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EDITORIAL

ASSESSING AND DOCUMENTING PROFESSIONAL ATTITUDE AMONG UNDERGRADUATE MEDICAL STUDENTS

Tehseen Iqbal
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Father's most important gift to his child is good manners. Prophet Muhammad صلی اللہ علیہ وسلم

Ultimate outcomes of undergraduate medical education is a doctor who has knowledge, skills, and professional attitude. Medical students had already partly formed professional attitudes before they started studying medicine. We, at medical college, just have to apprise or remind students that they have learned basic ethics/attitudes in premedical years. A scoring system is proposed to assess Professional Attitude of MBBS students. Positive or negative professional attitude/professional behaviour during the session will be closely monitored by the faculty. Positive behaviours increase the score; negative behaviours decrease the score. On the basis of this score, proper word will be entered in the relevant sentence on DMC or Character Certificate. Total Five year Marks: More than 85%= Excellent, 75.1–84.9= Very Good, 75%= Good, 70–74.9%= Fair, 65–69.9%= Satisfactory, Less than 65%= Poor. Assessment drives learning, so assessing students will guide their learning.

Keywords: Professional attitude, behaviour traits, MBBS students, PAS-Pak
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Professional values, ethics, and attitudes are the characteristics that identify a professional as member of a profession. The relevant ethical requirements ordinarily set out five fundamental principles, i.e., integrity; objectivity; competence and due care; confidentiality and professional behaviour.[1] Medical education produces a doctor who has knowledge, skills, and professional attitude. A graduate should be polite, considerate, trustworthy and honest, act with integrity, maintain confidentiality, respect patients' dignity and privacy, and understand the importance of appropriate consent.[2] General Medical Council reviewed its 'Outcomes for Graduates' in 2018 and put 'Professional Values and Behaviours' at number one[3] which were previously placed at number three in its document of 2009, i.e., Tomorrow's Doctors.

A professional student is punctual (to class and laboratory meetings), follows the teacher's instructions; respects private and public property; arrives appropriately dressed and ready to work, armed with his/her tools. A professional is observant and sees what needs to be done; is responsible and helps maintain a safe workplace with a civilized atmosphere. A professional always acts in a manner that reflects favourably on that community. A professional asks a question rather than risk making a serious mistake with an unfamiliar scientific instrument.[4,5] Medical students had already partly formed attitudes toward professionalism before they started studying medicine. These attitudes were largely based on their own experience with the health care system and physicians.[3] They develop their professional attitude further in medical college.[6] We, at medical college, just have to apprise or remind students that during twelve years of pre-medical education and five years of family training, they have developed basic behavioural/ethical traits. These behaviour traits were taught to them at school.[5] When students' behaviour assessment is instituted they will learn ethical and social skills of a good medical student and law abiding citizen as 'assessment drives learning'. Extracurricular activities such as sports, debates, hospital voluntary service, politics, the arts or community service can build skills in leadership, responsibility, and cooperation.[5]

There is no formal system for assessing and documenting professional attitude of undergraduate medical students in Pakistan. We are proposing a system of Professional Attitude Score for Pakistan (PAS-Pak) for MBBS classes. While preparing this scoring system, important points considered were:

- It should be simple to use
- It should clearly convey to students which behaviours are considered 'positive' and which behaviours are considered 'negative'
- It should not increase the burden on faculty
- To decrease the inter-personal bias and to ensure inter-rater objectivity, all faculty of particular session will be involved to evaluate the students
- It can be utilized to clearly elaborate the words (Excellent, Very Good, Good etc.) in the existing 'Character Certificate' and/or the 'Detailed Marks Certificate (DMC)' issued by the college.

Professional Attitude Score (PAS-Pak)

Twenty Marks for each Professional year; hundred in total for five years' MBBS course. Twenty Marks

each are allocated for all subjects in a Professional year. Head of the Department of each subject will calculate PAS-Pak for each student of the class during the session according to the given tables. The scores are then forwarded to the "In charge HoD" of the session (Senior most HoD of the session or as designated by the Principal of the College) who will calculate average of the session and report it to the Principal Office. On the basis of the total five year marks/score, proper word will be entered in the relevant sentence on DMC or Character Certificate. A Red Entry will be for: 1, Misbehaving with some teacher (Head of the department is authorized to give a red entry to student after investigation). 2, Punishment by the Disciplinary Committee. Each red entry will deduct 20 marks from the cumulative score at the end of the five-year session.

Total Five year Marks: More than 85%= Excellent, 75.1–84.9= Very Good, 75%=Good, 70–74.9%= Fair, 65–69.9%= Satisfactory, Less than 65%= Poor. 'During his/her stay at medical college, his/her professional attitude score was ……..'

Calculation for first year MBBS Class

At the start of the first year MBBS class, each admitted student will have fifteen (15) marks which can be increased or decreased on the basis of their positive or negative professional attitude/professional behaviour/ ethical behaviour during the session, as mentioned in Table-1 and Table-2. Students will be closely monitored by the faculty and will report to the head of the department about their attitude/ behaviour/ethics. Positive behaviours increase the score more than fifteen; negative behaviours decrease the score less than fifteen. Positive behaviours not covered in the tables given can be covered under the heading 'Behaviour befitting of a good medical student' and negative behaviours not covered in the tables can be covered under the heading 'Behaviour unbecoming of a good medical student'.

Calculation for 2nd, 3rd, 4th and Final Year Classes

At the start of the session, all students passing in first attempt will have baseline of 15 marks. Students passing in supplementary examination will be at 14 (minus one), students passing as detained students will be at baseline marks 13 (minus two).

Table-1: Positive Behaviour traits/Attitude

University position in last professional exam	2 marks
Published a research paper during the session	One mark
Class tests; First position 2 marks; second one marks; third 0.5 mark	0.5–2 marks
Worked in arranging college convocation 1 mark	One mark
Respecting and behaving according to the local cultural traditions	One mark
Took position in Qirat, Na'at or Debate competition 1 mark	One mark
Served in the hospital volunteer service for helping the students/patients	One mark
Donated blood during this session	One mark
Attendance more than 85%, 2 marks; 76%–84%, one mark	1–2 marks
Behaviour befitting of a good medical student (HOD)	Plus 1
At the start of the Session	**15 marks**

Table-2: Negative Behaviour Traits/Attitude

At the start of the Session	**15 marks**
Behaviour unbecoming of a good medical student (HOD)	-1
Misbehaved with a teacher or college staff	-1
Missing one test minus one; two tests minus 2	1–2
Delay in vacating hostel room after new allotment	-1
Throwing waste in college premises	-1
Punished by a teacher on breaching discipline in the classroom etc.	-1
Quarrelled/misbehaved with fellow students	-1
Caught cheating in a test minus one	-1
Damaged college property minus one	-1
Not respecting and not behaving according to the local cultural traditions	-1

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ORIGINAL ARTICLE

CARDIAC AUTONOMIC NEUROPATHY IN TYPE 2 DIABETES MELLITUS AND CONTROL SUBJECTS

Najla Shore, Miraa Qutab*, Maham Ijaz, Taha Farooq, Nabiha Saeed, Rida Fatima, Hina Sikandar

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Background: Cardiac autonomic neuropathy (CAN) is a common but frequently undiagnosed complication of diabetes mellitus (DM). Up to 15% of the patients have been reported to have CAN at the time of diagnosis of DM. The objective of this study was to determine correlation between glycaemic control, microalbuminuria (MAU) and cardiac autonomic neuropathy in type 2 diabetes mellitus (T2DM). **Method:** This was a cross-sectional comparative study conducted on 100 subjects (50 diabetics, 50 controls) at Services Institute of Medical Sciences, Lahore. Glycated haemoglobin (HbA1c) levels were measured by quantitative caloric method, MAU was quantified by using QuicKey human microalbuminuria ELISA kit and cardiac autonomic functions were assessed using PowerLab® 26T Teaching System. **Results:** All the diabetic subjects in our study had cardiac autonomic neuropathy. No significant correlation between duration of diabetes and glycaemic control (p=0.230), duration of diabetes and microalbuminuria (p=0.891), and glycaemic control and microalbuminuria (p=0.698) was found. **Conclusion:** Cardiac autonomic neuropathy and microalbuminuria are highly prevalent in T2DM. Duration of diabetes, glycaemic control, and microalbuminuria are not significantly correlated to each other.

Keywords: CAN, Microalbuminuria, T2DM, PowerLab, Neuropathy, Autonomic, HbA1c

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INTRODUCTION

Diabetes mellitus (DM) is a global health problem. Approximately 462 million people corresponding to 6.28% population of the world are affected by type 2 diabetes mellitus (T2DM).[1] The pooled prevalence of diabetes in Pakistan is 13.7% with a slight preponderance towards male gender and urban living.[2] Despite this high prevalence, more than half of the patients remain unaware of their disease due to which complications are often present at the time of the diagnosis.[3] Damage to various body organs including blood vessels, eyes, kidney, nervous tissue and heart has been observed with chronic hyperglycaemia of T2DM.[4] The damage to nerve fibres both somatic and autonomic including cardiac autonomic nerve fibres is a feature of DM and is responsible for various neuropathies.[5] Cardiac autonomic neuropathy (CAN) is often found in T2DM patients but it frequently remains undiagnosed.[6]

The *CAN Subcommittee of Toronto Consensus Panel on Diabetic Neuropathy* defines CAN as an "impairment of cardiovascular autonomic control in patients with established diabetes after excluding other causes". It has been proposed that autonomic nerve fibres that innervate heart and blood vessels are damaged with long-standing diabetes which in turn cause abnormalities in heart rate and vascular dynamics.[7] Based on the available data, the reported prevalence of CAN is highly variable and ranges from 25% to 75% in T2DM.[8]

The pathophysiology of CAN in DM is still unclear but it has been proposed that diabetes triggers multiple reactions that promotes neuropathic changes.[7] Microvascular changes of diabetes, including retinopathy and albuminuria, are associated with progression of CAN based on the results from the EURODIAB study.[9] Microalbuminuria (MAU) has been defined as 'albumin excretion rate (AER) of $\geq$30 mg/ 24 hour of urine sample collection'.[10] A review of literature indicates strong association between MAU and increased risk of cardiovascular complications.[11]

Most of the studies reporting this association have been done in type 1 diabetes but similar studies in type 2 diabetes are relatively few in number.[12] Pakistan ranks at sixth position in the world regarding the burden of DM.[13] Although CAN manifests approximately after 20 years of disease initiation, its pathology starts in early years of DM.[5] Increased urinary albumin excretion in diabetic patients with CAN has been observed. However, little is known about correlation between glycaemic control, microalbuminuria and CAN in our local population. The present study aimed at determining correlation between glycaemic control, microalbuminuria and cardiac autonomic neuropathy.

METHODOLOGY

This was a cross sectional comparative study carried out in the department of Physiology, Services Institute of Medical Sciences, Lahore. A sample size of 72 was calculated at 95% confidence level, and 5% margin of error while taking probability of CAN in 29% of the diabetic patients and zero in control subjects.[5] However, we enrolled a total of 100 subjects (50 cases

and 50 controls) by non-probability consecutive sampling based on inclusion and exclusion criteria. The subjects having type 2 DM ≥5 years of duration were included as cases while controls were non-diabetic subjects. Subjects with known ischemic heart disease, malignancies, renal failure, limb amputation and cerebrovascular stroke were excluded from the study. Height of all the subjects was measured in centimetres from the highest point of the vertex while the subject was standing in the anatomical position. Weight was measured in kilograms by using weighing machine floor type model RGZ-160 (SERICO, China) while all the subjects were bare-footed and wearing minimal clothing. Body mass index (BMI) was calculated from height and weight using formula:

$$\text{BMI} = \frac{\text{Weight (Kg)}}{[\text{Height (m)}]^2}$$

HbA1c levels were measured by quantitative caloric method using diabetes chemistry reagents developed by Stanbio laboratory, USA. The diagnosis of the diabetes was based on American Diabetes Association recommended glycated haemoglobin (HbA1c) level of ≥6.5%.[14] MAU was quantified by using QuicKey human microalbuminuria ELISA kit by Elabscience®, USA. MAU was categorized into mild (30–50 mg/24 hr), moderate (50–100 mg/24 hr), and severe (100–300 mg/24 hr) categories.[15] The cardiac autonomic functions were assessed using PowerLab® 26T Teaching System ADInstruments, UK. The diagnosis of CAN was established if two or more of the following cardiac autonomic functions were abnormal.[9]

Beat to beat heart rate variation (HRV) was measured by monitoring heart rate on ECG while patient was at rest and lying supine. The patient was then asked to breathe in and out at 6 breaths/minute (deep breathing). R-R interval during three successive breaths was measured and mean value was obtained. The difference between beats per minute during rest and deep breathing was calculated. A difference of >15 bpm between rest and deep breathing was taken as normal.

Heart rate response to standing was recorded using continuous ECG monitoring and the subject was asked to stand up. R-R interval on ECG was calculated at beats 15 and 30 after standing. The 30:15 of >1.03 was taken as normal.

