllerian hormone levels[13] and improvement of metabolic dysfunction in PCOS women.[14]

Our findings also suggest lower VD levels in all infertile women; however, they were significantly less in PCOS as compared to non-PCOS women. VDD is associated with ovulatory dysfunction, insulin resistance, hyperandrogenism and higher dehydroepiandrosterone sulfate (DHEAS) levels in PCOS women.[15] VD also affects lipid metabolism, so its deficiency results in lower HDL-C levels and higher LDL-C and cholesterol levels in women with PCOS.[16] Additionally, increased BMI, total cholesterol, and LDL-C, are considered risk factors of VDD in PCOS women.[17] Since PCOS women are usually obese and VD is a fat-soluble vitamin, higher body fat leads to more VD being accumulated in the fat and thus, lower serum VD levels.[18] It is for all these reasons that vitamin D supplementation can result in reduced androgen levels, improved glucose metabolism and reduced LDL-C and cholesterol levels in PCOS women.[19]

PCOS women have a reduced number of oocytes compared to non-PCOS women. According to a study[20], the participants with sufficient VD levels in the follicular fluid had a significantly greater number of retrieved oocytes compared to the participants with lower VD levels in the follicular fluid. This is explained

by evidence from other studies that VD sufficiency can result in decreased anti-Müllerian hormone levels[12], allowing for improved FSH sensitivity of growing follicles and a reduced inhibitory effect on the recruitment of growing ovarian follicles.[14] However, other studies have also stated the contrary.[7] The number of oocytes retrieved from PCOS women has been significantly higher than that in non-PCOS women, though, despite the difference in number, there was not any significant difference noted between the quality of oocytes in the two groups.[21] However, another study reported that the number of 'good quality' oocytes was lesser in the PCOS group, explaining the lower fertilization rate in those women which is in agreement with our findings except that number of oocytes were also less in our PCOS population.[10]

Our findings also indicate a significant positive correlation of VD with the maturity of oocytes. One study, upon comparing the VD deficient patients with VD sufficient patients found that the latter had better quality embryos and higher clinical pregnancy rate. Higher serum VD levels lead to better chances of ovulation.[3] VD administration induced ovulation in women before going for *in vitro* fertilization.[7] It was also noted that women successfully giving live births had significantly higher serum VD levels than those failing to do so. Lower serum VD levels also lead to higher chances of pregnancy loss.[5] However, some studies have suggested completely contradictory results. A study states that follicular fluids, which are a reliable indicator of the body's VD stores, with lower VD levels were associated with more successfully fertilized oocytes, positive hCG, better clinical pregnancy and live birth rates as compared with the follicular fluid containing higher VD levels.[22]

The negative correlation has been explained by the finding that higher follicular fluid VD levels were associated with lower glucose levels in the fluid which negatively impacted the oocyte maturation and thus, its quality.[23] The opposite correlation in our study may be due to the smaller sample size. The explanation behind the conflicting studies can be that it is only the physiological concentrations of serum VD levels that are beneficial for improving fertility by increasing endometrial receptivity. Excess VD can have detrimental effects on ovarian homeostasis, quality, and development.[21] This can be due to the ability of excess VD to antagonize the effects of other steroid sex hormones since it down-regulates oestrogen receptor (ER)-α, progesterone receptor (PR) -A and -B and steroid receptor coactivator (SRC) expression in human uterine leiomyoma cells.[24] It is because of all these reasons that VD supplementation should be done extremely cautiously by first evaluating the baseline VD levels if they are above 50 ng/mL, before any treatment for sub-fertility is started.[25]

CONCLUSION

The findings indicated significant correlation of VD with number of total and mature oocytes as well as with BMI and all lipid parameters in PCOS and non-PCOS sub-fertile women, suggesting that VD may increase fertility through the number of mature oocytes. The possible causality of the relationship between VD and infertility deserves further investigation.

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ORIGINAL ARTICLE

HEPATOPROTECTIVE EFFECT OF SEA BUCKTHORN BERRY SEED OIL IN CYCLOPHOSPHAMIDE-INDUCED HEPATIC TOXICITY IN BALB/c MICE

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Background: Chemotherapy-induced hepatotoxicity can be mitigated by use of antioxidants, which may help the liver to recover its endogenous antioxidant mechanism. The objective of this study was to determine the effectiveness of sea buckthorn berry seed oil (SBO) in attenuating cyclophosphamide-induced changes in liver enzymes and liver histology in BALB/c mice. **Methods:** This experimental study was conducted in Physiology Department of Foundation University School of Health Sciences (FUSH), in collaboration with Pathology Department, FUSH and National Institute of Health, Islamabad from Jan 2018 to Jun 2019. Thirty healthy male BALB/c mice were divided into 3 groups of 10 each. Group-1 served as control. Group-2 received cyclophosphamide (25 mg/Kg body weight) intraperitoneally for 10 days. Group-3 was co-administered cyclophosphamide (same dose) with of sea buckthorn berry seed oil (40 mg/Kg body weight) orally for ten days. All animals were sacrificed on 11th day. Serum levels of liver enzymes as liver injury biomarkers were assayed. Liver histopathology was carried out for evidence of hepatic injury and recovery. **Results:** Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) increased significantly in Group-2 (p<0.05). In Group-3, the rise in hepatic injury serum markers was significantly less than Group-2 (p<0.05). Upon histological examination, Group-2 was grade 5 according to Kleiner's scoring system for steatohepatitis, and had grade 2 sinusoidal dilatation on Rubbia-Brandt grading system. These changes were significantly less in Group-3 (p<0.05). **Conclusion:** Co-administration of SBO mitigated the CP-induced rise in hepatic injury biomarkers and sinusoidal injury.

Keywords: Cyclophosphamide, sea buckthorn berry seed oil, chemotherapy-induced hepatotoxicity, cellular antioxidants, oxidative stress

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INTRODUCTION

Traditional cytotoxic agents remain the mainstay of cancer treatment, despite recent progress in cancer therapeutics with the advent of targeted therapies and immunotherapies.[1] Cyclophosphamide (CP) is a synthetic alkylating agent commonly used as a chemotherapeutic and immunosuppressive drug.[2] It is an inactive cytostatic, which undergoes metabolic activation catalysed by the hepatic cytochrome P450 (CYP450) monoxygenase systems into active metabolites, phospharamide mustard and acrolein. Phospharamide mustard is responsible for anti-tumour effects while acrolein contributes to cytotoxicity and carcinogenicity of CP.[3]

During metabolic activation, reactive oxygen species (ROS) are also formed. Side effects of CP, mediated by generation of ROS, direct depletion of cellular antioxidants and the resultant oxidative stress, form the major limitation of using CP.[4] These side effects include pulmonary fibrosis, haemorrhagic cystitis, gastrointestinal bleeding, and irreversible azospermia in men, etc. Microvascular fatty changes in liver also form a part of toxicity spectrum.[5] CP is used in most myeloablative regimen, and when combined with total body irradiation, has been reported to result in veno-occlusive disease (VOD) in 38% of the patients. The hepatotoxicity appeared to be greatly potentiated by radiation.[6]

Chemotherapy-induced hepatotoxicity can be mitigated by use of antioxidants, which may help the liver to recover its endogenous antioxidant mechanism (reduced glutathione- GSH).[7] Consequently, combining a treatment regimen with effective and safe antioxidants could be the appropriate approach to allay cancer therapy-induced toxicity.[8]

Sea buckthorn is a natural source of antioxidants with documented free radical scavenging abilities.[9] Sea buckthorn seed and pulp oil are rich source of mono- and polyunsaturated fatty acids, carotenoids, phytosterols, vitamins E, K and 28 trace elements like zinc, iron, sulphur, calcium, magnesium, selenium, copper etc.[10] Selenium present in sea buckthorn extract may help in biosynthesis of glutathione peroxidase, which is crucial for the degradation of lipid hydroperoxides.[8–11]

Previous research has documented significant antioxidant effect of sea buckthorn extract against free radical production, oxidative damage, and lipid peroxidation resulting from acetaminophen, chromium, sodium nitroprusside, nicotine, CCl_4, hypoxia and radiation.[12–14] Few studies have been published till now to assess the antioxidant effect of sea buckthorn berry

seed oil (SBO) on CP-induced hepatic damage in mice. This study was designed to evaluate the influence of sea buckthorn berry oil on cyclophosphamide-induced rise in liver injury serum markers and cytoarchitecture damage in BALB/c mice.

MATERIAL AND METHODS

Healthy male BALB/c mice from inbred colony maintained at National Institute of Health (NIH) Islamabad were used for the study. Sample size was calculated using Resource Equation method[15]. Thirty adult mice with average weight of 30±5 g were maintained in well ventilated polypropylene cages (with 5 animals each) containing wood shavings. Animals were kept at 25±2 °C with relative humidity of 50–60% and on 12-hour light/12-hour dark cycle. Rodent feed was given to animals. The pellet diet consisted of 65% carbohydrate, 25% protein and 10% fat. Feed and tap water were given *ad libitum*. The study and all procedures were approved by the Institutional Ethical Review Committee (Ref: 217/FF/FUMC/ERC). The animals were cared for in accordance with Guide for the Care and Use of Laboratory Animals (8th ed., 2010).[16]

Injection cyclophosphamide 1 gm (Cyclomide 1,000 mg) was procured from Pharmedic Laboratories, Pakistan. Commercially available preparation of sea buckthorn seed oil was procured from SIBU Sea Berry Therapy, Utah, USA. Phosphate-buffered saline (PBS) (0.1M, *p*H 7.4) that contained KCl (1.17% w/v) was freshly made in Physiology Lab at FUSH. All other chemicals used for experiment were of analytical grade.

