PAKISTAN PHYSIOLOGICAL SOCIETY

Pakistan Journal of Physiology

VOLUME 18 NUMBER 3 (JUL–SEP 2022)

CONTENTS

TECHNICAL DATA

Page size= 8.5×11.0 inch, 4 colour and B/W, matt paper 90 gm/m^2, Cover= art card 250 gm/m^2, 4 colour printing, laminated, side stapled and glue binding. All margins= 1.0 inch, printed area 6.5×9 inch, 2 columns of 3 inch and 0.5 inch between columns.

EDITORIAL

MYTHS AND MYSTERIES ABOUT COVID-19: LESSONS FOR PAKISTAN

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The COVID-19 pandemic is an ongoing global pandemic. Although experts first identified coronaviruses in the mid-1900s, they have likely been circulating in human populations for thousands of years. The major countries have been accused by each other of spreading this virus in the world intentionally or by accident. Some studies reported that this virus has a potential to become a bioweapon. Professor Sachs said that origin of COVID-19 should be looked into. Sir Jeremy Farrar suspected that the virus was man-made. During this debate, origin of COVID-19 remains a mystery. The Sane Advice by World Health Organization is that countries need a public health system that can respond to the deliberate release of chemical and biological agents. Present day war is fought on many fronts. So, all sections of society should be vigilant and respond effectively when need arises.

Keywords: Corona virus, COVID-19, Bioweapon

The COVID-19 pandemic is an ongoing global pandemic of coronavirus disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). This novel virus was first identified from an outbreak in the Chinese city of Wuhan in December 2019, and attempts to contain it there failed, allowing it to spread across the globe. The World Health Organization (WHO) declared it a Public Health Emergency of International Concern on 30 January 2020 and a pandemic on 11 March 2020.[1] As of August 25, 2022, the pandemic had caused more than 596,119,505 cases and 6,457,101 deaths around the globe, making it one of the deadliest in human history.[2] In Pakistan 1,550,298 cases and 30,467 deaths are recorded as on 23 July 2022 and cases are once again increasing in the country**.**[3]

Myth about origin of COVID-19

Although experts first identified coronaviruses in the mid-1900s, they have likely been circulating in human populations for thousands of years. Some coronaviruses, such as SARS, MERS, and SARS-CoV-2, have a greater impact on populations.[4] As the great development in the field of genetic technologies and the production of vaccines collided with the emergence of new pathogens, the latest of which is the emerging SARS-CoV-2 virus that caused the COVID-19 pandemic around the world. The major countries have been accused by each other of spreading this virus in the world intentionally or by accident.[5] Specifically, these allegations have targeted research conducted at the Wuhan Institute of Virology in Wuhan, China, funded through a subaward from NIAID grantee EcoHealth Alliance. The naturally occurring bat coronaviruses studied through this subaward were significantly, genetically different from SARS-CoV-2 and, therefore, could not have caused the COVID-19 pandemic.[6] A renowned Economist Jefferay Sachs from America

claimed that the SARS-CoV-2 that caused the Covid-19 pandemic did not break out of natural reasons, instead came 'out of a US biotechnology lab'. He negates WHO and also claims that COVID-19 virus was not a natural spillover.[7] As on August 25, 2022, Covid-19 cases reported from China were 6,224,767 and deaths reported were 24,557. While from USA Cases reported were 92,615,784 and deaths reported were 1,031,062.[2] If it were from China, cases and deaths would have been more in China. Possibly, infection affects the most the area where infection starts!

Mystery: Is Corona virus a bioweapon?

Why was this virus studied in laboratories of different countries? Answer given was 'for its vaccine development.' But the next question is that before COVID-19 pandemic, who knew that its vaccine would be required. So, another theory is that this virus was being studied to develop a bioweapon. Some studies reported that this virus has a potential to become a bioweapon. The Centers for Disease Control and Prevention (CDC) define bioterrorism as 'the intentional release of viruses, bacteria, or other germs that can sicken or kill people, livestock, or crops.'[8] Many viral agents have been studied and/or weaponized, including some of the Bunyaviridae (especially Rift Valley fever virus), Ebolavirus, many of the Flaviviridae (especially Japanese encephalitis virus), Machupo virus, Coronaviruses (SARS-Cov-2 that causes COVID-19), Marburg virus, Variola virus, and yellow fever virus.[9] Washington Times in its January 26, 2020 issue said, "Coronavirus may have originated in lab linked to China's biowarfare program." Famous US economist Jeffrey Sachs, who led a two-year probe into the pandemic's origins, said he was 'pretty convinced' the virus was the result of 'US Lab Biotechnology.' Professor Sachs admitted 'We don't know for sure. But there's enough evidence that it should be looked into

and it's not being investigated —not in the US, not anywhere.'[10] Sir Jeremy Farrar, an eminent British expert admitted in a private email in February 2020 that a 'likely explanation' was that the virus was man-made. But the British scientist was shut down by his counterparts in the US who warned further debate about the origins of the virus could damage 'international harmony'. Now, as per new evidence, fever carrying mosquitoes that were studied in Ukraine biolabs were used to spark dengue pandemic in Cuba. Despite not denying its involvement in the Ukrainian laboratories' cooperation, Washington did not acknowledge that it had conducted biological weapon research there. Moscow asserted that Washington and Kiev (Ukraine) had planned to develop biological weapons components near Russia with the purpose of using them to attack the Nation (Russia).[11] During this debate, origin of COVID-19 remains a mystery.

Lessons for Pakistan

The sane advice by World Health Organization (WHO) is that countries need a public health system that can respond to the deliberate release of chemical and biological agents. Regrettable though this message may be, the use of poison gas in the war between Iraq and the Islamic Republic of Iran in the 1980s, the recent anthrax incidents in the United States, and the attack with sarin nerve agent, 6 years earlier, on the Tokyo underground, illustrate why it is necessary to prepare.[12] Present day war is fought on many fronts. So, all sections of the society should be vigilant and respond effectively when need arises. Attention of the readers is drawn towards a few incidents in Pakistan: Dengue causing mosquitoes, Cotton pest attack, and Lumpy Skin Disease in Cows may be bio-attacks. On the economic front, loss of agricultural land for housing schemes, and mango trees cutting for developing housing projects are alarming. Mangoes export earns us foreign exchange. All of the above may harm our country economically. Medical field has also been a target for economic strangulation of Pakistan as doctors send remittances in large amount.

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ORIGINAL ARTICLE

EFFECT OF GHRELIN IN ALLEVIATING NICOTINE INDUCED OXIDATIVE STRESS IN BALB/C MICE

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Background: Ghrelin, a hormone released from GIT mucosa is known to have antioxidant properties by increasing the levels of antioxidant enzymes and by preventing lipid peroxidation. The current study was designed to demonstrate the protective role of ghrelin against nicotine induced oxidative stress and lipid peroxidation in BALB/c mice. **Methods:** Ninety healthy, male BALB/c mice selected through non probability convenient sampling were sorted out into three groups having 30 mice each. Group I (control group) was given intraperitoneal injection of normal saline (i.p.). Group II was given nicotine at a dose of 2.5 mg/Kg body weight (i.p.), while Group III was given nicotine at a dose of 2.5 mg/Kg body weight (i.p.) along with ghrelin at a dose of 10 µg/Kg (i.p.) on alternate days for 4 weeks. On 30th day sampling was done for assessment of serum levels of oxidative stress enzymes (superoxide dismutase, glutathione reductase and catalase levels) and lipid peroxidation marker (malondialdehyde) on ELISA. Data was analysed using SPSS-24. ANOVA followed by post Hoc Tukey test were applied and $p \leq 0.05$ was considered significant. **Results:** Nicotine group showed significant decrease in serum antioxidant enzymes along with significant increase in lipid peroxidation marker. Administration of ghrelin significantly raised the antioxidant enzymes and caused decline in levels of lipid peroxidation marker. **Conclusion:** Ghrelin appears to be an antioxidant due to its ability to increase the levels of antioxidant enzymes and decrease lipid peroxidation marker in nicotine-induced oxidative stress.
Keywords: Nicotine, Ghrelin, Oxidative stress, Antioxidant enzymes, Lipid peroxidation

INTRODUCTION

Cigarette smoking is one of the leading cause of increasing morbidity globally.[1] Nicotine is the predominant chemical amongst the constituents in cigarette smoke. Consumption forms include smoke (cigarettes, pipes, and cigars) and smokeless tobacco (chewable tobacco).[2] Nicotine is also present in insecticides, which can lead to its accidental or intentional poisoning. Nicotine damages many organs of our body like lungs, heart, liver, brain and blood vessels by increasing the production of Reactive Oxygen Species (ROS) and by causing lipid peroxidation.[3] These ROS are responsible for causing oxidative damage which includes DNA/RNA damage, oxidation of proteins and inactivation of membrane enzymes and receptors.[4–6] Smokers are at greater risk of cardiovascular diseases, chronic obstructive lung diseases, cancers, peptic ulcer, infertility and hepatotoxicity. Nicotine disrupts multiple cellular processes. Nicotine exposure produces oxidative stress in tissues by causing depletion of glutathione content and decreasing the activity of oxygen free radical scavengers, like superoxide dismutase and catalase.[7]

Limited amount of lipid peroxidation occurs naturally due to activity of reactive oxygen species (ROS) produced as a result of normal metabolic processes. High levels of ROS directly damage membrane bound lipids which results in decrease in the cell membrane fluidity and an increase in membrane permeability resulting in impairment of function of cell membrane.[8]

'Oxidative stress' results from an imbalance between the formation of ROS and the antioxidant defences. Mitochondrial enzymatic antioxidant defences like catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD) play a significant role in abolishing the oxidative effects of ROS by scavenging them and thus preventing tissue damage.[9]

Ghrelin, a peptide hormone having 28 amino acids is released primarily by the stomach gastric cells during hunger and starvation.[10] Ghrelin is also released from other tissues like pancreas, jejunum, lungs, urogenital organs and pituitary gland.[11] Ghrelin levels peak before eating and decline after meal.[12]

Ghrelin performs multiple functions, which includes appetite stimulation, Growth Hormone secretion, increase in GI motility and secretion. Ghrelin has also shown to have anti-fibrotic, anti-inflammatory, anti-oxidant, and anti-apoptotic actions.[13,14] Ghrelin performs its antioxidant function by increasing the levels of antioxidant enzymes and by preventing lipid peroxidation.[15] Ghrelin decreases the expression of inducible nitric oxide synthase and NF-κB, both of which are responsible for production of ROS.[16]

Systemic administration of ghrelin alleviates oxidative stresses. It has been hypothesized that intraperitoneal administration of ghrelin may have protective role in alleviating oxidative stress in animals who are exposed to nicotine. The current study was

designed to determine role of ghrelin in nicotine induced oxidative stress and lipid peroxidation in BALB/c mice by estimating and comparing antioxidant enzymes (GR, CAT, SOD) and lipid peroxidation marker (MDA) levels.

MATERIAL AND METHODS

This experimental study was conducted at Foundation University School of Health Sciences, Islamabad in collaboration with National Institute of Health, Islamabad, Pakistan after approval from Ethics Review Committee of Foundation University Islamabad. The duration of study was 18 months (2019–2021).

Ninety (90) healthy male BALB/c mice of 6–10 weeks age, in the range of 25–40 g body weight were selected through non-probability convenient sampling and were divided into three groups having 30 mice each. Sample size was calculated using G power method.[17] Animals were kept at animal house of NIH a week prior to study for acclimatization to the environment (Room temperature 22±2 ºC, and 12/12 hour day/night cycle). Group I (control group; n=30) was given standardized pellet diet and 0.65% normal saline at a dose of 1 ml/Kg intraperitoneally (i.p.), Group II (Nicotine only group, n=30) was given pellet diet and i.p. nicotine injection obtained from Alfa Aesar, Johnson Matthey Company, Great Britain at a dose of 2.5 mg/Kg body weight(b.w.)/day for 4 weeks.[18,19]

Group III (Nicotine plus ghrelin group, n=30) was given pellet diet, nicotine i.p. injection at a dose of 2.5 mg/Kg b.w./day for 4 weeks along with ghrelin obtained from Abbexa Ltd Cambridge, UK at a dose of 10 μg/Kg body weight i.p. on alternate days for 4 weeks.[18–20]

On 30th day, mice were euthanized and intracardiac sampling was done for assessment of antioxidant enzymes (Superoxide dismutase, Glutathione reductase and Catalase) and lipid peroxidation marker (Malondialdehyde) in serum by ELISA.

Data was analysed on SPSS-24. Parametric test One-way Analysis of Variance (ANOVA) was applied for significant difference of means between the groups followed by post HOC Tukey test, and $p \leq 0.05$ was considered significant.

RESULTS

Group II (nicotine only group) showed evident oxidative damage with significant decrease in antioxidant enzymes levels (CAT, SOD and GR) and increase in lipid peroxidation marker (MDA) in serum ($p<0.001$) as compared to group I. (Table-1).

In Group III mice, receiving ghrelin along with nicotine, serum antioxidant enzymes and MDA levels were significantly more as compared to Group II ($p<0.001$ for each marker) (Table-1).

Statistically there was no significant difference between group I and group III in antioxidant enzymes and MDA levels ($p=0.279$ for CAT), ($p=0.517$ for SOD), ($p=0.589$ for GR) and ($p=0.978$ for MDA) (Table-1).

Table-1: Effects of nicotine, and nicotine plus ghrelin on hepatic tissue levels of glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA)

Variable	Group (Mean±SD)				Group-wise comparison using post-Hoc Tukey test		
	Control (I)	Nicotine (II)	Nicotine+Ghrelin (III)	ANOVA *p*-value	Control *vs* Nicotine (I *vs* II)	Nicotine *vs* Nicotine+Ghrelin (II *vs* III)	Control *vs* Nicotine+Ghrelin (I *vs* III)
GR pg/ml	960.40±98.82	591.80±76.89	927.17±89.76	<0.001*	<0.001*	<0.001*	0.589
SOD ng/ml	8798.58±243.75	4677.94±165.92	8712.87±198.42	<0.001*	<0.001*	<0.001*	0.517
CAT ng/ml	9.45±2.82	5.05±1.16	8.26±1.72	<0.001*	<0.001*	<0.001*	0.279
MDA ng/ml	498.15±105.63	4799.17±169.33	488.15±106.04	<0.001*	<0.001*	<0.001*	0.978

The results are Mean±SD. The group means were compared with one way ANOVA and post-hoc Tukey's HSD test. *Significant

DISCUSSION

Nicotine effects most of our body organs.[21] It has been used widely as a model agent for inducing free radical damage in animal models.[19,22] In the present study, nicotine was used to induce oxidative stress in animal model and ghrelin was evaluated for antioxidant activity against nicotine induced oxidative stress in male BALB/c mice. The results demonstrate that ghrelin significantly protected the animals from nicotine-induced oxidative stress.

In our study, manifestations of oxidative stress were observed which included significant decline in serum levels of antioxidant enzymes CAT, GR and SOD and rise in lipid peroxidation marker MDA.

Nicotine administration significantly lowered the levels of antioxidant enzymes in serum as compared to the control group. Co-administration of ghrelin with nicotine significantly attenuated the decline in the antioxidant enzymes caused by nicotine. The rise in antioxidant enzymes by ghrelin proves its protective role against oxidative stress.[23]

Lipid peroxidation is one of the major manifestations of oxidative stress induced by ROS.[8] One of the markers for lipid peroxidation is MDA, which is produced by decomposition of fatty acids and is responsible for the bio molecular changes produced by lipid peroxidation. In this study, lipid peroxidation induced by nicotine administration was manifested by

increased levels of MDA in the nicotine group in serum as compared to the control group. Co-administration of ghrelin prevented the rise in MDA levels in nicotine plus ghrelin group. Several studies support the potential role of ghrelin to curtail lipid peroxidation.[24,25]

Cetin Ebru et al conducted a study to assess the hepatoprotective effect of ghrelin on CCl₄ induced acute liver injury in adult male Sprague-Dawley rats. In their study a single injection of CCl₄ was given to induce oxidative stress which resulted in increased MDA levels in serum and hepatic tissue. Administration of ghrelin (10 ng/Kg daily for 5 days) resulted in significant decline in MDA levels in both serum and hepatic tissue. The authors concluded that ghrelin significantly lowered MDA levels.[26] This is in line with our results of decreased MDA levels after ghrelin treatment in nicotine induced oxidative stress. It may be expected that ghrelin preserves the membrane of cells against lipid peroxidation by decreasing the concentrations of MDA due to decrease production of ROS.

Another study, conducted by Alireza Lotfi et al[27] to observe the effect of ghrelin on serum levels of MDA in newly hatched chicken. The authors concluded that ghrelin administration significantly lowers the levels of MDA which is in line with our study. Mahmoud Elsawy et al[28] studied the effect of ghrelin on antioxidant enzymes SOD and GPx in serum and hepatic tissue of a male albino diabetic rats. They concluded that in diabetes, the levels of antioxidant enzymes are reduced while treatment with ghrelin significantly raised their levels. This positive result is in line with our results of increased antioxidant enzymes levels after ghrelin treatment in nicotine induced oxidative stress. In that study only SOD and GPx were estimated however, we estimated the levels of SOD, GR, CAT and MDA levels.

Basra Deniz Obay et al conducted a study to observe the antioxidant effect of ghrelin on pentylenetetrazole induced oxidative stress in rat. Pentylenetetrazole decreased the levels of reduced glutathione (GSH), CAT and SOD while treatment with ghrelin prevented the fall in their levels.[29] The results of the above mentioned studies are comparable to our study in which ghrelin administration improved the levels of the antioxidant enzymes after nicotine induced oxidative stress.

To summarize, our data showed that ghrelin treatment prevents nicotine induced oxidative stress and lipid peroxidation. This deduction can be used effectively to combat oxidative stress induced by pathological conditions. Smokers are at great risk of developing oxidative stress induced injury, and they may be benefited from recombinant ghrelin supplementation to prevent the damage.

CONCLUSION

Evident effect of ghrelin in attenuating nicotine induced oxidative damage illustrates that ghrelin exhibits antioxidant properties. Co-administration of ghrelin partially restored the antioxidant enzyme levels (SOD, CAT GPx with corresponding decline in lipid peroxidation.