In heart rate response to the Valsalva manoeuvre, subjects were asked to exhale forcibly into the mouthpiece of a manometer at a pressure of 40 mmHg for 15 sec while ECG was monitored. Healthy subjects develop tachycardia during the manoeuvre while bradycardia with release. The ratio of R-R interval after release and during Valsalva manoeuvre of >1.2 was considered normal.

Systolic blood pressure (SBP) response to standing was checked by measuring SBP of the subject in supine position. The patient was then asked to stand up and blood pressure was again monitored after two minutes. A fall of <10 mmHg is normal, 10–29 mmHg is border line while a fall of >30 mmHg on standing was considered abnormal.

Diastolic blood pressure (DBP) response to isometric exercise was evaluated by first taking the blood pressure of the subject in one arm. The subject was then asked to squeeze a handgrip dynamometer to his/her maximum force and value was calibrated on PowerLab®. The patient was then asked to squeeze the same dynamometer with 30% of the maximum for 5 minutes. A rise of >16 mmHg in diastolic blood pressure on opposite arm was taken as normal.

RESULTS

A total of 100 study subjects divided in two equal groups (diabetic and controls) were enrolled for the study. More than half of the study subjects (56%) were male while remaining (44%) were females. Mean age of the diabetic patients was 39.5±1.07 Years and that of the controls was 38.5±3.03 Years. The BMI of diabetics was 26.55±1.16 Kg/m^2 while it was 25.12±2.67 Kg/m^2 in healthy controls (Table-1).

Comparison of mean values for parasympathetic (HRV and hear rate response to standing) and sympathetic (systolic blood pressure response to standing and diastolic blood pressure response to isometric exercise) functions showed significant differences between cases and controls, while heart rate response to Valsalva manoeuvre was similar in both groups (Table-2).

Chi-square analysis of sympathetic and parasympathetic parameters indicated highly significant differences between diabetics and controls (Table-3).

Microalbuminuria was found to be of mild, moderate, and severe nature in 6, 14 and 30 cases respectively. None of the sympathetic or parasympathetic parameters were significantly correlated with microalbuminuria. Significant correlations were observed between HRV and heart rate response to standing in relation to duration of diabetes. Only heart rate variation during deep breathing had significant correlation with glycaemic control (Table-4).

We did not find any significant correlation between duration of diabetes and glycaemic control (p=0.230), duration of diabetes and microalbuminuria (p=0.891) and glycaemic control and microalbuminuria (p=0.698).

Table-1: Demographic characteristics of the study population (n=100)

Variables	Cases
HbA1c (%)	10.67±1.67
Duration of diabetes (Months)	83.96±15.21
Microalbuminuria (mg/24 hour)	140.13±76.26

Table-2: Comparison of cardiac autonomic functions between cases and controls by Student's *t*-test (Mean±SD)

Cardiac autonomic neuropathy test	Cases	Controls	*p*
Heart rate response to deep breathing	9.84±6.62	23.74±5.56	<0.001*
Heart rate response to standing	0.95±0.16	1.20±0.14	<0.001*
Heart rate response to the Valsalva manoeuvre	1.45±0.38	1.44±0.14	0.823
Systolic blood pressure response to standing	32.40±11.65	4.72±2.25	<0.001*
Diastolic blood pressure response to isometric exercise	8.60±7.24	21.20±3.9	<0.001*

Table-3: Chi-square analysis of CAN among diabetic and control subjects [n (%)]

Cardiac autonomic neuropathy test	Cases	Controls	*p*
Heart rate response to deep breathing	32 (64)	0 (0)	<0.001*
Heart rate response to standing	37 (74)	0 (0)	<0.001*
Heart rate response to the Valsalva manoeuvre	14 (28)	1 (2)	<0.001*
Systolic blood pressure response to standing	35 (70)	0 (0)	<0.001*
Diastolic blood pressure response to isometric exercise	37 (74)	0 (0)	<0.001*

*Highly Significant, 0 cells have expected count <5

Table-4: Pearson correlation analysis for various sympathetic and parasympathetic parameters with microalbuminuria, duration of diabetes and glycaemic control

Cardiac autonomic neuropathy test	Microalbuminuria		Duration of diabetes		Glycaemic control	
	r	*p*	*r*	*p*	*r*	*p*
Heart rate variation during deep breathing	-0.108	0.454	-0.303*	0.03	-0.416*	0.003
Heart rate response to standing (30:15)	-0.043	0.765	-0.297*	0.04	-0.220	0.125
Heart rate response to Valsalva manoeuvre	0.017	0.907	-0.007	0.96	0.143	0.323
Systolic blood pressure response to standing	-0.232	0.105	0.102	0.48	-0.015	0.915
Diastolic blood pressure response to isometric exercise	-0.216	0.131	-0.265	0.06	0.063	0.663

*Correlation is significant at 0.05 level (2-tailed)

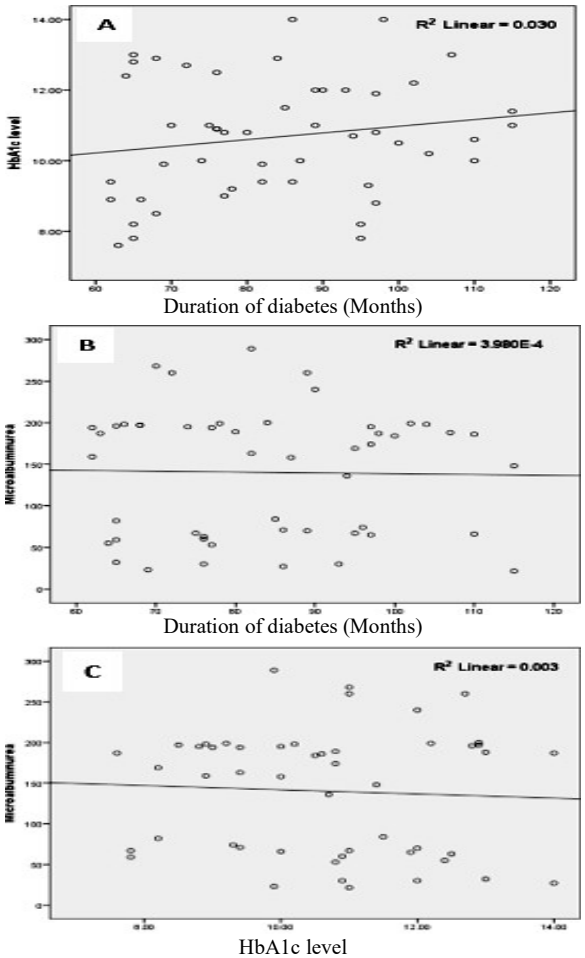

Figure-1: Correlation between A) duration of diabetes and glycaemic control, B) duration of diabetes and microalbuminuria, C) glycaemic control and microalbuminuria

DISCUSSION

Diabetes mellitus is a global health problem having significant impact on quality of life of the patients. Up to 15% of the patients have been reported to have CAN at the time of diagnosis of diabetes.[16] Moreover, before CAN is symptomatic and evident clinically in diabetic patients, sub-clinical CAN may persist for several years.[7] Thus, *Toronto Consensus Panel on Diabetic Neuropathy* has recommended that CAN screening must be done in all asymptomatic T2DM patients at the time of diagnosis.[17]

All of the diabetic subjects in our study were diagnosed with CAN with a mean duration of diabetes and HbA1c level of 83.96±15.21 months and 10.67±1.67% respectively. This indicates a very high prevalence of cardiac autonomic neuropathy in our population. The reported prevalence of CAN from different studies is highly variable. In a recent study, prevalence of CAN in newly diagnosed cases was reported to be 15.3%.[16] while another study reported a prevalence of 20.67% in newly diagnosed cases.[18] Fawwad *et al* reported CAN in 68% and 50% of the subjects with poor and good glycaemic control after 5 years.[19] Another study reported, that not a single case of CAN was found in patients with less than 5 years of diabetes but when the duration was increased to 15 years, 100% of the subjects developed cardiac autonomic neuropathy.[20,21]

Despite good glycaemic control, more than two-third of the newly diagnosed cases of diabetes in Pakistan have some degree of cardiac autonomic neuropathy within one year.[22] This variability can partly be explained by risk factors including age, gender, type and duration of diabetes and glycaemic control.[17] The other possible factors may be non-inclusion of factors,

e.g., obesity, smoking, hypertension, distal polyneuropathy, nephropathy, and retinopathy which were not taken into consideration in our study.[23]

A number of factors including age, gender, ethnicity, glycaemic control, duration of diabetes and microvascular complications play a role in the development of CAN.[24] However, duration of diabetes and glycaemic control have been identified as two of the most prevalent risk factors for development of CAN. In turn, increased risk of cardiovascular diseases, kidney diseases and mortality has been reported with development of CAN.[25] Duration of diabetes is an independent risk factor while chronically elevated levels of HbA1c are associated with increased risk of cardiovascular and renal complications.

In our study, all the patients had poor glycaemic control. Glycaemic control is an independent risk factor for the development of CAN in both types of diabetes.[21] Measurement of glycated haemoglobin (HbA1c) is an important diagnostic and prognostic marker for disease progression.[26]

Most of the diabetic subjects (80%) in our study had moderate to severe microalbuminuria. MAU has been identified as an early sign of cardiovascular and renal diseases in diabetic and non-diabetic individuals.[27] Studies have reported association between glycaemic control and microalbuminuria, but we did not find any significant association between the two variables. Ullah *et al* reported mild association between microalbuminuria and glycaemic control in diabetic patients.[28] In another study, microalbuminuria was reported in type 2 diabetics with poor glycaemic control and elevated blood pressure. On the other hand, no microalbuminuria was reported in cases with poor glycaemic control only.[29] Still another study reported association between microalbuminuria and glycaemic control but the association was significant only at HbA1c levels of >11%.[30] Thus, the differences can be explained on the basis of duration for which diabetes remain undiagnosed in the subjects and degree of uncontrolled hyperglycaemia. The other possible factors for the above differences can be ethnicity, susceptibility of nephropathy and method of assessment of microalbuminuria.

To summarize, none of the sympathetic or parasympathetic parameters were significantly correlated with microalbuminuria. However significant correlations were observed between heart rate variation during deep breathing and standing and diastolic blood pressure response to isometric exercise with duration of diabetes. Beat to beat variation was also associated positively with glycaemic control. Symptoms and signs of autonomic dysfunction, including resting HR, BP responses to standing, and time and frequency measures of HRV in response to deep breathing, standing and Valsalva manoeuvre, should be conducted on all patients with diabetes to allow early detection and intervention.[31]

CONCLUSION

Cardiac autonomic neuropathy and microalbuminuria are highly prevalent in T2DM. Duration of diabetes, glycaemic control and microalbuminuria were not significantly correlated to each other.

RECOMMENDATION

Further large-scale studies with more refined subject selection must be designed to determine the true prevalence of CAN in our population. Moreover, prospective cohorts must also be planned to discover the optimal level of glycaemic control, duration of diabetes and microalbuminuria in order to reduce morbidity and mortality in T2DM.

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ORIGINAL ARTICLE

PROGNOSTIC CATEGORIZATION OF PRIMARY MYELOFIBROSIS PATIENTS OF PESHAWAR

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Background: Primary myelofibrosis (PMF) is a least common type of myeloproliferative neoplasm (MPN) and is clonally derived stem cell disorder classified as Philadelphia chromosome negative MPN. The Dynamic International Prognostic Scoring System (DIPSS) utilizes five variables including age, haemoglobin level, white blood cells count, peripheral blood blasts and other symptoms for characterization of myelofibrosis patients. The objective of this study was to categorize primary myelofibrosis patients of Khyber Pakhtunkhwa. **Methods:** This cross-sectional analysis was carried out from June 2018 till May 2019. Blood samples and other information were collected from 50 PMF patients enrolled at the assigned health care facilities of Peshawar. Non-probability convenience sampling technique was used, and an informed and written consent was obtained from the participants. DIPSS was utilized for the prognostic categorization of PMF patients. **Results:** Majority (26, 52%) of the patients were in the age group II (41–60 years) and most (34, 68%) of them were male. Participants were categorized into four risk groups (low, intermediate-1, intermediate-2, and high) by applying DIPSS scoring system. Among the 50 patients, 3 (6%) were in low-risk group, whereas only one was in the high-risk group. Thirteen (26%) patients fell in intermediate-1 risk category and 33 (66%) were placed in the intermediate-2 risk group. **Conclusion:** Most of the myelofibrosis patients in Peshawar fall in intermediate-risk group while small percentage was included in high-risk group.