Animals were divided into 3 groups of 10 mice each. Group-1 was negative control; group-2 was positive control and group-3 was experimental group. Group-1 mice were given normal saline (0.65%) 1 ml/Kg body weight (i.p.) daily for 10 days. Group 2 mice were given CP 25 mg/Kg body weight (i.p.) for 10 consecutive days to induce the liver damage.[8] Group 3 animals were given CP 25 mg/Kg body weight (i.p.) along with SBO 40 mg/Kg body weight (orally) for 10 consecutive days.[17]

On 11th day, after ensuring proper anaesthesia, intra-cardiac blood sampling was done for assessments of liver injury serum markers. Samples were centrifuged at room temperature at 4,000 rpm for 15 minutes. Serum was used to assess alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin by commercial kits on auto-analyser (Selectra E Fully Automatic Chemistry Analyser).[7] A one-centimetre sample of each liver was preserved in a 10% formalin solution for 24 hours before slides were prepared for histological examination. Five μm thickness sections were cut and fixed on slides, stained with haematoxylin and eosin and observed under compound light microscope for histopathological analysis and comparison among the three groups.[18] The grading for hepatic injury, as depicted by hepatocellular and sinusoidal damage, was done by using NAFLD Activity Score (NAS) proposed by Kleiner *et al*[19], for hepatocellular damage, and grading system of sinusoidal dilation given by Rubbia-Brandt *et al*[20], for sinusoidal damage, as adapted from Vauthey *et al*[21].

The statistical analysis was done using SPSS-24. Values were expressed as Mean±SD. The statistical significance of the differences of various quantitative changes between the experimental and control groups were evaluated using one-way ANOVA followed by Tukey's HSD (Honestly Significant Difference) post hoc test for multiple comparisons. The difference was regarded statistically significant at $p \leq 0.05$.

RESULTS

The liver enzymes, AST, ALT and ALP, levels increased significantly in Group-2. Serum alkaline phosphatase (ALP) was more than 3 times the control average value and ALT/ALP ratio was less than 2. In Group-3, the rise in hepatic injury serum enzyme markers was significantly less than Group-2. A significant increase in the serum bilirubin was seen in Group-2 compared to Group-1 after CP administration (89.75%, $p<0.001$). In Group-3, the increase in the serum bilirubin was significantly less as compared to Group-2 (18.6%, $p<0.001$). The bilirubin levels of Group-1 and Group-3 were not significantly different ($p=0.242$). (Table-1).

Microscopic examination of the sections of Group-1 showed normal histological structure like hepatic lobes containing cords of hepatocytes with sinusoids between these cords. The central vein was normal, and the portal triads also appeared normal. In Group-2, there was diffuse fatty infiltration, central vein congestion, loss of hepatocyte architecture, damage to endothelium of sinusoids with sinusoidal congestion and dilatation. Upon grading of steatohepatitis by the NAFLD Activity Score (NAS)[19], Group-2 had a score of 5 and was graded as steatohepatitis. Sinusoidal injury was graded as moderate based on grading system of sinusoidal dilation given by Rubbia-Brandt *et al*[20]. Based on these observations, Group-2 was classified as having hepatic injury, defined as steatohepatitis Kleiner score ≥4, and/or grade 2 to 3 sinusoidal dilation, as adapted from Vauthey *et al*[21].

All these changes were less in Group-3 compared to Group-2, with NAFLD Activity Score (NAS) of 3 and was graded as borderline steatohepatitis. Sinusoidal injury was scored as grade 1 (mild)[20]. With steatohepatitis NAS equal to 3 and grade 1 sinusoidal dilation, Group-3 did not qualify as having hepatic injury. The photomicrographs of histological changes in Group-2 and Group-3 are given in Figure-1 and Figure-2 respectively. The parameters used for grading are given in Table-2 and Table-3.

Table-1: The effects of CP, and CP+SBO on serum levels of total bilirubin, ALT, AST, and ALP

Groups	Total bilirubin (mg/dL)	ALT (SGPT) (u/L)	AST (SGOT) (u/L)	Alkaline phosphatase (u/L)
Group-1 (Control)	0.39±0.09	56.5±11.37	140.2±5.02	257.5±2.36
Group-2 (CP)	1.02± 0.14[a]	115.8±7.84[a]	221.9±19.45[a]	788.1±5.66[a]
Group-3 (CP+SBO)	0.47±0.06[b]	74.9±7.73[b]	177.4±5.79[b]	380.7±4.24[b]

a=p<0.05 Group-1 *vs* Group-2, b=p<0.05 Group-2 *vs* Group-3

Table-2: Grading of steatohepatitis by NAFLD Activity Score (NAS)

Parameters	Group-1	Group-2	Group-3
Steatosis (Kleiner score)	0 (≤5%)	1 (5%–35%)	0 (≤5%)
Lobular inflammation (foci/×200 field)	0 (no foci)	2 (2–4 foci)	1 (≤2 foci)
Ballooning	0 (none)	2 (many cells)	1 (few cells)
NAFLD Activity Score	0	5	3
Grading	none	Steatohepatitis	Borderline steatohepatitis

NAFLD: nonalcoholic fatty liver disease

Table-3: Grading of sinusoidal injury

Parameter	Group-1	Group-2	Group-3
Sinusoidal dilatation	0 (absent)	2 (moderate-centrilobular involvement extending in two-thirds of the lobular surface)	1 (mild-centrilobular involvement limited to one-third of the lobular surface)

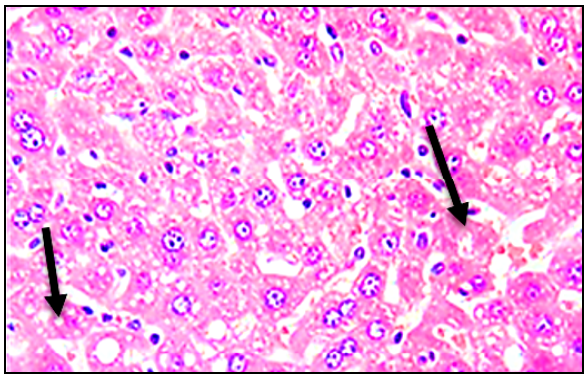

Figure-1: Photomicrograph of histological changes in hepatic tissue in Group-2

Sinusoidal dilation with congestion and fatty changes with disruption of hepatic architecture in liver tissue (×400)

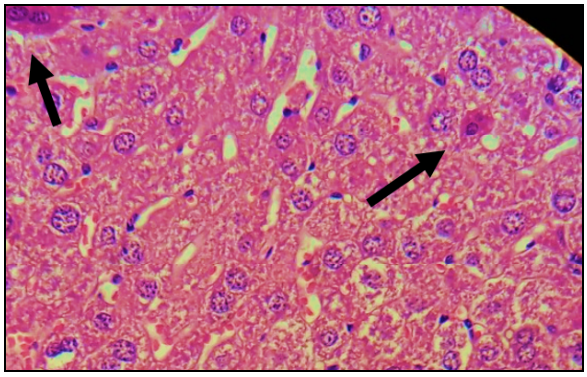

Figure-2: Photomicrograph of histological changes in hepatic tissue in Group-3

Multinucleated giant cells as a sign of regenerative changes in liver tissue (×400)

DISCUSSION

Recently there has been a revival of interest in chemoprotective and antioxidant activities of plant extracts used in traditional medicine.[22] A study was conducted by Nafees *et al*[23] to demonstrate the protective effects of rutin, a naturally occurring bioflavonoid derived from buck wheat, against the hepatotoxicity induced by CP. They concluded that the hepatoprotective effects of rutin were associated with up-regulation of antioxidant enzyme activities that counter the increased amounts of oxidants (ROS) generated by CP and resulted in down regulation of serum toxicity markers.

In the present study, sea buckthorn seed oil (SBO) was evaluated for the antioxidant activity against the CP-induced oxidative stress, specifically hepatotoxicity, in male BALB/c mice. CP administration causes oxidative injury to the liver tissue and leads to the leakage of the marker enzymes such as AST, ALT, ALP and bilirubin in serum because of its metabolite acrolein, which damages cell membrane and inhibits transport proteins. The rise in ALP was around 3-times more than the upper limit of normal/control value and ALT/ALP ratio was <2, which is suggestive of cholestatic damage.[24] Co-administration of SBO with CP significantly reduced/haulted the rise in serum AST, ALT, ALP, and bilirubin levels found in CP group, indicating a protective effect.

Our findings are supported by previously published work. Gupta *et al*[25] reported that sea buckthorn (SBT) extract (500 mg/Kg for 10 days) was hepatoprotective in arsenic toxicity (induced with 25 ppm in drinking water for 3 months) with significant reversal of AST, ALT and ALP, although not to the control levels. In their study, as well as ours, the reversal of hepatic injury markers was not complete. As the duration of SBT administration is same, it suggests that full benefits of SBT are not realized in short term use.

A 2011 study[12] investigating the effect of SBT administration on CCl_4 induced oxidative stress, reported CCl_4 induced elevation of serum AST (171%), ALT (215.3%) and bilirubin (232.6%). Pre-treatment by oral administration of SBT extract (25–75 mg/Kg body weight) for 7 days significantly protected from CCl_4 induced elevation in serum ALT, AST and bilirubin.[12] When compared to our study, there was a greater elevation (more than twice) in the serum levels of liver enzymes upon induction of hepatic injury. The reason for this may be the toxin itself. CCl_4 toxicity is a research model used to produce acute hepatocellular injury, while CP in therapeutic doses is reported to cause more sinusoidal and ductal damage and relatively less hepatocellular damage.[12]

Hepatoprotective effect of SBT has also been studied on chromium-induced oxidative stress in Sprague-Dawley rats by Geetha *et al*[26]. They reported

that potassium dichromate treatment for 30 days significantly increased serum AST and ALT levels. Co-administration of SBT leaf extract in 100 and 250 mg/Kg of body weight doses for the same duration preserved the serum ALT and AST levels at control values. They concluded that the leaf extract of SBT protected the rats from the chromium induced oxidative injury.[26] The fact that in their study the liver enzymes remained unchanged in the face of oxidative stress when given SBT supplementation, suggests that a higher dose than that used in the current study, given for a longer duration, may curtail oxidative stress damage.

The mechanism of injury is probably due to damage to sinusoidal endothelium of liver because of toxic metabolites of CP, with generation of ROS and depletion of glutathione from sinusoidal endothelial cells, causing their necrosis, obstruction and obliteration of hepatic veins.[5] Initial injury to the sinusoidal endothelial cells leads to extravasation of RBCs into the sub-endothelial space of Disse, emboli formation and blocking of venous out-flow, resulting in hepatic congestion and sinusoidal dilatation. At later stages, fibrosis occurs which causes destruction of central venules, leading to hepatic sinusoidal obstructive syndrome (SOS) or veno-occlusive disease (VOD).[27]

Intensive chemotherapy, often with cyclophosphamide, is closely associated with endothelial cell injury leading to rapidly progressive, occlusive disease of small hepatic venules.[28] When high doses of CP are used, as chemotherapy in cancer or as myeloablation therapy in combination with total body irradiation, in preparation for bone marrow haemopoietic cell transplantation, acute liver failure and death can occur.[5] The observation of sinusoidal endothelial damage with sinusoidal congestion in Group-2 (grade-2) and concurrent steatohepatitis in our study correlates with the observations made in the clinical setting and earlier animal studies.