ACKNOWLEDGMENT

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HPK: Conception and design, acquisition, analysis, interpretation of data and drafting of article
SA: Substantial contribution to concept and design of study and final approval
MI: Acquisition, interpretation of data
HA: Drafting and revision of article

ORIGINAL ARTICLE

DIAGNOSTIC ACCURACY OF FOCUSED ASSESSMENT WITH SONOGRAPHY IN TRAUMA FOR VISCERAL INJURIES IN BLUNT ABDOMINAL TRAUMA PATIENTS KEEPING CT AS GOLD STANDARD

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Background: Blunt abdominal trauma is common and is associated with intra-abdominal injury. Focused assessment with sonography in trauma (FAST) is extremely sensitive and specific test for the diagnosis of visceral injury in such patients with added benefit of quick scan and bedside availability. This study was conducted with an aim to establish the diagnostic accuracy of FAST for diagnosing visceral injuries in blunt abdominal trauma patients. **Methods:** This study involved 247 patients of both genders aged 18–60 years, referred to Department of Diagnostic Radiology with history of blunt abdominal trauma. All these 247 patients underwent FAST and CT abdomen. The results of CT scan were taken as gold standard and those of FAST were judged accordingly as true/false positive/negative. **Results:** Visceral injury was diagnosed in 167 (67.6%) patients on FAST while CT scan confirmed visceral injury in 165 (66.8%) patients. There were 155 true positive, 12 false positive, 10 false negative and 70 true negative cases which yielded 93.94% sensitivity, 85.37% specificity, 91.09% accuracy, 92.81% positive predictive value and 87.50% negative predictive value for FAST in detecting visceral injury. **Conclusion:** FAST is fairly sensitive, specific, and accurate in diagnosing visceral injury among blunt abdominal trauma. Its non-invasive, radiation-free nature, and widespread bedside availability advocate its preferred use in place of CT in diagnosis of visceral injury in blunt abdominal trauma cases.
Keywords: Accuracy, Sonography, Trauma, Abdominal, Visceral, Scan

INTRODUCTION

Trauma is 3rd commonest cause of death in all ages and is first commonest cause of mortality in people of 5–25 year age. It inflicts huge resources in healthcare system.[1] Prevalence of intra-abdominal injury after blunt trauma has been reported as high as 13%. Commonest causes of blunt abdominal trauma include vehicle accident, fall, assault, and industrial mishaps, liver being the commonest injured organ followed by spleen, gut, retroperitoneal haematoma, and other organs. Males are more frequently involved compared to females.[2]

Computed Tomography (CT) is gold standard in evaluation of intra-abdominal injury in blunt abdominal trauma patients for parenchymal organ injuries as well as detecting small bowel and mesenteric injuries, and associated hemoperitoneum. It provides an accurate evaluation of visceral injuries, thus helping the surgeons to select the correct initial clinical emergency management.[3] But it requires unavoidable time delay, needs patient transfer, and is inappropriate for hemodynamically unstable patients.[4] Increased rate of mortality and morbidity in trauma patient is mostly due to delay in early diagnosis or misdiagnosis.[5]

Focused assessment with sonography in trauma (FAST) is used in evaluation of trauma patients since past 30 years. Identification of free fluid within the peritoneal cavity, pleural spaces and pericardium can be detected promptly with FAST on patient arrival in Accident and Emergency Department. It is targeted on dependent intra-peritoneal sites where blood is most likely to collect: the hepatorenal space (i.e., Morrison's pouch), the inferior portion of the intra-peritoneal cavity (including pouch of Douglas) and lienorenal recess. Other advantages of FAST include evaluation of solid organ injury, fractures, pneumothorax, sequential examinations, as well as use in pre-hospital transport and multiple casualty settings as a triage tool.[6] FAST takes less time, is cost-effective, non-invasive, repeatable and easily accessible. It can also be used for unstable patients in resuscitation area. Moreover, surgeons and emergency physicians with limited experience in ultrasound can also perform FAST after a brief training.[4] FAST is becoming the common earliest screening investigation in majority of Accident and Emergency Departments worldwide, and is also part of the Advanced Trauma Life Support program for assessment of the hypotensive trauma patient.[6] The sensitivity and specificity of FAST to detect visceral injuries after blunt abdominal trauma has been reported as 93.5% and 84.4% respectively; CT scan revealed visceral injury in 44%.[7]

Trauma leading to visceral injuries is very common in our population and early diagnosis and early treatment is of extreme importance to reduce the morbidity and mortality. The present study aims to detect the diagnostic accuracy of FAST in the assessment of visceral injuries in blunt abdominal trauma patients keeping CT scan as gold standard.

PATIENTS AND METHODS

The study was conducted after approval from Hospital Ethical and Research Committee. All patients presenting to Emergency Department with suspicion of visceral injury after blunt abdominal trauma were included in the study after written informed consent. The inclusion criteria were patients with clinical suspicion of visceral injury after blunt abdominal trauma who were hemodynamically stable, with age range of 18–60 years. Exclusion criteria were pregnant patients, penetrating trauma and burns, already operated, and hemodynamically unstable patients.

Detailed history was taken from the patients followed by clinical examination, FAST, and CT. All scan were performed under a single standard technique and were reported/reviewed under supervision of a single competent radiologist. Data were recorded on a pre-designed proforma, entered on and analyzed using SPSS-20, and 2×2 tables were generated to calculate sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of FAST against CT scan as the gold standard.

RESULTS

Mean age of the patients was 34.1±11.7 years. There were 186 (75.3%) male and 61 (24.7%) female patients with a male to female ratio of 3:1. Road traffic accident was the most frequent cause and was observed in 59.1% patients followed by fall from height (32.4%) and assault (8.5%).

Visceral injury was diagnosed in 167 patients on FAST while CT scan confirmed visceral injury in 165 patients (Table-1). No statistically significant differences were observed in the frequency of CT confirmed visceral injury in several subgroups based on patient's age (p=0.898), gender (p=0.814) and mode of injury (p=0.619) (Table-2).

When cross-tabulated diagnosis of visceral injury on FAST with that of CT scan, there were 155 true positive, 12 false positive, 10 false negative and 70 true negative cases (Table-3). The sensitivity, specificity, accuracy, positive predictive value and negative predictive value for FAST in detecting visceral injury are tabulated as Table-4. Similar tabulation was done based on age, gender and mode of injury (Table-5, 6).

Table-1: Diagnosis of visceral injury on FAST and abdominal CT scan (n=247)

Modality	Visceral Injury	Frequency	Percent (%)
FAST	Yes	167	67.6
	No	80	32.4
CT Scan	Yes	165	66.8
	No	82	33.2

Table-2: Stratification of visceral injury confirmed on CT scan across various subgroups (n=247)

Subgroups	n	Visceral injury [n (%)]	p
Age			
18–39 years	176	118 (67.0%)	0.898
40–60 years	71	47 (66.2%)	
Gender			
Male	186	125 (67.2%)	0.814
Female	61	40 (65.6%)	
Mode of Injury			
RTA	146	97 (66.4%)	
Fall from height	80	52 (65.0%)	0.619
Assault	21	16 (76.2%)	

Chi-square test, Observed difference was statistically insignificant

Table-3: Cross-tabulation of FAST and CT scan for diagnostic performance in visceral injury after blunt trauma abdomen (n=132)

FAST	CT Scan		Total
	Visceral injury	No	
Visceral injury	155[a]	12[c]	167
No	10[b]	70[d]	80

[a]True Positive= 155, [b]False Negative= 10, [c]False Positive= 12, [d]True Negative= 70

Table-4: Diagnostic performance of FAST in visceral injury after blunt abdominal trauma

Statistical Parameter	Formula	Value
Sensitivity	$\frac{a}{a+b}$	93.94%
Specificity	$\frac{d}{c+d}$	85.37%
Accuracy	$\frac{a+d}{a+b+c+d}$	91.09%
Positive Predictive Value	$\frac{a}{a+c}$	92.81%
Negative Predictive Value	$\frac{d}{b+d}$	87.50%

Table-5: Cross-tabulation of FAST and CT scan for diagnostic performance in visceral injury after blunt trauma abdomen across age groups (n=247)

Age group	FAST	CT Scan		Total	Diagnostic Performance
		Visceral Injury	No		
18–39 years (n=176)	Visceral Injury	111	8	119	SN=94.07%, SP=86.21%, PPV=93.28%, NPV=87.72%, AC=91.48%, PR=67.05%
	No	7	50	57	
40–60 years (n=71)	Visceral Injury	44	4	48	SN=93.62%, SP=83.33%, PPV=91.67%, NPV=86.96%, AC=90.14%, PR=66.20%
	No	3	20	23	

SN= Sensitivity, SP= Specificity, AC= Accuracy, PPV= Positive Predictive Value, NPV= Negative Predictive Value, PR= Prevalence

Table-6: Cross-tabulation of FAST and CT scan for diagnostic performance in visceral injury after blunt trauma abdomen across gender groups (n=247)

Gender	FAST	CT Scan		Total	Diagnostic Performance
		Visceral Injury	No		
Male (n=186)	Visceral Injury	118	9	127	SN=94.40%, SP=85.25%, PPV=92.91%, NPV=88.14%, AC=91.40%, PR=67.20%
	No	7	52	59	
Female (n=61)	Visceral Injury	37	3	40	SN=92.50%, SP=85.71%, PPV=92.50%, NPV=85.71%, AC=90.16%, PR=65.57%
	No	3	18	21	

SN= Sensitivity, SP= Specificity, AC= Accuracy, PPV= Positive Predictive Value, NPV= Negative Predictive Value, PR= Prevalence

DISCUSSION

Blunt abdominal trauma is among the commonest causes of injuries and is mainly caused due to automobile accidents. The rapid rise in number of automobiles and its aftermath has caused rapid rise in victims to blunt abdominal trauma. Motor vehicle accidents cause 75 to 80% of blunt abdominal trauma.[1] Other causes of blunt abdominal trauma include fall from height, being hit with blunt objects, sport trauma, industrial accidents and bomb blasts.[1,2] Blunt abdominal trauma is usually not apparent thus often missed unless meticulously and repeatedly looked for. Delay in diagnosis and inadequate treatment of the abdominal injuries causes increased mortality.[1] Even in the presence of the best techniques and advancements in diagnostic and supportive care, the morbidity and mortality rates are still high. The reason for increased mortality and morbidity can be interval between trauma and hospitalization, inappropriate and lack of adequate surgical treatment, delayed diagnosis, postsurgical complications and accompanying trauma specially to head, thorax and pelvis.[2]

The early assessment of unstable trauma patients must be rapid and unequivocal to accurately guide the diagnostic and therapeutic interventions in accordingly.[1–3] CT scan is the gold standard in assessment of intra-abdominal injury in blunt abdominal trauma patients but it demands unavoidable time delay, needs patient transfer, and is inappropriate for hemodynamically unstable patients.[3] Plain radiography of the chest and pelvis and FAST produce early results that has direct influence on further management. The FAST targets to determine any abnormal abdominal fluid collection within the peritoneal, pericardial, and other potential spaces. In hemodynamically unstable trauma patients, any free fluid suggests FAST-positivity and is assumed to represent hemorrhage.[4–7] Recent studies claimed that FAST was an extremely sensitive and specific test for the diagnosis of visceral injury with added benefit of quick scan and bedside availability.[7] However, the available evidence contains controversy.

In the present study, the mean age of the patients was 34.1±11.7 years —the most productive time of life. Similar mean age has been reported by Mohsin *et al*[8] among blunt abdominal trauma patients presenting at Liaquat National Hospital, Karachi. Latif *et al*[9] from Combined Military Hospital, Lahore, and Janjua *et al*[10] from Pakistan Institute of Medical Sciences, Islamabad also reported similar results. A relatively lower mean age of 30.5±11.2 years has been reported by Bano *et al*[11] among patients presenting at Civil Hospital Karachi while much higher mean age of 39.4±12.1 years has been reported by Mushtaq *et al*[12] from Nishtar Hospital Multan. Waheed *et al*[13] reported comparable mean age among such patients in KSA.

There was a male predominance among our patients. This is in line with Janjua *et al*[10], Latif *et al*[9], and Mohammadi *et al*[14] from Iran. This younger age and male predominance can be attributable to mode of injury (RTA, 59.0% cases) which frequently involves young males.[2]

Road traffic accident was the most frequent cause in our patients followed by fall from height, and assault. Latif *et al*[9] from CMH Lahore, Bano *et al*[11] from Civil Hospital Karachi, and Musiitwa *et al*[15] from Uganda reported similar frequencies of RTA, fall from height and assault among such patients.

In the present study, visceral injury was diagnosed in 66.8% of patients with blunt abdominal trauma on CT scan. Our observation is similar with that of Gul *et al*[16] who reported visceral injury among 68.0% of blunt abdominal trauma patients presenting at DHQ Hospital, Mirpur, AJK. Mohsin *et al*[8] have reported the frequency of visceral injury to be 74.0% among such patients at Liaquat National Hospital, Karachi. Comparable frequency of visceral injury as 63.6% and 63.8% has been reported by Janjua *et al*[10] in local population and Waheed *et al*[13] in KSA respectively. FAST was found to have 93.94% sensitivity, 85.37% specificity, 91.09% accuracy, 92.81% positive predictive value, and 87.50% negative predictive value in detecting visceral injury. Our results are in agreement to those of Hamid *et al*[7], Mohsin *et al*[8], and Latif *et al*[9] who observed similar sensitivity of FAST in patients with blunt abdominal trauma. Much lower sensitivity of 36.6% has been reported by Kanai *et al*[17] in Iran while much lower specificity of 67.0% has been reported by Nnamonu *et al*[18] in Nigeria. A possible explanation for this conflict among studies can be the hardware and operator dependent nature of ultrasound.

The present study further adds to the already available evidence on the topic. The results of the present study are in line with the other studies already published in local population and establish the role of FAST in the emergency evaluation of victims of blunt abdominal trauma. Owing to non-invasive and radiation free nature of ultrasound along with widespread and bedside availability FAST appears to be superior to CT scan which exposes the patients to radiations, requires patients transfer causing unavoidable delay, and is not available at many district setups.

The strengths of our study are large sample size of 247 patients and that we stratified the data to address effect modifiers. A very strong limitation to the present study is that it was a single centre experience and considering the hardware and operator dependent nature of ultrasound which is generally agreed to be a source of inter-observer variability. There is need for a multi-centre trial to further establish the role of FAST in the diagnostic evaluation of patients presenting in emergency with blunt abdominal trauma.

CONCLUSION

FAST was found to be 93.9% sensitive, 85.4% specific and 91.1% accurate in diagnosing visceral injury among patients with blunt abdominal trauma. Its non-invasive, radiation-free nature, and easy bedside availability advocate its preferred use in place of CT in diagnosis of visceral injury in blunt abdominal trauma cases.

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ORIGINAL ARTICLE
EFFECT OF TAURINE ON BIOMARKER OF OXIDATIVE STRESS IN SERUM OF HIGH FAT DIET-LOW DOSE STREPTOZOTOCIN INJECTED MODEL OF TYPE 2 DIABETIC RATS

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Background: Oxidative stress plays a major role in the development of insulin resistance and pancreatic beta cell dysfunction. Taurine and beta-alanine have been documented to act as antioxidants. The aim of this study was to compare the antioxidant effect of taurine and beta-alanine in type 2 diabetic rats. **Methods:** This Laboratory based experimental study was conducted in Department of Physiology at Army Medical College, Rawalpindi, in collaboration with National Institute of Health, Islamabad, from July to Sep 2018. Ninety male Sprague-Dawley rats were randomly divided into three groups; diabetic control (DC), diabetic beta-alanine (DBA), and diabetic taurine group (DTau). All ninety rats were fed with taurine free-high fat diet for a period of four weeks, and administered low-dose streptozocin on the 14th day for the induction of type 2 diabetes mellitus. Also, DC rats were supplemented with 0.02% (w/v) taurine, DBA rats with 3% (w/v) beta-alanine, and DTau rats with 3% (w/v) taurine in their respective drinking water, for a period of four weeks. At day 21, plasma glucose levels and insulin resistance were measured to confirm development of type 2 diabetes mellitus in the three groups. At the end of four weeks, terminal intracardiac sampling was done to measure 8-isoprostane levels. **Results:** 8-isoprostane levels were significantly increased in diabetic group. Taurine supplementation ameliorated these effects as compared to beta-alanine. **Conclusion:** Taurine reduces oxidative stress in type 2 diabetes mellitus.
Keywords: High fat diet, Oxidative stress, Taurine supplementation

INTRODUCTION

Oxidative stress (OS) is a major direct pathway in the development of insulin resistance (IR) and type 2 diabetes mellitus (T2DM) in both rodent and human studies.[1,2] An important consequence of excessive reactive oxygen species and OS, especially linked with IR and diabetes, is lipid peroxidation (LPO). LPO can be measured by its primary (lipid hydroperoxides and conjugated dienes) or secondary (thiobarbituric reactive substances and F_2-isoprostanes) end products. Among these, the F_2-isoprostanes (F_2-IsoPs) have surfaced as a direct and reliable measure of OS. F_2-IsoPs are prostaglandin-like compounds derived non-enzymatically, through free radical, such as superoxide anion (O_2^-)-catalysed peroxidation of arachidonic acid. F_2-IsoPs are first formed in esterified form in tissues, and thereafter enter the circulation in the free form. 8-isoprostane-PGF2α (8-IP), the most abundant F_2-IsoPs, has proved to be highly precise lipid biomarker for *in vivo* OS in both animal and human studies. Elevated levels of 8-IP are reported in biological fluids in T2DM patients.[2,3]

Tau is a methionine and cysteine-derived amino acid found in mammalian tissues. Reduced Tau levels have been reported in diabetes.[4] An inverse correlation exists between plasma Tau levels and diabetic complications, while, dietary Tau supplementation reduced OS and alleviated diabetic complications, implicating Tau as a conditionally essential amino acid in disease states associated with high OS, such as DM and metabolic syndrome.[5]

With regard to its antioxidant property, Tau is responsible for ensuring normal electron transport chain (ETC). In doing so, Tau protects mitochondria from excessive formation of O_2^-.[5] Tau forms a conjugate with uridine on mitochondrial transfer RNA (tRNA) and forms 5-taurinomethyluridine [$\tau m^5(S^2)U$] for adequate formation of mitochondrial encoded proteins (MEPs), which are functional subunits of respiratory chain complexes. Since, the $\tau m^5(S^2)U$ content of tRNA is dependent on Tau levels, Tau deficiency causes a paucity of MEPs (ND5 and ND6) and disruption in activities of complexes I and III of the respiratory chain. This diverts electron to the acceptor oxygen resulting in excessive O_2^- generation and hence, increased OS.[6]

Beta alanine (BA) is another emergent class of therapeutic agent, reported to positively affect metabolic control in various animal models of diabetes, as well as, in human subjects with T2DM, on account of antioxidant properties. In the body, BA combines with L-histidine to form the dipeptide carnosine (β-alanyl-L-histidine).[7–9] Carnosine (CAR) is a non-enzymatic free-radical scavenger. At physiological concentrations, CAR alters the reactivity of O_2^-, by directly reacting with, and forming a charge-transfer complex with O_2^- radical. Being a natural dipeptide, CAR also reduces LPO.[7,9]

Diabetes-associated increased OS in various organs is followed by micro- and macrovascular complications.[1] Therefore, mitigating OS is a major target for preventing, managing, and treating T2DM. This has steered the path for investigation of new compounds having strong antioxidant properties.[5] The current study aimed to compare the antioxidant effect of Tau and BA, in type 2 diabetic Sprague-Dawley rats.