Keywords: Myeloproliferative neoplasms, Primary myelofibrosis, DIPSS, Prognosis, Peshawar

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INTRODUCTION

Primary myelofibrosis (PMF) is a clonally derived stem cell disorder, classified as Philadelphia negative myeloproliferative neoplasm (MPN). Polycythaemia Vera (PV) and essential thrombocythaemia (ET) are also included in this category.[1] PMF has the most heterogeneous clinical presentation among the MPNs which may encompass anaemia, hepatosplenomegaly, leukocytosis or leukopenia, thrombocytosis or thrombocytopenia and disease related constitutional symptoms.[2]

PMF is the least common of MPNs with an annual incidence of 0.5–1.5 cases per 100,000 individuals.[3] It usually affects older individuals (median age of 65 years at the time of diagnosis) but young people are not necessarily spared.[4] Reported median length of survival is 3.5–5.5 years with worst prognosis compared to PV and ET.[5] The main causes of death are infections, bleeding, thromboembolism and transformation to acute leukaemia. Allogenic stem cell transplantation is the only chance of cure.[6] Advanced age, anaemia, red blood cells transfusion needs, leukopenia, leukocytosis, thrombocytopenia, peripheral blast count and constitutional symptoms are associated with poor outcome in PMF patients.[8]

Previously, based on recommendations of an International MDS Risk Assessment Workshop (IMRAW) in 1997 a prognostic scoring system called as International Prognostic Scoring System (IPSS) was developed to estimate the survival of PMF patients. This model is applied at the time of diagnosis and utilizes five risk factors for survival (age older than 65 years, haemoglobin level <10 g/dL, WBC count >25×10^9/L, peripheral blood blasts ≥1%, and presence of constitutional symptoms). It identifies 4 risk categories by assigning 1 adverse point to each one of these risk factors. The presence of 0, 1, 2, and ≥3 adverse points defines low, intermediate-1, intermediate-2 and high-risk disease, respectively.[9] However, acquisition of additional risk factors during the disease may substantially modify the patient's survival.

The Dynamic International Prognostic Scoring System (DIPSS) can be applied any time during the disease course and utilizes the same prognostic variables as IPSS.[10] It, however, allocates 2, instead of 1, adverse points for haemoglobin concentration lower than 10 g/dL. The resulting risk categories are low (score=0), intermediate-1 (score=1 or 2), intermediate-2 (score=3 or 4) and high (score=5 or 6) with corresponding survival rates.[11] A refined version, i.e., DIPSS-Plus, has been developed in which platelets count abnormalities, karyotype deformities and blood transfusion needs are addressed.[12] Beside these, two recently developed scoring systems are developed, i.e., MIPSS70 (Mutation-enhanced International Prognostic Score System) and GIPSS (Genetically Inspired International Score System). MIPSS70 is based upon assessment of genetic mutations and other clinical risk factors. MIPSS70-Plus

and MIPSS70-Plus version 2.0 are latest forms which included cytogenetic and haemoglobin assessment in relation to age and sex. GIPSS rely on karyotype deformities and genetic mutations such as presence of *CALR*, *JAK2*, *MPL* and others.[13] Based on DIPSS, the aim of this study was to prognostically categorize the PMF patients in Peshawar.

MATERIAL AND METHODS

This cross-sectional analysis of PMF patients was conducted at the Department of Haematology, Institute of Basic Medical Sciences, Khyber Medical University Peshawar, from June 2018 to May 2019. Blood samples and other information were collected from already diagnosed PMF patients enrolled at different health care settings in Peshawar, i.e., Haematology/Oncology Department of Hayatabad Medical Complex, Institute of Radiotherapy and Nuclear Medicine (IRNUM), and Blood Diseases Clinic, Peshawar. A total of 50 patients were observed and consecutive non-probability sampling technique was used while selecting the patients. Sample size was calculated according to WHO formula for sample size determination.[14]

Patients were enrolled based on pre-set inclusion and exclusion criteria. Diagnosed patients of primary myelofibrosis (PMF) and all patients of PMF, of either gender were included in study. Patients with secondary myelofibrosis due to other conditions like tuberculosis, fungal infections, Hodgkin or Non-Hodgkin lymphoma, other variants of MPNs (PV, ET, Chronic Myeloid Leukaemia) and post-PV and post-ET myelofibrosis and patients suffering from other neoplastic disease were excluded. Study was initiated after endorsement from Ethical Board of Khyber Medical University, Peshawar. Informed written consent was obtained from each patient. Data was collected through a specially designed proforma which included demographic details, clinical history, physical examination and investigations profile of the patient.

Blood samples were collected from the diagnosed patients of PMF at specified centres by trained phlebotomists applying standard techniques. The samples were transported immediately to laboratory, applying standard protocols for blood sample transportation. Blood smear was prepared and examined using light microscopy to look for anaemia, white blood cells, platelets, blasts, total blood cells count, and mean haemoglobin concentration. The data was primarily recorded on Microsoft Excel Spreadsheet. Statistical analysis was performed using SPSS-23. Simple arithmetic analyses (mean, standard deviation and/or percentages) were deduced for each parameter. Age and gender wise stratification was also performed.

RESULTS

A total of 50 diagnosed PMF patients were included in the current study. Mean age of the study population was 50.60±13.01 years. Out of these 50 patients, 34 (68%) were male and 16 (32%) were female. Patients were distributed into three different age groups, i.e., 13 (26%) were in the age group I (20–40 years), 26 (52%) in age group II (41–60 years), and group III (61–80 years) comprised of 11 (22%) patients. The commonest presenting clinical features were pallor, splenomegaly and disease related constitutional symptoms (fatigue, fever, night sweats and weight loss), whereas hepatomegaly was observed in only 3 (6%) patients (Table-1).

Mean total leukocyte count (TLC) was 19.5×10^9 cells/L with a minimum of 1.19×10^9 cells/L, and a maximum of 77.60×10^9 cells/L. The mean haemoglobin concentration was 9.42 g/dL (Range: 5.5 g/dL–18.8 g/dL), whereas mean platelets count was 272.64×10^9 cells/L (Range: 10×10^9 cells/L–656×10^9 cells/L) (Table-2).

Peripheral blood blasts observed in all patients were more than 1%. The participants were categorized into four risk groups (Low, Intermediate-1, Intermediate-2, and High) by applying Dynamic International Prognostic Scoring System (DIPSS). Among the 50 patients, 3 (6%) were in low-risk group, whereas only one was in high-risk group. Thirteen (26%) patients fell in intermediate-1 risk category while 33 (66%) were placed in intermediate-2 risk group (Table-3).

Table-1: Clinical characteristic of study patients (n=50)

Constitutional Symptoms	Patients	Pallor	Splenomegaly	Hepatomegaly
Yes	43	31	48	03
No	7	19	2	47

Table-2: Descriptive statistics of haematological parameters (n=50)

Parameters	Hb (g/dl)	TLC (cells/L)	Platelets (cells/L)
Mean	9.426±3.08	19.5×10^9	272.64×10^9
Minimum	5.5	1.19×10^9	10×10^9
Maximum	18.8	77.60×10^9	656×10^9

Table-3: Categorization of study participants (n=50)

Risk Category	Frequency	Percentage
Low Risk	3	6
Intermediate-1	13	26
Intermediate-2	33	66
High Risk	1	2
Total	50	100

DISCUSSION

The current study was conducted to prognostically categorize PMF patients in Peshawar. A total of 50 patients of primary myelofibrosis were analyzed and majority of the patients were male (68%). These

findings are supported by a study conducted on 1,000 US PMF patients which identified male predominance (male:female=3:2) and a strong correlation with old age (median age 60 years).[15] The higher median age, however, may pertain to the overall higher life expectancy of US population in comparison to Pakistani population.

The median length of survival for PMF patients is 3.5–5.5 years.[16] The disease course is complicated by progressive anaemia, symptomatic splenomegaly and severe constitutional symptoms.[17] International Prognostic Scoring System (IPSS) was developed to estimate the survival of PMF patients.[9] This model is applied at the time of diagnosis and identifies four risk categories. However, acquisition of additional risk factors may substantially alter the disease course. So Dynamic International Prognostic Scoring System (DIPSS) was developed which can be applied any time during the disease course. DIPSS also utilizes the same five prognostic variables (age older than 65 years, haemoglobin level <10 g/dL, WBC >25×10^9/L, peripheral blood blasts ≥1%, and presence of constitutional symptoms) as IPSS, however it allocates 2 instead of 1 adverse points to Hb level <10 g/dL. The current study provides valued data regarding the percentage of PMF patients who present with adverse risk factors including age, haemoglobin concentration, leukocytosis, and presence of disease related constitutional symptoms. More than two third patients (36, 72%) had anaemia whereas, leukocytosis was identified in 32 (64%). Disease related constitutional symptoms were observed in 43 (86%) patients while 7 (14%) were lacking them. Similar observations were made by others[18,19].

By applying DIPSS model for survival, the participants of the current study were categorized into four risk groups (Low, Intermediate-1, Intermediate-2, and High). Among the 50 PMF patients, majority (66%) of them were placed in Intermediate-2 risk category while 26% patients were categorised in Intermediate-1 risk group. A low percentage of patients (6% and 2% respectively) were in low and high risk categories. This may be because as many PMF patients are elderly at diagnosis or present with several co-morbidities, death is the ultimate result due to poor prognosis. Thus, a high degree of prognostic certainty is desired to permit aggressive therapeutic procedures and enable better therapeutic planning, especially for patients who are young and eligible for bone marrow transplantation.[20]

CONCLUSION

The intermediate risk group of myelofibrosis patients was most common in our study population followed by low risk group and then high risk group. The poor survival rate of high-risk patients may be the reason behind our findings.

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ORIGINAL ARTICLE

EARLY POSTOPERATIVE COMPLICATIONS OF CATARACT SURGERY IN DIABETIC PATIENTS

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Background: Progressions in technology and expertise has made cataract surgery a common and safe procedure now but this might be associated with early postoperative complications in diabetics. The objective of this study was to determine the occurrence of early postoperative complications after cataract surgery among diagnosed cases of diabetes mellitus. **Methods:** This clinical observational analytical study was carried at Department of Ophthalmology, Liaquat University Eye Hospital, Jamshoro from May to Oct 2018. Diagnosed diabetic patients who underwent cataract surgery, were enrolled for this study. The patients with early senile cataract and diagnosed cases of diabetes mellitus with normal arterial pressure and better macular function were included. Patients were enquired for early postoperative findings after cataract surgery. Data was analysed using SPSS-22. Mean±SD for quantitative variables and frequency (%) for qualitative were calculated. **Results:** Total 91 diabetic patients were included in the study. Early postoperative complications of cataract surgery in diabetic patients were compared according to duration of diabetes mellitus. Visual acuity of most patients remained between 6/6 and 6/24. Diabetics for >5 years were more prone to early postoperative complications as compared to those with <5 years. Statistically significant differences between early postoperative complications in the two groups were observed (p=0.01). **Conclusion:** Though frequency of complications after cataract surgery has decreased due to advances in surgical techniques, better preoperative assessment, and understanding of diabetes control, still early postoperative complications are common in individuals with longer duration of diabetes.

Keywords: Cataract Surgery, Complications, Diabetes mellitus

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INTRODUCTION

Cataract is one of the most common causes of blindness globally. Among all eye diseases, frequency of blindness owing to cataract was found to be 50% or more in underprivileged/distant regions and 5% among the developed countries. According to national survey on blindness in 2004, proportion of blindness was found to be 13% due to occurrence of postoperative complications of cataract surgery. Cataract with diabetes mellitus (DM) bears huge health as well as financial load, predominantly in developing countries where DM management as well as cataract surgery is found to be frequently unreachable. Cataract in diabetics must be taken as an issue worth emphasis by academics, researchers and the policymakers.[1] Frequency of type 2 DM and pre-diabetes has profusely raised than earlier supposed to be in Pakistan.[2,3] The prevalence of DM was reported as 26.3% in Pakistani populace by National Diabetes Survey of Pakistan (NDSP) 2016–2017.[4] The hazard of developing cataract among the cases of DM is 5 times greater than the general population. Cataract is found to be 2-fold as often among diabetic patients.[5] This is attributed mostly due to the speedy production of sorbitol in diabetic patients as related to non-diabetics. Inside the cell, due to polar property of sorbitol diffusion becomes difficult; this way, hyper osmotic effect is generated by accumulation of sorbitol that leads to infusion of fluid. Consequently, inside the cell polyols accumulate and lead to opacification of lens.[6] Rise in the incidence of cataract in DM patients has been observed. Progress in technology and expertise has made cataract surgery a common and safe technique now. Nevertheless, the diabetic patients bear still enhanced threat to sight-threatening problems, i.e., diabetic macular oedema, post-surgery macular oedema, advance in diabetic retinopathy as well as posterior capsular opacification.[7]

This study was carried out to determine the frequency of early postoperative complications after cataract surgery among diagnosed cases of DM in a tertiary care eye hospital.

METHODOLOGY

This clinical observational analytical study was carried at Department of Ophthalmology, Liaquat University Eye Hospital Hyderabad, and Liaquat University of Medical and Health Sciences, Jamshoro from May to Oct 2018 on diagnosed cases of diabetes mellitus, who underwent cataract surgery.

Patients with early senile cataract and diagnosed as cases of diabetes mellitus with controlled glycaemic status monitored with HbA1c, having normal arterial pressure and good macular function were

included in this study. Patients were enquired for postoperative findings after cataract surgery. Cataract patients with history of other eye surgery, secondary glaucoma, trauma to eye, posterior capsular tear or vitreous loss were not included. No preoperative medication was used.