Khan *et al*[5] looked at the effect of CP on the micro-anatomy of liver at various doses. They reported that CP-induced histological changes like central vein congestion and fatty infiltration in the liver were dose related and manifested earlier when larger doses were used and later when low doses were given for longer durations. Other studies looking at the effect of antioxidant supplement on toxin-induced steatohepatitis and sinusoidal injury support the lessening of damage seen in our study.

A study by Sheweita *et al*[7] aimed to investigate the role of essential oils extracts (fennel, cumin and clove) as a source of natural antioxidants in the mitigation of CP-induced hepatotoxicity. They treated male mice with CP (2.5 mg/Kg body weight/day) for 28 days, and on histological examination of livers, inflammation in portal tract and hepatocytes, swelling and dilation in sinusoidal space, as well as hyperplasia in Kupffer cells were observed.[7] They found that co-administration of the essential oils with CP lessened the changes caused by CP to a significant extent. They observed that quercetin present in essential oils was found to act as scavenger of ROS/RNS radicals, inhibiting the oxidative DNA damage induced by H_2O_2 and preventing free radical-mediated cytotoxicity. This observation supports the protective effect of SBO seen in our study, as SBO is documented[29] to be rich in flavonols like quercetin and isorahmnetin.

Deleve *et al*[30] investigated the cellular mechanism of action of CP in rodent liver along with the consequences of supplementation with methionine and serine on CP toxicity. They observed that CP caused hepatic VOD, and the damage was inflicted indirectly because CP requires activation by the hepatocytes with generation of acrolein. In co-culture of hepatocytes and endothelial cells, sinusoidal endothelial cells (SEC) were significantly more susceptible to CP toxicity with acrolein-induced depletion of cellular glutathione (GSH) preceding the cell death. They also probed the consequences of supplementation with serine and methionine on CP toxicity in co-culture. Serine is precursor of methionine, which is precursor for cysteine, a thiol-containing, semi-essential proteinogenic amino acid in hepatocyte GSH. They reported that hepatocytes in supplemented medium when exposed to CP maintained GSH levels at ~80% of the control level and both hepatocytes and SEC were protected from CP toxicity. They concluded that the protection afforded by the serine/methionine supplement was due to increase in hepatocyte GSH level.[30] This observation supports our results of lesser sinusoidal damage (grade-1) seen in SBO supplemented group compared to grade-2 in CP group, with high levels of antioxidants (vitamins A, C, E, and K, carotenoids and trace elements copper, zinc and selenium) in SBO probably replenishing the endogenous cellular GSH.

CONCLUSION

Our study demonstrates the protective effect of SBO against CP-induced liver damage, which reflects its antioxidant properties. This evidence can be used to counter the hepatotoxic potential of CP, especially in myeloablative regimes of cancer therapy.

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ORIGINAL ARTICLE

EFFICACY OF ORAL AZITHROMYCIN AND ORAL DOXYCYCLINE IN PATIENTS WITH ACNE VULGARIS

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Background: Acne vulgaris is a long-term skin disease characterized by blackheads or whiteheads, pimples, oily skin, and possible scarring. Systemic antibiotics have long been used as a treatment option in patients with acne. The aim of this study was to compare the efficacy of oral doxycycline *vs* azithromycin in patients with acne vulgaris. **Method:** It was a randomized controlled trial conducted in the Department of Dermatology, Lady Reading Hospital, Peshawar from 1st February to 30th July 2021. A total of 60 patients were enrolled in the study. Patients were randomized into 2 groups with Group A receiving azithromycin and Group B receiving doxycycline. Patients' number of lesions was counted and followed for 4 months. A reduction in 80% of lesions was considered effective. **Results:** The mean age of the patients was 21.24±3.84 years. There were 16 female and 14 male patients in group A and 17 female and 13 male patients in group B. The majority of patients in both groups were found to have responded to the treatment showing good efficacy. **Conclusion:** Oral doxycycline and azithromycin were highly effective in patients with acne vulgaris. Efficacy of both drugs was similar to each other.

Keywords: Acne vulgaris, Azithromycin, Doxycycline, Efficacy

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INTRODUCTION

Acne vulgaris (AV) is a common dermatological disorder resulting from clogging of the hair follicles with dead keratinocytes and sebum from the sebaceous glands.[1] It is characterized by blackheads or whiteheads, pimples, oily skin, and possible scarring.[2,3] Face, upper chest, back and those areas of the skin that are rich in sebaceous glands are primarily affected. Since it commonly involves the face in the young population, it is usually associated with low self-esteem, depression, and in extreme cases, thoughts of suicide.[4]

Acne vulgaris is a global health problem, affecting almost 630 million people worldwide in 2015 alone.[5,6] In the United States, it is considered to affect 80% of the population any time in their life.[7]

The most common age of onset is between 13 and 20 years, and it is estimated that 80–90% of the patients affected in the West are adolescents.[8,9] The disease continues to affect nearly half of these patients even in adult life in their twenties, with a smaller proportion of patients continuing to have the disease even in the middle ages.[3]

Acne vulgaris presents as whitehead or blackhead comedones, papules, pustules and nodules primarily affecting the face. Comedone is differentiated from the rest of the lesions based on the absence of inflammation in their lesions. Pustules are raised lesions that are characterized by the presence of pus, while papules do not have pus in their lesions. In addition to these lesions, patients may complain of pain, tenderness, and redness, and may develop scars on their face.[10,11]

Numerous pharmacological and non-pharmacological measures have been described in the literature that helps manage AV. Reducing daily sugar intake may reduce the overall burden of the disease.[12] Local application of azelaic acid, benzoyl peroxide, salicylic acid and retinoids are reported to have a significant effect in improving AV. Oral treatments which include antibiotics and retinoids also have proven efficacy in multiple trials in AV especially in moderate to severe inflammatory AV.[13] Among the systemic antibiotics, the commonly prescribed medications are tetracyclines including doxycycline and minocycline.[14] Macrolides, especially oral azithromycin are also used.[15]

Multiple studies have been performed worldwide to compare the efficacy of azithromycin with oral doxycycline in the treatment of acne. In one study conducted in Pakistan, the efficacy of azithromycin was found to be 22.8% compared to 55.4% in patients receiving oral doxycycline.[16] Multiple dosing regimens have been applied to treat acne vulgaris in different areas. The aim of this study was to compare the efficacy of daily oral doxycycline 100 mg with oral azithromycin 250 mg once a day for 120 days in patients with active acne vulgaris.

MATERIAL AND METHODS

It was a randomized control trial conducted in the Department of Dermatology, Lady Reading Hospital, Peshawar from 1st February to 30th July 2021 after approval from the Ethical Review Board vide Ref No. 50/LRH/MTI).

The sample size was calculated using WHO software with the following assumptions: significance level=5%, statistical power=80%, anticipated population treated with oral doxycycline=55.4%[16], and anticipated population treated with oral azithromycin=22.8%.[16] Patients' data were collected after informed consent from all patients with acne vulgaris presenting in the Outpatient Department of Dermatology Lady Reading Hospital Peshawar. Non-probability consecutive sampling technique was used and a total of 60 patients (30 each in Group A and Group B) were enrolled. All patients with acne vulgaris, aged 13–40 years, and both genders were included. Patients previously treated with oral doxycycline, oral retinoids, steroids, or taking antibiotics for other purposes, patients with folliculitis of the face, with acne fulminans, and sebaceous hyperplasia were excluded.

Patients were considered to have acne vulgaris if they had open and/or closed comedones, papules, pustules, nodules and cysts on their faces. Doxycycline was given orally as a 100 mg once daily medication for 4 months while azithromycin was also given orally for 4 months in a dose of 250 mg once daily. The efficacy of the drug was calculated by counting the number of lesions. An 80% reduction in the number of lesions was considered to be effective.

A detailed history was taken from the patients or their attendants and a complete physical examination was performed including examination of the face, and the number of lesions was counted. The patients were divided into 2 groups using blocked randomization. Oral doxycycline 100 mg once daily was given to patients in Group A, and Group B patients were put on oral azithromycin 250 mg once daily. All patients were followed for 4 months for efficacy, with the number of lesions on the face being counted at the end of each month. Data was recorded on a proforma.

Data was analysed using SPSS-20. Quantitative variables like age were described as Mean±SD. Categorical variables like gender and efficacy were described as frequencies and percentages. Stratified analyses were done on the basis of age and gender. Chi-square test was done for differences in groups, and $p \leq 0.05$ was taken as significant.

RESULTS

This study enrolled 60 patients presenting to Department of Dermatology, Lady Reading Hospital, Peshawar. Thirty-three (55%) of these patients were female, and 27 (45%) were male. The mean age of the study cohort was 21.24±3.84 years (Range: 14–31 years). There were 16 female and 14 male patients in group A and 17 female and 13 male patients in group B. (Table-1).

Out of 30 patients in each group, 25 patients in Group A, and 26 patients in Group B responded to the treatment showing good efficacy. (Table-2, 3).

Table-1: Age distribution of the study groups (n=60)

Group	N	Age (Years)		Mean±SD
		Minimum	Maximum	
A	30	16	30	21.43±3.03
B	30	14	31	21.40±4.42
Total	60	14	31	21.24±3.84

Table-2: Correlation of efficacy of oral azithromycin and doxycycline

Effective	Group A		Group B		*P*
	N	%	N	%	
Yes	25	83.3	26	86.7	0.001*
No	5	16.7	4	13.3	

*Significant

Table-3: Frequency of % reduction in lesions

% Reduction	Group A		Group B	
	N	%	N	%
≥80	25	83.3	26	86.7
50–79	1	3.3	0	0
20–49	4	13.2	4	13.2

DISCUSSION

Acne vulgaris is a chronic dermatological disease with a propensity to affect the face. The disease is so common that almost every individual develops at least some comedones and/or papules on their face in their life.[17,18] Since it has the tendency to cause facial scars which may have a significant cosmetic and psychological impact on the patient, it is very essential to have effective timely treatment. Oral retinoids and antibiotics have been observed to be effective in managing AV.[19]

Our study found that the efficacy of azithromycin is similar to that of doxycycline in the management of acne vulgaris. It was observed that contrary to popular belief, the disease is equally common in males, and the results of the drugs are not affected by gender.