METHODOLOGY

This laboratory-based experimental study was done at Department of Physiology, Army Medical College, Rawalpindi, and National Institute of Health, Islamabad, from July to Sep 2018, after approval of the Ethical Committee of the College.

Preliminary assessment of plasma glucose was done to exclude rats with pre-existing derangement in glucose metabolism. Ninety male Sprague-Dawley rats, aged 60–90 days, and weighing 250±50 grams, were selected for the study. Rats were kept in 2×3 feet steel cages, fitted with clean water bottles, in a well-ventilated room at 20–22 ºC, on 12-hour light/dark cycle.

Rats were divided randomly into 3 groups: diabetic control (DC), diabetic BA (DBA) and diabetic Tau (DTau). All rats were fed for 4 weeks, with Tau free-high fat diet (Table-1). DC rats were supplemented with 0.02% (w/v) Tau which represented Tau content of standard rat chow, DBA rats with 3% (w/v) BA, and DTau rats with 3% (w/v) Tau in their respective drinking water for a period of 4 weeks.

Table-1: Composition of taurine free-high fat diet

Ingredients	Weight (g/Kg)
Animal fat	310
Casein	250
Cholesterol	10
Vitamin and mineral mix	60
DL-Methionine	3
Yeast powder	1
Sodium chloride	1
Powdered taurine free diet:	365
i. Cornstarch	122.2385
ii. Casein	78.11
iii. Dextrised cornstarch	40.365
iv. Sucrose	36.5
v. Soybean oil	51.1
vi. Cellulose	18.25
vii. Mineral mix	12.775
viii. Vitamin mix	3.65
ix. L-cystine	1.095
x. Choline bitartrate	0.9125

On 14th day of study, a single, low-dose (35 mg/Kg body weight) injection of streptozotocin (STZ) (Calbiochem, USA) was given intraperitoneally, in the lower-right quadrant of the abdomen of all rats, to induce T2DM with insulin resistance. At day 21st, tail vein blood samples were obtained to measure plasma glucose and insulin resistance, to confirm development

of T2DM.[10] The same diet and supplementation were continued for another week.

On completion of 4 weeks of study, rats were euthanized by overdose of ether anesthesia. Terminal blood samples were collected by intracardiac sampling, in sodium fluoride tubes for plasma, and in gel separator tubes for serum. The samples were centrifuged and stored at -80 °C until assayed. Plasma glucose was measured by glucose oxidase method (Linear Chemicals, Spain), serum levels of insulin were measured by Sandwich ELISA (Cayman Chemical Company), and HOMA-IR was calculated from these values. Serum 8-IP was measured by enzyme immune assay (Cayman Chemical Company).

Data was entered and analysed on SPSS-21 to calculate Mean±SD of all variables. ANOVA and Post-hoc Tukey test was applied for comparison between groups, and $p<0.05$ was considered significant.

RESULTS

At the completion of 3rd week, T2DM was confirmed according to criteria. At the end of the study, Post hoc Tukey's HSD revealed statistically significant difference in the levels of 8-IP between control and Tau ($p=0.00$), and between BA and Tau ($p<0.001$). Non-significant difference in 8-isoprostane levels was found between control and BA groups ($p=0.07$) (Table-2).

Table-2: Comparison of 8-isoprostane levels among groups of diabetic rats, by Post Hoc Tukey's Test at completion of the study

8-IP (pg/ml)	DC (n=30)	DBA (n=30)	DTau (n=30)
Mean±SD	219.83±64.15	190.79±54.35	141.33±23.26
p	I & II= 0.07	II & III <0.001*	I & III= 0.00*

*Significant

DISCUSSION

In this study the high fat diet-low dose STZ model of T2DM was used because this model closely resembled the human T2DM in its natural history and metabolic profile.[10] The effect of Tau and BA supplementation on oxidative stress related parameter were examined in diabetic rats. Tau significantly ($p=0.00$) ameliorated OS in supplemented rats in comparison to rats supplemented with BA.

Tau has been documented to reduce OS indices in other experimental studies. In one such study, STZ-induced diabetic Wistar dams were orally supplemented with Tau (1 g/Kg bodyweight/day) from 5th till 12th gestation day, and euthanized on the 13th day. The authors found that after the 8 days of treatment, Tau reduced the OS marker, malondialdehyde (MDA) in diabetic dams ($p<0.05$) as well as in mitochondrial and cytosolic fractions of embryonic milieu ($p<0.05$). The authors further found Tau to be embryo-protective in pregnant diabetic dams, and suggested Tau to be used as

a supplemental therapeutic in diabetic pregnancies.[11] In a study by Zhang *et al*, mice were injected once daily with iron, 5 days a week, for a total of 13 weeks, to produce iron overload with consequent hepatic dysfunction and increased OS. Tau treatment elevated hepatic Tau levels (40%), improved hepatic function, and reduced ROS formation, liver LPO, and OS. The authors suggested Tau as a therapeutic agent with potential to reduce iron overload-induced hepatic damage.[12]

In contrast to animal studies, the results of the few clinical trials that have explored the effect of Tau consumption on OS, are contradictory. In one such trial in which type 2 diabetic patients consumed Tau (3,000 mg daily for 4 months), no effect on OS status was reported. It could be because the sample size of the study was small due to the recruitment of only freshly-diagnosed cases of T2DM, and hence, lack of significant alterations in OS marker.[13] On the other hand, Maleki *et al*, conducted a novice controlled clinical trial in type 2 diabetics and found that Tau supplementation (1,000 mg, thrice daily, for 8 weeks) significantly decreased levels of serum MDA (26.33%, $p=0.00$). The authors attributed these findings to the suppression of superoxide generation in the mitochondria, as well as to stimulatory effect of Tau on the activities of antioxidant enzymes superoxide dismutase (5.1%, $p=0.00$) and catalase (4.22%, $p=0.00$), culminating in markedly reduced generation of ROS.[5]

Significant reduction in OS was also noted in Tau supplemented diabetic rats as compared to BA treated rats ($p<0.00$). The difference in antioxidant capacity of Tau and BA could be because the dosage of BA (3%) was too low or the duration of BA supplementation was too short to cause beneficial effect, as opposed to Tau given in the same dose and for the same duration. Or it could be because 3% BA, being structurally similar to tau, competitively inhibits Tau uptake at transporter site, producing 50% reduction in plasma and tissue Tau levels.[14]

BA also eliminates mitochondrial Tau content by 60%.[15] Taken together, this resulted in increased mitochondrial superoxide generation with resultant increased LPO and OS. Similar to our finding, immersion of isolated neonatal cardiomyocytes of Wistar rats for 2 days, in a 5 mM BA-containing medium produced a 45% decrease in Tau content along with increased mitochondrial OS. Co-administration of 5 mM Tau with BA prevented these effects. BA inhibited the post-transcriptional modification of tRNA by tau, thereby negatively impacting MEP synthesis and assembly of respiratory chain complexes. This ultimately resulted in accumulation of harmful oxidants (Figure-1)[16].
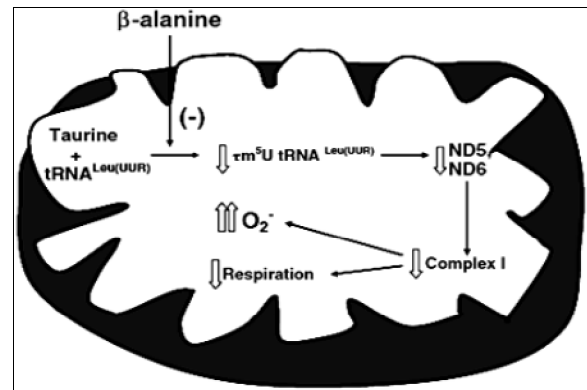
Figure-1: Scheme for BA-mediated increase in OS[16]

In the present study, 8-IP levels of diabetic BA supplemented rats were comparable ($p=0.07$) with those of control rats. This could be because of insufficient dose of BA used in the present study (3%), or to the absence of nutritional control during supplementation, which may have resulted in insufficient CAR concentration in the body, to be effective as antioxidant and bring serum 8-IP levels down to the value where difference with serum 8-IP levels of control rats became significant. This could also be due to the BA-induced Tau deficiency. It could also be that the duration of supplementation was too short to cause any beneficial effect. In corroboration with our study, Gibbons *et al*, also documented that feeding mice for four weeks with BA supplemented diet had no effect in reducing OS.[17] Kerai *et al* also demonstrated that rats fed on BA (3%) supplemented diet remained susceptible to ethanol-induced hepatic LPO, as a result of Tau depletion.[18]

CONCLUSION & RECOMMENDATIONS

Tau as compared to BA, significantly improved the OS indices in type 2 diabetic rats. Therefore, it can be used as a supplement for the treatment of T2DM. It is suggested that combined supplementation of alanine and taurine should have also been investigated to assess if together these could be more beneficial as an effective therapeutic approach rather than when given independently. Plasma levels of CAR in BA supplemented rats should have been measured to ascertain the amount of CAR generated.

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ORIGINAL ARTICLE

ASSOCIATION OF *Nrf2* GENE POLYMORPHISM WITH POLYCYSTIC OVARY SYNDROME —A CASE-CONTROL STUDY

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Background: Polycystic Ovary Syndrome (PCOS) is considered as one of the most common endocrine disorder in women of reproductive age. Exact cause of PCOS is still unknown. However, evidence for genetic basis has been reported. Reactive oxygen species and antioxidants have been documented as key factors involved in ovarian physiological metabolism. *Nrf2* is a key transcription factor that regulates the expression of antioxidant proteins, therefore provides protection against oxidative stress. Objective of this study was to find the association of *Nrf2* (rs6721961) gene polymorphism with pathogenesis of PCOS among Pakistani population. **Methods:** This case-control study was conducted in Pakistan Railway Hospital, Rawalpindi, from Oct 2020 to Sep 2021. The study included 200 PCOS patients diagnosed according to Rotterdam diagnostic criteria and 200 healthy controls. Blood samples of all participants were collected and DNA was extracted by Chelex Method. Polymerase Chain Reaction (PCR) was performed to find respective allelic frequencies of *Nrf2* (rs6721961) genotype using specific primers. **Results:** The frequency of CC, CA and AA genotypes of rs6721961 polymorphism of *Nrf2* gene were 72.5%, 27.5%, 0% in controls and 56.5%, 39.5%, 4% in cases. Significant association of CA genotype of *Nrf2* gene polymorphism (rs6721961) and allele A were found with PCOS (OR: 0.54, 95% CI: 0.35–0.82, p=0.004), (OR: 0.61, 95% CI: 0.42–0.89, p=0.01) respectively. **Conclusion:** *Nrf2* gene polymorphism (rs6721956) is significantly associated with PCOS among Pakistani population.
Keywords: Polycystic ovary syndrome, *Nrf2* gene, Rotterdam diagnostic criteria

Pak J Physiol 2022;18(3):15–8

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a common hormonal disorder and one of the most prevalent endocrinopathy in women of reproductive age.[1] This disorder tremendously affects the quality of women's life during reproductive years.[2] Evidence suggests that it is a multifactorial disease caused by genetic[3] and environmental[4] influences. Environmental influences include diet, socioeconomic status, geography and environmental toxins.[4] Moreover, polymorphisms and differential regulation of genes are also believed to affect the pathogenesis of PCOS.[5] The recent studies carried out in different geographical locations in Pakistan showed that prevalence of PCOS is increasing day by day.[6] A significant association has been found between PCOS and genetic factors in various populations all over the world.[7]

Reactive Oxygen Species (ROS) are produced as by-products during ovarian physiological metabolism and antioxidants act as factors that can maintain the balance between ROS production and clearance.[8] Previous work has established that the balance between ROS and antioxidants greatly influences the reproductive activities in female mammalian animals such as endometrial changes in luteal phases, implantation, ovulation and fertilization.[8,9] However, under oxidative stress conditions, compromised reproduction and fertility may be induced including impaired ovarian functions, deteriorated oocyte quantity and infertility.[8] Therefore, antioxidants are crucial for maintaining the redox balance in the ovaries to support normal ovarian function.[10]

Paraoxonase 1 (PON1) gene encodes for antioxidant enzymes. Q192R, C108-T, and L55M polymorphisms in *PON1* gene are associated with the risk of developing PCOS.[11] A16V polymorphism in *Superoxide dismutase 2 (SOD2)* gene was also found to be associated with PCOS.[12] Nuclear factor erythroid 2-related factor 2 (*Nrf2*) is an important transcription factor that regulates the expression of antioxidant proteins, therefore provides protection against oxidative stress.[13] Polymorphism in *Nrf2* gene has been found to be associated with various diseases.[14] A number of genetic studies have established the association of *Nrf2* gene polymorphism (rs6721961) with conditions linked to oxidative stress such as respiratory diseases, cardiovascular diseases and male infertility.[15] However, no research to our knowledge has been done to date to find out association of polymorphism in *Nrf2* gene with PCOS. The objective of this study was to assess the association of *Nrf2* gene polymorphism (rs6721961) with PCOS.

MATERIAL AND METHODS

In a case-control design, a total of 200 patients with PCOS were recruited from the Gynaecology Department of Pakistan Railway Hospital, Rawalpindi between October 2020 and April 2021. The Hospital caters for patients from all over Pakistan. All cases were diagnosed with PCOS as per Rotterdam diagnostic criteria.

A total of 200 healthy, premenopausal women were recruited as the control group from the local community. All cases and controls had no history of hyperprolactinemia, Cushing's syndrome, thyroid dysfunction, androgen secreting tumour, pregnancy, hormonal therapy including oral contraceptives for at least 3 months prior to the study. Participants provided written informed consent for the study. The study was approved by the Ethical Review Committee of Islamic International Medical College, Rawalpindi (Appl. # Riphah/IRC/20/235. October 19, 2020).

Clinical assessment was done with a questionnaire-based interview regarding socio demographic factors, menstrual cycle and characteristics of PCOS. Detailed physical examination included measurement of height, weight (to determine BMI), waist and hip circumference (waist to hip ratio), systolic and diastolic blood pressure, hirsutism (mFG score), and distribution of acne.

After obtaining written informed consent from the participants, 2–3 ml fasting venous blood samples were collected for serum measurements and genomic DNA isolation. Hormonal evaluation included levels of total testosterone, serum hormone-binding globulin (SHBG), DHEAS, LH, FSH and E2. Fasting glucose and insulin levels were also determined. Genomic DNA extraction from whole blood was done by Chelex Method and stored at -70 °C until use. The rs6721961 polymorphism was identified by multiplex Tetra-primer ARMS-PCR by using specific primers (Table-1).

Table-1: Primer sequence of *Nrf2* gene

Primers	Primer Sequence 5′ to 3′
Nrf2 FP 1	CTCCGTTTGCCTTTGACGAC
Nrf2 RP 1	GGGGAGATGTGGACAGCG
Nrf2 FP 2	GCGAACACGAGCTGCCGGA
Nrf2 RP 2	CCCTGATTTGGAGTTGCAGAACC

The PCR reaction was performed in a tube containing forward and reverse primers specific to *Nrf2* gene (rs6721961). The final total volume for PCR reaction was 24.5 μl. PCR reaction mixture constitutes 8.5 μl water by Invitrogen™, 12.5 μl 2× Thermoscientific™ Master Mix which consists of 0.05 U/μL Taq DNA polymerase, reaction buffer, 4 mM of each dNTP (dATP, dCTP, dGTP and dTTP). Each primer vial contained 30 nmol of respective primer which was diluted to 0.01 nmol/μl working solution.

Primer were supplied by M/S Macrogen™ (Korea). A final volume of 0.5 μl was added to reaction mixture from 4 primers. 3 μl of DNA was added from sample to be genotyped. Extracted DNA was subjected to multiplex tetra ARMS-PCR and respective allelic frequencies were recorded.

PCR was done by DNA denaturation at 96 °C (5 min) accompanied by 35 cycles of 96 °C (1 min), annealing at 63 °C (1 min), extension at 72 °C (1 min) and an ultimate extension step at 72 °C (5 min). Resolution of PCR amplicons was done on two percent agarose gel to envision the DNA bands under ultra violet light in Syngene gel imaging system (Gene Box™).

SPSS-21 was used for data analyses. The differences in demographic and clinical characteristics were compared using independent sample *t*-test between cases and controls. Frequencies and percentages were determined for descriptive statistics. Associations of *Nrf2* genotypes with PCOS were determined by calculating the odds ratio (OR) and 95% confidence intervals (CIs), and $p \leq 0.05$ was considered statistically significant.

RESULTS

Cases and controls were divided into two groups on the basis of age, 16–30 years and 31–60 years. Mean age of PCOS cases was 35.66±11.12 years, which was not significantly different from that of the control group 33.46±10.95 (p=0.04). Anthropometric characteristics revealed significant differences (p<0.01) for BMI (Kg/m^2), waist (inches), hip (inches), LH/FSH, total testosterone (0.004) values between age groups 16–30 years and 31–60 years in PCOS cases. Phenotypic characteristics including acne were also found to be significantly associated with the two age groups in PCOS cases (p=0.01). Fasting glucose level, systolic and diastolic blood pressure were significantly different between the age groups 16–30 years and 31–60 years in cases. Among controls, significant differences (p=0.01) was found for LH/FSH ratio in the two age groups.

The PCR products size for rs6721961 polymorphism were 113 bp for C allele, 205 bp for A allele and 282 bp for control on a 2% agarose gel. The amplicons derived are shown in Figure-1.

The allele and genotype frequencies were compared between cases and controls. We found that the frequency of CA genotype as well as allele A were significantly different in PCOS cases compared to controls, where the heterozygote genotype CA as well as the variant allele A seemed to confer significant protection against developing PCOS (OR: 0.54, 95% CI: 0.35–0.82, p=0.004, (OR: 0.61, 95% CI: 0.42–0.89, p=0.01) respectively. (Table-3).