The surgical procedures performed were extra capsular cataract extraction and phacoemulsification. After surgery on first postoperative day, all patients were evaluated for early postoperative complications by best corrected visual acuity BCVA (using Snellen's chart), slit lamp examination for anterior and posterior segment and measurement of intraocular pressure (IOP) with Goldman Applanation tonometer. The frequency of early complications like endophthalmitis, uveitis hyphema, raised IOP, macular oedema, and progression of retinopathy were noted. Fundal findings were well-thought-out as significant on the basis of occurrence of proliferative diabetic retinopathy, and clinically important macular oedema rendering to Early Treatment Diabetic Retinopathy Study Classification (ETDRS). The FFA and B-scan was done to support the diagnosis.

All data was recorded on a pre-designed proforma, and entered and analysed using SPSS-22. Mean±SD was calculated for quantitative variables, and frequency and percentage for qualitative variables. Qualitative variables were compared by applying Chi-square test, and $p<0.05$ was considered statistically significant.

RESULTS

Total 91 diabetic patients who underwent cataract surgery were included in this study. Their descriptive statistics are shown in Table-1.

Early postoperative complications of cataract surgery in diabetic patients were compared in two groups, one with diabetes <5 years and other group with diabetes for ≥5 years. Visual acuity of most patients remained between 6/6 and 6/24. Changes in fundus, raised IOP, Macular oedema, and progressive retinopathies were more frequent in patients with diabetes for >5 years than those with diabetes of <5 years duration, and the differences were significant ($p=0.01$) (Table-2).

Table-1: Descriptive statistics of study population

Variable	n (%)
Mean Age (Years)	38.14±4.06
<40 years	49 (53.8)
>40 years	42 (46.2)
Gender	
Male	33 (36.3)
Female	58 (77.3)
Duration of Diabetes Mellitus	
≥5 years	52 (57.1)
<5 years	39 (42.9)

Table-2: Association of early postoperative complications of cataract surgery with duration of diabetes mellitus (n=91) [n (%)]

Early postoperative complications of cataract surgery	Duration of diabetes mellitus		Total	*p*
	<5 years	≥5 years		
Visual acuity 6/6–6/12 and no complications	21 (23.1)	9 (9.9)	30 (33.0)	0.01*
Visual acuity 6/18–6/24	6 (6.6)	11 (12.1)	17 (18.7)	
Visual acuity 6/36–6/60	2 (2.2)	6 (6.6)	8 (8.8)	
Visual acuity <6/60	0	3 (3.3)	3 (3.3)	
Significant fundus findings	1 (1.1)	5 (5.5)	6 (6.6)	
Uveitis	7 (7.7)	8 (8.8)	15 (16.5)	
Hyphema	1 (1.1)	0	1 (1.1)	
Raised IOP	1 (1.1)	5 (5.5)	6 (6.6)	
Macular oedema	0	3 (3.3)	3 (3.3)	
Progression to retinopathy	0	2 (2.2)	2 (2.2)	
Total	39 (42.9)	52 (57.1)	91 (100)	

*statistically significant

DISCUSSION

Diabetes mellitus is a proved risk factor for developing cataract and to treat the affected eye cataract extraction is frequently performed. Cataract extraction among DM patients is allied with threat of capsular contraction with opacification, postoperative deterioration of macular oedema, and progression of diabetic retinopathy.[8,9]

In our study, majority of cataract patients were <40 years age and having diabetes mellitus for >5 years. There was significant association of early postoperative complications with longer duration of diabetes mellitus. According to recent reports by WHO, out of 51% of the worldwide visual impairment, 20 million individuals were found with loss of sight due to cataract.[10] As the figure of type 1 and 2 DM patients is on the upsurge, here is also an affiliated rise in the cataract surgery and related complications in DM cases.[10] Pascolini D *et al*[11] revealed cataract as first cause of blindness. Patients of DM develop cataracts earlier and so go for cataract surgery on earlier age as equated with healthy non-diabetics.[7,12] Hadad *et al*[8] and Heesterman *et al*[13] reported that diabetic patients undergoing cataract surgery have increased risk of developing postoperative complications compared to non-diabetics.

In this study, on first postoperative day, visual acuity of majority patients remained 6/18–6/24 and 6/6–6/12; while VA <6/60 was seen only in 3 patients. Ostri *et al*[14] expressed that VA improved significantly subsequent to phacoemulsification in DM patients irrespective of the grade of diabetic retinopathy. Oyewole K *et al*[15] found achievement of VA 6/12 or better on the first postoperative day among 76% of operated cases.

None of our patients was found with endophthalmitis in early postop period but according to Kiziltoprak H *et al.* [7] endophthalmitis has been reported as severe complication due to cataract surgery

specifically in DM patients and is allied with a deprived visual prognosis.

In this study, postoperative complications were significantly associated with >5 years duration of DM. Similarly, Yang *et al*[16] revealed that the duration, severity, type of diabetes, as well as the hardness of the lens, and HbA1c levels are threats for development of macular oedema after cataract surgery in diabetic patients. Ivancic *et al*[17] described about most common post-surgery complications, i.e., keratopathy, fibrinous uveitis as well as posterior capsular opacification among the DM patients underwent cataract surgery. In diabetic patients, endothelium might be more susceptible to trauma and have weaker compensatory capabilities. Cataract surgery in diabetic patients consequences in greater endothelial cell loss compared to non-diabetics.[18]

CONCLUSION

Though the frequency of complications after cataract surgery has decreased due to advances in surgical techniques, better preoperative assessment and understanding of diabetes control, still early postoperative complications are frequent in individuals with longer duration of diabetes.

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ORIGINAL ARTICLE

BITTER COFFEE FOR SWEET DIABETES: A RANDOMIZED CONTROLLED TRIAL FOR TREATMENT OF TYPE 2 DIABETES WITH BLACK COFFEE IN BALB C ALBINO MICE

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Background: Diabetes Mellitus is a chronic metabolic ailment which slowly but surely harms the human body if left untreated. The objective of this study was to determine the effect of black coffee on HbA_{1C}, fasting and postprandial blood sugar levels in mice model of type 2 diabetes mellitus. **Methods:** This was an experimental, randomized control study performed at the Pharmacology Laboratory, Multidisciplinary Research Laboratory at Islamic International Medical College and National Institute of Health (NIH) Islamabad Pakistan. The study comprised a total of 30 male Balb/c albino mice and diabetes was induced in experimental group (n=20) by using low dose streptozotocin (40 mg/Kg). After confirmation, diabetic mice were further divided into two groups of 10 each. Group 2 was diabetic control and Group 3 was treated with black coffee for 45 days. Blood samples were taken from lateral tail vein for fasting and post prandial blood sugar levels and by intracardiac puncture for HbA1c. Statistical analysis was done on SPSS-21. Comparisons between the groups were analyzed using one way ANOVA (post hoc tuckey test), and $p<0.05$ was considered significant. **Results:** Black coffee treated mice (Group 3) had significantly decreased serum HbA_{1C} levels (6.02±0.29) fasting (116.8±4.92) and postprandial (173.6±18.3) blood glucose levels in comparison with those found in diabetic control mice (Group 2). **Conclusion:** Black coffee significantly decreases serum HbA_{1C}, fasting and postprandial blood glucose levels in diabetic mice.

Keywords: Black Coffee, Diabetes, HbA_{1C}, Pancreatic islets cells

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INTRODUCTION

Diabetes mellitus is a complicated, multifactorial global health burden that has achieved pandemic extents, fuelled by collaborations between several environmental and social components. It is a chronic catabolic disease that is host to many devastating complications. Type 2 Diabetes is the most widely recognized type of Diabetes represented by hyperglycaemia, insulin resistance, and relative insulin insufficiency.[1–3]

The International Diabetes Federation (IDF) data delivered an estimate of 381.8 million adults in 219 nations and regions with diabetes for 2013; and anticipated the number to ascend to 591.9 million in 2035. Conferring to report of International Diabetes Federation (IDF) in 2015, in Pakistan 7 million individuals were diabetics.[4,5]

With change in lifestyle patterns and lack of physical activity, type 2 diabetes mellitus is becoming the foremost type of diabetes which is estimated to affect 642 people by the year 2040. Among the many risk factors, obesity has become the major culprit to cause type 2 diabetes.[6] Type 2 diabetes is increasing at alarming rate despairingly the incidence is stirring up at earlier age in both adults and children.[7] Diabetes related complications, especially cardiovascular impairment, have become the chief basis of morbidity and mortality.[8] Every 6 seconds a diabetic patient pays the penalty of this disease with his life.[9] Nearly 5 million deaths were prompted by type 2 diabetes in year 2017.[10]

The distinctive treatment option of oral hypoglycaemic agents, however offer great glycemic control, yet over a long course of time, somehow fails to halt the progression of complications eventually. Plant materials which are being utilized as customary medication for the treatment of diabetes for quite a long time are considered to turn into the great choices as new treatment innovations.[11] To alleviate this well-being liability, there is an urgent and immediate need to use effective therapies.[12]

A couple of agents used in our routine diet, like black coffee can ensure a magnificent effort in lowering the glucose levels of diabetic patients. Black coffee, being the rich source of antioxidants, fights with the oxidative stress which is believed to be one of the underlying mechanisms in pathogenesis of complications imparted by diabetes.[13]

Long-term coffee consumption is associated with increase in insulin sensitivity and decrease in risk of diabetes. Many investigators have established an inverse relationship between coffee intake and development of type 2 diabetes.[14,15]

Although there are numerous studies done to highlight the eloquent role of black coffee on development of diabetes, yet very scanty data is

available about black coffee as treatment option of type 2 diabetes. In this randomized control trial, the effects of black coffee in Balb/c albino diabetic mice are seen on fasting blood glucose, postprandial blood glucose and serum HbA_{1C}.

MATERIAL AND METHODS

This comparative randomized control experimental study was carried out at Pharmacology Laboratory, Multidisciplinary Research Laboratory at Islamic International Medical College and National Institute of Health (NIH) Islamabad Pakistan. Before starting the study, a formal approval by the Ethics Review Committee of Islamic International Medical College, Riphah International University, was obtained. The duration of this study was 12 months (April 2017 to April 2018). A total of 30 healthy, 6–8 weeks old male, weighing 30–50 g albino Balb/c mice, having normal baseline parameters were included in the study.

All the mice were accommodated in standard cages made up of plastic and placed on metallic racks, at the Animal House of NIH, Islamabad. The mice had free access to tap water through the inverted bottles fixed on top of the cages. Animal house atmosphere was maintained at 20±2 °C with relative humidity of 50–70% and a light and dark cycle of 12 hours each. After the acclimatization for 1 week, the mice were randomly divided into two groups, 10 mice were allocated to Group 1 and 20 mice were allocated to the experimental Group. Group 1 was labelled as Normal Control and was given normal diet for 5 days whereas the Experimental group was given normal diet plus streptozotocin (40 mg/Kg/day)[16] intraperitoneally for consecutive 5 days. After 5 days, confirmation of diabetes was done in experimental group by measuring fasting blood glucose levels. Experimental group was later on divided into two groups, i.e., Group 2 and 3. Group 2 mice were labelled as Diabetic Control and were given normal standard diet only. Group 3 mice were given normal diet mixed with Black Coffee (5 g/Kg/day)[17] orally for 45 days.

A mid cycle sampling was done after 20 days to see the progress of drugs on fasting and postprandial blood glucose levels. After 45 days of treatment, final sampling of the experiment was done from lateral tail vein in all groups which included fasting and postprandial blood glucose levels, and HbA_{1C} by cardiac puncture.

Statistical analysis was done using SPSS-21. Results were documented as Mean±SEM. Comparisons of quantitative parameters among the three groups were analysed by using the one way ANOVA (post hoc tuckey test), and $p<0.05$ was considered significant.

RESULTS

Table-1 shows the comparison of Mean±SEM of all the groups. The results of group 3 are comparable to group 2. The diabetic control and significant value of $p<0.05$ is seen.

In the below mentioned table, the significant results are verified which are certainly comparable with the disease control group. In black coffee treated group, there was a substantial drop in hyperglycaemia which displays the positive effect of black coffee as a treatment tool for type 2 diabetes.

Table-1: Comparison of Mean±SEM in all groups

		Group 1 Normal control		Group 2 Diabetic control		Group 3 Black coffee treated		
Sampling	**Variables**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	***P***
Initial sampling (day 15)	Fasting (mg/dl)	91.9	3.61	371.4	38.6	305.5	32.6	0.61
Mid-term Sampling (day 36)	Fasting (mg/dl)	81.0	4.07	337.6	35.3	116.8	4.92	0.000
	Random (mg/dl)	107.1	2.23	374.6	23.4	265.1	21.2	0.000
Terminal Sampling (day 61)	Fasting (mg/dl)	81.0	4.07	337.6	35.3	116.8	4.92	0.000
	Random (mg/dl)	139.1	7.35	443.8	21.5	173.6	18.3	0.000
	HbA_{1C} (%)	4.75	0.13	7.82	0.11	6.02	0.29	0.000

$p<0.05$=statistically significant

DISCUSSION

The results of present study confirm that hyperglycaemia induced by streptozotocin, is ameliorated by black coffee. In present study, the anti-diabetic effect of black coffee is seen in group 3.