Maleszka *et al*[20] compared the efficacy of 2 drugs in 2011. They administered azithromycin 500 mg for 3 day for the 1st week followed by 500 m weekly to one group and 100 mg doxycycline to the other group. They reported similar efficacy with azithromycin and doxycycline treatment in both groups which is inline with our study.

Our study results are in agreement with Parsad D *et al*[21] who observed that oral doxycycline daily was as effective as oral azithromycin used only for 4 days in a month. Their dosing regimen was different from ours. Singhi MK *et al*[22] observed that oral azithromycin was superior to oral doxycycline in patients with AV. Their findings are in contrast to our results. One reason for this may be because they used higher doses of azithromycin compared to us. Moreover, they added topical erythromycin to all patients which may have some added effect in the oral azithromycin group compared to the doxycycline group. The results of daily oral doxycycline and azithromycin were superior to the

results shown in above mentioned studies which might be due to the prolonged duration and once daily dosing regimen compared to other studies.

Our findings were similar to Gruber *et al*[23] who reported that azithromycin was not inferior to tetracycline. They studied minocycline instead of doxycycline. Their dosing strategy was also different. Instead of following the daily dose routine, They gave azithromycin in cycles, with 10 days drug free intervals. Such regimens need to be evaluated further for efficacy.

LIMITATIONS

The sample was small and side-effects profiles of the prescribed drugs were not taken into account. Though these drugs are generally safe with little side-effects, it is recommended to keep track of any side-effects.

CONCLUSION

Both oral doxycycline and azithromycin are effective in the treatment of acne vulgaris, and have similar efficacy.

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ORIGINAL ARTICLE

JOB-RELATED DEPRESSION AMONG ICU AND NON-ICU HEALTHCARE WORKERS IN A TERTIARY CARE HOSPITAL

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Background: A higher levels of job stress among healthcare staff showing an inverse relationship between job stress and job performance among healthcare workers has been reported. The aim of current study was to compare the frequency of depression among intensive and non-intensive unit healthcare workers in a tertiary care hospital in Peshawar. **Methods:** This was a cross-sectional study conducted in Hayatabad Medical Complex, Peshawar. A purposive questionnaire was designed to collect data from hospital staff working at intensive care unit (ICU) and non-ICU specialties while another questionnaire was used based on 'Beck's Depression Inventory' for determination of depression levels. Sample size was calculated using WHO sample size calculator. The data was analysed on SPSS-21. Shift duration and unit of working were analysed through univariate and multivariate logistic regression for depression status. Odds ratio was calculated with 95% confidence interval by applying Chi-square test. **Results:** The study included 214 participants. Average age of the included study participants was found to be 28.37±5.94 years. A significant difference was found in healthcare workers having shift duration more than 8 hours and in those working in ICU than non-ICU specialities (p<0.05). On multivariate logistic regression, shift duration of more than 8 hours (OR: 2.58, p=0.03) and being working in ICU (OR: 13.89, p<0.001) were significantly associated with depression. **Conclusion:** Healthcare workers working at ICU and with shift duration of more than 8 hours are more prone to be mentally depressed.

Keywords: Healthcare, ICU, non-ICU, Depression, Peshawar

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INTRODUCTION

Occupational stress is an emerging global health issue and is currently reported as the 2nd most common occupational disorder in most parts of the world.[1] The WHO labels major depression as the major cause of disability. Depression is a chronic illness that has conspicuous effects in terms of quality-adjusted life year and disability-adjusted life year.[2] It causes 30% of psychiatric disabilities in women while 12.6% in men.[3] Doctors and nurses are the two major victims of occupational depression.[4,5] The globalization and emergence of the economies led the population toward a higher depression.[6]

Researchers still are trying to quantify depression-related variables in different sectors in business. Unfortunately, the health sector is still unable to receive attention from researchers.[7] Nurses and doctors are at a high depression risk edge.[8] Most of the research related to the healthcare sector entertained the attitudes and behaviours of doctors and nurses related to patients.[9] The most prevalent risk factor for depression is the number of working hours. Some researchers determined that in professional work environment, working hours can be related with depression while some studies contradict any association between working hours and work related depression.[10] In developing countries, it is worthwhile considering working hours in relation to depression. The study in Taiwan pin-points that working hours were strongly associated with depression amongst nurses and doctors.[11]

The staff of the healthcare unit is sensitive to many other stressors like the upholding of miraculous professional behaviour, a very high degree of patient care, prevalence of special friendly relationships with patients, clinical audits and a zero error working attitude.[12] Many researcher had limited their study towards the less experienced and general medical doctors and nurses while undermining the rest.[13] Therefore, such studies have less utility for the policy makers for physicians. Social support acts as a better treatment for depression.[14] It has not yet been proved whether the social factor is significant for the physicians who work in a continually tedious and high emotional environment.[15]

A study in intensive healthcare units of hospitals in the UK suggested that one in every five doctor needs special psychiatric distress treatment. While the result of the same study about nurses showed that every sixth nurse is a victim of depression.[16] In the developing countries the high skills and experience required amongst workers in intensive care units makes it impractical for the management to rotate the medical staff.[17] The highly demanding service of ICUs makes it near impossible to suggest free vacations for doctors and nurses. Such dilemmas present in underdeveloped countries are examples of triggers for depression in ICU medical staff in underdeveloped countries as compared to the developed ICU medical staff.[18] The level of

depression has been linked with factors like fatigue and work efficiency.[19] The cardinal causes behind such serious levels of depression are the highly demanding work environment where the death ratio is at a peak in daily routines.[20] The increased population rate in developing countries makes serious challenges for management in the healthcare sector. To overcome these challenges one of the primary suggestions is to create friendly and efficient work environment. Nurses and doctors in intensive care units are trained to face depression related challenges in the UK hospitals.[21,22] Occupational stress and its role in developing psychological illnesses among healthcare workers is a neglected area in developing regions, including Pakistan.

There is an immediate need to identify the factors responsible for this alarming rise in occupational health related problems among healthcare providers particularly related to those working in intensive healthcare units. The purpose of the current study was to compare the frequency of depression among intensive and non-intensive unit healthcare workers in a tertiary care hospital in Peshawar.

METHODOLOGY

This cross-sectional study was conducted in Hayatabad Medical Complex, Peshawar, from 1st September 2021 to 30th June 2022. Non-probability purposive sampling was used. Sample size was calculated by using WHO sample size calculator. Confidence level (1-α%) was taken as 95%, with precision (d) of 0.05 and a power of 80%. Prevalence of depression in the population (P1), working in non-intensive care unit areas was taken as 39% and for population (P2) working in intensive healthcare unit the prevalence is assumed to be 60%.[23] The calculated sample size was 214.

Only healthcare workers working as Physician or Registered Nurse in any of two stratified in-patient healthcare areas were included in the study. Healthcare workers of either gender working in the same in-patient healthcare unit for past 3 months were included in the study while any healthcare worker with a recent history of divorce (in last 3 months), death of close relative, separation, injury or acute illness, pregnancy and change in living conditions were excluded from the study.

Selected participants were approached, preferably during break hours or at the end of a shift duty. The eligible participants were invited to participate in the study and an informed consent was taken. A questionnaire was designed to collect information regarding socio-demographic and work-related information including work hours, shift of work and other personal information regarding substance use and family medical history while another questionnaire based on 'Beck Depression Inventory', a well-known tool for screening and measurement of levels of depression, was used to study work-related depression among healthcare workers of intensive and non-intensive healthcare units. Individual having a score of 17 or more on Beck Depression Inventory scale was considered as 'depressed'.

The collected data were analysed on SPSS-21. Frequencies were calculated for proportion of depression among intensive care unit and non-intensive care unit health workers and then statistically compared. Shift duration and unit of working were analysed through univariate and multivariate logistic regression for depression status. Odds ratio was calculated with 95% confidence-interval by applying Chi-square test.

RESULTS

Data was collected from 214 participants, i.e., 139 (65%) participants were female while 75 (35%) participants were male. Average age of all the included study participants was found to be 28.37±5.94 years. Majority of the participants belonged to Muslim ethnicity (87.4%). Among the participants, 149 (69.6%) were single while 55 (25%) were married, 2 (0.9%) were divorced and 8 (3.7%) were engaged. 45 (21%) participants had more than 2 children with maximum number of 3. About 70% of the study participants had completed their graduation while around 25% had completed intermediate education. Experience in the same unit was reported to be 5.25±4.82 years. Duration of shift ranged from 5–12 hours with an average of 8.11±1.47. Around 33% of participants had spent more than 5 years in their respective unit. Around 31% of the participants had shift durations of more than 8 hours.

The ratio of doctors to nurses within the study participants was almost equal. Only 2% of the study participants had co-morbid conditions of hypertension. The number of participants from ICU and non-ICU units was almost the same.

The history of depression was reported by 14% of the study participants. On beck's depression inventory, about 64% of all the participants were found to have some form of depression. Average score on Beck's Depression Inventory scale for all the participants was found to be 22.17±10.10. Among all the categories of depression, moderate depression and severe depression were common, i.e., 31% and 19% respectively. Stratification of depression with respect to socio-demographic variables is shown in Table-1.

On Univariate logistic regression for depression status, only shift duration and unit was found to be significantly associated with the outcome variable (Table-2).

Similarly, on multivariate logistic regression, Shift duration of more than 8 hours was (OR: 2.58, p=0.03) and being working in ICU (OR: 13.89, p<0.001) was significantly associated with depression (Table-3).