Table-2: Demographic, clinical and hormonal characteristics of the study population

Variables	PCOS (n=200)		p	Controls (n=200)		p
Age group (Years)	16–30	31–60		16–30	31–60	
BMI	26.51±4.15	28.48±3.20	<0.001	23.06±1.68	22.96±1.89	0.70
Waist	35.11±2.36	36.57±1.51	<0.001	31.64±2.62	31.79±2.89	0.69
Hip	39.82±3.15	41.28±2.23	<0.001	36.64±2.40	36.88±2.58	0.50
WHR	0.87±0.03	0.88±0.03	0.54	0.85±0.02	0.85±0.03	0.84
mFG	7.69±1.41	7.75±1.89	0.08	3.70±1.81	3.56±1.91	0.60
LH	12.90±4.69	11.64±4.40	0.56	7.14±2.37	7.40±2.48	0.44
FSH	3.38±1.52	3.90±1.66	0.25	4.76±2.42	5.34±2.33	0.88
LH/FSH	4.32±2.14	3.31±1.72	<0.001	1.72±0.66	1.50±0.47	0.01
TT	4.00±0.88	4.43±1.12	0.004	1.64±0.68	1.57±0.69	0.49
SHBG	21.02±17.72	18.86±18.05	0.40	59.39±22.56	63.65±19.84	0.16
Fasting insulin	111.48±45.52	99.66±43.31	0.06	65.72±18.96	64.95±17.26	0.76
Fasting glucose	4.63±0.37	4.82±0.31	<0.001	4.75±0.47	4.64±0.49	0.12
Systolic blood pressure	117.77±8.04	124.48±9.69	<0.001	114.49±10.12	115±9.03	0.44
Diastolic blood pressure	73.39±6.97	79.78±8.65	<0.001	70.96±7.66	72.29±8.38	0.24
Acne	0.17±0.38	0.33±0.47	0.01	0.13±0.33	0.57±0.23	0.08
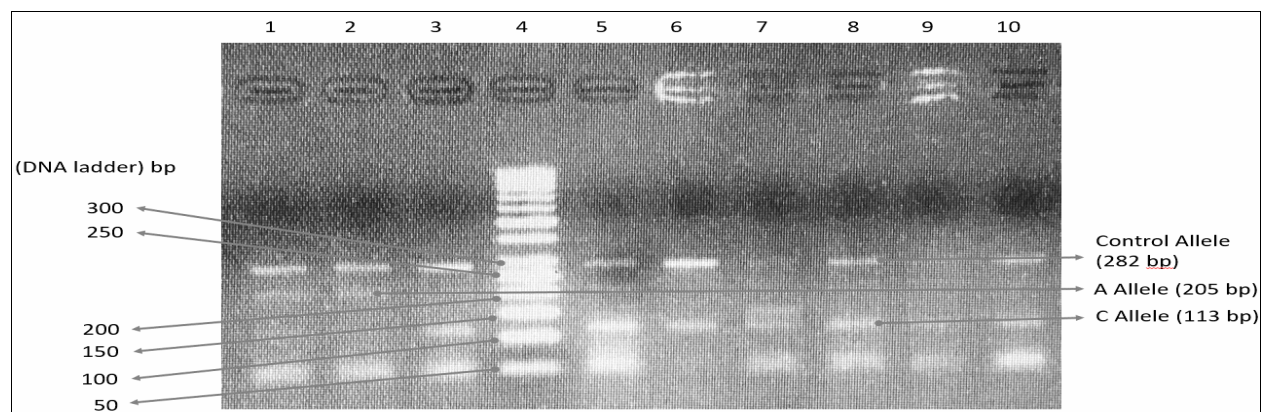
Figure-1: Electrophoretogram on 2% agarose gel showing amplified control, C & A bands separated on electrophoresis

A bands at 205 bp are present in 1st, 2nd, 3rd and 5th wells. C bands at 113 bp are present in 3rd, 5th, 6th, 7th, 8th, 9th and 10th wells.
Nrf2 gene bands at 282 bp are present in all samples as internal control. Gene Ruler TM 50 bp ladder is in 4th well.

Table-3: Association of genotypes of *Nrf2* gene polymorphism (rs6721961) with cases and controls

Genotypes	Controls n=200 (%)	Cases n=200 (%)	OR (95% CI)	p
CC	145 (72.5)	113 (56.5)	Refl	
CA	55 (27.5)	79 (39.5)	0.54 (0.35-0.82)	0.004
AA	0 (0)	8 (4)	-	
Alleles				
C	345 (86.25)	305 (79.43)	0.61 (0.42-0.89)	0.01
A	55 (13.75)	79 (20.57)	-	

DISCUSSION

This study was conducted to see the influence of *Nrf2* gene polymorphism (rs6721961) in Pakistani population to see its possible role in aetiology and pathogenesis of PCOS. To our knowledge, this is the first study to report that -617C/A SNP, which is located in the promoter region of the *Nrf2* gene, is associated with PCOS. Individuals with CA heterozygote had a significantly lower risk of PCOS than those with CC homozygotes. This correlates with the study done by Yamaguchi Y *et al*[16] where patients of renal cell carcinoma carrying CA or AA genotype of rs6721961 at the promoter region, showed elevated expression of *Nrf2* protein as compared to CC genotype. In contrast to this study, Shimoyama Y *et al*[17] suggested that individuals carrying the rs6721961 CA genotype showed increased incidence of acute lung injury.

This study suggests that the SNP of rs6721961 in *Nrf2* gene conferred a protective role against PCOS. However, whether this SNP is a direct causal factor or it is a parallel phenomenon due to linkage disequilibrium with other genetic mutations is not known. It is essential to elucidate the mechanisms by which the rs6721961 polymorphism can exert its protective effect in our population. Evidence suggests that this SNP also put forth protective role against other oxidative linked diseases such as diabetes. A study conducted in Mexico showed that rs6721961 (-617 C/A) polymorphism of the gene *Nrf2* was related to diabetes in male subjects, where A carriers have lower risk of developing diabetes.[18] Jiancheng Wang *et al*[15] also found protective effect of -617C/A (rs6721961) polymorphism for diabetic nephropathy in Chinese Han population. This study highlights the importance of early identification of people at risk to undergo genetic testing for *Nrf2* gene polymorphism (rs6721961) and their careful follow up

for early detection and treatment of the disease in our population. The finding of this study can be combined with other diagnostic modalities currently available to reliably diagnose and manage PCOS patients in Pakistan.

LIMITATIONS

We have genotyped only -617C/A SNP variant of *Nrf2* gene promoter region and we could not eliminate the chance that other SNPs in this region might be associated with PCOS. Further studies are needed to see the combined effect of various SNPs of *Nrf2* gene in PCOS.

CONCLUSION

Nrf2 gene polymorphism (rs6721961) is significantly associated with PCOS among Pakistani population.

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AR: Proofreading and critical analysis
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BK: Data analysis and logical reasoning
TSH: Data analysis and logical reasoning

ORIGINAL ARTICLE

RADIOGRAPHIC COMPARATIVE REGENERATIVE EFFECTS OF HYALURONATE AND PIROXICAM IN OSTEOARTHRITIS MODELS OF RATS

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Background: Osteoarthritis is the most common degenerative joint disease that affects commonly in old age. Standard treatment of this disease is still underdeveloped, however multiple drug groups delay progression or reduce the symptoms. Objective of this study was to assess and compare the regenerative effects of hyaluronate and piroxicam in a rat model of osteoarthritis at the radiographic level. **Methods:** This laboratory-based randomized trial was conducted at Department of Pharmacology Army Medical College, Rawalpindi, from May to July 2019. Resection of the medial meniscus and anterior cruciate ligament resulted in osteoarthritis in the right knee joints of 24 rats. They were separated into 3 groups of 8 rats each. For 4 weeks, groups I, II, and III received intra-articular saline, hyaluronate, and piroxicam, respectively. After one week, radiographs of the anaesthetized rats' matching knee joints were collected. **Results:** Comparison of radiograph of control group with drug treated group confirmed regenerative effects of hyaluronate and piroxicam (p=0.001). However comparison of hyaluronate and piroxicam treated groups had p=0.335 that professed both drug have equal regenerative effects in rat model of osteoarthritis. **Conclusion:** In a rat model of osteoarthritis, intra-articular injection of hyaluronate acid and piroxicam had regenerating benefits at the radiological level. Both administered medicines had similar regeneration effects.
Keywords: Regeneration, Hyaluronate, Piroxicam, Osteoarthritis, Rat model, Intra-articular

INTRODUCTION

Osteoarthritis (OA) is one of the most common chronic joint disorders that is a prominent cause of disability globally. It is a disease of old age with in 10% men and 18% women, aged over of 60 years. Sometimes it also affects individuals in young age with a history of trauma to the affected joint. It is a multifaceted joint disease of degenerative nature, its initiation and progression mechanisms are only partly understood. Abnormal cartilage loss is a conspicuous feature of OA with perpetuated abnormal cartilage restoration and bone remodelling as contributory factors. As OA ages, joint space is also gets narrowed and compromised. Patients of OA usually present with pain (that can be severe to moderate intensity), stiffness and decreased mobility of affected joint.[1,2] Definite treatment of OA is not discovered yet. But there are large range of non-pharmacological interventions (Standard exercises, Yoga sessions, manual therapies, and joint wears) to Pharmacological interventions available to relieve symptoms and slow down the disease progression. Viscosupplement substance, Non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids are three big drug groups that are largely prescribed to patients of OA.[3,4]

Hyaluronate is an endogenous substance that is richly present in articular cartilage and synovial fluid. It consists of repeating β-1,4-D-glucuronic acid and β-1,3-N-acetylglucosamine units. Hyaluronate ensures a significant role in the biomechanics of healthy synovial fluid, where it is to some extent accountable for lubrication, viscoelasticity, shock absorbing and joint structure stabilization.[5] It is one of the favourite investigational viscosupplement used in the management of OA. Intra articular (IA) hyaluronate not only improves joint function but also alleviates pain in patients of OA. Multiple *in vitro*, animal and human researches illustrated regenerative effects of exogenous hyaluronate. Proposed mechanisms by which hyaluronate employs its chondroprotective effects and regenerative effects include increase proteoglycan and glycosaminoglycan synthesis, direct anti-inflammatory effect and viscoelasticity maintenance. Its effects on inflammatory mediators, i.e., cytokoines, prostaglandins and proteases are distinguished. Anti-oxidant property of hyaluronate provides chondrocyte protection against the harm induced by oxygen-derived free radicals.[6]

Piroxicam is an NSAID of Oxicam group. It non-selectively and reversibly inhibits

cyclooxygenase (COX), and thus exhibits anti-inflammatory and analgesic activity by decreasing the synthesis of prostaglandins, prostacyclins and thrombaxanes. Its anti-inflammatory and analgesic properties make it a suitable choice for chronic disease like OA. Besides COX inhibition it diminishes vasodilation in response to bradykinin and histamine. Piroxicam has properties to lessen the central component of nociception. Due to its gastointestinal upset prolonged use of piroxicam is not feasible. Thus intra-articular route of administration is a substitute route for OA patients.[7–9]

Presently there is no definitive cure for OA. Hyaluronate, piroxicam, and a variety of additional viscosupplement substances, and NSAIDs are under research and are used to relieve pain and delay disease progression. The goal of this study was to examine the restorative effects of hyaluronate and piroxicam in murine models of OA and determine whether this medication has regenerative benefits.

MATERIAL AND METHODS

This randomized control study was carried out in a laboratory setting. It was conducted in partnership with the National Institute of Health (NIH) in Islamabad at the Department of Pharmacology and Therapeutics, Army Medical College Rawalpindi. This study was approved by the Ethical Review Committee of the Centre for Research in Experimental and Applied Medicine.

The animal intervention lasted two months, i.e., May to July 2019. The animals were kept in the experimental animal facility of National Institutes of Health. Initially, 24 mature male or non-pregnant female Sprague Dawley rats aged 8–10 weeks and weighing 400–500 g were chosen using a non-probability handy sampling technique. They were randomly assigned to 3 groups, of 8 rats each, the disease control, hyaluronate, and piroxicam groups (Group I, II, and III, respectively). Free access to fresh drinking water and standard rodent feed was provided throughout the study.

Osteoarthritis was induced in the right knee joint of the rats by standard surgical procedure. The rats were anaesthetized with 5% xylazine and 1% ketamine prior to surgery.[10] The skin around the joint was shaved aseptically, and the joint was totally exposed by a para patellar incision on the medial side. Anterior cruciate ligament was explored and transacted followed by identification and resection of medial meniscus. Thereafter, the wound was aseptically closed using a surgical stapler.

After surgery, the animals were allowed 3 weeks to roam freely inside the cage.[11] The rats' disease-induced joint was treated with intra-articular medicines. For four weeks, rats were given 100 μL of saline water, 30 μL of Hyaluronate (HA), and 70 μL of Piroxicam (PIRO) respectively to group I, II, and III respectively.[12–14]

The rats were then given intraperitoneal injections of 10% xylazine and 1% ketamine before being transported to the Radiology Department of a private hospital for radiographs of their knee joints. With the help of radiologist, Kellgren and Lawrence grading system was used to assess the severity of OA. The grading system used by Kellgren and Lawrence is as follows:[15,16]

Grade 0: No OA radiographic characteristics seen
Grade 1: Possible osteophyte lipping and dubious joint space narrowing (JSN) on anteroposterior weight-bearing radiograph
Grade 2: Obvious osteophytes and potential JSN
Grade 3: Multiple osteophytes, confirmed JSN, sclerosis, and potential bone deformity
Grade 4: Large osteophytes, JSN, severe sclerosis, and evident bone deformity

Animals were killed with a lethal dose of chloroform after radiographic grading.

Data was analysed on SPSS-23. ANOVA followed by the Post Hoc Tukey test was used to compare differences in groups considering $p \leq 0.05$ as statistically significant.

RESULTS

Radiographs from illness group I were rated as grade 4, grade 3, and grade 2 on 2 (25%), 4 (50%), and 2 (25%) radiographs respectively. Figure-1 shows an X-Ray image of a control group rat with joint deformity and sclerosis and a grade 3 JSN. The radiograph of group I is characterised by osteophytes and bone deformities.

Following injection of HA, radiographic alterations in group II revealed no OA in 2 (25%), dubious changes in 4 (50%), and moderate changes in 2 (25%) radiographs with grades 0, 1, and 2, respectively. The X-Ray in Figure-2 shows a rat from the HA group with very minor changes, displaying the OA phenotype. This radiograph has a grade of 2. Radiographs of this group indicated no or ambiguous OA changes.

Group 3 received IA piroxicam once weekly for 4 weeks and had small alterations of grade 2 and 1 in half (50%) of the rats and doubtful changes in another half (50%) of the animals respectively. Figure-3 depicts an X-ray of a Group 3 rat with minimal OA changes of grade 2. In this group, majority of the radiographs revealed probable JSN and osteophyte lipping.

ANOVA was used to compare the three groups. HA and PIRO had regeneration effects at the radiographic level ($p < 0.001$ between groups 1 and 2, < 0.001 between groups 1 and 3, and 0.335 between groups 2 and 3). This indicated regenerative effects of both drugs when compared with non-treated group and equal effects when compared with each other.
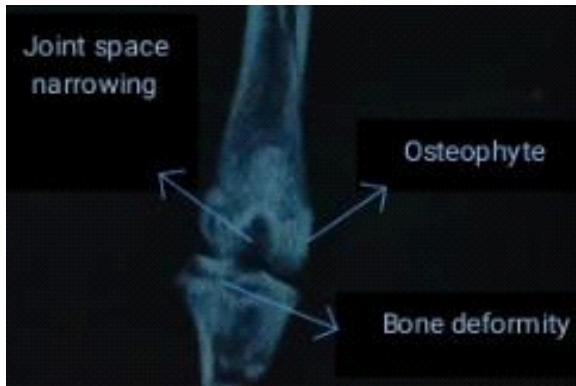
Figure-1: Radiograph of a rat of group 1 (Control)

Figure-2: Radiograph of a rat of group 2 (HA group)

Figure-3: Radiograph of a rat of group 3 (piroxicam group)
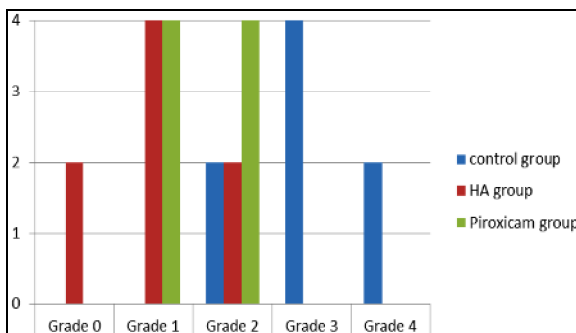
Figure-4: Kellegren and Lawrence grading of OA knee joints of rats of all groups

DISCUSSION

Osteoarthritis (OA) is a disease of old age that can involve any joint of the body but commonly involves hand, knee, hip and spinal facets. Irregular pain is the main problem with which patients usually present. Cartilage degeneration is the pathological factor of OA that is followed by joint inflammation. Different drug groups are used to alleviate symptoms and delay the cartilage degeneration. Viscosuplement substances, NSAIDs and corticosteroids are three major drug groups that are used to manage patients of OA.[17,18] When the treatment groups' radiographic grades were compared to the disease group, significant regeneration benefits of hyaluronate and piroxicam were confirmed (p=0.001). In the case of hyaluronate, Arafat and Kamel's investigation yielded identical findings. In albino rats models, they discovered a substantial (p<0.02) regeneration impact of Hyaluronate.[19] Our findings are supported by Zhiwei Zhang's work who found that hyaluronate decreases radiographic osteophytosis grade (p<0.05) when compared to a saline-treated rat model of osteoarthritis.[20] Our results are corroborated by Li Jung Kang *et al*[21] who established a surgically induced OA mice model and found that HA had regenerative effects when compared to a vehicle-treated group.

Regarding regenerative effects of piroxicam, similar results were found in a study by Park *et al* who reported that there was a statistically significant difference of joint swelling and PGE2 level in IA piroxicam treated rats as compared to IA saline treated rat models of OA.[22] Research work of Aziza[23] revealed that therapy of piroxicam significantly reduces joint oedema and arthritic index in Freud adjuvant induced arthritis models of rat that also supports our findings.

Although both drugs are often prescribed by rheumatologists for the treatment of OA, no *in vitro*, animal, or human studies have yet been undertaken to compare the regeneration benefits of these two drugs. Both hyaluronate and piroxicam had equivalent regeneration effects in rat models of OA after comparing the two groups in our study.

CONCLUSION

As compared to saline treated group, both hyaluronic acid and piroxicam intra-articular exhibit equal regenerative effects in a rat model of OA.