Improvement of biochemical markers like HbA_{1C}, fasting and postprandial blood glucose levels in this study, is supported by the study of Sasha Jin *et al*[17] who studied the effect of chlorogenic acid (CGA, one of the major phenols present in black coffee) on glucose and lipid metabolism in late diabetic db/db mice for 12 weeks. They established that CGA could reduce the levels of fasting plasma glucose and HbA_{1C} during diabetes and improve kidney fibrosis through the modulation of adiponectin receptor signalling pathways in db/db mice.[17]

The reduction in biochemical and histopathological parameters were found by Kobayashi M *et al*[18] who used black coffee, caffeine extract, decaffeinated coffee against different sets of experiments to analyze the preventive part of black coffee on development of STZ-induced diabetes in male C57 BL/6J mice. They demonstrated that continuous black coffee ingestion prevented the development of STZ-induced diabetes mellitus. Black coffee also

increased pancreatic insulin output and suppressed the STZ-induced decline in pancreatic insulin content.[18]

Hui Cao *et al*[19] endorsed that significant evidence from epidemiological investigations showed that dietary polyphenols manage and prevent type 2 diabetes. Their review summarizes human studies and clinical trials of polyphenols as anti-diabetic agents. In prospective cohort studies, higher coffee consumption has been associated with a lower risk of Type 2 diabetes. It is demonstrated that ingestion of chlorogenic acid could significantly reduce early fasting glucose and insulin responses in overweight men during an oral glucose tolerance test. Polyphenols from different foods like coffee showed anti-diabetic effects in T2D patients through increasing glucose metabolism, improving vascular function as well as reducing insulin resistance and HbA1c level.[19]

Hesti Riany *et al*[20] concluded that different species of coffee (arabica, robusta and liberica) cultivated in Jambi province of Indonesia, have the ability to counter the hyperglycaemia induced by streptozotocin in mice. According to this intervention, the liberica species has offered the most promising results regarding biochemical analysis. There is marked decrease in fasting and postprandial blood glucose levels in streptozotocin induced diabetic mice given liberica species of coffee for 30 days as compared to mice given other species. There is another interesting aspect of this study is that when the histological parameters were examined; the mice treated with arabica species of coffee for 30 days, showed the lowest degenerative changes in liver.[20] That study embarks the finding in accordance with the previous studies.

Sake Juli Martina *et al*[21] determined that the use of Arabica coffee gayo bean and leaf extract showed a greater decrease in blood glucose levels in healthy mice after a glucose challenge. The biochemical parameters, i.e., fasting and postprandial blood glucose levels were markedly reduced to prove the evidence which are consistent with the outcomes of contemporary study. Blood sugar levels can be reduced by using Arabica coffee gayo bean extract as an alternative treatment. Taking coffee on regular basis not only reduces the risk of diabetes mellitus but also has ability to treat it. The components in coffee beans like caffeine stimulate lipolysis in adipose tissue. Arabica coffee gayo beans have ample amount of chlorogenic acid. Chlorogenic acid is a beneficial agent in the treatment of diabetes due to its antioxidant and anti-inflammatory effects which are very favourable in counteracting its effects.[21]

In the present study, improvement in biochemical parameters in Groups 3, which was given black coffee, was observed indicating that black coffee can be used in treatment of type 2 diabetes mellitus.

CONCLUSION

Black coffee significantly lowers HbA_{1C}, fasting and postprandial blood glucose levels in diabetic mice model. Therefore black coffee can be used as adjunct treatment for type 2 diabetes.

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ORIGINAL ARTICLE

TRIMESTER SPECIFIC REFERENCE RANGES OF SERUM TSH, FT3 AND FT4 DURING LAST SEMESTER OF PREGNANCY

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Background: Pregnancy is a stress for thyroid gland. After decades of research it is recommended that normal reference ranges of thyroid hormones test during different phases of pregnancy are necessary. The objective of this study was to establish serum TSH, FT3 and FT4 levels during last trimester of pregnancy. **Methods:** This was a cross-sectional analytical study. Simple convenient sampling technique was applied and sample size was calculated using classical sample size calculation formula of Cochran. Serum TSH, FT3 and FT4 were estimated by ELIZA method. **Results:** Maternal group showed serum TSH levels of 1.79±0.85 mIU/L. Maternal serum FT3 levels were 1.711±2.089 pmol/L. Mean serum FT4 of Maternal group were 1.31±1.07 ng/dL, (Range: 0.78–5.20 ng/dl). **Conclusion:** Our study population trimester specific ranges of TSH, FT4 and FT3 were lower than latest international levels according to Guidelines of American Thyroid Association of 2017. These values may be used to compare thyroid dysfunction in pregnancy.

Keywords: Thyroid hormones, TSH, T3, T4, Pregnancy, Pakistan, Reference value, trimester

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INTRODUCTION

Thyroid hormones are essential for foetal development during prenatal and postnatal period. During pregnancy, increased synthesis of thyroid hormones is needed to meet foetal requirements, which leads to high requirement of iodine.[1] Pregnancy is a stress for thyroid gland; it affects 3% women during pregnancy and 10% women of child bearing age. About 18% of Australian women have thyroid antibody positive test during first trimester of pregnancy.[2]

Establishment of normal reference ranges of thyroid hormones during different phases of pregnancy is essential. Non-pregnant references values should not be used for interpretation of laboratory results of pregnant women.[3] Trimester specific references of thyroid functions tests are not considered in the laboratories. Intensive research has been conducted worldwide to develop reference ranges of thyroid hormones during each trimester of pregnancy.[4]

During pregnancy, thyroid adapts through changes in thyroid economy and to meet the increased metabolic demands of the body. Thyroid function test of healthy pregnant women are different from those of non-pregnant women. Pregnancy specific, and ideally trimester specific, reference intervals for thyroid function are needed to be practiced.[5]

The Endocrine Society and American Thyroid Association have recommended population based, trimester-specific reference intervals and assay specific values to assess thyroid function during pregnancy.[6,7] The reference intervals for thyroid function tests should be based on 2.5th and 97.5th percentile of respective iodine deficient population. The studied population should comprise of healthy, selected women having singleton pregnancy and without any complication. Women with no history of thyroid disease, TPO-Abs-negative women, and women who are not taking anti-thyroid drugs are usually deemed healthy.[8]

In case of non-availability of population-based thyroid function tests reference levels, trimester specific TSH reference intervals should be practiced as recommended by the international guidelines of the Endocrine Society, irrespective of laboratory method. TSH reference interval for first trimester is 0.1–2.5 mU/L, for 2nd trimester 0.2–3.0 mU/L, and for 3rd trimester it is 0.3–3.0 mU/L respectively.[9]

The TSH cut-off values may be misleading to over diagnosis and treatment of thyroid disease in pregnant women because the recommended TSH cut-off value of 2.5–3 mU/L is less than most population-based studies showing higher reference values of TSH.[10] Currently researchers are not using trimester specific reference levels. Männistö *et al*[7] used population based calculated reference intervals of Northern Finland Birth Cohort (NFBC 1986 population) in their research. NFBC reference intervals for TSH were 0.07–3.1 mIU/L during first trimester and 0.1–3.5 mIU/l during second trimester respectively. Population-based free T4 reference intervals were found to be 11.4–22.4 pmol/L in first trimester and 11.1–18.9 pmol/l in second trimester respectively.[11]

FT4 immuno-assays in the market are method specific, for normal pregnancy related reference intervals. It is necessary that gestation specific reference intervals should be derived in appropriate reference population, otherwise it may lead to misinterpretation of thyroid status. Tandem mass spectrometry is a gold standard methodology for standardization of thyroid hormones values.[12]

Many researchers have reported that serum TSH reference ranges are lower throughout the pregnancy as compared with age matched non-pregnant women. During first trimester of pregnancy, serum TSH level decreases continuously. Both lower as well as upper normal limits of serum TSH level are lower about 0.1–0.2 mU/L and 1.0 mU/L respectively compared with non-pregnant women.[13,14]

Serum TSH level is the best indicator of thyroid function during 2nd and 3rd trimester. Reliable trimester specific, population-based reference intervals for TSH are available, based on adequate sample size of singleton pregnancies in iodine sufficient and antibody free population.[15,16] This study was designed to estimate baseline reference values of serum TSH, FT3 and FT4 levels in normal singleton pregnancy from a tertiary care hospital.

MATERIAL AND METHODS

This was a cross-sectional, hospital-based study. This study was conducted at the Department of Physiology, Institute of Molecular Biology & Biotechnology (IMBB), University of Lahore in collaboration with Departments of Gynaecology and Obstetrics, DHQ Teaching Hospital, Dera Ghazi Khan during Jan–Mar 2020. The study protocol was approved by Ethical Review Committee of Ghazi Khan Medical College, Dera Ghazi Khan and Board of Advanced Studies and Research, University of Lahore. Written informed consent was obtained from subjects. Simple convenient sampling technique was applied.

The study participants were thirty (n=30). Study participants were pregnant mothers with singleton pregnancy attending antenatal clinic during last trimester of pregnancy. All subjects were examined for any symptoms and signs of hypothyroidism and hyperthyroidism. Thirty (30) subjects fulfilling the selection criteria were included in the study. Sample size was calculated using classical sample size formula. Subjects having goitre were evaluated on clinical examination for hypothyroidism, hyperthyroidism excluded from the study. Subjects were selected during last trimester of pregnancy. A pre-tested structured questionnaire was used as data collection tool for interview and Clinical examination method by two-member research team of medical specialists. Family history of goitre and thyroid surgery and anti-thyroid medication was taken. Age, educational status, socioeconomic status, area of residence, duration of stay and gestational age was also recoded. Blood samples of the subjects were taken aseptically through venepuncture. Serum TSH levels, FT3 and FT4 levels were estimated using Chemi-illumination micro particle assay (C MIA) ARCHITECT fully automated ELIZA. Data was analysed using SPSS-18 for basic statistics. Mean±SD and percentile analysis for TSH, FT3 and FT4 was done.

RESULTS

A maternal group of thirty normal healthy, euthyroid pregnant women (n=30) was selected. Mean age of pregnant women of maternal group was 25.77±5.10 years. Mode of delivery in 16 (53.3%) women was Spontaneous Vaginal Delivery and 14 (46.7%) had to undergo caesarean section for delivery.

Mean serum TSH level was 1.79±0.85 mIU/l (Range: 0.63–3.84 mIU/l), serum FT3 levels was 1.711±2.089 pmol/l (Range: 0.770–12.09 pmol/l). Serum FT4 of maternal group was 1.31±1.07 ng/dl (Range: 0.78–5.2 ng/dl) (Table-1).

Serum TSH levels of maternal group on 5th, 50th, and 95th percentiles were 0.669, 1.790, and 3.304 mIU/l respectively. Serum FT3 levels of maternal group 5th, 50th, and 95th percentiles were 0.842, 1.120, and 3.574 pmol/l respectively. Serum FT4 levels of maternal group were 0.785, 0.990, and 5.09 ng/dl respectively (Table-2).

Table-1: Comparison of group parameters of Maternal Group (n=30)

Parameter	Mean±SD	SEM	Range
TSH	1.785±0.847	0.155	0.630–3.480
FT3	1.711±2.089	0.381	0.770–12.090
FT4	1.314±1.065	0.194	0.780–5.200

Table-2: Percentile of serum FT3, FT4, and TSH of study group (n=30)

	Percentile						
Variables	**5th**	**10th**	**25th**	**50th**	**75th**	**90th**	**95th**
Serum FT3	0.842	0.900	0.900	1.120	1.615	3.574	7.519
Serum FT4	0.785	0.842	0.900	0.990	1.068	2.144	5.090
Serum TSH	6	0.708	1.047	1.790	2.367	3.122	3.304

n=number of participants, 50th Percentile=Median

DISCUSSION

This study was performed to establish reference levels for maternal TSH, FT3 and FT4 during last trimester of pregnancy. It was imperative to establish reference levels from study area as no reference levels of maternal serum TSH, FT3 and sFT4 were available for Pakistani pregnant women, for comparison of maternal TSH, FT3 and FT4 levels of

normal pregnant ladies. Many countries have not yet established trimester specific reference ranges of thyroid hormones among normal pregnant women. Rare data is available among South Asian Region countries (SARC) on trimester specific reference ranges of thyroid hormones for pregnant women, which is required for early diagnosis and management of thyroid disorders.[17]

Pakistan is also having no trimester specific reference ranges of serum T3, TSH, FT3 and FT4 among healthy pregnant women which are taken as standard for diagnosis and treatment of thyroid disorders during pregnancy. It has been recommended that every tertiary care hospital should establish its own trimester specific reference ranges of thyroid hormones levels based on population of that area.[18] Our study population was also from a tertiary care hospital which had no trimester specific reference ranges for serum TSH, T3, T4, FT3, and FT4 for comparison. These reference ranges are also not available on national level. So our study established reference ranges from Maternal Control Group of study for compared with other countries reference ranges and recommended levels of TSH, FT3, and FT4 by American Thyroid Association.[19]

Elhaj *et al* reported trimester specific reference ranges of serum TSH, Serum FT3 and FT4 after a longitudinal study on pregnant Sudanese women. Their study was hospital based with sample size of sixty-three pregnant women (n=63). Our study was also hospital based and sample size thirty pregnant women (n=30) during third trimester. Our study design and sample size were in accordance with Elhaj *et al*. Our results for serum TSH and FT3 levels were significantly higher (p<0.00) than Elhaj *et al*[18].