Table-1: Demographics data and comparison

Variable	Depression Status		*p*
	Yes	No	
Gender			
Male	45	93	0.314
Female	30	46	
Age			
<30 yrs	99	56	0.761
>30 yrs	39	20	
Religion			
Muslim	121	66	0.860
Non-Muslim	17	10	
Marital Status			
Single	102	47	1.00
Engaged	5	3	
Married	31	24	
Divorced	0	2	
No. of children			
0–1	110	28	0.721
2 or more	28	17	
Years of experience			
0–5 yrs	89	53	0.437
> 5 yrs	49	23	
Shift Duration			
Up to 8 hrs	104	43	0.05
>8 hrs	34	33	
Designation			
Doctors	66	43	0.22
Others	72	33	
Unit			
ICU	92	12	<0.05
Non-ICU	46	64	

Table-2: Univariate logistic regression

Variables	Odds Ratio	Confidence Interval (95%)	*p*
Shift Duration (Up to 8 hrs *vs* More than 8 hrs)	2.347	1.293-4.262	0.005
Working Unit (ICU *vs* Non-ICU)	10.667	5.239-21.716	0.000

Table-3: Multivariate logistic regression

Variables	Odds Ratio	Confidence Interval (95%)	*p*
Shift Duration (Up to 8 hrs *vs* More than 8 hrs)	2.583	1.096-6.086	0.03
Working Unit (ICU *vs* Non-ICU)	13.895	6.296-30.665	0.000

DISCUSSION

The higher risk of depression among healthcare professionals is related to the psychosocial and organizational characteristics of their job including long and irregularly timed work shifts.[24,25] Literature reports that healthcare workers operating in intensive care units where work is very exhaustive due to constantly dealing with critically ill patients, leads to a higher burden of psychosocial stress and psychological morbidity.[26] In Pakistan, occupational stress is due to organizational factors; moreover, a lack of proper training and illiteracy have their own impacts on the coping mechanisms and capacities of the working population. Studies conducted in the healthcare settings of Pakistan, report higher levels of job stress among healthcare staff showing an inverse relationship between job stress and job performance among healthcare workers. A study conducted by the Family Physicians of Karachi, reports the prevalence of depression as high as 39%.[27]

In current study, a very high frequency of depression amongst healthcare personnel was observed. This finding is important from two perspectives. First, the mental health of healthcare personnel is severely affected negatively. It also acts as a promoting factor for other non-communicable diseases such as cardiovascular diseases, diabetes etc. It can lead to further issues such as job dis-satisfaction and job rotations which in turn further create more issues in the healthcare workforce from a healthcare system perspective. It also leads to anxiety and dis-satisfaction of patients and patient attendants. Second, it can lead to increasing numbers of medical errors, thus compromising patient care.[28]

In this study, we found that the ICU had a significant association with depression as compared to non-ICU units. This finding is similar to other studies done previously.[29,30] There are several reasons for this finding. The overall environment of the ICU is usually very negative and not optimistic, generally promoting depressed moods. Second, the patients in the ICU are very much critical. Patients need intensive care and even minor mistakes may cost a patient their life. This puts extra responsibility on the shoulders of ICU healthcare personnel. Studies have also shown that attendants of patients are also depressed and anxious[31], which also leads to staff in ICU becoming depressed.

Besides this, we also found that prolonged working hours, specifically beyond 8 hours had a significant association with depression. This finding is in line with other related studies which shows a strong biological plausibility for our finding. Prolonged working hours leads to reduced sleep and exhaustion and thereby leading to depression.[32] The odds of having depression are 2.58 times higher in those healthcare workers whose shift duration is more than 8 hours as compared to those whose shift duration is up to 8 hours.

The odds of having depression are 13.98 times higher in those healthcare workers who work in ICU as compared to those who work in units other than ICU. In our study, we did not find any relationship between depression and gender, or age group, designation, ethnicity, education status etc. These findings were somewhat non-consistent in the literature as well. There are several reasons for these findings. We selected a very tightly matched target population through rigorous inclusion and exclusion criteria which helped decrease the differences in many socio-demographic characteristics of the population leading to no significant association with depression.

CONCLUSION

Healthcare workers working at ICU and shift duration of more than 8 hours are more prone to be mentally depressed.

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ORIGINAL ARTICLE

FREQUENCY OF HEPATITIS C SEROCONVERSION IN CHRONIC KIDNEY DISEASE PATIENTS ON HAEMODIALYSIS

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Background: Haemodialysis increases Hepatitis-C seroconversion risk adversely affecting Chronic Kidney Disease (CKD) prognosis. This study aimed to determine the frequency of Hepatitis C seroconversion in chronic kidney disease patients on haemodialysis. **Method:** This cross-sectional study was conducted at Dialysis Unit of Ayub Teaching Hospital, Abbottabad from March 2016 to January 2019. A pre-designed proforma was used for data collection. A sample of 121 patients of dialysis dependent CKD was enrolled for the study through non-probability consecutive sampling. The Glomerular Filtration Rate (GFR) was re-estimated from serum creatinine to confirm the diagnosis using Modification of Diet in Renal Disease (MDRD) study equation. Patients who were Hepatitis C virus (HCV) negative at the start of dialysis, and became positive 3 months post-dialysis were considered having undergone seroconversion. Data were analysed using SPSS-16. **Results:** Out of 121 participants, 80 (66.1%) were male and 41 (33.8%) were female. The mean age of the patients was 51.0±6.22 years with range from 41 to 61 years. Hepatitis C seropositivity was recorded in 40 (33.06%) of the study participants during the study period. Statistically significant (p<0.05) associations were observed between HCV seropositivity and age and gender of the patients. **Conclusion:** Hepatitis C infection is a common complication of haemodialysis. Rigorous screening of patients for Hepatitis C and separate machines for Hepatitis C patients can lead to a decrease in the burden of this disease.

Keywords: Hepatitis C, Chronic Kidney Disease, Haemodialysis, Seroconversion, Renal Replacement Therapy

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INTRODUCTION

Chronic kidney disease (CKD) is defined as an irreversible, significant and long-standing loss of kidney function.[1] Chronic kidney disease is a growing public health problem. Its prevalence is estimated to be 8–16% globally. The complications consist of increased all-cause and cardiovascular mortality, renal disease progression, anaemia, cognitive decline, mineral and bone disorders and pathological fractures.[2] Patients with CKD are at a higher risk of death from cardiovascular disease than the general population.[3] The proper treatment of CKD patients is to have renal transplant but due to lack of donors, issues of immunosuppression, and cost associated with renal transplant leaves haemodialysis a more acceptable modality of treatment.

Hepatitis C is a liver disease caused by Hepatitis C virus which can cause both acute and chronic liver infection. The infection ranges in severity from a mild illness lasting a few weeks (acute hepatitis) to a serious lifelong illness (chronic hepatitis) leading to cirrhosis and hepatocellular cancer.[4] Hepatitis C infection is a public health problem, with an estimated worldwide prevalence of 3%, affecting approximately 180 million carriers. About 4 million people get infected annually. The prevalence of hepatitis C infection in dialysis patients is in general much higher than general population. Studies conducted in dialysis centres from different countries show that prevalence of Hepatitis C ranges from 1% to 84.6% and this is of particular concern because chronic Hepatitis C infection accounts for significant morbidity and mortality in patients undergoing hemodialysis.[5]

Currently, in Pakistan, around 10 million people are suffering from hepatitis C which accounts for 6% of total population of Pakistan. A higher prevalence of Hepatitis C antibodies (38% weighted average) has been attributed to patients undergoing chronic haemodialysis in Pakistan.[6] Numerous risk factors have been identified for Hepatitis C infection among patients undergoing haemodialysis and these include number of prior blood transfusions, period of chronic kidney disease, type and duration of dialysis, concurrent prevalence of Hepatitis C in the dialysis unit[7] and adherence to universal infection control practices.[8–10]

The aim of this study was to determine the causes associated with high frequency of hepatitis C infection among patients undergoing haemodialysis. This study will generate local data which will help in proposing suggestions to reduce the associated risk of transmission of virus, thus helping in alleviation of morbidity and mortality associated with concurrent liver disease due to Hepatitis C infection in CKD patients.

METHODOLOGY

This cross-sectional study was conducted at Dialysis Unit of Ayub Teaching Hospital Abbottabad, from Mar 2016 to Jan 2019. The sample size was calculated for population proportion estimation with the specified absolute precision with World Health Organization software for sample size determination in health studies. Keeping the prevalence of Hepatitis C in dialysis patients from Khyber Pakhtunkhwa to be 28%[11], an absolute precision of 8% and confidence level of 95%, the sample size was estimated to be 121. The sampling technique was non-probability consecutive sampling.

Patients aged 18–60 years with chronic kidney disease who were receiving haemodialysis from Ayub Teaching Hospital Abbottabad and those who tested positive for HCV after 3 months of dialysis were included in the study irrespective of gender discrimination. Patients who tested positive for HCV before the start of haemodialysis were excluded from the study. A patient was declared having Hepatitis C infection if serum/plasma of the subjects tested positive for anti-hepatitis C antibodies (IgG, IgM and IgA) by HCV-Ab rapid test cassette.

After obtaining approval from the Hospital Ethical Committee, the study commenced with obtaining an informed consent from patients followed by data collection. The information was recorded on a pre-designed proforma. After recording the biographical data, presence of CKD was confirmed by checking previous medical records. The GFR was re-estimated from serum creatinine by the Modification of Diet in Renal Disease (MDRD) study by the equation given below:

$$\text{GFR (mL/min/1.73 m}^2\text{)} = 175\ (\text{S. Cr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ for females}) (1.212 \text{ for blacks})$$

Hepatitis C status of patients was confirmed using HCV-Ab rapid test cassette in use at the Ayub Teaching Hospital microbiology laboratory for screening of CKD patients before starting haemodialysis. A detailed history regarding the risk factors for transmission of Hepatitis C was taken from the patients. Patients were specifically asked about the dis-infective measures taken by the staff during hemodiaysis.[12]

The data was analysed using SPSS-16. Quantitative variables such as age, GFR, number of blood transfusions and duration of haemodialysis were described as Mean±SD. Qualitative variables such as gender, HCV seropositivity and adoption of dis-infective measures were described as percentages and frequencies. Data were stratified by age, gender, number of blood transfusions, duration of dialysis and adoption of dis-infective measures with respect to outcome variable. Chi-square test was applied to see the significance of difference by age, gender, number of blood transfusions, duration of dialysis and dis-infective measures with respect to outcome variable, i.e., seropositivity for Hepatitis C virus.

RESULTS

This cross-sectional study enrolled 121 patients (80, 66.12% males; 41, 33.88% females) with dialysis-dependent chronic kidney disease. The mean age of the patients was 51.02±6.22 years ranging from 41 to 61 years. The descriptive statistics of the study population are given in Table-1.

Hepatitis C seropositivity was recorded in 40 (33.06%) of study participants during the study period as given in Table-2.

When the outcome variable, i.e., HCV seropositivity was cross-tabulated with gender, age, adoption of dis-infective measures, number of blood transfusions and duration of dialysis dependent chronic kidney disease, statistically significant associations were observed between HCV seropositivity and age and gender of study participants ($p<0.05$) (Table-3).