ACKNOWLEDGEMENT

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NI: Study design, Animal intervention
SB: Study design, Critical review
SMQ: Study design, Proof reading
HMIA: Biostatistics, Writing
MWAK: Animal intervention
SJ: Biostatistics

ORIGINAL ARTICLE

SAFETY AND EFFICACY OF A SINGLE-ROD SUB DERMAL CONTRACEPTIVE ETONOGESTREL IMPLANT: SIX MONTH FOLLOW-UP EXPERIENCE IN WOMEN OF REPRODUCTIVE AGE

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Background: Etonogestrel implant is an effective and healthier contraceptive method for women in developing countries because of its convenience, price, long duration, and safety of use while breastfeeding. It has high possibility of return to fertility after removal. The objective of the study was to determine the efficacy in term of frequency of compliance of etonogestrel implant and side-effects in women receiving it as reversible contraception. **Methods:** A descriptive case series was designed and was conducted in Family Planning Centre, Federal Government Polyclinic, Islamabad. The study was carried out in 265 women of age 15–45 years with regular menstrual cycle, normal pelvic and systemic examination, and willing for long term reversible contraception. Non-probability consecutive sampling was used for collection of subjects. After informed consent etonogestrel implant was inserted and followed for its efficacy and side effects. Data were analysed using SPSS-16. Qualitative variables like side effects, efficacy and compliance were measured as frequency and percentage. Quantitative variables like age of patient were measured as Mean±SD. **Results:** Mean age of the patients was 27.86±6.67 years. Compliance to, acceptance, and efficacy of etonogestrel implant as reversible contraception was found in 249 (93.96%) patients while rest of the patients had removed the etonogestrel implant due to any side-effects. **Conclusion:** Etonogestrel implant demonstrated excellent contraceptive efficacy and was well tolerated. The vaginal bleeding pattern was variable and was characterized by relatively few bleeding events, but proved acceptable to most subjects.
Keywords: Etonogestrel implant, efficacy, polymenorrhagia, frequency, acne, amenorrhea
Pak J Physiol 2022;18(3):23–6

INTRODUCTION

Regardless of significant advances in contraceptive methods unplanned pregnancies remain a health problem worldwide. Globally, approximately 208 million pregnancies occur each year of which 41% are unplanned and about 21% results in elective, induced abortion.[1,2] The rate of abortion of unplanned pregnancies can be significantly reduced by proper accessibility and utilization of contraceptives. The United States has one of the highest unplanned pregnancy rate among developed countries. In Pakistan out of estimated nine million conceptions, unplanned and unintended pregnancy rate is 46%, and out of them 54% ends up in intended abortions.[3] Healthier pregnancies and infants can result if the women are properly helped and counselled in planning whether they should have more children and when to have them. This can be achieved by increasing their access to contraceptives. The women are mostly motivated immediately after birth or an abortion, so it's the important time to start contraceptive.[4]

Contraception is an important public health device.[5] It is responsible for playing a major role in decreasing maternal and neonatal morbidity and mortality which often occur as a result of unplanned pregnancy along with the socio-economic burden usually related to it.[6]

Availability of different family planning methods also provides useful substitutes for those having bad experiences with their existing methods in addition to helping those who need and want proper contraception.[7] Currently among the family planning programs the use of hormonal implants has gained considerable attention. Long-acting reversible contraceptives (LARCs) including intrauterine devices (IUDs) and subdermal contraceptive implants are methods that offer successful long-term contraception without any action by the user. Usage of hormonal implants as family planning method has different advantages in terms of long-term effectiveness, availability and improved compliance of user.[8]

Nowadays sub-dermal contraceptive implants are popular contraceptive methods due to reduced need to take protection during sexual activities. The sub-dermal contraceptive implants are of a matchstick size and stimulate release of hormones which prevent pregnancy.[9] The etonogestrel-releasing implant contains 68 mg etonogestrel which is surrounded by ethylene-vinyl-acetate rod which is marketed as Implanon® and Nexplanon® in the United States. Etonogestrel is the biologically active metabolite of desogestrel which is

used in some progestogen-only and combined contraceptive pills. The etonogestrel-releasing implant is presently designed for 3 years of use. Contraceptive implants perform their action by binding to their specific receptors present in various target cells which are dispersed along the hypothalamic-pituitary-gonadal-genital tract axis. The implant interferes with several important processes necessary for gamete union and fertilization. Progestins inhibit ovulation and cause thickening of the cervical mucus.[10] This implant is inserted into the arm, unlike intrauterine devices, and provide contraception for a period of approximately 3–5 years.[11,12]

Insertion of implants is generally even easier than insertion of IUDs, but removal can be more challenging than insertion. Problems with removal tend to occur in rare cases when the implant breaks or is difficult to locate. Self-removal is not a feasible option for implants. After implant removal, women can expect a rapid return to fertility. Within three weeks of removal, ovulation resumes in more than 90% of women.[13]

Mostly women, during the middle age go for subdermal implant for safe and secure long-term reversible contraception in order to avoid surgical or medical intervention used for family planning.[14] However, the women who desire to use this device, must be eligible for it to avoid future hazard of health outcomes. It is contraindicated in liver diseases, blood disorders, allergy, diabetes, hypertension, and pregnancy.[15]

The objective of this study was to determine the efficacy in term of frequency of compliance of etonogestrel implant and side-effects in women receiving it as a method of reversible contraception.

PATIENTS AND METHODS

This descriptive case series study was conducted in Family Planning Centre, Federal Government Polyclinic, Islamabad (FGPC). The duration of study was 6 months from 23 Feb to 22 Aug, 2018. World Health Organization (WHO) software for sample size determination in health studies was used to calculate the sample size. Sample size was calculated with 95% confidence level, 4.5% Population Proportion of amenorrhea, 2.5% Precision, and 5% level of significance.[11,16] Sample size thus calculated was 265.

Non-probability consecutive sampling was used after approval from the Ethical Committee of FGPC Islamabad. Patients were selected from influx of women visiting Out-patient Department in antenatal clinic and Family Planning Centre fulfilling eligibility criteria. After informed written consent, healthy female volunteer of reproductive age (15–45 years) with regular menstrual cycle, normal pelvic and systemic examination, and willing for long term reversible

contraception were selected. Women who were pregnant, diabetic, hypertensive, had liver disease, ovarian or breast cancer, or history of thromboembolism, sexually transmitted disease, and congenital uterine anomalies were excluded. Details about past medical and surgical history were recorded, necessary systemic, and specifically pelvic examination was done. Visiting cards were issued on which date of insertion and date of removal of etonogestrel implant were mentioned for the convenience of patient and doctor. Efficacy was measured by variables like compliance of patient and side-effects in 6 months follow-up.

Data was analysed using SPSS-16. Results were presented in table form. Qualitative variables, i.e., side effects, efficacy, and compliance were measured as frequency and percentage. Quantitative variables like age of patient were measured as Mean±SD.

RESULTS

Mean age of the patients was 27.86±6.67 (15–45) years. The patients were categorised into 3 age groups. Most of the patients receiving etonogestrel implant were in age group 35–45 years. (Table-1).

Acceptability of etonogestrel implant as reversible contraception was 93.96%, while the rest of patients had removed the etonogestrel implant due to side-effects. The side-effects were found only in 16 (6%) of patients. Frequency and percentage of compliance and side-effects in different age groups is shown in (Table-2).

Amenorrhea and polymenorrhagea were the leading side-effects. Parity had no role over efficacy and common side-effects. In primipara women, the efficacy was 94.4%, while in multipara women it was 93.9%. (Table-3).

Table-1: Age-wise distribution of patients

Age (Years)	Frequency	Percentage
15–20	61	23.0
21–30	69	26.0
31–45	135	50.9
Total	265	100.0

Table-2: Compliance and side-effects in different age groups [n (%)]

	Age Groups (Years)		
	15–20	21–30	31–45
Compliance and Acceptability			
Yes	59 (96.7)	65 (94.2)	125 (92.6)
No	2 (3.3)	4 (5.8)	10 (7.4)
Side-effects			
Polymenorrhagia	0	1 (1.4)	4 (3.0)
Amenorrhea	2 (3.3)	2 (2.9)	2 (1.5)
Irregular vaginal spotting	0	0	3 (2.2)
Weight gain	0	0	1 (0.7)
Acne	0	1 (1.4)	0
None	59 (96.7)	65 (94.2)	125 (92.6)

Table-3: Efficacy and common side-effects based on parity of the subjects [n (%)]

	Parity	
	Primipara	**Multipara**
Compliance and Acceptability		
Yes	34 (94.4)	215 (93.9)
No	2 (5.6)	14 (6.1)
Side Effects		
Polymenorrhagia	0	5 (2.2)
Amenorrhea	1 (2.8)	5 (2.2)
Irregular vaginal spotting	1 (2.8)	2 (0.9)
Weight gain	0	1 (0.4)
Acne	0	1 (0.4)
None	34 (94.4)	215 (93.9)

DISCUSSION

In our study 94% participants continued etonorgestral implant at 6 months. This is in agreement with a community-based prospective cohort study by Akilimali PZ et al[17]. That study included 531 subdermal implant users who were 18–49 years old and were followed at 6, 9 and 12 months. Their reported rate of implant removal was 5.5% at 6 months, 8.4% at 12 months, 10.1% at 12 and 20% at 24 months from the date of insertion. Most of the women discontinue due to side-effects (72.3%), most common of which was heavy bleeding (30.0%).[17]

In contrast, lower continuation rates have been reported by Lakha and Glasier[18] which were 89% at 6 months, 75% at 1 year and 59% at 2 years. Commonest side-effect they observed was irregular vaginal bleeding in 27% cases. However, only 5% cases got subdermal implant removed due to this disorder. Prolonged spotting was reported in 23% cases but only 1% cases had implant removed due to this disorder. Amenorrhea was seen in 24% cases but only 4.5% cases got removal due to amenorrhea. Polymenorrhagia was observed in 22.5% and 16% cases got removal due to this.[18] In our study polymenorrhagia was found in 5 (1.9%), amenorrhea in 6 (2.3%), and 3 (1.1%) had irregular vaginal spotting.

Harrison-Woolrych and Hill have reported approximate failure rate of 1 per 1,000 insertions (218 out of 204,486).[19] Pregnancy due to subdermal implant failure has also been reported by Hamontri and Weerkul.[20] One case of ectopic pregnancy following implant failure has been reported by Mansour et al.[21] There was no failure of contraception observed in our study.

Mrwebi KP et al in their descriptive cross-sectional study reported 27.2% discontinuation rate of etonogestrel implant in the first 6 months of use. That study involved 188 women, 67.3% of whom removed the implant in the first year of use. Implant was discontinued by 71.3% of the participants because of side-effects out of which 75 (39.9%) participants had heavy bleeding.[22]

Parkpinyo N et al[23] in a retrospective cohort study involving 1,030 women having etonogestrel contraceptive implant, reported that 1.7% of the women had removal of their etonogestrel implant by 6 months. Most common reason (32%) for early removal was their wish to get pregnancy, and in 49 (22.5%) it was their menstrual disturbances.[23]

Dagnew et al[24] in a facility-based cross-sectional study observed that among 537 women about 37% of etonogestrel implant users had discontinued this method before the planned time. About 86% of them discontinued implant before two years of insertion, and about 13.6% discontinued at 6 months. The reasons for discontinuation in their subjects were the side-effects (mainly polymenorrhagia) followed by a desire for pregnancy.

CONCLUSION

Etonogestrel implant demonstrated excellent contraceptive efficacy and was well tolerated. Compliance to, acceptance, and efficacy of etonogestrel implant as reversible contraception was found in majority of users while only 6% users had removed the etonogestrel implant due to its side-effects. The discontinuation rates can be significantly lowered with sufficient counselling of the women about the expected bleeding pattern before insertion of subdermal implant.

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RIK: Data analysis, References
HB: Tables, Data Interpretation, References
SK: Data entry

Conflict of Interest: None
Funding: None to declare

ORIGINAL ARTICLE

PREVALENCE OF MALARIA IN BLOOD DONORS IN A TERTIARY CARE HOSPITAL IN PESHAWAR

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Background: Malaria is a protozoan parasitic infection of humans resulting from one or more of the five species of the genus Plasmodium. A transfusion transmitted infection (TTI) is a virus, parasite, or other potential pathogen that can be transmitted in donated blood through transfusion to a recipient. The aim of this study was to find out the frequency of malaria in blood donors at a tertiary care hospital in Peshawar. **Methods:** A total of 218 patients were observed in this descriptive cross-sectional investigation from 16 April to 16 October 2021, at Department of Pathology, Hayatabad Medical Complex, Peshawar. Informed consent was obtained from the blood donors justifying the inclusion criteria. Three ml venous blood of each donor was collected in EDTA containing vacutainer. The complete blood count was done on CELL-DYN Ruby® analyzer. Geimsa stained thick and thin blood films were made from each donor blood sample and was examined under ×100 objective lens (oil immersion) using a light microscope for Plasmodium. **Results:** Mean age of the subjects was 32 ± 11.46 years; 88% donors were male and 12% were female. The frequency of malarial parasite was 4% in blood donors. The transmission of malarial parasite was found significant with a history of fever among the blood donors ($p<0.05$). **Conclusion:** The frequency of malarial parasite was 4% in blood donors presenting at a tertiary care hospital of Peshawar, Pakistan.
Keywords: Blood donors, Malaria, Plasmodium, Peshawar, Pakistan

INTRODUCTION

A transfusion-transmitted infection (TTI) is a virus, parasite, or other possible pathogen that can be communicated to a recipient through blood transfusion.[1] Malaria is a protozoal infection of humans caused by one or more of the five Plasmodium species, i.e., *P. falciparum, Plasmodium vivax, P. ovale, P. malariae* and *P. knowlesi*.[2] Malaria can spread through transfusion of cellular components of the blood, and it is responsible for majority of transfusion-transmitted infections worldwide.[3] Prior screening of donated blood ensures safe blood transfusions. The TTIs such as the HIV-AIDS, hepatitis, syphilis, and malaria are all possible with each unit of blood transfused. TTIs are more likely to be transmitted by commercially compensated blood donors than by voluntary donors, posing a serious infection risk. The morbidity and mortality caused by infected blood transfusions have deleterious effects on both recipients and their families.[4]

There has been scarcity of data on distribution and potential impact of different Plasmodium species in transfusion-related malaria cases, particularly among young children and pregnant women who are the most frequent recipients of blood transfusions in Pakistan.[5] In endemic regions, transfusion malaria is very common. The donor may remain infective for years after a malaria infection, i.e., up to 3 years with '*P. falciparum*', up to 4 years with '*P. vivax*', and up to 50 years with '*P. malariae*'.[6]

Infections are more likely in transfusions of blood retained for fewer than 5 days, while transmission is uncommon in infusion of blood preserved for more than 14 days. Malaria, on the other hand, is not reported to be transmitted by frozen plasma.[7] Malaria can spread quickly and cause severe morbidity and mortality when spread through blood transfusion to a non-immune recipient, especially if detection is delayed. Pakistan is classified as a country with moderate malaria endemicity, with a National API (Annual Parasite Index) of 1.08 with considerable variation within and between provinces. *P. vivax* and *P. falciparum* are the only parasite species found, with *P. vivax* accounting for more than 80% of reported cases in the country.[8] The incidence of transfusion-transmitted malaria is about 0.25 cases per million blood units donated by donors.[9] Three hundred (300) blood donors from Jamila Sultana Foundation Rawalpindi were evaluated and screened for the presence of infectious disease in another study. Four (1.6%) of these donors were infected with malaria.[10] The aim of the current study was to find the frequency of malarial parasite in donated blood in a tertiary care hospital.

MATERIAL AND METHODS

This was a single centred, simple cohort study constructed and conducted after the approval of Institutional Review Board of the Hospital and by the College of Physicians and Surgeons Pakistan. The study

was conducted from 16th April to 16th October 2021 at Department of Pathology, Hayatabad Medical Complex. Sample size was calculated using WHO sample size calculator with 3.5% margin of error and 95% confidence interval keeping 7.5% prevalence of malaria in donors.

A total of 218 blood donors, after informed consent, were included as per inclusion criteria and consecutive, non-probability sampling technique was used. The inclusion criteria were blood donor of either gender with age between 18–65 years. A blood donor with body weight less than 50 Kg, history of blood donation within 3 months and pregnant or lactating woman were excluded from the study.

Three mL venous blood of the donor was collected in an EDTA containing vacutainer, after following aseptic techniques of blood collection. The complete blood count was obtained on CELL-DYN Ruby® analyzer. Geimsa stained thick and thin blood films were made from each donor's blood and were examined for malarial parasite under ×100 objective lens (oil immersion lens) using a light microscope.

The demographic and clinical data of all patients including age, gender, address, occupation, and presence or absence of malarial parasite were recorded on a pre-designed proforma. The data were entered and analyzed using SPSS-23. Malaria was stratified among age, gender, a history of blood transfusions, and any history of fever in the previous six months. Chi-square test was applied for determination of association between blood transfusion and history of fever with transmission of malarial parasite, and *p*≤0.05 was considered statistically significant.

RESULTS

Mean age of the subjects was 32±11.46 years. The age distribution among 218 donors showed that 74 (34%) were between the ages of 18 and 30 years, 70 (32%) of donors were between the ages of 31 and 40 years, whereas 52 (24%) donors were between the ages of 41 and 50 years, 22 (10%) donors were in age range 51–65 years. Regarding gender distribution 192 (88%) donors were male and 26 (12%) were female.

Twenty-eight (13%) out of 218 donors had a history of receiving a blood transfusion themselves, and 179 (82%) donors didn't receive any blood transfusion. In the preceding 6 months 48 (22%) donors had a history of fever while 170 (78%) donors did not have fever in the preceding 6 months. Nine (4%) donors had malarial parasite while 209 (96%) donors didn't have malarial parasite in their blood smear. The demographic data is shown in Table-1.

Stratification of malarial parasite with respect to blood transfusions and history of fever in past six months is tabulated as Table-2.

Table-1: Demographic data of blood donors (n=218)

Demographic	Observations	Frequency	Percentage
Age (Yrs)	18–30	74	34
	31–40	70	32
	41–50	52	24
	51–65	22	10
Gender	Male	192	88
	Female	26	12
History of receiving blood transfusion	Yes	39	18
	No	179	82
History of fever	Yes	48	22
	No	170	78
Malarial parasite in blood smear	Yes	9	4
	No	209	96

Table-2: Stratification of malarial parasite with demographic data (n=218)

Observations	Malarial Parasite		*p*
	Yes	No	
History of blood transfusion	2	37	0.73
History of fever	5	43	0.013

DISCUSSION

Because of poor transfusion techniques, 'Transfusion Transmissible Infections (TTIs) threaten millions of people around the globe. Blood and blood component should be analysed to assess the prevalence of TTIs such as HIV, HBV, HCV, syphilis, and malaria in the blood donors.[11] This ensures safety of the blood transfusion. There has been a paucity of information concerning the distribution and potential role of the different Plasmodium species in transfusion-related malaria cases.[12] Most infections occur in case of transfusion of blood stored for less than 5 days and it is rare in transfusions of blood stored for more than 2 weeks. Frozen plasma is not known to transmit malaria. The blood transfusion system in Pakistan is decentralized, demand-driven, and primarily based on uncontrolled transfusion techniques. This is especially prevalent in small towns and rural areas.[13]

Recently, Naeem *et al*[14] found that 0.1% of blood donors had malarial parasite in the northern region of Pakistan. However, in current study, malarial parasite was detected in 4% blood donors. Olawumi *et al*[15] have reported malaria parasitemia in 27.3% of blood donors in Ilorin, Nigeria. *Plasmodium falciparum* was identified in greater numbers (85.7%) than *Plasmodium malariae* (14.3%). Malaria parasitemia did not differ by age or gender (*p*>0.05). However, the malaria parasitemia was reported to be higher in donors with blood group 'O' than the donors with blood groups A and B.[16] Another study from Kaduna, Nigeria reported 27 (7.5%) positive cases of malarial parasites among the blood donors; only the *P. falciparum* was found while no other parasite was reported in the donated blood.[17] Bahadur *et al*[18] found only 3 (0.03%) blood unites infected with malarial parasite upon malaria rapid detection test (RDT) of 11,736 units of donated blood.