In 2017, Alexander *et al*[19] updated the guidelines of American Thyroid Association (ATA) first published in 2011 for diagnosis and management of thyroid disease during pregnancy and post-partum period. Maternal serum FT3 concentration during 3rd trimester was decreased and was considered more precise and qualitative test. ATA recommended upper TSH level reference limit of 2.5 mU/l in first trimester, 4.0 mU/l during 2nd and 3rd trimesters, in case of non-availability of population based trimester specific reference ranges (5th–98th percentile). FT4 levels between 2.5–97.5 percentile were recommended as 7.4–12.2 ng/dl. Serum FT3 levels were recommended as 4.1–4.4 pg/ml during first and 4.0–4.2 pg/ml during 2nd trimester respectively.

Serum TSH and FT3 levels in our study were lower as compared to standards of ATA[19]. This could be because our study population, geographical distribution and dietary habits were different.

Yang *et al*[20] reported thyroid functions reference ranges during pregnancy in large Chinese Population and compared with guidelines of American Thyroid Association. Their study was hospital-based, and of longer duration of three years (2013–2016). Their study sample size was 46,262. Our study was also hospital-based with lesser duration and sample size of thirty (30) pregnant women in last trimester of gestation. Our results for serum TSH and FT4 during third trimester of pregnancy are in agreement with the Chinese women.

CONCLUSION

Trimester specific reference ranges of TSH, FT4 and FT3 of our study population were lower than international recommenced levels of 2017 Guidelines of American Thyroid Association. These values may be used to compare thyroid disease in pregnancy in our location.

RECOMMENDATIONS

More studies should be conducted in different regions of Pakistan to establish normal range of trimester specific values for thyroid function tests during pregnancy.

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ORIGINAL ARTICLE

ATP LEVELS AND ITS DEGRADATION PROCESS AS A POST-MORTEM INTERVAL INDICATOR

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Background: There are many scientific methods in practice to determine the time of death, but no methods alone will precisely state it. Researchers are trying direct and indirect methods to estimate the post-mortem interval (PMI) by gathering evidence to find a more accurate way out to measure time since death. Adenosine triphosphate (ATP) transfers energy within the body, and is a crucial indicator in estimating PMI. This study aims to find out the levels of blood ATP after death at certain periods to help determine the PMI. **Methods:** This experimental study was carried out at the Department of Forensic Sciences, University of Health Sciences, Lahore. Thirty-six rabbits with good health were taken and divided into six groups. Blood samples were obtained at regular intervals. The relationship between PMI and ATP degradation levels was analysed statistically. **Results:** At 0 hours, the mean ATP levels were recorded as 44.59 which declined linearly with the time recorded until 72 hours (1.51) post-mortem. The mean ATP levels at different post-mortem intervals were significantly different (p<0.001). **Conclusion:** ATP levels decline in blood post-mortem and can be used to determine PMI.

Keywords: Post-mortem, Post-mortem interval, ATP degradation,

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INTRODUCTION

The time of death holds great importance, especially in forensic sciences, as it is the most sought-after information that medical and legal experts try to find. In forensic sciences and criminal investigations, a tad bit of mistake or uncertainty of results may change the course of any murder case.[1] Soon after the death, the deceased body starts experiencing numerous physical as well as chemical variations. While there is no way to revert or evade these changes, it can be declared that these changes take place with order and can be somewhat predicted using constancy.[2] The rate at which expected changes will take place depends greatly on a variety of conditional and ecological attributes. The research lays its foundation on a solid understanding of how the time of death can be estimated as well as factors that can affect this interval.[3]

Many scientific methods include biochemical reactions and fluid concentrations levels that indicate time since death by changing their temperature and molecular concentration level in blood. Pericardial fluids, *p*H level, cerebrospinal fluid, vitreous humor, lipid concentrations in bone, synovial fluids, and Adenosine Triphosphate (ATP) levels are some indicators of post-mortem interval time since death.[4]

Adenosine Triphosphate is widely employed in determining the time elapsed since death. In a live body, ATP transfers the energy to muscles for voluntary and involuntary actions and to perform any physical activities.[5] When death occurs, metabolism in the body gradually decreases, hence the loss of ATP. It is crucial to take into account the significant role of ATP level to find a definitive method or technique to estimate Post-Mortem Interval (PMI) and hence our experiment was to determine the levels of ATP in blood after death at certain periods to help determine the PMI precisely.

Ninety-six percent of ATP comes from erythrocytes. When the death of human occurs, an imbalance of production and consumption of ATP starts. When the cause of death is unknown, then the breakdown of ATP is a very useful marker for measuring time since death because ATP gets utilized in metabolism and other chemical reactions actively. ATP level of blood starts to fall, and the equilibrium is disturbed after death.[6] The rate at which ATP changes into adenosine diphosphate (ADP) and then adenosine monophosphate (AMP) is the time since death when ATP unbalances the equation of production and decomposition of its molecules in the human body.[7]

Chinese researchers extensively conducted studies to measure the relation between ATP blood levels with the time passed since death. The process of breakdown of ATP molecules after death becomes slow as the time after death passes. Hence, it is found that there is a direct relation between blood ATP levels with the post-mortem interval time. In comparison with other research methods, the tendency of ATP to change and degrade may prove to be a better way for the estimation of PMI in medico-legal cases.[6] This study aimed to explore the correlation between blood ATP levels and post-mortem interval and to study this at different temperatures to come up with a reliable correlation between the two.

MATERIAL AND METHODS

This experimental study was conducted at the Department of Forensic Sciences, University of Health Sciences, Lahore. Adult, healthy rabbits of both genders were included in this study. Blood from the heart was taken for analysis.

The calculated sample size was 6 for each group. Thirty-six rabbits were used for the proposed study. The rabbits were sacrificed following the guidelines of the Ethical Committee of UHS and the international Public Service Guide for care and use of laboratory animals. All rabbits were placed in the supine position on the bench and sacrificed at the same time under anaesthesia with intraperitoneal injection.

The animals were divided into 6 groups of 6 each at different time intervals 0 hrs, 6 hrs, 12 hrs, 24 hrs, 48 hrs and 72 hrs respectively. Blood was drawn from the hearts of the rabbits by direct laparotomy/ autopsy procedure.

The interaction of the fluorescent reagent with ATP in the blood sample leads to fluorescence, whose intensity was luminometrically detected and recorded as Relative Light Unit (RLU). The ATP concentrations corresponding to the RLU were calculated from the standard equation involving RLU values and ATP levels.

The concentration of ATP (ηmol/µL or µmol/mL or mM) in the test samples was calculated as:

ATP concentration= (BV×D)×DDF

Where B=amount of ATP in the sample well calculated from the standard curve (ηmol or mM).
V=sample volume added in the sample wells (µL).
D=sample dilution factor if a sample is diluted to fit within the standard curve range (before the reaction well set up)
DDF=deproteinization dilution factor

Statistical analysis of data was performed with SPSS-20 and MATLAB software for data interpolation, fitting and curve plotting, testing the regression equations and interpolation functions.

RESULTS

The mean ATP levels at each post-mortem interval are mentioned in table 2. At 0 hours, the mean ATP levels were recorded as 44.59 which declined linearly with the time recorded at 6 hours (32.35), 12 hours (28.35), 24 hours (22.34), 48 hours (1.98), and 72 hours (1.51) post-mortem.

Data were normally distributed as assessed by the Shapiro Wilk test. A one-way ANOVA test was applied to compare the mean ATP levels among different post-mortem interval. It was found that the mean ATP levels at different post-mortem intervals were significantly different (p<0.001) (Table-1).

Table-1: Comparison of ATP levels in the blood at different post-mortem intervals (ηmol/µL)

PMI (Hrs)	ATP (Mean±SD)	Range	*p*
0	44.59±3.38	40.62–48.48	<0.001
6	32.35±2.30	29.67–35.31	
12	28.35±1.99	26.26–31.10	
24	22.34±1.56	20.61–24.74	
48	9.03±1.98	6.36–11.66	
72	1.51±0.92	0.26–2.62	

DISCUSSION

This study was done to determine the levels of ATP in blood at different time intervals at room temperature. Our results found a linear decrease in blood ATP level starting at 0 hrs to 72 hrs post-mortem. Interpolation of ATP levels in the blood is an appropriate choice for PMI estimation which is weakly influenced by ambient temperature.[8] Sun T, *et al*[8] experimented to find a relationship between ATP changes of rabbit blood and post-mortem interval. Blood ATP levels were measured by using the ATP fluorescence rapid detection technique, every 4 hours till 72 hours. They found that the blood ATP levels slightly increased early after death and then declined constantly at all temperatures. The ATP levels in blood showed relatively stable and regular degradation changes within 72 hours after death. Our results also showed a linear regression in ATP levels in blood being highest at 0 hrs (mean 44.59) and lowest at 72 hrs (mean 1.52). We didn't counter any hike in ATP levels in early hours, may be because our first-time interval was 6 hrs, not 4 hrs.

Sun TY, *et al*[5] also found that blood ATP level decreased with the extension in PMI. There are many studies available on determining the PMI by levels of ATP in muscles and its degradation products but quite a few studies available to discuss ATP levels in blood making our discussion minimal and hence limiting our study to this conclusion only.

In forensic medicine, determination of time of death has always been challenging to determine the time of death, both theoretically and practically as post-mortem changes are certain and they keep on progressing influenced by various external factors. Among all these factors, the most important factor is environmental temperature, affecting the estimation of PMI.[9] Our experiment was done at ambient room temperature and no temperature changes were recorded.

CONCLUSION

Under controlled experimental conditions, ATP level in blood degrades post-mortem, following a certain pattern. ATP levels in blood decline as the PMI extends. This decline in ATP level in blood may be used as a potential marker for the determination of PMI more accurately. The temperature change may have a lesser effect on ATP level decline and is linear in change.

LIMITATIONS

In animal studies, data available on PMI is very limited or restricted. Interpretation of the results obtained from different animal data is of substantial disparity in terms of research methods and species studied, making it hard to generalize the conclusions regarding the relativity of the findings of the experiments performed on animals.

FUTURE ASPECTS AND UTILIZATION

ATP detection in blood for estimation of PMI is convenient and reliable and may be easily taken up by the forensic scientists to use in real-time scenarios. Further studies are required to establish a set of average value drop in ATP at certain time periods.

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ORIGINAL ARTICLE

ASSOCIATION OF HEADACHE WITH THE DURATION OF WEARING FACE MASK DURING COVID-19 PANDEMIC

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Background: Many personal problems are experienced after wearing face masks during current pandemic. This study was designed to determine the association of headache with duration of wearing face mask. **Methods:** This cross-sectional study was conducted from Apr to Oct 2020. Non-probability purposive sampling was done. Non-health care professionals and health care professionals, including doctors, nurses, and paramedical staff wearing face masks were included. Individuals, not using facemask and suffering from respiratory, neurological dysfunction, migraine, or any other cause of headache were excluded. All volunteer participants were provided with self-designed proforma regarding facemask usage and duration of wearing face mask and working environment. Variables studied were gender, age, profession and duration of wearing face mask. **Results:** Mean age of study population (n=126) was 40.96±7.31 years; 68 (54.0%) were health professionals and 58 (46.0%) were non-health professionals. Among 126 participants, 94 (74.6%) were wearing facemasks for ≥6 hours and 32 (25.4%) for <6 hours. Headache was most common in those wearing mask for >6 hours while those wearing face mask for lesser duration were less likely to develop headache. Those wearing face masks for longer duration, complained of mental fatigue, anxiety and breathlessness as compared to those wearing face masks for lesser duration with (p=0.01). Headache was more prevalent in health professionals (p=0.01). **Conclusion:** There is significant positive association of headache with wearing face mask for longer duration and mostly affected are health professionals.