Table-1: Descriptive statistics of study population

Variable	Mean±SD	Min	Max
Age of patients (Years)	51.02±6.22	41	61
Blood transfusions	5.57±1.75	3	8
Duration of dialysis dependent CKD (Months)	6.48±1.78	4	9
Glomerular Filtration Rate (mL/min)	36.87±7.02	25	48

Table-2: HCV seropositivity in study population

HCV Seropositivity	Frequency	Percentage
Yes	40	33.06
No	81	66.94

Table-3: Cross-tabulation of HCV seropositivity with study parameters ($p<0.05$)

HCV Seropositivity	Gender of patients			Age of patients (Yrs)			Adoption of dis-infective measures			Number of blood transfusions			Duration of Dialysis dependent CKD (months)		
	Male	Female	Total	≤50	≥51	Total	Yes	No	Total	Up to 5	>5	Total	Up to 6	>6	Total
Yes	33	7	40	27	13	40	38	2	40	16	24	40	25	15	40
No	47	34	81	39	42	81	75	6	81	46	35	81	36	45	81

DISCUSSION

The prevalence of Hepatitis C infection among patients with dialysis dependent kidney disease is much higher than general population. A variable prevalence of Hepatitis C seropositivity has been reported, reaching up as high as 84.6%.[5]

The frequency of Hepatitis C seropositivity in this study was quite high. Recently a considerably high prevalence of Hepatitis C in patients with chronic kidney disease who are not on renal replacement therapy has been reported by Shafi, *et al*[13]. They reported that of all patients with CKD, 49 (27.2%) had Hepatitis C test positive by ELISA.

Our results are in agreement with a study from India[14] which reported that 33.5% of dialysis dependent patient population was positive for Hepatitis C. The authors conducted a retrospective hospital record based study and included records of dialysis dependent chronic kidney disease patients for a period of two years. They observed that out of the total 262 patients who underwent haemodialysis for chronic kidney disease during this period, 88 (33.5%) were found to be positive for HCV infection. Out of these 88 HCV positive patients, 59 were males and 29 were females. The highest prevalence was found in the age group of 41–60 years (43.18%) and lowest prevalence was observed in the age group of <20 years (2.27%) and >80 years (1.17%).[14] Our results are also in concordance with the study done by Stuyver *et al*[15] in which 25 (36.7%) out of 68 haemodialysis patients seroconverted to HCV. Our results are also in agreement with Khalaf *et al*[16].

A study by Ashuntantang *et al*[17] reported a 25% seroconversion rate for Hepatitis C in dialysis dependent chronic kidney disease patients. These results are slightly lower than the results of our study probably because of small sample size of 40 patients in that study.[17] A study by Bhaumik and Debnath reported frequency of new Hepatitis C infections in dialysis dependent chronic kidney disease patients as 10.9%.[18] They reported male predominance among their study population, and 100% history of blood transfusion in newly acquired Hepatitis B and C infections.

A study from Vietnam identified increased blood transfusions and frequency of haemodialysis among a number of risk factors for acquiring Hepatitis C.[19] That study reported 6% seroprevalence of Hepatitis C. Ismail *et al*[20] found HCV seroconversion rate of as 48.9 %. That seroconversion rate is somewhat higher as compared to our study probably because of a slightly higher number of patients enrolled in that study.

Further large-scale studies are required to generate more data on the frequency of hepatitis C seroconversion in chronic kidney disease patients during haemodialysis.

LIMITATIONS

This was a single centred small study and its results cannot be generalized. Number of haemodialysis sessions completed before Hepatitis C seroconversion was not recorded. Infection with HBV either alone or concomitant with Hepatitis C was not recorded. Other possible sources of Hepatitis C transmission were not studied.

CONCLUSION

Dialysis dependent chronic kidney disease patients are at a higher risk of acquiring Hepatitis C infection which is a preventable cause of morbidity and mortality. Rigorous implementation of Hepatitis C screening before starting haemodialysis, and separate haemodialysis of Hepatitis C positive dialysis dependent patients can effectively reduce the rates of Hepatitis C seroconversion.

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ORIGINAL ARTICLE

MYOCARDIAL INFARCTION AMONG HOSPITALIZED PATIENTS WITH COMMUNITY ACQUIRED PNEUMONIA: A RETROSPECTIVE OBSERVATIONAL STUDY

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Background: Pneumonia combined with influenza is a leading cause of death worldwide. There is an increased risk of acute myocardial infarction in patients with respiratory tract infection during hospitalization and after discharge. This study aimed to determine the frequency of myocardial infarction (MI) in hospitalized patients with community acquired pneumonia (CAP). **Methods:** A retrospective observational study was conducted at a tertiary care hospital in Islamabad, Pakistan. A total of 209 adult patients admitted from May 2018 to Dec 2020 with primary diagnosis of CAP were identified using local ICD code. Paper chart review for clinical parameters including symptoms, laboratory values, and radiological findings was performed. Severity of pneumonia was determined using CURB-65 score. Acute MI was diagnosed on elevated troponin and electrocardiogram findings. Univariate and multivariable analyses was performed for risk factors, co-morbidities, demographics, CAP severity at the time of admission, lab values and radiological findings and $p<0.05$ was considered statistically significant. **Results:** Males were 121 (58%) and females were 88 (42%) in the study. Incidence of acute MI in CAP patients was 10.52%. Only one patient experienced ST-elevation myocardial infarction (STEMI); the rest had non-ST-elevation myocardial infarction (NSTEMI). There was significant association between MI and history of ischemic heart diseases, angina, cardiomyopathy, acute kidney injury and pulmonary oedema ($p\leq0.05$). A significant increase in trend of acute MI was observed across the CURB-65 scores. **Conclusion:** The incidence of acute MI among hospitalized patients with CAP was 10.52%. Early recognition and prompt treatment will improve outcomes.

Keywords: Community-Acquired Pneumonia, Acute Myocardial Infarction, Ischemic heart diseases, CURB-65

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INTRODUCTION

Globally, pneumonia is the third leading cause of death and is associated with several complications such as pleurisy, empyema, and lung abscess.[1] The current literature has reported an increased risk of major adverse cardiovascular events, such as acute myocardial infarction (MI), in patients suffering from respiratory tract infection[2] both during hospitalization and after discharge, and this is associated with increased short-term mortality[3]. Any acute inflammatory state, including Community Acquired Pneumonia (CAP) can contribute in acute worsening in pre-existing cardiac conditions and can trigger new cardiac events.[4]

Musher *et al*[5] were the first to report this association, as 12 (7%) out of 170 had acute MI with pneumococcal pneumonia at the time of hospital admission. Acute cardiac events are more frequently reported in patients admitted to hospital with diagnosis of pneumonia and are associated with a worse prognosis.[3] Since MI and CAP can present with similar symptoms (for example shortness of breath, palpitations, and chest pain) this can potentially mask underlying acute MI.[5]

A recent meta-analysis[6] based on a global perspective of cardiovascular complications after CAP revealed the incidence of cardiovascular complications in 13.9% of patients admitted with CAP. That updated systematic review and meta-analysis, encompassing 92,188 patients, reported that cardiovascular complications such as acute coronary syndrome (ACS), new or worsening heart failure, new or worsening arrhythmias, and acute stroke are commonly seen among patients admitted with CAP. In patient with coronary artery disease (CAD), the risk of acute MI is reported to be correlated with the severity of pneumonia.[6] Scarce data is available to assess the prevalence of acute MI in patients admitted with CAP in local population of Pakistan. This study was done to determine the incidence and risk factors for acute MI among hospitalized patients with CAP in north western region of Pakistan.

METHODS AND MATERIAL

This was a cross-sectional, observational, retrospective study conducted on adult patients admitted with a diagnosis of CAP from May 2018 to Dec 2020 in a

large tertiary care centre. This study was approved by the Institutional Review Board of Shifa International Hospital, Islamabad.

All adult patients of age 18 years and old, admitted with principal diagnosis of CAP were identified using hospital discharge database code. Retrospective chart review was done independently. Patients with pertinent findings of pneumonia on chest X-Ray (presence of reticulonodular opacities or lobar consolidation) were selected for the study. Patients with history of frequent visits to healthcare facilities, who received intravenous antibiotics in last 3 months, or developed pneumonia after 48 hours of hospitalization were excluded from the study. Patients with normal X-Ray, with neutropenia (<2,000/mm^3), on chemotherapy, or who had leukaemia/lymphoma were also excluded from the study.

Demographic features (such as age, gender, co-morbid medical conditions), risk factors (history of Diabetes Mellitus (DM), Hypertension (HTN), smoking, Chronic obstructive pulmonary disease (COPD), asthma, presence of angina, CAD, prior history of percutaneous intervention (PCI) or coronary artery bypass graft (CABG), baseline ejection fraction (EF), clinical parameters (including presence of fever, cough, shortness of breath, chest pain, altered level of consciousness) and laboratory variables, presence of acute kidney injury (AKI), radiographic findings (Pleural effusion, lung collapse and/or pulmonary oedema), electrocardiogram findings (new ST-segment elevation or depression, T-wave inversions, or no new change), CURB-65 score and presence of acute MI (at the time of hospital admission or during the hospital stay in the first 3 days) were assessed.

CAP was defined as presence of cough with associated chest X-Ray findings suggestive of Pneumonia. The severity of CAP was categorized as low risk with a (CURB score of 0 or 1), intermediate risk (CURB score of 2), and high risk (CURB score ≥3).

Acute MI was defined as the development of chest pain or palpitation or dyspnoea, along with ST-elevation or depression of at least 1 mm in 2 consecutive leads on electrocardiogram (EKG), the development of pathological Q-waves or observation of new left bundle branch block (LBBB) on EKG with or without positive cardiac enzymes (CKMB >3.4 ng/ml in females and >7.2 ng/ml in males, and raised troponin-I level >15.6 ng/ml in females and >34.2 ng/ml in males), or echocardiographic evidence of new regional wall abnormality or hypokinesia. Acute MI in the form of either ST-elevation myocardial infarction (STEMI) or non-ST-elevation myocardial infarction (NSTEMI) was diagnosed and affirmed by a certified cardiologist/ critical care consultant.