The statistically insignificant higher seroprevalence of malaria antibody in donors having history of fever within the last 3 months (22%) as compared with that in normal donors (16.9%) does not provide enough evidence at this stage to prove or disprove usefulness of such criteria, and results need to be confirmed on a larger sample study to prevent unnecessary donor deferrals. Dubey *et al*[19] have reported that in patients suffering from thalassemia with history of fever, the prevalence of malarial antibodies was 17.4%. In our study, 22% of the blood donors had a history of fever in the past six months. However, only 5 blood donors had a history of fever with malarial parasite detected in their blood.

CONCLUSION

The frequency of malarial parasite was found to be 4% in blood donors presenting at a single centre. A significant association was found between the transfusion related transmission of malarial parasite and history of fever in the blood donors. Large, multi-centre study is recommended to elaborate our results in general population.

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ORIGINAL ARTICLE
NOMOPHOBIA, SLEEP DEPRIVATION AND LEISURE ACTIVITIES IN ADOLESCENTS AND YOUNG ADULTS

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Background: Nomophobia is mobile phone addiction, characterized by an intense and irrational fear of being without a mobile phone, which affects the sleep and leisure activities of youth. The study examined the relationship between nomophobia, sleep deprivation, and leisure activities. It also measured the mediating role of nomophobia between sleep deprivation and leisure activities and find out demographic differences (age, gender, education, socioeconomic status) on nomophobia, sleep deprivation, and leisure activities. **Methods:** Conveniently selected sample of 500 adolescents and young adults from Khyber Pakhtunkhwa, after informed consent, was measured on Nomophobia Questionnaire, Athens Insomnia Scale, and Pittsburg Enjoyable Activities Scale. The data were collected from March to August 2019. The sample was further divided based on age (adolescents 250) (young adults 250). **Results:** Nomophobia has a significant positive correlation with sleep deprivation and has a significant negative correlation with leisure activities. Nomophobia significantly mediated between sleep deprivation and leisure activities. Significant differences of age, socio-economic status, and education on nomophobia, sleep deprivation, and leisure activities were found. Furthermore, significant gender differences were found on nomophobia and leisure activities, while non-significant gender differences were concluded on sleep deprivation. **Conclusion:** Nomophobia and sleep deprivation are greater in adolescents who belong to lower social class, while involvement in leisure activities is higher in young adults who belong to upper social class. Females have a higher level of nomophobia and involve less in leisure activities than males.

Keywords: Nomophobia, sleep deprivation, leisure activities, adolescents

INTRODUCTION

As the world's digital transformation continues, we now live in an era of wireless communication. The smartphone has a significant impact on human existence as soon as it was invented. Nomophobia has also been referred to as mobile phone dependency or addiction.[1] Nomophobia is a modern-day condition that has only lately been used to explain the stress or anxiety induced by the absence of a mobile phone (MP), personal computer (PC), or any other online communications system in those who use them regularly.[2]

Yildirim and Correia, on the other hand, stated that it is a dread of not being able to utilize a smartphone or a mobile phone and/or the services it provides.[3] It refers to the dread of not being able to communicate, of losing the connectivity that smartphones offer, of not being able to access information via smartphones, and of foregoing the ease that smartphones provide. Adolescents are more likely to experience nomophobia.[4] Females have a higher level of nomophobia than males.[5] A review of the literature revealed that after age and demographic factors, educational degree was the most efficient predictor of nomophobia levels. It was discovered that as the degree of knowledge increases, the amount of nomophobia experienced by young individuals is

reduced. Individuals from the lowest socio-economic status are more likely to be smartphone addicts.[6]

Insufficient sleep and sleeplessness are other names for sleep loss. It's a disorder that develops as a result of insufficient sleep. A person is at risk if they have any of the following: sleep condition that stops someone from obtaining enough sleep or causes poor quality sleep, affecting the capacity of that person to do their daily tasks and feel good when they are awake depends on how much sleep he or she gets in total, as well as how much of each kind of sleep he or she gets, also relies on whether or not he sleeps at a time when his body is ready and willing to do so.[7]

Adults need 7 to 8 hours of sleep each night to be in excellent health. Lack of sleep may lead to a variety of health issues, including sluggishness, sleepiness, tiredness, decreased immunity, physical weakness, and impaired memory. Acute and chronic sleep deprivations are the two types. Acute insomnia occurs when a person misses one or more nights of sleep. The chronic type is when a person misses out on those seven (or more) hours per night consistently (weeks or even months). As compared to their younger counterparts, elderly individuals spend more time in bed but are sleepless, and they have a greater amount of sleep fragmentation, which is often followed by daytime drowsiness. Other research has shown that sleep duration tends to grow with age, according to the

findings.[8] Exploring gender differences in sleep quality, higher prevalence of poor sleep quality is found in females than males.[9] Studies have shown that the economic situation and family income are associated with sleep deprivation.[10] Also, the literature showed sleep complaints are reported more in adults with lower levels of education, unemployment, or those living in poverty.[11]

Leisure is the time spent on activities other than work or obligations related to one's job or one's self that is not required for basic maintenance or self-care. With leisure, you have the freedom and flexibility to do anything you choose, and it can be utilized in several ways to fulfil your requirements for contemplation, self-enrichment, and relaxation. When people have spare time in the evenings or weekends, they engage in extracurricular facilities. They're never focused on work and don't include activities like cleaning the home or sleeping. Biking, bowling, curling, horseback riding, golfing, hiking/walking, skating, skiing, and swimming are some examples of leisure activities. Active leisure and passive leisure are the two types of leisure available. Activities such as volunteering, hobbies, and exercise all fall under the category of active leisure since they require both exerting physical and mental energy. These activities often take place outside of the house and with other people. Passive leisure is time spent resting and requiring minimal effort at home, such as reading or watching television.[12]

Several theories have been proposed on nomophobia and sleep deprivation. Recently mother-infant attachment theory of Bowlby was extended to attachment of humans to others objects such as mobile phone.[13] This attachment has different styles: secure, anxious and avoidant, and two of these styles anxious and avoidant attachment are related to problematic use of technology, mobile phones, internet, social media and video games. This may lead towards anxiety and problematic behaviors.[14] A previous longitudinal study developed a model on nomophia by concluding that nomophobia has strong positive correlation with sleep deprivation.[15]

No published work specifically in Pakistan is available on relationship of nomophobia with sleep deprivation and leisure activities. The purpose of the present study was to find out the association between nomophobia, sleep deprivation, and leisure activities in adolescents and young adults. The current study will be beneficial for clinical psychologists and other health workers by providing information about these variables.

METHODOLOGY

This was a correlational and cross-sectional study. A survey method was used to collect data. A sample of 500 participants (220 men and 280 women) was selected from different colleges and universities of Khyber Pakhtunkhwa with convenient sampling technique.

A Demographic Sheet including demographic variables like age, gender, education, socio-economic status and institutes was used. Nomophobia was assessed by Nomophobia Questionnaire (NMP-Q); Cronbach's alpha of NMP-Q is 0.91.[3] Sleep difficulty was measured by Athens Insomnia Scale; the alpha reliability ranges from 0.87 to 0.89.[16] Pittsburgh Enjoyable Activities Test was used to measure leisure activities. The alpha reliability of this scale is 0.73.[17]

Ethical approval from the Ethical Review Board of the Psychology Department, Hazara University and relevant authorities was obtained. The required information was gathered through a demographic sheet that included age, gender, socio-economic status, and education. After written informed consent and instructions, Nomophobia Questionnaire, Athens Insomnia Scale, and Pittsburgh Enjoyable Activities test were used for individual participants.

RESULTS

The sample was divided on the basis of age (adolescents 250 (50%), young adults 250 (50%), educational level (undergraduate 298 (59.6%), postgraduate 202 (40.4%), and socio-economic class (lower class 150 (30%), middle class 208 (41.6%), upper class 142 (28.4%). Alpha values for NMPQ, AIS, and PEAT were 0.91, 0.80, and 0.82 respectively. Significant item-total correlation of all of these three scales indicated that all of these scales have a satisfactory level of construct validity.

Table-1 shows that nomophobia has significant positive correlation with sleep deprivation and has significant negative correlation with leisure activities. Sleep deprivation has significant negative correlation with leisure activities. The strength of relationship exists between sleep deprivation, and involvement in leisure activities was altered because of mediating effect of nomophobia.

Sleep deprivation was indirectly related to involvement in leisure activities, through its relationship with nomophobia. Sleep deprivation reported high relationship with nomophobia (a=3.6889, p=0.001). There was significant negative association of nomophobia with involvement in leisure activities (b= -0.0836, p=0.001). Result also showed significant negative impact of sleep deprivation on involvement in leisure activities (c= -0.4933, p=0.001). The indirect effect (ab= -0.3085) was totally above zero (-0.4322 to -0.1993). Sleep deprivation lead to less involvement in leisure activities after taking into account sleep deprivation indirect effect through nomophobia (C′= -0.8018, p=0.001) (Table-2).

Table-3 shows significant differences in scores of adolescents and young adults on nomophobia, sleep deprivation, and leisure activities. Nomophobia and sleep deprivation are greater in adolescents, while involvement in leisure activities is greater in adults.

Table-4 shows significant gender differences in nomophobia and leisure activities, while non-significant gender differences on sleep deprivation. These results indicate that nomophobia is greater in females while leisure activities are greater in males.

Table-5 indicates significant differences of socioeconomic status on nomophobia questionnaire, Athens Insomnia Scale and Pittsburg Enjoyable

Activities Test. Nomophobia and sleep deprivation are greater while involvement in leisure activities is less in people who belong to lower socio-economic class as compared to other classes.

Table-1: Correlation matrix for nomophobia, sleep deprivation and leisure activities among adolescents and adults (n= 500)

Scales	1	2	3	Mean±SD
Nomophobia	-	0.48*	-0.39*	91.22±28.77
Sleep deprivation	-	-	-0.35*	5.83±3.76
Leisure activities	-	-	-	18.77±8.46

*Significant

Table-2: Summary of mediation analysis for leisure activities with sleep deprivation as independent variable and nomophobia as mediating variable

DV	M	Effect of IV on M	Effect of M on DV	Direct effect	Indirect effect	Total effect	LL	UL
Leisure Activities	Nomophobia	3.6889*	-0.0836*	-0.4933*	-0.3085*	-0.8018*	-0.4322	-0.1993

DV= dependent variable (leisure activities); IV= independent variable (sleep deprivation); M= mediating variable (nomophobia); *$p<0.001$

Table-3: Mean±SD and *t*-values of adolescents and adults on nomophobia, sleep deprivation and leisure activities

Scale	Mean±SD		*t* (498)	*p*	CI 95%		Cohens' d
	Adolescents (n=250)	Adults (n=250)			LL	UL	
NMP	101.84±25.88	80.60±27.61	8.87	0.001	16.53	25.94	0.79
SD	6.71±3.85	4.96±3.468	5.352	0.001	1.11	2.40	0.47
LA	16.68±8.68	20.86±7.70	-5.68	0.001	-5.61	-2.73	0.50

Note. NMP=Nomophobia; LA= Leisure Activities; SD = Sleep Deprivation.

Table-4: Mean±SD and *t*-values of gender on nomophobia, sleep deprivation and leisure activities

Scale	Mean±SD		*t* (498)	*p*	CI 95%		Cohens' d
	Females (n=280)	Males (n=220)			LL	UL	
NMPQ	93.54±28.54	88.37±28.87	1.99	0.04	0.08	10.25	0.18
SD	5.77±3.57	5.92±4.01	-0.44	0.65	-0.81	0.51	0.03
LA	18.10±8.48	19.57±8.37	-1.99	0.04	-3.01	-0.02	0.17

NMP=Nomophobia; LA=Leisure Activities; SD=Sleep Deprivation

Table-5: One-way ANOVA for difference of scores of socio-economic classes on nomophobia, sleep deprivation and leisure activities (n=500)

Variable	Mean±SD			F	p	Tukey's Post Hoc	η²
	Lower class (n=150)	Middle class (n=208)	Upper class (n=142)				
NMPQ	97.68±26.10	93.29±27.05	81.35±31.40	13.30	0.001	1>2>3	0.050
SD	6.61±3.74	6.18±3.73	4.51±3.52	13.48	0.001	1>2>3	0.051
LA	16.22±8.70	18.22±7.85	22.26±7.97	20.84	0.001	3>2>1	0.077

NMP=Nomophobia; LA=Leisure Activities; SD=Sleep Deprivation

DISCUSSION

The findings of the current study indicate a significant positive correlation between nomophobia and sleep deprivation and a significant negative correlation between nomophobia and leisure activities. The findings of the current study are in line with previous researches. Peszka[18] reported that the severity of nomophobia was correlated with the severity of maladaptive sleep behaviours. Another research concluded that individuals practicing physical and recreational leisure activities have less smartphone addiction/nomophobia.[19]

Nomophobia is a significant mediator in the relationship between sleep deprivation and leisure activities. A study concluded that mobile phone addiction (nomophobia) impacts a person's leisure time.[20] Sleep problems lead to less leisure time physical activities.[21]

Adolescents and young adults scored significantly different on nomophobia, leisure activities,

and sleep deprivation. Previous research has found that adolescents are more prone than adults to feel nomophobia, or fear and worry about being without their phone.[4] Another survey found that older people spend more of their leisure time watching television, reading, relaxing, and thinking than those between the ages of 15 and 19. The findings of previous research suggest that sleep deprivation has grown increasingly common in the adolescent years, the years between the age of 13 and 18, and include the time before, during, and after puberty.[22]

There were significant gender differences in nomophobia and leisure activities, but not in sleep deprivation. Females have a higher level of nomophobia and engage in fewer leisure activities than males. These findings are aligned with the findings of a previous study.[23] Regarding non-significant gender differences on sleep deprivation, one of the previous studies reported that the overall prevalence of insomnia was 26.6%, with little difference between males (26.3%) and females

(27.0%).[24] The previous studies have contradictory findings regarding gender differences in sleep deprivation. Women reported more sleep loss than men.[25] A recent study found that men are more sensitive to sleep deprivation than women.[26] One possible reason for non-significant gender differences can be the changes in life style and social roles of both genders. Currently life style of females has changed; both males and females are employed now-a-days and have numerous responsibilities they need to handle. This reason may lead to sleep deprivation equally in males and females.

The findings of the current study revealed significant socioeconomic differences in nomophobia, leisure activities, and sleep deprivation. People from lower socioeconomic origin have a higher level of nomophobia, engage in fewer leisure activities, and have shorter sleep durations and more sleep issues than people from higher socioeconomic background. The findings of the current study are in line with previous research which suggested that the students with the lowest socioeconomic status were more likely to be smartphone addicts or nomophobic.[27] Another research concluded that individuals with lower level of socioeconomic status experience short sleep duration and higher sleep disturbances.[28]

LIMITATIONS AND SUGGESTIONS

The current study was conducted in limited areas of Pakistan. Future researchers on this topic should include other areas of Pakistan too. We studied only studied age, gender, socio-economic status, and educational differences on nomophobia, sleep deprivation, and leisure activities, while ignoring other demographic variables. Future work should include other demographic variables like work status, marital status, etc. too. The current study only looks into the connections between nomophobia, sleep deprivation, and leisure activities. It is proposed that future studies look into the causal inferences for these correlations. Other psychological variables, which can affect these variables, should also be used in further studies.

IMPLICATIONS OF THE STUDY

Our findings provide a significant contribution to the field of clinical psychology by providing information about nomophobic subjects and their problematic sleep patterns. The findings of this study can help psychologists in developing preventative and interventional strategies for this population. The main goal of these interventions should be to raise awareness of the negative psychological effects of nomophobia and problematic phone use on one's health, everyday life, and social activities, as well as to improve human interaction. Also, the findings obtained from the current study have added to the existing literature on nomophobia.

CONCLUSION

Nomophobia and sleep deprivation are greater in adolescents who belong to lower social class, while involvement in leisure activities is higher in young adults, who belong to upper social class. Females have a higher level of nomophobia and involve less in leisure activities than males.

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AZ: Conceptualization, Rationale and Literature review
HB: Objectives, Hypothesis and Methodology
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SNA: Discussion, Limitation, Suggestions and Implications

ORIGINAL ARTICLE

ASSOCIATION BETWEEN AND COMPARISON OF SELF-EFFICACY AND CURIOSITY AMONG VISUALLY IMPAIRED PATIENTS: A CROSS-SECTIONAL STUDY

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Background: The objective of this study was to find out the relationship between self-efficacy (SE), Curiosity and Demographic variables among visually impaired (VI) patients, and to assess the difference on SE and Curiosity among VI patients from rural and urban areas. **Method:** This was a cross-sectional study. A sample of 100 visually impaired patients (50 males and 50 females) was selected through purposive sampling technique. General self-efficacy scale, and curiosity and exploration inventory were used to measure SE and curiosity among study participants. Pearson product moment correlation, regression analysis, and independent sample *t*-test were run for data analysis. **Result:** The results depicted a significant correlation among curiosity, self-efficacy, gender, age, residence area, socioeconomic status (SES), and visual impairment. Regression analyses for patients of VI showed that curiosity and residence were important predictor of SE. The patients living in urban areas had a significantly high level of SE and curiosity as compared to those living in rural areas. **Conclusion:** Curiosity, SE, demographic variables and VI are associated with each other. SE can be predicted by curiosity and residence in patients with VI. Significant differences exist in VI patients in SE and curiosity with reference to their areas of residence.