Keywords: COVID-19, corona virus, face masks, duration, Health professionals, Front liners, Hospital

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic stances an abrupt upsurge in hospitalizations for pneumonia with multi-organ disease and has created panic in general population worldwide. COVID-19 is caused due to emergence of a novel virus in December 2019, known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus was initially identified in Wuhan city of China, when a huge group of individuals presented in hospitals due to pneumonia with unidentifiable notorious causative agent. As per 1st July 2020, SARS-CoV-2 was the cause that pretentious more than 200 countries, and out of affected more than 10 million known cases, there were about 508,000 confirmed deaths.[1] Identifying the COVID-19 infection amongst health professionals and determent risk agents that threat towards worse outcomes is the need of the time for portraying viral transmissions as well as for averting future infections and for updating and apprising preventive control measures for health care professionals and also the general populace.[2]

Throughout the intensification in cases of COVID-19 in different countries, all health control authorities documented as well as implemented the mandatory usage of Personal Protective Equipment (PPE) by the health professionals in contact with positive cases. This PPE is generally composed of protective suit, medical gloves, defensive goggles, face shield and face mask. There are several types of face masks, and the most recommended include, highly effective with type FPP2 (Europe), N95 (USA) and KN95 (China).[3] Other types of masks including surgical masks are also commonly used by general public and health warriors not in direct contact with Covid-19 patients.[4]

Wearing the face masks for an extended duration may lead to physiological as well as psychological problems and may decline the working efficiently. Capability of performing work proficiently declines while wearing face mask. In fact, the time edge that an activity can be continued is diminished after wearing face masks and also PPEs.[5] Adverse impacts have been observed while wearing N95 and surgical masks for prolonged time such as headache, skin problems (rashes and acne) as well as the weakened cognition in majority of inspections and surveys.[6]

Although, chances for the subsequent waves of COVID-19 are looking so nearby; that's why preparation for prevention and control measures for upcoming expected pandemic are required to be able to combat the anticipated adverse effects. Recurrent skin issues may be resolved by improving the level of hydration and rest, skin care, and so on; if possible, well designed comfortable masks are one of the potential recommendations for appropriate

management of adverse effects associated with prolonged face mask usage.[6] Wearing the face masks with good hygiene of hands has been considered as one of the effective and acceptable measures for prevention of SARS-CoV-2 transmission.[7] Discomfort has been reported due to usage of tight-fitting PPE for extended duration.[8] Facemask associated headache has been reported due to wearing face masks for longer durations.[9]

According to National Institute of Health in the United State, inhaling higher levels of CO_2 might be life threatening if they exceed the tolerable range, as CO_2 is possible to build up in the face mask. Carbon dioxide at lower level has little toxic and noxious effects; nonetheless this is well known to be perilous when >10% in living atmosphere. At advanced level, >5%, may lead to hypercapnia and respiratory acidosis. CO_2 toxicity may cause headache, inability to focus and concentrate well.[10] Re-breathing CO_2 for extended time wearing facemask has not been taken into attention.

Hypercapnia because of inhalation of CO_2 has been suspected for developing mental exhaustion, fatigue, muscular weakness, headache and also drowsiness. Inhaling repeatedly the exhaled air enriched with CO_2 may cause upward surges in arterial CO_2 concentration that may be the fundamental mechanism for headache and other attributes like palpitation, drowsiness, and dyspnoea. This study was designed to determine the association of headache with duration of wearing face mask during the COVID 19 pandemic.

METHODS

This cross-sectional study conducted by Physiology Department in collaboration with Medicine Department, Indus Medical College, Tando Muhammad Khan from 7th April to 7th October 2020 by nonprobability purposive sampling. Healthcare professionals (n=68), including doctors, nurses, paramedical staff wearing face mask were included and for comparison non-healthcare professional (n=58) were included. The professionals not using facemask, suffering from respiratory or neurological dysfunction, migraine, or any other disorder causing headache were excluded from this study. All volunteer participants were provided with self-designed proforma regarding facemask usage, duration of wearing face mask and working environment. Data were entered and analysed on SPSS-20. Results were tabulated as Mean±SD.

RESULTS

Descriptive statistics of study population are shown in Table-1.

Presence of headache was compared with duration of wearing face mask and there were statistically significant differences (p<0.01) found for the duration of mask wearing. Headache was common in those wearing mask for longer duration (>6 hours) while among those wearing face mask for shorter duration (<6 hours) it was found less likely to develop headache (Table-2).

Presence of complaints other than headache were also observed among face mask users and were compared according to duration of wearing face mask. Complaints of mental fatigue, anxiety and breathlessness were more common among those wearing face mask for longer duration compared to those wearing face masks for lesser duration that is statistically highly significant (Pearson Chi-square=19.92, df=5 and p=0.01) (Table-3)

In this study, when frequency of headache was compared between health professionals and non-health professional, headache was more prevalent in health professionals with significant difference (p=0.01, Pearson Chi-square 6.426, df=1) (Table-4).

Table-1: Descriptive statistics of study population (n=126)

Variable	Number (%)
Age in years (Mean±SD)	40.96±7.31
Gender	
Male	104 (82.5%)
Female	22 (17.5%)
Profession	
Health professionals	68 (54.0%)
Non-health Professional	58 (46.0%)
Duration of wearing face mask (hours)	
>6 hours	94 (74.6%)
<6 hours	32 (25.4%)
Headache	
Yes	88 (69.2%)
No	38 (30.2%)

Table-2: Association of headache with duration of wearing face mask (n=126)

Duration	Headache		Total
	Yes	No	
>6 hours	77 (61.1%)	17 (13.5)	94 (74.6%)
<6 hours	11 (8.7%)	21 (16.7%)	32 (25.4%)
Total	88 (69.8%)	38 (30.2%)	126 (100%)

Table-3: Complaints other than headache according to duration of wearing face mask (n=126)

Duration	Complaints other than headache						Total
	Hearing loss	Anxiety	Palpitation	Difficulty breathing	Mental fatigue	Halitosis	
>6 hours	4 3.2%	28 22.2%	6 4.8%	16 12.7%	28 22.2%	12 9.5%	94 74.6%
<6 hours	2 1.6%	9 7.1%	3 2.4%	3 2.4%	1 0.8%	14 11.1%	32 25.4%
Total	6 4.8%	37 29.4%	9 7.1%	19 15.1%	29 23.0%	26 20.6%	126 100%

Table-4: Association of headache among health professional (n=68) and non-health professional (n=58) wearing facemasks

	Headache		Total
Profession	**Yes**	**No**	
Health professional	54 (42.9%)	14 (11.1%)	68 (54.0%)
Non health professionals	34 (27.0%)	24 (19.0%)	58 (46.0%)
Total	88 (69.8%)	38 (30.2%)	126 (100%)

DISCUSSION

In this study, headache among face mask wearers is significantly associated with duration of wearing face mask and profession (including both health and non-health professionals). Significant association has been observed between other complaints than headache among face mask wearers with duration of wearing face mask.

The findings of this study are supported by Rapisarda *et al*[9] and Ramirez-Moreno *et al*[11] that personal protective equipment (PPE) has been required for health professionals in directive to cover the outbreak of pandemic COVID-19. Slight neurological instabilities such as headache had been associated to extensive utilization of facemask.

Healthcare providers are vital resources for every country. Their health and safety are crucial not only for continuous and safe patient care, but also for control of any outbreak.[12] Recent face masks with shielding eyewear designs rely on flexible skull straps for ensuring a tight-fit, often leading to headache, facial pain or ear lobe discomfort because of tensional forces on head.[8]

Healthcare professionals are more prone to develop headaches following prolonged use of face mask. Furthermore, it was also suggested that wearing facemasks for shorter durations might decline the frequency and severity of face mask related headaches.[11] Our study is in agreement with it.

There is a lack of data regarding the period for which the same face mask might be continuously used, and none of guidelines addresses this. Available data suggest that respirators may be used intermittently or continuously for around 8 hours and that adverse effects of facemasks increase with more than 8 hour use.[13]

Restrictive airflow due to face mask is the main cause of hypercapnia that can lead to respiratory failure with symptoms of tachycardia, flushed skin, dizziness, papilledema, seizure and depression. According to latest updates face shield and social distancing could be better substitute of face mask.[14] Choudhury *et al*[15] also revealed various physiological effects among health care workers wearing face masks or PPEs for prolonged duration in ICUs. These changes highlight the need for institutional policies for better working conditions for healthcare workers, shorter working shifts or suitable breaks during the shifts to sustain hydration as well as rest, and research on better quality PPE as these health professionals are frontline workers on whom the medical care rests in the pandemic.

CONCLUSION

There is significant positive association of headache and other attributes with wearing face mask for longer duration and mostly affected were the health professionals. Monotonous long term execution of physical measures to interject or lessen the spread of respiratory viruses might be tough but numerous modest and little cost interventions might be possibly useful in reducing the spread. Possibly, shorter duty shifts and shortening the interval of PPE use, might be a healthier approach to evade adversative influences of PPE usage.

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ORIGINAL ARTICLE

SERUM VISFATIN IN NON-OBESE MALE NORMAL AND CORONARY ARTERY DISEASE PATIENTS

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Background: Mechanism of direct cardioprotective action of visfatin is still unclear. The aim of this study was to detect the serum visfatin concentration in healthy males and compare it with non-obese male patients of coronary artery disease to access the cardioprotective role of visfatin. **Methodology:** It was a cross-sectional, comparative study. Data was collected from July to December 2018 after obtaining informed consent of the subjects. The participants included 20 non-obese healthy males, and non-obese males angiographically confirmed having coronary artery disease. All participants were non-smoker, non-diabetic, and age matched from 35–55 years. Serum visfatin was analyzed using ELISA. Anthropometric measurements including waist circumference, hip circumference, and body mass index was evaluated and correlated with serum visfatin. Statistical analysis was done using SPSS-20. The values were considered significant at $p<0.05$. **Results:** Serum visfatin levels were significantly lower (3.90 ng/ml) in non-obese coronary artery disease group as compared to healthy males (4.85 ng/ml). No significant correlation was found with anthropometric measurements. **Conclusion:** Significant lower level of serum visfatin in non-obese male coronary artery disease patients depicts its probable cardioprotective role that is independent of anthropometric measurements.

Keywords: Visfatin, waist circumference, hip circumference, body mass index

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INTRODUCTION

The adipocytokine visfatin that simulates the glucose decreasing effect like insulin, and initiates phosphatidylinositol-3-OH kinase (PI3K) pro-survival kinases-protein kinase B and mitogen-activated protein kinase 1 and 2 (MEK1/2)-extracellular signal-regulated kinase 1 and 2 (Erk 1/2)[1] may have an anti-apoptotic effects[2]. Inhibition of mitochondrial permeability transfer pore (mPTP), activation of these kinases reverses cardioprotection. Mechanism of direct cardioprotective action of visfatin is still unclear. Visfatin can minimize myocardial damage within murine heart and insulated murine cardiomyocytes when directed during myocardial recovery. The mechanism has a tendency to cover MEK1/2, the PI3K pathways and mPTP.[3] This may be due to the lack of serum visfatin in male patients with non-obese coronary artery disease. Initiation of the above-mentioned kinases maintains powerful cardioprotection at the time of myocardial reperfusion[4,5], and that inhibition of the mitochondrial permeability transition pore (mPTP)[6] is the second consequence. mPTP is a non-specific mitochondrial channel whose initiation of myocardial reperfusion in the first few minutes is a major contributing factor to cardiomyocyte death.[7]

The visfatin gene is impaired by hypoxia-inducible factor[8,9] increasing the likelihood of up-regulation of visfatin in response to myocardial ischemia. Visfatin was previously known as a pre-B cell colony enhancing factor (PBEF)[10], a growth factor associated with a number of cellular processes for early B cells, with studies showing that PBEF acts as a biomarker of acute pulmonary injury[11,12] up-regulated in infected foetal membranes[13], neutrophil apoptosis is prevented by laboratory inflammation and clinical sepsis[2] and is involved in the growth of vascular smooth muscle cells through a mechanism based on nicotinamide adenine dinucleotide (NAD)[14]. The enzyme nicotinamide phosphoribosyltransferase (Nampt), the rate-limiting enzyme in NAD biosynthesis that mediates the conversion of nicotinamide into nicotinamide mononucleotide[15], was also known as PBEF/visfatin. It is uncertain if visfatin contributes in clinical setting to cardioprotection. Recent studies have related visfatin to MAPK-mediated angiogenesis[16] and to a possible pro-inflammatory facilitator in atherosclerotic plaques, indicating that endogenous visfatin may have a negative effect on coronary artery disease.[17]

Visfatin plasma concentrations have no significant variations in visfatin *m*RNA expression between human visceral and subcutaneous adipose tissue.[18] The levels of circulating visfatin were decreased in morbidly obese men after gastric banding.[19] In acute and chronic cases, these effects of visfatin vary. The aim of this study was to detect the serum visfatin concentration in healthy males and compare it with non-obese male patients of coronary artery disease to access possible cardioprotective role of visfatin.

METHODOLOGY

This was a cross-sectional study including 20 non-obese healthy males without coronary artery disease and 20 non-obese male patients with angigraphically confirmed coronary artery disease. The sample size was calculated to be 20 in each group.

Waist and hip circumferences were recorded (Cm). Waist to hip ratio was calculated. Height (m) and weight (Kg) in all subjects were recorded wearing usual clothes, without shoes. Body mass index (BMI) was calculated as:

BMI=Weight in Kg/(Height in m)²

Subjects were categorized as normal, BMI=18.0–22.9, over weight, BMI=23.0–24.9, and obese, BMI≥25 (Table-1).

Serum visfatin levels were determined by Nampt/PBEF, Human ELISA kit manufactured by Enzo Life Sciences (ELS) AG Switzerland, with an analyzer STAT FAX 303 Reader. The intensity of the colour reaction was measured at 450 nm after acidification and was directly proportional to the concentration of Nampt in the samples. Statistical analysis was done using SPSS-20, and $p<0.05$ was taken as significant.

RESULTS

There was no significant difference between BMI, Waist circumference, and Waist-Hip ratio in group A and group B (p=0.979, 0.126, and 0.978 respectively). The difference between group A and group B was significant (p=0.048) in hip circumference (Table-1).