A sample size of 205 patients was calculated according to the WHO calculator[7], using a 95% confidence level, 7% anticipated population proportion, and 3.5% absolute precision. A total of 209 patients were finally included to adjust for missing data.

Data were analysed on SPSS-24. Effect modifications of presence of MI in patients admitted with CAP was obtained against the risk factors, including age, gender, comorbid conditions (DM, HTN, COPD, asthma) and risk factors for CAD (smoking, history of angina, history of PCI including CABG and presence of cardiomyopathy) were controlled by stratification. Post-stratification Pearson Chi-square test was applied and $p<0.05$ was considered significant.

RESULTS

Out of the 209 patients, 121 (58%) were males. The Mean±SD age of presentation was 66±14 years. At the time of hospital admission, acute MI was present in 22 (10.52%) patients with severe CAP. Among these patients, only one patient experienced STEMI. Chest pain was present in 33% of the patients with CAP, while fever (73%), cough (83%), and shortness of breath (84%) was also seen in of patients. HTN was the most common comorbid condition (59%), followed by DM (51%). Although asthma and COPD was not frequently observed in the patients, cardiomyopathy was found in 29% of the patients.

Baseline demographics, cardiac status, co-morbidities, symptoms, and clinical findings of the patients at the time of admission are outlined in Table-1 while radiological features and pneumonia severity scores (CURB-65) are illustrated in Table-2.

Our study did not show any gender-related difference in the prevalence of co-morbid conditions, symptoms, or complications such as acute kidney injury (AKI), except for smoking (Table-3).

Table-1: Baseline characteristics of patients admitted with community-acquired pneumonia (n=209)

Baseline Characteristics	Frequency	Percentage
Hypertension	124	59.3
Diabetes Mellitus	108	51.0
COPD	27	12.9
Asthma	41	19.6
Cardiomyopathy	49	29.0
History of angina	43	20.0
Smoking	52	24.8
History of PCI	20	9.6
History of CABG	12	5.8
Chest pain	70	33.0
Shortness of breath	177	84.0
Cough	182	87.0
Fever	155	73.0
ALOC	70	32.0
AKI	97	46.0

COPD: Chronic obstructive pulmonary disease; PCI: Percutaneous coronary intervention; CABG: Coronary artery bypass graft; ALOC: Altered level of consciousness; AKI: Acute kidney injury

Table-2: Radiological presentation and CURB-65 score of patients admitted with community-acquired pneumonia (n=209)

	Frequency	Percentage
Radiological presentation		
Collapse	7	3.3
Effusion	38	18.0
Pulmonary oedema	39	19.0
CURB-65 score		
I	59	30.0
II	68	32.0
III	45	21.0
IV	18	8.0
V	4	1.0

Table-3: Baseline characteristics of the patients with respect to gender

Baseline characteristics	Male (n=121)	Female (n=88)	*p*
Age (Years)	66	63	0.14
Hypertension	60	62	0.02
Diabetes Mellitus	54	45	0.2
COPD	29	9	0.4
Asthma	21	25	0.33
History of Angina	32	27	0.2
Cardiomyopathy	31	16	0.2
Smoking	44	14	0.000
AKI	67	44	0.72

COPD: Chronic obstructive pulmonary disease; AKI: Acute kidney injury

Our study revealed the incidence of acute MI in 10.52% of the patients admitted with CAP. Clinical features such as chest pain (50%), premorbid conditions such as a history of ischemic heart diseases (50%), and radiologic presence of pulmonary oedema (63%) suggest the presence of underlying MI. A significant correlation was seen between the acute MI and a history of ischemic heart diseases, angina, cardiomyopathy, the presence of AKI, and pulmonary oedema ($p \leq 0.05$). Characterization of pneumonia patient with respect to presence or absence of acute MI are summarized in Table-4.

Table-4: Co-morbid conditions and clinical and radiographic features of patients in the presence or absence of myocardial infarction

Clinical features	MI present (n=22)	MI absent (n=187)	*p*
Diabetes mellitus	13	86	0.26
Hypertension	15	108	0.52
COPD	4	24	0.7
Asthma	4	35	0.56
Cardiomyopathy	18	30	0.000
Smoking	8	43	0.34
History of angina	11	32	0.001
History of PCI	6	13	0.03
History of CABG	2	9	0.9
Shortness of breath	21	151	0.11
Chest pain	11	37	0.004
Pulmonary oedema	14	24	0.000
Presence of AKI	18	93	0.014

COPD: Chronic obstructive pulmonary disease; PCI: percutaneous coronary intervention; CABG: Coronary artery bypass graft; AKI: Acute kidney injury

DISCUSSION

CAP is the most common infectious disease-related cause of death worldwide.[1] Majority of patients who present to the hospital with CAP in the United States have pre-existing chronic cardiac conditions[8], and with advancing age, this association becomes more significant[9]. Acute inflammatory states including CAP, can affect cardiovascular system in numerous ways sand has been recognized as precipitant of acute cardiac events.[10] This is also relevant especially for incidences of heart failure and ACS since their symptoms can overlap with those of CAP and other associated conditions (e.g., Acute lung injury/ARDS). A meta-analysis of large pool of studies[3] revealed the incidence of overall cardiac complications, including heart failure, ACS, and incident arrhythmias in hospitalized patients with CAP as 17.7%, 14.1%, 5.3%, and 4.7% respectively.

Acute infections like CAP can trigger life threatening cardiac complication by multitude of different mechanisms.[11] One proposed patho-physiological phenomenon is induction of biomechanical stress as result of increased sympathetic activity and other haemodynamic changes (Alteration of circulatory volume and systemic coronary vascular tone), prompting plaque rupture.[11,12] Pre-existing CAD that is insufficient to produce myocardial ischemia under baseline condition can also results in significant ischemia in setting of increased myocardial oxygen demand, especially in the first few days after CAP diagnosis.[13] In the light of above facts, high frequency of MI (10%) in our sample can be justified.

Studies have suggested the association of cardiac complications in CAP with development of other medical conditions including acute renal failure, respiratory failure and shock.[14] We found that AKI is more prevalent in the patient population with acute MI, streamlined with other studies.[4,15]

Regarding predicting factors for MI, a study by Aliberti *et al*[15] reported severe sepsis and a previous history of liver disease as a strongest association with this condition. In contrast to that study, our study found a prior history of cardiomyopathy to be the strongest predictor of acute MI ($p<0.000$). Moreover, in symptoms, we found chest pain to be a depicting marker for acute MI in CAP.

It has been proposed that vaccination against respiratory infections in patients with established cardiovascular disease could serve as potentially cost-effective intervention to improve their clinical outcomes.[10,15,16] Limited evidence has been available to establish weather influenza vaccination has a role to play in primary prevention of CAD.[17] Nevertheless, the potential benefits in high-risk CAD populations is reflected in current recommendations. According to World Health Organization, influenza vaccination aims

primarily at protecting against severe pneumonia specially in vulnerable high-risk groups, including those of advance age, or with severe chronic illness.[18]

LIMITATIONS

Due to the retrospective design of the study, some variables could not be extracted from medical records. Beta Natriuretic Peptide was included in our analysis, and was not available for all patients. Patient did not have PSI score calculated which is the most used scale for the assessment of pneumonia. This study was conducted in a single hospital serving in urban area. It would be interesting to extend these observations in a large and multicentre sample.

CONCLUSION & RECOMMENDATIONS

Our study reports higher incidence (10%) of acute MI in hospitalized patients with CAP. Strategies should be employed to recognize MI early in the course of CAP to improve clinical outcomes. These can be introduction of clinical scoring system, biomarker-based approaches, non-invasive cardiac imaging, or a combination of the above. Emphasis on vaccination as an inexpensive and safe intervention which may become a first-line strategy for prevention of avoidable infections and their cardiovascular complications. There is urgent need to divert research and systemic efforts towards this area to reduce the continuing high mortality from CAP. Further studies and randomized control trial are required for better risk stratification and guidelines development.

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ORIGINAL ARTICLE

SEQUELAE OF COMPLETE HEART BLOCK PATIENTS COMING TO RAWALPINDI INSTITUTE OF CARDIOLOGY IN ASSOCIATION WITH PRESENTING COMPLAINTS

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Background: Complete heart block (CHB) is considered as a medical emergency and can be fatal if left untreated. Most patients with acquired complete heart block require a permanent pacemaker and their condition improves after implantation. Objective of this study was to determine the types and frequency of various presenting complaints and their correlation with treatment modality used. **Methods:** This prospective cohort study was conducted at Rawalpindi Institute of Cardiology, Rawalpindi for a duration of six months. A total of 153 patients who were diagnosed having CHB were enrolled. The patients were extensively worked up for history, physical examination, blood tests, chest X-ray, electrocardiography and echocardiography. A pre-designed proforma was filled for each patient to record demographic details, risk factors, past medical and surgical history, vitals and other parameters. The treatment given to each patient was also recorded. **Results:** The most common presenting complaint was dizziness (37.9%), followed by chest pain (31.4%), and syncope (22.8%). Other presenting complaints (17.6% of patients) include shortness of breath, palpitations, generalized fatigue, tiredness and nausea. There was a negative correlation (p=0.003) between chest pain and pacemaker implantation. A negative correlation between syncope and death (p=0.050) was also observed. **Conclusions:** The most common presenting complaint was dizziness and majority (58.6%) of these patients were implanted with a pacemaker. Majority of patients who presented with chest pain were treated medically. No death occurred in group of patients who presented with syncope.