Keywords: Visual impairment, curiosity, self-efficacy, association, blindness, low vision, urban, rural

Pak J Physiol 2022;18(3):35–8

INTRODUCTION

Visual impairment (VI) was classified into two groups by International Classification of Diseases that are: distant vision impairment and near vision impairment. Worldwide, the major causes of VI are: trachoma, diabetic retinopathy, uncorrected refractive errors, age-related macular degeneration, cataract, glaucoma, corneal opacity.[1] According to a nationwide survey by Jadoon *et al*, the prevalence of VI in Pakistan in the age of 30 years and above is about 2.7%. This survey described that 1.7% million VI are adults in Pakistan. Almost 86% of the patients suffer from avertable causes of VI while 14% adults suffer from totally blindness. The result of this survey depict that younger adults hurt expressively less from VI as equated to older people.[2]

Self-efficacy (SE) is operationally well-defined as a person's believe to be able to achieve his/her goals and have ability to perform a given task, people who realize that they have capacities and able to successfully plan their activities are those who have high level of SE, while persons who are not able to realize their abilities and unable to perform their assignment are those who have low level of SE.[3] A study indicated that SE and perceived social support are those two factors which can help to diminish the effects of VI in academic goal achievement.[4] Due to their vast command of how to manage their condition, people with visual impairment exhibit more self-efficacy than the general population. Self-efficacy appears to be

crucial for living the greatest life possible.[5] Regarding the practical influence on day-to-day activities such as personal and household management, travel reading and writing, career and leisure, the direct effectiveness of the visual loss has been explored. The degree to which there is a lack of or loss of independence in any of these areas affects the levels of self-esteem for either congenitally blind or adventitiously blind people.[6]

Curiosity, the craving to identify, is a motivating distinctive. Curiosity consists of several related elements, counting craving exploration for its own sake, scarcity kindliness or wanting to decline slits in information, and strain acceptance at what time confronted with innovation.[7] Curiosity is an imperative motivational factor that associate indications reflecting innovation and contest with growing prospects. A prime moderator of individual development is understanding to its fundamentals. Curiosity prods energetic, planned actions in answer to incentives besides action through the subsequent belongings: innovation, difficulty, indecision, and struggle.[8,9]

Age and educational level are identified as the most significant factors connected to visual impairment. That study offered the first information about the prevalence and causes of visual impairment in Taiwan.[10] With age, the main contributing factors to visual impairment change. For the early diagnosis and treatment of eye diseases as well as, where necessary, the referral for rehabilitation, recognition of these patterns is essential.[11]

Strong gender inequalities have been observed, with higher prevalence of visual impairment and associated cataracts in women, which may be related to gender inequalities in access to health care, and underdiagnosis in men. Glaucoma with undiagnosed eye disease may be related to gender socialization. It leads to lower utilization and effectiveness of health care services.[12] The objectives of this study were to find out the relationship between SE, Curiosity, and Demographic variables among visually impaired patients, and to assess the difference on SE and Curiosity among VI patients from rural and urban areas.

METHODOLOGY

This was a cross-sectional study conducted from 1st Oct 2019 to 30th Feb 2020 in Faisalabad, Pakistan. The purposive sample of 100 VI patients was collected from the Madina Teaching Hospital, Faisalabad with equal number of males (n=50) and females (n=50) with mean age 47.12±16.09. Post hoc power analysis was done using G*Power-3.9.1.2. Input parameters for two-tail independent sample t-test were effect size of 0.8, alpha 0.05, and sample size 50 for each group. Based on the above-mentioned assumptions, desired sample size 100 contained a statistical power (1-β err prob) 0.99 with critical t=1.66. Input parameters for one-tail point biserial model were effect size of 0.5, alpha 0.05, and total sample size 100. According to the above-stated expectations, the anticipated sample magnitude 100 comprises a statistical power (1-β err prob) 0.99 with critical t=1.66.

Inclusion criteria were the adolescent and adults with VI who had been taking treatment and willing to participate. Small children under 12 year age were not eligible even if they were taking treatment from a hospital.

Curiosity was examined using 10-items curiosity and exploration inventory (Kashdan *et al*[13]). Participant answers on the 5-point Likert scale ranging from 1 (very slightly) to 5 (extremely). The score ranged between 10 and 40. Reliability of this scale is 0.85.[14]

Self-efficacy was examined using the 10 items of General self-efficacy (Ralf Schwarze[15]). The score range of general self-efficacy is 10–40, maximum score describes more self-efficacy. It is a four-point likert scale in which 1 is a minimum response rate and 4 is a maximum response rate on each item of the self-efficacy scale. Urdu version of General self-efficacy was used in this study with reliability range from 0.76 to 0.90 in different studies[15,16].

Informed consent was obtained from all participants. Demographic information was recorded for each subject and they completed the scales. Questionnaires were administered to each participant and help offered in any difficulty in filling the proforma. Results were analysed using SPSS-21.

RESULTS

The demographic information is tabulated in Table-1. The reliability index and Pearson product moment correlation coefficient is given in Table-2. The results depicted a significant correlation among Curiosity, SE, gender, age, residence area SES and VI.

In Table-3 regression analysis is summarised. Curiosity and residence are important predictors of SE. A multiple regression analysis run to predict SE from Curiosity and residence. These variables statistically significantly predicted SE, $F_{(2, 97)}$=12.814, p<0.0001, R^2=0.209. All variables added statistically significantly to the prediction, p<0.05. SE was outcome variable, whereas curiosity and residence were the predictor variables.

Table-1: Demographic characteristics (n=100)

Variable		Number	%
Age	14–50	73	73
	51–85	27	27
Gender	Female	50	50
	Male	50	50
Residence	City	71	71
	Village	29	29
Family System	Neutral	58	58
	Joint	42	42
Treatment	Government	28	28
	Private	72	72
Other Disease	Yes	33	33
	No	67	67
Heredity	Yes	57	57
	No	43	43
Occupation	Business	36	36
	Job	46	46
	Student	18	18
Education	Graduation	39	39
	Intermediate	17	17
	Matric	44	44

Table-2: Correlation between SE, Curiosity, and demographic variables (gender, age, residence area VI and SES)

Variables	SE	Curiosity	Gender	Age	Residence	VI	SES
SE	1	0.45**	0.01	-0.07	-0.21*	0.03	0.12
Curiosity		1	0.03	-0.32**	-0.36**	-0.23*	0.23*
Gender			1	0.29**	-0.11	0.06	-0.12
Age				1	0.11	-.03	-0.01
Residence					1	00.07	-0.31**
VI						1	0.01
SES							1
Cronbach's Alpha	0.87	0.85	–	–	–	–	–
Mean±SD	30.43±5.7	31.62±7.91	–	–	–	–	–

**p<0.01, SE: Self-Esteem, VI: Visual impairment, SES: Socioeconomic status

Table-3: Summary of linear regression analysis of residence and curiosity as predictors of self-efficacy

Variable	Model		
	B	SE	B
Curiosity	0.314	0.070	0.435*
Residence	-0.680	1.212	-0.054
R^2	–	0.209	–

*$p<0.05$

Student's t-test was applied to find out the differences across residence. The results in Table-4 describes the significant difference ($t=2.140$, $p<0.05$) on SE among those who live in urban and rural area. Same findings exist for Curiosity where ($t=3.834$, $p<0.0001$) significant difference found among rural and urban patients with VI. Participants living in urban area have higher level of SE and curiosity as compared to participants living in rural areas.

Table-4: Independent sample t-test for residence on SE and MHI

Variable	Residence	n	Mean±SD	t	p	Cohen's d
SE	Urban	71	31.20±5.474	2.140	0.035	0.46
	Rural	29	28.55±5.932			
Curiosity	Urban	71	33.44±7.287	3.834	0.000	0.84
	Rural	29	27.17±7.723			

DISCUSSION

The current study aimed to examine the association amongst Curiosity and SE as well as to explore the difference in Curiosity and SE across the area of residence. There were significant differences across the residence on SE among VI patients. It depicts that those who live in cities have different level of SE compared to those who live in villages. Another study also concluded that countryside patients had low level of SE than the city dwellers.[17]

According to Almeida *et al*[18], there was an optimistic relationship of curiosity and SE on the population of the healthy and the drug addict people which support our findings that the relationship of curiosity and SE is optimistic. Further findings of the study showed that residence is a noteworthy predictor of SE. Another study[19] claimed that SE and curiosity both are positively linked among internal medicine residents. Tthis positive link indicates prediction of SE from residence. This important finding leads to explore further that it varies across different context. Which factors are associated that cause prominent level of SE with VI patients who lived in city as compared to those who live in village? A recent study from China confirms that SE in adult students from rural areas is lower than the urban students.[20] Reasons may vary; may be because of their lifestyle or the patients who live in city area have more facilities or may be other geographical reasons. In a Japanese study on older adults with VI, the performances of multifaceted actions reduced in precise categories, nonetheless completely, and this may cause by poor agility and more submissive attitudes in their

routine actions.[21] Level of curiosity may also differ according to level of VI. A study from last century stated that the test of perceptual curiosity disclosed the sighted had higher level of curiosity than the blind.[22]

CONCLUSION

The results depicted a significant correlation among Curiosity, SE, gender, age, residence area SES and VI. Curiosity and residence as important predictors of SE. People with VI living in urban areas have elevated level of SE and curiosity compared to people living in rural areas.

ACKNOWLEDGMENT

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ORIGINAL ARTICLE

COMPARISON BETWEEN ONLINE AND ON CAMPUS CBL SESSIONS: PERCEPTIONS OF STUDENTS IN HITEC-IMS, TAXILA

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Background: Medical colleges and educational institutions around the world underwent a major shift of teaching methodology from regular classroom environments to virtual classrooms during the COVID-19 pandemic. While many instructional methodologies were easily conducted in both formats, interactive activities requiring group participation like CBLs presented a big challenge. The objective of this study was to compare Online and On-campus formats of CBL sessions through students' feedback and identify the areas in both formats that require amelioration. **Methods:** A cross-sectional, descriptive study was conducted at HITEC-IMS, Taxila including 185 students from 1st year and 2nd year MBBS who had attended at least 4 CBL sessions online and on campus formats. The questionnaire comprised of statements evaluating 5 major constructs: Acquisition of knowledge (AK), Critical thinking (CT), Communication skills (CSKILL), Presentation skills (PS), and Physical Environment (PE). Responses were assessed on a 5-points Likert scale. Data was analysed on SPSS-28. The two formats were compared using Chi-square test, and $p \leq 0.05$ was considered significant. **Results:** Responses for acquisition of knowledge (74.6% *vs* 69.2%) and critical thinking (75% *vs* 70%) were comparable for both formats while online format was lacking in attainment of communication skills (67% *vs* 80%) and presentation skills (56.8% *vs* 71.4%). **Conclusion:** Respondents perceived On-campus format as a better option for conduction of CBL.

Keywords: CBL, Online format, On-campus format

INTRODUCTION

The concept of virtual learning is known since 1990 and is evolving rapidly with advancement in digital technologies. Online teaching and learning has been promoted by many countries[1] for years but its implementation in educational institutes especially in the developing countries remained a challenge. The issues of administration, infrastructure requirement, access, availability of internet facility and lack of trained staff have been a major setback.[1,2] Keeping the students motivated and inclined to use e-learning materials have been a problem at individual student level.[3,4]

The current situation regarding the novel coronavirus (COVID-19) pandemic has led the educational institutes worldwide to shift their teaching and learning from traditional to online formats.[5] This has proved to be a testing time for developing countries like Pakistan, as many medical institutes were not prepared for such a drastic change in their teaching methodology.[6,7] There was a lack of digital expertise at the universities following traditional On-campus teaching programs. Apart from the technical and financial difficulties encountered the students and faculty faced social and mental health issues.[8]

Case Based Learning (CBL) has always been an active small group learning tool in medical schools.[9] This tool evokes clinical reasoning, critical thinking and problem solving along with building generic skills like communication skills, time management and leadership among the medical students.[10] Active learning occurs

when students interact with each other.[11] In HITEC-IMS, Taxila, Case Based Learning has been an effective teaching as well as learning approach for all basic sciences. CBLs used to be organized in 4 to 5 groups where students were provided case scenarios in advance along with their learning objectives. A pre-CBL meeting was conducted before each CBL among faculty members in order to keep the essence of the discussion on the same lines while facilitating the designated groups. The CBL group members assigned their team leader, scribe, and time-keeper. The leader involved all group members in the discussion under supervision and guidance of a faculty member as a facilitator.

The group interactions were marked by the instructor on an assessment form developed by our department which appraised their preparation, hypothesis generation, keywords identification, interpersonal skills and time management as well. A post CBL test followed the dialogue and debate. Such forums conducted in physical classrooms enhance intercommunication and establish equal and just environment for active participation.[11] The pandemic restricted this one-to-one interaction.

At HITEC-IMS, the undergraduate program was in mid-session when the students had to vacate the campus due to COVID-19 pandemic and the whole teaching schedule was shifted online.[12] This shift to online format mandated by the medical institute resulted in transitioning from On-campus to Online CBLs. The utility of this tool is, however, controversial. The data on

studies addressing the effectiveness of online CBL as teaching strategy is scarce.[13] Only a few studies have evaluated the effectiveness of teacher-student interaction and students assessment through online format. The CBL assessment is multidimensional, where students are assessed not only for their knowledge but generic skills like leadership, presentation and communication skills. At HITEC-IMS, the CBL are designed not only for acquisition of learning outcomes but also to achieve these generic skills which is represented in our CBL assessment form (Figure-1). This study will enable us to identify the areas that need improvement in both formats. This study was designed to analyse and compare the perceptions of students regarding both formats of Case Based Learning sessions through feedback and to identify the areas that need improvisation for use in future as an effective learning methodology.

		SUMMATIVE ASSESSMENT					**FORMATIVE ASSESSMENT**				
SR #	**Student ID/Name**	**Identification and Explanation of key and new words 1 - 5**	**Generates hypothesis and explains mechanism 1 - 5**	**Comes prepared with objectives and details of the case 1- 10**	**Post-tutorial Test 20**	**Total 40**	**Manages time 1-5**	**Interpersonal skills 1 -5**	**Maintains group dynamics 1 - 5**	**Provides feedback 1 - 5**	**Total 20**
1											
2											
3											

HITEC-Institute of Medical Sciences
CBL ASSESSMENT FORM
PHY-form-01   ISSUE # 01   ISSUE DATE: 01-12-2020

CBL number:_____   Session Date :_____   Tutor:_____

Department:_____   Group:_____   Class:_____

Instruction for facilitator: For the CBL session you are conducting rate each student on a scale of 1-5. Minimum marks for each student = 8, Maximum = 40 where 1 = poor, 2 = fair, 3 = fairly good, 4 = good, 5 = very good. Multiply the marks in 4th column with 2.

Figure-1: CBL Assessment form, HITEC-IMS

MATERIAL AND METHODS

This was a cross-sectional descriptive study conducted by Physiology Department, HITEC-IMS, Taxila. The study duration was six months. The sample was obtained through non-probability purposive sampling. Written informed consent from all study participants was taken on consent forms. The study was approved by Ethical Review Board (ERB) of HITEC-IMS. All the undergraduate medical students who attended at least four CBL sessions of both Online and On-campus formats were included in the study. Students who were not willing to participate were excluded. A questionnaire comprising 19 statements related to Online and On-campus CBL sessions was developed by Physiology Department HITEC-IMS and gotten validated from a team of medical educationists.[14] This questionnaire was filled for both Online and On-campus CBL formats.

The statements were designed to assess the Acquisition of knowledge (Q1–Q5), Study skills and Critical thinking (Q6–Q8), Communication skills (Q9–Q13), Presentation skills (Q14 and Q15) and Physical environment (Q16–Q18). Students were also asked for suggestions and future recommendations. Each student had to respond to every statement on a 5-points Likert scale[15] where '1' represented 'strongly disagree' and '5' represented 'strongly agree'. Questionnaires were circulated among the undergraduate medical students of 1st and 2nd year at HITEC-IMS and responses were recorded separately for both formats. Data was analysed using SPSS-28. Likert scale points 1–3 were taken as 'Agreed' (Good Perception), and points 4–5 were included in the 'Disagreed' (Bad Perception) category. Chi-square test was applied, and $p \leq 0.05$ was taken as statistically significant. The results for categorical variables were expressed as percentages.

RESULTS

A total of 185 responses were received from the students. Among them 94 students belonged to 1st year MBBS, while 91 students were from 2nd year MBBS. From 1st year MBBS 56% respondents were female and 44% were male, while 49% females and 51% males submitted their responses from 2nd year MBBS. The mean age of 1st year students was 19.69 years, and that of 2nd year students was 20.61 years. The percentages of responses for Online and On-campus formats is tabulated as Table-1.

The results revealed the comparison between Online and On-campus Case Based Learning sessions. The communication skills ($p=0.007$) as well as presentation skills ($p=0.005$) conducted On-campus were significant, i.e., more effective as compared to Online mode, while Acquisition of knowledge, critical thinking and physical environment were insignificant (Table-2). Results also showed that respondents recommended On-campus format for future as effective learning strategy which was found significant ($p=0.001$) when both formats were compared.

Table-1: Individual question responses on the questionnaire (%)

Constructs	Question	Online Agreed	Online Disagreed	On-campus Agreed	On-campus Disagreed
Acquisition of Knowledge	1. The session is useful in making me understand the problem based case scenario	72.9	11.3	79.5	10.8
	2. The session has positive impact on learning of applied Physiology	73.3	14.5	79.5	10.8
	3. The session has helped me to enhance my retention of knowledge	69.2	13.5	78.4	9.7
	4. The session helped me to achieve the learning objectives of CBL in a better way	69.7	11.4	79.4	8.6
	5. My queries are better answered by this format	64.9	14	74.1	10.8
Critical Thinking	6. The session has helped me to promote critical thinking and reasoning skills	68.2	11.9	73.5	9.8
	7. The session has motivated me to study better prior to the session.	63.8	12.4	71.9	13.5
	8. This format has improved my self-directed learning skills	69.7	10.3	73.6	11.9
Communication Skills	9. This format helped me develop active listening skills	66	13.5	76.8	12.4
	10. This format allowed better exchange of ideas.	66	14.6	75.7	7.1
	11. This format gives me more chance to interact with each other and the facilitator	64.9	17.2	80	9.2
	12. This format has improved my communication skills with peers.	70.3	17.9	77.3	11.9
	13. This format has improved my communication skills with teachers.	58.3	18.4	75.2	9.8
Presentation Skills	14. This format has improved my self confidence	62.7	11.9	75.1	11.3
	15. This format has improved my presentation skills	65.4	14.1	75.1	10.2
Physical Environment	16. The physical environment made me more receptive to the session	64.3	14.5	73.5	13
	17. Internet connectivity is not a demotivating factor for me	59.4	21	65.4	17.3
	18. The environment is less intimidating/Conducive in this format	61.7	14.1	64.4	16.2
Recommendations	19. I would like to recommend this format of CBL in future for class	57.3	21.1	74.1	15.1

Table-2: Comparison of On-campus and Online formats of CBL sessions

	Poor perception	Good perception	p
Acquisition of knowledge and critical thinking			
On-Campus format	25.4%	74.6%	0.298
Online format	30.8%	69.2%	
Communication skills			
On-Campus format	20.0%	80.0%	0.007*
Online format	33.0%	67.0%	
Presentation skills			
On-Campus format	28.6%	71.4%	0.005*
Online format	43.2%	56.8%	
Physical environment			
On-Campus format	31.9%	68.1%	0.101
Online format	38.9%	61.1%	
Future Recommendations			
On-Campus format	25.9%	74.1%	0.001*
Online format	42.7%	57.3%	

*Significant

DISCUSSION

Due to the COVID-19 pandemic the educational institutions shifted to online teaching. In wake of this sudden shift to online format, training sessions of faculty as well as students were conducted at HITEC-IMS prior to commencement of e-learning program to orient them with the new online system involving google classrooms and ZOOM for video conferencing. In this study we assessed the perception of students regarding the Online and On-campus experience of CBL sessions. We compared the two formats in terms of acquisition of knowledge, critical thinking, communication skills, presentation skills and the physical environment.