Keywords: Correlation, presenting complaints, outcome, complete heart block, pacemaker

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INTRODUCTION

Diseases of heart and blood vessels are recognized as the prime cause of death worldwide and one of the major contributors towards consequential health problems. A significant part of cost of medical care is spent on treatment of cardiovascular diseases which cause morbidity, mortality, lower quality of life and average life expectancy.[1] In 2016, the cause of death in approximately one third of population was found out to be cardiovascular diseases (CVD).[2]

CVDs are long standing diseases that slowly and silently develop with time and do not cause any symptom. It's only when the disease process has progressed to a certain extent that it causes symptoms and if the disease has caused substantial damage, the advance disease can directly manifest as sudden death.[3]

Defects in conduction system of heart is one of the significant part of CVDs. Atrio-ventricular blocks are especially frequently seen in general population. First degree heart block is most common, subsequently second degree and lastly complete heart block (CHB). CHB is the most far-reaching and can cause death at times.[4]

CHB occurs when atrial impulses do not reach the ventricle and atria and ventricles operate independently. In adults, the cause can be ischemic or non-ischemic. Non-ischemic causes include excessive vagal tone, fibrosis, sclerosis of conducting system, electrolyte imbalance, infiltrative diseases etc. The manifestation of CHB depends on the extent of block and resultant escape rhythm. Escape rhythm in turn is dependent on the intrinsic rate of tissue distal to the block. Presenting complaint is mostly tiredness, dizziness, palpitation, shortness of breath or syncope.[5] Only sometimes, the patient is asymptomatic and is diagnosed on a routine clinical visit or visit to hospital due to some other ailment. The clinical presentation is also dependent on collateral disease. When heart block results from acute myocardial infarction, the ischemic symptoms like chest pain and dyspnoea predominate.[4] When CHB occurs consequent to vasospastic angina, the patient presents with dizziness, dyspnoea, cardiac arrest or sudden death. Holter monitoring is indicated in these patients to identify the cause of these symptoms.[6] The diagnosis of CHB is confirmed when dissociation between atrial and ventricular function is observed on 12-lead ECG or echocardiography.[7]

The electrical activity in the heart originates in the sino-atrial node and spreads inferiorly into the conducting system. Malfunctioning of conducting system reduces the heart rate and makes heart inadequate to reinforce circulation requiring some medical

intervention.[8] For disturbances in the conducting system of heart, permanent pacemaker implantation is one of the prime therapies used now a days.[9] The first pacemaker implantation in a living person was conducted by Dr. Ake Senning in 1958. This implant lasted for some hours only. From then onwards heart block and bradyarrhythmia are primarily treated using pacemakers. Artificial pacemaker is a tiny electric machine having a weight of up to 50 grams and size is close to that of a match box. This electrical machine recognizes the intrinsic rhythm of heart and has the ability to pass on electrical impulses if required for heart stimulation and replacement of natural defective pacemaker.[10]

Use of pacemakers has risen greatly in past few years particularly in old age group. The rise in pacemaker use is attributed to many factors like technological improvements in the device, geriatric age group, better diagnosis and more clinical indications. Approximately 1.25 million permanent pacemakers are implanted per year in the whole world.[11] The use of this device has resulted in an increase in life expectancy of a patient who has no major co-morbidities to that of a healthy individual, and has improved quality of life.[12]

The aim of the present study was to determine the types and frequency of various presenting complaints in complete heart block patients presenting to a tertiary care hospital and their correlation with the outcome in terms of medical treatment, pacemaker implantation, or death.

MATERIAL AND METHODS

The present study was a prospective cohort study conducted from January to July 2018 at Rawalpindi Institute of Cardiology, Rawalpindi, after obtaining approval from Ethical Review Committee of Rawalpindi Institute of Cardiology.

The sample size was calculated on WHO sample size calculator assuming confidence level of 95%, alpha error of 5%, study power of 80%, anticipated population proportion with acute myocardial infarction of 8% and desired precision of 4%.[13] The study included a total number of 153 patients, 20 to 96 years old, having complete heart block. A proforma was designed that was filled for each patient. The information recorded on proforma included demographic details, risk factors, presenting complaints, vitals taken at the time of admission, aetiology, haemodynamic status and outcome.

The inclusion criteria were patients presenting to emergency department with complaints of chest pain, vertigo, dizziness or loss of consciousness and having complete heart block manifested on electrocardiogram. Once the heart block was confirmed by ECG, a detailed history of patients was taken to identify the risk factors. Vitals (pulse, arterial blood pressure, temperature and respiratory rate) were monitored regularly during the admission of patient to monitor haemodynamic stability. The tests performed to determine aetiology were blood complete picture, urea and electrolyte concentration, renal function tests, liver function tests, and chest X-ray. Echocardiography was done to evaluate left ventricular dysfunction.

Data was analysed using SPSS-21. Frequencies of all qualitative variables were analysed and expressed as percentages. Quantitative variables were expressed as Mean±SD. Comparison of outcome in different groups was evaluated using Chi-square test, and $p<0.05$ was considered statistically significant.

RESULTS

In our study, 153 patients were evaluated to estimate the frequency of presenting complaints of complete heart block. Correlation of each presenting complaint with the outcome (pacemaker implantation, no pacemaker implantation/medical treatment or death) was studied.

Most common presenting complaint in emergency department for complete heart block was dizziness (37.9%), followed by chest pain (31.4%) and syncope (22.87%). Other presenting complaints (in 17.6% of patients) included shortness of breath, palpitations, generalized fatigue, tiredness and nausea (Table-1).

Each presenting complaint was studied for its outcome. Forty-eight (48) patients presented with chest pain out of which 16 (33%) were implanted with a pacemaker, 26 (54%) were given medical treatment and 6 (12.5%) patients died. Out of 58 (37.90%) patients presenting with dizziness 34 (58%) were implanted with a pacemaker, 22 (38%) were given medical treatment and 2 (3.4%) died. In 35 patients, the presenting complaint was syncope and 21 (60%) were implanted pacemaker, 14 (40%) were given medical treatment, and no patient died. (Table-2).

The comparison of treatment modality in CHB patients with different presenting complaints revealed a negative correlation ($p=0.003$) between chest pain and pacemaker implantation. The other significant finding was a negative correlation between syncope and death ($p=0.05$). (Table-3).

Table-1: Frequency of presenting complaints in CHB patients (n=153)

Presenting complaint	No. of patients	Percentage
Chest pain	48	31.40
Dizziness	58	37.90
Syncope	35	22.87
Others	27	17.64

Table-2: Outcome in CHB patients with different presenting complaints

Presenting complaints	Pacemaker	No pacemaker	Death
Chest pain (n=48)	16	26	6
Dizziness (n=58)	34	22	2
Syncope (n=35)	21	14	0
Others (n=27)	15	8	4

Table-3: Pearson's correlation between presenting complaints and outcome in CHB patients

Presenting Complaints	Pacemaker		No Pacemaker		Death	
	p	*r*	*p*	*r*	*p*	*r*
Chest Pain	0.003*	-0.243	0.041*	0.166	0.167	0.112
Dizziness	0.110	0.130	0.527	-0.052	0.116	-0.128
Syncope	0.195	0.105	0.873	-0.013	0.050*	-0.159
Others	0.552	0.048	0.181	-0.109	0.139	0.120

*Significant

DISCUSSION

The most frequent presenting complaint in our patients was dizziness, followed by chest pain, and syncope. The greatest percentage of patients who presented with syncope were treated by pacemaker implantation. Patients with chest pain were the least percentage implanted with pacemakers. CHB is mostly symptomatic due to a significant decrease in heart rate and it is an indication for implantation of pacemaker. Congenital CHB, however, can be asymptomatic and requires prophylactic pacemaker implantation to avoid any complications.[14]

CHB can present with a variety of symptoms. In a study conducted in patients with PPM in Nigeria[15], the medical records were retrospectively studied for the presenting complaints. It was found that 86% of the patients had CHB and the most common presenting complaint was easy fatigability (46%) followed by syncope (32%). The procedure was considered as safe and no death was reported in this study.[15]

In a study from Central Africa[7], 26 enrolled patients having CHB presented with a combination of symptoms that were mainly fatigue, dizziness, lethargy, and dyspnoea on slight physical exertion, while 3 were asymptomatic. Pacemaker was implanted in 15 patients (58%). The follow-up of patients showed death in 45% of patients who were not implanted with a pacemaker. No death was recorded in PPM implanted patients. The mode of treatment in such countries depends on availability of resources. The health of the patients is vulnerable due to high cost and non-affordability of implantation procedures.[7] The results of that study are different from our study where a single presenting complaint was predominantly present.

Our results do not correspond with a study conducted in Jakarta[16] whereby all patients undergoing PPM implantation were included. Most patients in that study presented with syncope (52%) followed by dizziness (16%). Only 8% of patients presented with chest pain. The major indication of implantation was CHB (56%). Their study population was different from ours and included cases other than CHB. The treatment modality had resource and financial limitations.[16]

There is scarcity of data related to CHB presenting complaints. Most studies available are case reports. In a study[17] where cardiac sarcoidosis led to CHB, a 49-year-old male patient presented with complaint of dizziness for one week. He was implanted with a PPM and the treatment was successful.[17] In another study[18], an elderly woman presented with dizziness along with chest pain. She had multiple co-morbidities as well. Initially temporary pacing was done that failed to relieve her symptoms. Her symptoms were relieved when a PPM was implanted. The treatment was successful after a follow up of 15 months.[18]

The second common presenting complaint in our patients was chest pain, and 33% patients presenting with chest pain were implanted with pacemakers. The cause of chest pain can be cardiac or non-cardiac. If the cause is acute myocardial infarction, it should be properly identified and promptly treated. Hsu *et al*[19] have reported case of a 56-year-old woman presenting to emergency with chest pain and CHB was diagnosed after investigations. She was treated conservatively and was successful. Transient cases can be treated conservatively and PPM is not required in all cases.[19]

A smaller number of patients in our study presented with syncope. However, it is a common presenting complaint in CHB patients and should be worked up thoroughly as the underlying cause can be neurological as well as cardiac. Brownstein *et al*[20] have reported a 31-year-old man presenting with syncope only as the presenting complaint. He had history of anxiety and depression and excessive smoking. He used marijuana to sleep. CHB was diagnosed and PPM was implanted. The pacemaker was removed after 3 months and his ECG showed normal conduction. The underlying cause was Lyme disease and antibiotic therapy improved his condition.[20]

In another study[21] a 39-year-old woman had recurrent seizures since early age. Syncope in this patient was diagnosed being due to CHB, and subsequently PPM was implanted. This was a challenging case as the focus was on nervous system pathology. The treatment was successful and the 2-year follow-up did not show recurrence of symptoms.[21]

Majority of our patients who presented with syncope were implanted with pacemakers. Use of pacemakers in patients presenting with syncope shows mixed results. In a study, 70% symptomatic relief was observed in patients who were implanted with pacemakers as compared to control group who did not receive pacemaker. They also reported non-significant (27%) symptomatic relief in other patients after pacemaker insertion in CHB patients presenting with syncope.[22]

CONCLUSION

The most common presenting complain in our patients having CHB was dizziness, and majority of these patients were implanted with a pacemaker. The next frequent presenting complaint was chest pain and majority of patients were treated medically. No death occurred in patients presenting with syncope.

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