The data regarding the analysis of interactive teaching methodology such as the CBL is scarce. Our students perceived that both formats were almost equal in terms of acquisition of knowledge and development of critical thinking (74.6% on campus *vs* 69.2% Online).

Chi-square test was used to compare the difference between the two formats which was found to be insignificant (*p*=0.298). Majority of the students were satisfied with the online conduct of CBL. This shows that the medical colleges can attain these objectives (acquisition of knowledge and critical thinking) through Online teaching as well. A qualitative study at Monash University, Australia in 2016 gathered responses of 68 students regarding a Remote online (RO)-CBL session where 78% agreed that RO-CBL was effective in reaching their learning objectives.[16] A slight difference in our findings is due to the use of different software used at Monash University, i.e., Google hangouts for RO-CBL[16] whereas we used basic ZOOM for the web based CBL.

There is no one standard method of measuring critical thinking but, there are numerous ways to assess it. Critical thinking can be deemed as a process rather than an endpoint.[17] This emphasizes the need of self-directed learning during CBL sessions[11] We focused this attribute in our study by inquiring about improvement in reasoning skills, motivation to study better prior to the session and improvement in self-directed learning. The students in this study did not show any inclination to a specific format.

Sumandeep Vidyapeeth University in India conducted a non-randomized interventional study on undergraduate medical students of first year to assess their perception of an e-CBL via Google groups. Their feedback was positive and significant (*p*<0.01) for the e-learning approach to CBL as it strengthened their critical thinking and integration with clinical sciences.[13]

Eighty percent (80%) of students in our study agreed that face to face CBL provide more opportunity to communicate with the faculty and peers (*p*=0.007). It presented a healthy environment for discussion in contrast to online conferencing where internet

connectivity issues had been a major setback resulting in frequent interruptions in discussions. This factor thwarted the exchange of ideas, active listening skills and communication with peers and facilitator. The flow of dialogue and discussion was interrupted as the scribe did not have the adequate space and time to perform his task. This compromised the role of the scribe enhancing the communication lag. The study at Monash University provided comparable results in this regard. Some students were uncomfortable to communicate on the e-portal and could not understand the body language of their instructor while others had bad internet connectivity in their region.[16] Social communication makes the foundation for interaction among the peers in a face-to-face environment. If not provided this can prove to be a major disadvantage to online learning. Turning off the cameras in a digital environment lessens the quality of communication among students and faculty. This was pointed out by Public Health students in a questionnaire based study regarding online education after lockdown.[2]

Students at HITEC-IMS believe that presentation skills were improved more with On-campus CBLs compared to Online (71.4% *vs* 28.6%). These cannot be more beneficial on an online format as compared to a real situation where one on one interaction enhances self-confidence and presentation skills.

Although the physical environment contributes to learning and face-to-face interaction allows better understanding, the virtual environment is less intimidating and informal making students relax and more expressive. The students faced challenges during Online learning due to ill-equipped housing situations particularly poor bandwidth which was a huge distraction and lessened their motivation to learn.[2]

The students did not appear to be bothered by their study environment whether it was online or on-campus. The results were insignificant (*p*=0.101) when they were asked to compare the physical classroom environment with a virtual one. They seem to have adapted to the online mode provided that they have good communication and internet connection. A survey was conducted at an Indian University on undergraduate medical students regarding their opinion of online education during the pandemic where 65.9% preferred the face to face format and 39.9% were comfortable with Massive Open Online Courses (MOOCs). They insisted that the online format had a bad impact on their social and mental health while they learned better in a traditional On-campus classroom.[1] Another study on e-learning in India suggested that the online mode does not provide the comfortable environment and social interaction of a face-to-face meeting between students and the teacher or among peers.[18]

More than half (56%) of the respondents (*p*=0.001) in our study favoured the use of face-to-face

CBL format in future as they found it more effective for learning and communication, and did not involve the hustle and bustle of internet connectivity and webcam. Some students at Monash University were also of the same view about RO-CBL. Difficulties related to technology and communication gaps in the online format made it less favourable to continue to be used.[16]

A study on e-learning in India suggested that medical institutions should take measures to reduce the gap between the privileged and non-privileged learners considering its not accessible to all.[18]

CONCLUSION

Both formats are equal for acquisition of knowledge and critical thinking, but the online format falls short in acquiring communication skills and presentation skills.

LIMITATIONS

Though online format has its benefits it still falls short of accountability and social interaction. Poor internet facilities in remote areas pose a threat to effective management of e-learning. Faculty training in e-learning is also the need of the hour.

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Contribution of Authors:
All authors contributed equally in conducting this study.

Conflict of Interest: None
Funding: None

ORIGINAL ARTICLE

DIFFICULTY INDEX, DISCRIMINATION INDEX, SENSITIVITY AND SPECIFICITY OF SINGLE BEST ANSWER QUESTIONS TO ASSESS MEDICAL STUDENTS' PERFORMANCE IN SEND UP EXAMINATION AT LAHORE MEDICAL & DENTAL COLLEGE

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Background: Item analysis of the single best answer question based assessment is a simple and valuable method that can help the examiners to check the reliability and validity of this examination tool. This study aimed to determine difficulty index and discrimination index of single best answer question (SBAQ) based send-up Physiology paper of 1st Prof MBBS, and to check sensitivity and specificity of SBAQs to predict undergraduate medical students' performance. **Methods:** This was an observational study. A retrospective review of undergraduates' examination performance was done after approval from Institutional Review Board. Theory paper consisted of 45 SBAQs. Each SBAQ comprised a stem followed by five options. One option was correct and rest four were distractors. Data was analysed on SPSS-22. Difficulty and discriminative index was calculated. Receiver operating characteristics (ROC) curve was generated. The sensitivity and specificity of SBAQ was determined. **Results:** Out of 45 items, 33 (73%) were having difficulty index within the acceptable range (30–70%), 3 (6.7%) were very easy with the difficulty index of more than 70%, and 9 (20%) very difficult with the index of less than 30%. Mean±SD difficulty index was 47.45±17.83. Discrimination index revealed 33 items within acceptable range, 10 were poor and 2 items revealed negative scoring. Mean±SD discrimination index was 0.30±0.19. ROC curve revealed AUC of 0.86 (p=0.000; CI= 0.803-0.90) with sensitivity of 84% and specificity of 89%. **Conclusion:** Mean difficulty index of send-up paper was within acceptable limits. However, discrimination index identified 12 items that need revision.

Keywords: Difficulty index, Discrimination index, Single best answer question, Sensitivity, Specificity

Pak J Physiol 2022;18(3):44–7

INTRODUCTION

Assessment —a key component of any educational system, is more than a tool to measure performance of the students. Competency assessment is essential in medical education as it can modify the learning approach of the students and improve their performance.[1,2] Properly designed assessment can increase the learning drive of students and check the cognitive, psychomotor and affective domains.[3] Assessment provides an effective feedback to students as well as facilitators. Students can improve their learning strategies to achieve learning objectives while it helps facilitators to identify the gaps between the teaching methods and learning objectives.[4] Inappropriately designed assessments can result in undesirable results of competencies.[5]

Recently, huge effort has been allocated by different medical education programs and authorities at under- and postgraduate levels to enhance authenticity and effectiveness of assessments.[1] Many methods have been designed to measure different aspects of competencies. Any single assessment method cannot be considered perfect as each format has its pros and cons. Assessment design should be reliable, valid, acceptable, feasible, and have educational impact to be authentic.[6] Assessments can be designed to check the four

components: knows, knows how, shows how, and does.[3] Single Best Answer Questions (SBAQs) have become a popular assessment design in many educational domains including medical education.[7] This method has an edge over other assessment designs as extensive part of the course can be assessed in a short time frame. Efficiently designed SBAQs can effectively identify strengths and shortcomings of students as well as discriminate between the best and weak. It can guide the facilitators on their educational strategies.[8] Properly designed SBAQs can be used to check interpretation and application of knowledge (high cognitive level) rather than simple recall of facts, although it can be challenging and demands a lot of effort by examiner.[9]

Item analysis of the SBA questions is simple and valuable method to help examiners to check the reliability and validity of this examination tool.[10] It can inform examiners about how difficult the SBAQ was (difficulty index) and its ability to discriminate between weak and strong students (discrimination index). Flawed multiple choice questions can affect the performance of high achievers as well as borderline students.[11]

This study aimed to determine difficulty index, discrimination index, sensitivity and specificity of the individual SBAQs of send-up Physiology paper of 1st

Prof MBBS for predicting our undergraduate medical students' performance.

MATERIAL AND METHODS

This was an observational study conducted at Department of Physiology, Lahore Medical and Dental College, Lahore from Nov 2021 to Feb 2022 after approval from Institutional Review Board (Ref No. LM&DC/1702830). A retrospective review of examination performance of 1st year MBBS students of session 2021 appearing in the send-up examination of Physiology theory was done.

The paper comprised of 45 SBAQs with one mark to each SBAQ (item) and no negative marking. Each SBAQ comprised of a stem and 5 options. One option was the correct option (key) and the rest 4 were incorrect (distracters). Pass percentage, difficulty index, and discrimination index of each SBAQ were calculated.

Difficulty index (also termed easiness index) is the proportion of students who attempted the item correctly. Its value ranges between 0 to 100%. Acceptable range for difficulty index is 30–70%. Items having values less than 30% or greater than 70% are considered as difficult/easy questions respectively.[12–15]

$$\text{Difficulty index} = (CN/TN) \times 100$$

where CN is number of students who correctly attempted the item and TN is total number of students who attempted the item.

Discrimination index is a measure to discriminate between the high and low achievers. The range for discrimination index is between -1.00 to +1.00. The expected response is that the high achievers should pick the correct response more frequently compared to the lower achievers. Items having discrimination index of ≥0.4 are considered as good items, 0.3–0.39 are reasonable, 0.2–0.29 are marginal or borderline items, ≤0.19 are poor and these items should be revised or removed from the assessment.[12–15]

$$\text{Discrimination index} = [(CH/NH)-(CL/NL)]$$

where CH and CL stand for number of correct responses in high and low achiever group, and NH and NL stand for number of students in high and low achiever group. Top and bottom ⅓ were taken as high and low achievers.

Data was analysed on SPSS-22. Difficulty and discriminative index was calculated. Receiver operating characteristics (ROC) curve was generated and sensitivity and specificity of SBAQ were determined. Area under curve (AUC) was calculated. AUC value of more than 0.7 was considered as an acceptable value for prediction and a value of >0.8 was considered as very good value. Correlation between discrimination and difficulty index was checked with the Pearson test.

RESULTS

A total of 147 candidates of 1st professional MBBS attempted the SBAQ paper in the subject of Physiology.

Out of 147 candidates, 72 (49%) passed and 75 (51%) did not pass. There were 45 items in the question paper; 33 (73%) items were having difficulty index within the acceptable range of 30–70%, 3 (6.7%) were very easy with the difficulty index of >70% and 9 (20%) items were very difficult with the index of <30%. Difficulty index of the SBAQ paper was 47.45±17.83. Discrimination index revealed 33 items within acceptable range, 10 were poor and 2 items revealed negative scoring and were defective items (Tables 1–3).

Discrimination index was 0.30±0.19. Receiver operating characteristics (ROC) curve analysis revealed AUC of 0.86 (*p*=0.000, CI=0.803–0.90) with sensitivity of 84% and specificity of 89% (Figure-1). There was no correlation between difficulty and discrimination index with Pearson test (*r*=0.155, *p*=0.350).

Table-1: Overall results of SBAQs (n=147)

SBAQs	Frequency	Percentage
Pass	72	49.0
Fail	75	51.0

Table-2: Difficulty index of SBAQs (n=45)

Difficulty index	SBAQ [n (%)]	Interpretation
30–70%	33 (73.3)	Acceptable
>70%	3 (6.7)	Very easy
<30%	9 (20)	Very difficult

Table-3: Discrimination index of SBAQs (n=45)

Discrimination index	SBAQ [n (%)]	Interpretation
>0.35	21 (46.7)	Excellent
0.25 to 0.35	9 (20)	Good
0.2 to 0.24	3 (6.7)	Acceptable
0-0.2	10 (22.2)	Poor
Negative value	2 (4.4)	Defective item

Table-4: Difficulty index and Discrimination index of each item of SBAQs

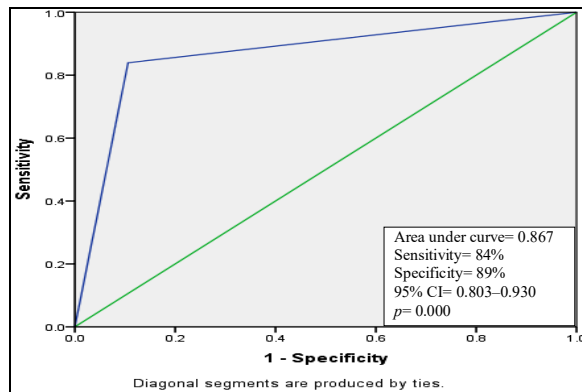| SBAQ	Difficulty index	Discrimination index	SBAQ	Difficulty index	Discrimination index
1	34.7	0.29	24	47.6	0.53
2	91.8	0.08	25	81	0.20
3	45.6	0.1	26	35.4	0.31
4	38.1	0.63	27	51.7	0.37
5	28.6	0.04	28	8.8	0.10
6	33.3	0.36	29	32.7	0.51
7	62.6	0.37	30	54.4	0.08
8	76.2	-0.1	31	54.3	0.46
9	29.9	0.35	32	59.2	0.28
10	47.6	0.51	33	66.7	0.40
11	23.8	0.14	34	63.9	0.21
12	48.3	0.25	35	45.6	0.37
13	59.2	0.53	36	68	0.6
14	39.5	0.48	37	31.3	0.10
15	45.6	0.48	38	50.3	0.43
16	56.5	0.01	39	42.9	0.15
17	48.3	0.64	40	25.9	0.27
18	51.7	0.77	41	26.5	0.11
19	64.6	0.46	42	68.7	0.35
20	64.6	0.10	43	29.9	0.29
21	68.7	0.35	44	38.10	0.27
22	53.7	0.41	45	23.10	0.06
23	16.3	-0.09			

Figure-1: Receiver operating characteristic curve analysis to predict sensitivity and specificity of SBAQs

DISCUSSION

In this study, difficulty index and discrimination index of SBAQs of the send paper of Physiology was determined. These indices were employed to post validate the items individually and also to assess whether the items were properly constructed or not. Currently in medical curriculum there is a rising trend to use multiple choice questions or SBAQs to assess knowledge. The individual items of SBAQs can check memory based core knowledge of the candidates and if constructed thoughtfully can also assess higher cognitive domains such as application and problem solving skills.[16–17] It is very essential to control the quality of assessment.

In the present study, 33 (73%) items were having difficulty index within the optimum range, however 9 (20%) items were very difficult and 3 (6.7%) were very easy. The easy items in this study were placed at the start of the theory paper as warm-up items and they were based upon core knowledge which is essential for the students. Therefore they were not removed. The difficult items were checked for incorrect key, controversial areas or any language flaws. The purpose of adding difficult item by the assessor was to identify the top scorers.[12–15] Higher the value of difficulty index, more easy the item is; and higher the value of discrimination index better it can discriminate between the knowledgeable and non-knowledgeable candidates.[18] There is reciprocal relationship between difficulty and discrimination index. However, when Pearson test was applied no significant correlation was observed between the difficulty and discrimination indices. Previous studies based upon item analysis in the subjects of Physiology and Pathology from India reported positive correlation between difficulty and discrimination index.[19,20]

On Discrimination index analysis, 33 (73%) items were having discrimination index within or above the acceptable range and 12 items out of 45 needed revision. There were 10 items (out of 12) having discrimination index within the poor range (0–0.2) and in

the remaining 2 items it was negative. Items having discrimination index between 0–0.2 were checked for the content, cognition level and the answer key. Eight out of the 10 items were primarily based upon the core knowledge. They were formulated to check recall and understanding of the candidates. Therefore, poor discrimination index between 0–0.2 was not considered a problem in these items, whereas 2 out of the 12 items where discrimination index was less than zero (negative value) were checked for the language error, typographical flaws, face validity and the answer key. As no major error was found, it was assumed that learning objectives were not properly conveyed to the students in the teaching session of the particular topics and students might have misunderstood. These points were planned to be clarified in the paper discussion session with students.

In this study sensitivity and specificity of SBAQs was determined by the ROC curve analysis and it was observed that SBAQs were 84% specific and 89% sensitive in predicting passed and failed candidates. This result is in concordance with another study based on psychometric analysis at Department of Medical Sciences, University Sains Malaysia, reporting discriminative ability of multiple choice question of more than 0.8 by ROC curve.[12] A study from India also reported multiple choice questions as an effective tool to assess students' performance.[19]

SBAQs are employed as an assessment tool for entry tests and also for under- and postgraduate pragmatic examinations.[21] They are used because of objectivity, reproducibility, decreased element of observer bias and comprehensive coverage of the subject in less time.[22,23] A well-formulated SBAQ based assessment can measure cognition level based upon Bloom's Taxonomy and also differentiate between high and low scorers.[24] While formulating an assessment a reliable and valid reference pool is required having multiple choice questions of known difficulty and discrimination index.[25] However, a cross-sectional study in 20 medical schools of UK concluded that there was pseudo-impression of competency with SBAQ based assessment. They found very short answer questions well correlated with students' performance than the SBAQs.[21]

One of the limitations of this study is that it is conducted in one institutional setting only. Therefore generalizability of the results to other educational institutions or programs is reserved.

CONCLUSION & RECOMMENDATIONS

Mean difficulty index of the send-up paper was within the acceptable limits. However, discrimination index identified 12 items that need revision. Discrimination index and difficulty index are useful tools to post-validate the assessment. In order to control quality of the future assessments, SBAQs with known difficulty and discrimination indices should be selected.

ACKNOWLEDGEMENTS

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AK: Idea, data collection, write-up, analysis
SZ: Data interpretation, write-up
FJN: Data collection, analysis, write-up
MAC: Data collection and interpretation with analysis

Pakistan Journal of Physiology

INFORMATION FOR AUTHORS

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Abstract: The second page of the manuscript must contain an abstract of not more than 250 words. This abstract should consist of four paragraphs, labelled **Background**, **Methods**, **Results**, and **Conclusions**. They should briefly describe, respectively, the problem being addressed in the study, how was the study performed, the salient results, and what did the author(s) conclude from the results.

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Journal article: Badar A. Launching a new journal in the era of publication accountability. Pak J Physiol 2005;1(1–2):1.

Book reference: Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Bremier BM, (Editors). Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: McGraw Hill; 1995. pp.465–78.

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