Median (IQR) of group A was 4.85 ng/ml (3.55–8.60), group B was 3.90 ng/ml (2.70–5.0) compared using Mann-Whitney U test as data was not normally distributed (Table-2).

Statistically non-significant negative correlation was observed with serum visfatin and BMI. (r= -0.19, p=0.419) in group A. No significant correlation was observed between serum visfatin and BMI in group B; waist circumference, hip circumference, and waist-hip ratio in group A and B (Table-3).

Table-1: Comparison of anthropometric measurements between groups A and B

Anthropometric Parameters	Group A Healthy (n=20)	Group B CAD (n=20)	*p*
BMI (Kg/m²)	22.00±0.73	22.07±0.89	0.979
Waist Circumference (Cm)	77.85±5.71	80.75±3.24	0.126
Hip Circumference (Cm)	89.00±6.37	92.85±3.66	0.048*
Waist-Hip Ratio	0.87±0.01	0.87±0.02	0.978

Mean±SD, *Statistically significant

Table-2: Comparison of serum visfatin between group A and group B using Mann-Whitney U test

Biochemical Parameter	Group A (n=20)	Group B (n=20)	*p*
Serum Visfatin (ng/ml)	4.85 (3.55–8.60)	3.90 (2.7–5.0)	0.046*

Values are given as Median (IQR), *Statistically significant

Table-3: Correlation of serum visfatin with anthropometric measurements

Serum visfatin & anthropometric measurements	Group A (n=20)		Group B (n=20)	
	r	*p*	*r*	*p*
BMI	-0.19	0.419	0.37	0.113
WC	0.13	0.591	0.40	0.083
HC	0.11	0.644	0.22	0.346
WHR	0.35	0.132	0.19	0.428

DISCUSSION

Serum visfatin concentrations are significantly lower in male subjects with coronary artery disease as compared to healthy non-obese male patients in our study. Serum visfatin shows no significant correlation with waist circumference, hip circumference, and body mass index.

Recent evidence indicates that visfatin can be responsible, depending on the cell type and length of treatment, for a variety of cardiovascular effects, one of which involves the ability to shield the myocardium from adverse effects of acute ischemia-reperfusion injury.[19] As such, visfatin may not only provide a potential new cardioprotection target but may also serve as an anti-diabetic agent with an exclusive mechanism of action to provide diabetic patients of an episode of acute myocardial ischemia-reperfusion injury with a potential new drug target. This is also confirmed by Lovren *et al*[20] who stated that visfatin-containing plasmid injection in a unilateral limb ischemia mouse model resulted in improved limb perfusion compared with untreated animals.

Results are contradictory on the relationship between anthropometric parameters and levels of visfatin. Some researchers found associations between visfatin and BMI, others showed no correlation[21] or even negative correlation[22]. There were no changes in the BMI or waist circumference between the patients and the controls in our study. The findings showed that visfatin and BMI levels were not significantly associated with coronary artery disease in non-obese male patients.

BMI plays a major role in the development of cardiovascular diseases and is shown in Table-3 in the current study. In healthy groups, there is a negative but not significant association. With respect to cardiovascular disease[20], visfatin has been reported to have many different effects. These involve endothelial dysfunction, angiogenesis, instability of the atherosclerotic plaque, and cardioprotection. The interesting finding is that visfatin can directly protect the myocardium at the cardiomyocyte level against the symptoms of acute ischemia-reperfusion injury.[23] The function of visfatin levels that are lowered in non-obese male patients with coronary artery disease is difficult to determine as being cardioprotective. Reduced levels of visfatin in non-obese male CAD patients may play a protective role in the heart. Visfatin's vascular function in chronic and acute cases is different.

High visfatin concentrations stimulate endothelial dysfunction, atherosclerotic plaque unstabilization, angiogenesis, such as in obesity and type 2 diabetes mellitus when exposed for longer time. Immediate visfatin administration stimulates endothelial nitrous oxide synthase expression and activity in endothelial cells and directly protects cardiomyocytes from the adverse effects of acute ischemia-reperfusion injury.[24] The role of visfatin as a cardioprotective agent may be observed in large-scale studies.

CONCLUSION

Significant lower levels of serum visfatin in non-obese male patients with coronary artery disease represent most likely its cardioprotective role and are independent of anthropometric measurements. Further studies on a larger scale are recommended including females subjects.

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ORIGINAL ARTICLE

CLINICAL PROFILE AND OUTCOME OF ACUTE KIDNEY INJURY IN PATIENTS WITH NEONATAL SEPSIS

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Background: Acute kidney injury (AKI) in patients with neonatal sepsis is associated with worse outcomes. This study was conducted with an aim of developing an insight into the magnitude of this problem in our region. **Methods:** This descriptive cross-sectional study was conducted at Neonatology Unit of Ayub Teaching Hospital from 1st Jul 2019 to 31st Dec 2019. All neonates diagnosed as cases of neonatal sepsis and having acute kidney injury were included in the study. Patient characteristics were recorded on a structured proforma and analysed using SPSS-20. **Results:** A total of 115 neonates were included in the study. Among the study population, 75 (65.2%) were male and 40 (34.8%) were female. A total of 77 (67%) patients were diagnosed as having early onset neonatal sepsis and 38 (33%) patients presented with late onset neonatal sepsis. History of delayed cry at birth was present in 57 (49.6%) patients. A total of 83 (72.2%) patients were discharged, 22 (19.1%) patients expired, 4 (3.5%) patients were referred and 6 (5.2%) patients left against medical advice. There was a statistically significant difference in outcome in relation to history of delayed cry (p=0.002) and involvement of other organ systems in addition to AKI (p=0.002). **Conclusion:** Acute kidney injury is quite prevalent in neonates with sepsis. Asphyxiated newborns with sepsis and AKI are more prone to adverse outcomes.

Keywords: acute kidney injury, neonatal sepsis, mortality

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INTRODUCTION

Acute kidney injury (AKI) implies failure of the renal system to get rid of the nitrogenous waste products and maintain fluid and electrolyte balance.[1] New-borns in general and preterm babies in particular have a predilection for developing acute kidney injury owing to the immaturity of the renal mechanisms in this age group.[2] Studies from the different regions of the world have reported an incidence of 8–24% AKI in neonatal population with the variation being based on the type of study population. AKI in neonates has recently been a focus of interest owing to the improvement in survival rates of critically ill neonates due to advanced management protocols. AKI in neonatal age group is postulated to be associated with development of chronic kidney disease later in life.[3] Both term and preterm sick admitted neonates, without pre-existing renal disease, quite frequently suffer from acute kidney injury.[4]

Pre renal acute kidney injury is the commonest form of renal injury in neonates. It occurs either due to poor perfusion or ischemic injury to the kidneys. There are a multitude of underlying aetiologies but most studies document birth asphyxia and sepsis to be the most common. The physiological qualities of the new-born renal system including high vascular resistance, high rennin activity, decreased glomerular filtration and low intra cortical perfusion rates predispose them to acute tubular necrosis.[5]

There are a myriad of features based on the severity of renal damage with the treatment options ranging from simple medical management to renal replacement therapy, peritoneal dialysis and hemodialysis.[6] Studies from different regions of the world have documented sepsis as the leading cause of acute kidney injury in neonatal population. Acute kidney injury secondary to septicaemia has also been considered a major determinant of poor neonatal outcome.[7,8]

Neonatal sepsis implies the clinical and systemic manifestations arising from bacterial, viral or fungal invasion of the body. Sepsis is considered to be a significant cause of morbidity and mortality in neonatal population. The disease manifestations are manifold ranging from mild presentations to severe systemic and multi-organ involvement. The exact incidence vary in different regions of the world[9,10], and is estimated to be 1 to 50 per 1,000 live births.[11] Recent estimates from a meta analysis documented 2,202 cases of neonatal sepsis per 100,000 live births with a mortality of 11–19%. Globally, these figures predict an estimated incidence of 3 million cases of sepsis in neonates.[12]

Worldwide neonatal sepsis is estimated to be responsible for 3% to 30% cases of paediatric mortality per year. Neonatal sepsis is categorized as early onset neonatal sepsis (EONS) and late onset neonatal sepsis (LONS) based on the disease

manifestations in neonates from birth to seven days of life and from second week of life to 28 days of life, respectively.[11]

Sepsis is a major cause of neonatal deaths in our region and acute kidney injury in these patients further worsens the prognosis. There is paucity of data on frequency, clinical profile and outcomes of AKI in septic neonates in our region. The present study is aimed at developing an insight into the magnitude of this problem.

PATIENTS AND METHODS

This was a descriptive cross-sectional study conducted at Neonatology Unit of Ayub Teaching Hospital from 1st July 2019 to 31st Dec 2019. Approval of the hospital ethical committee was obtained. A total of 1,028 neonates were admitted in Neonatology Unit with sepsis during this time period. Out of these, 115 patients fulfilled the criteria of acute kidney injury and were included in the study. Patients with deranged renal profile secondary to congenital anomalies of the urinary tract were excluded from the study. Acute kidney injury was defined as serum creatinine levels >1.5 mg/dL and/or urine output less than 0.5 ml/Kg/hr. Sepsis was defined either on the basis of clinical criteria (based on EMA Sepsis scoring)[13] and/or microbiological isolation of organisms on cultures.

Patient characteristics like age, gender, weight, gestational age, duration of hospital stay, mode of delivery, place of delivery, type of sepsis, and outcome were recorded on a structured proforma. Serum values of urea, creatinine, sodium and potassium were also recorded. Data was entered and analysed using SPSS-20. Descriptive statistics were used to calculate mean and standard deviation for age, weight, duration of hospital stay serum urea, creatinine and serum electrolytes. Categorical variables like gender, type of sepsis, gestational age, history of delayed cry at birth, mode of delivery, place of delivery and outcome were described as frequencies and percentages. Significance testing in case of categorical variables was done using Chi-square test, and $p<0.05$ was considered significant.

RESULTS

Out of a total of 1,028 neonates, 115 (11.1%) with sepsis were included in the study. Mean age of the patients was 6.49±6.855 days, and mean weight was 2.52±0.652 Kg. Mean duration of hospital stay was 7.54±4.105 days (Table-1).

Of the total patients with acute kidney injury, 75 (65.2%) were male and 40 (34.8%) were female. A total 77 (67%) patients were diagnosed as having early onset neonatal sepsis and 38 (33%) patients presented with late onset neonatal sepsis. Mode of delivery was vaginal delivery in 94 (81.7%) patients and caesarean section in 21 (18.3%). A total of 95 (82.6%) babies were born at full term while 20 (17.4%) were preterm. History of delayed cry at birth was present in 57 (49.6%) patients. A total of 61 (53%) patients presented with involvement of other organ systems in addition to acute kidney injury (Table-2).

Eighty-three (72.2%) patients recovered and sent home on treatment, 22 (19.1%) patients expired, 4 (3.5%) patients were referred to hospitals with facilities for dialysis and intensive care, and 6 (5.2%) patients left against medical advice. Patients were further categorized as survivors 87 (75.7%), and non-survivors 28 (24.3%) on the basis of outcome (presuming those who left against medical advice as non-survivors) (Table-2).

There was no statistically significant difference in outcome in relation to gender (p=0.706), type of sepsis (p=0.458), gestation (p=0.730) and mode of delivery (p=0.678). The difference was statistically significant when outcome was assessed in relation to history of delayed cry (p=0.002) and involvement of other organ systems in addition to AKI (p=0.002) (Table-3).

Table-1: Patient characteristics (n=115)

Patient characteristics	Mean±SD
Age (days)	6.49±6.855
Weight (Kg)	2.527±0.652
Duration of hospital stay (days)	7.54±4.105
Serum urea (mg/dL)	122.669±72.037
Serum creatinine (mg/dL)	2.128±0.966
Serum sodium (mEq/L)	137.973±12.788
Serum potassium(mEq/L)	5.081±1.128

Table-2: Demographic characteristics of participants (n=115)

Variables	No. of patients	Percentage
Gender		
Male	75	65.2
Female	40	34.8
Mode of delivery		
Vaginal delivery	94	81.7
C Section	21	18.3
Type of sepsis		
Early onset	77	67
Late onset	38	33
Gestational age		
Full term	95	82.6
Preterm	20	17.4
Delayed cry at birth		
Yes	57	49.6
No	58	50.4
Multi-organ failure		
Yes	61	53
No	54	47
Outcome		
Discharged on home treatment	83	72.2
Expired	22	19.1
Referred to other centres	4	3.5
Left against medical advice	6	5.2
Survival		
Survivors	87	75.7
Non-survivors	28	24.3

Table-3: Patient characteristics in relation to outcome

Variables	Survivors	Non-survivors	*p*
Gender			
Male	56	19	0.736
Female	31	9	
Mode of delivery			
Vaginal delivery	70	24	0.531
C Section	17	4	
Gestational age			
Full term	73	22	0.517
Preterm	14	6	
Type of sepsis			
Early onset	55	22	0.133
Late onset	32	6	
Delayed cry at birth			
Yes	36	21	0.002
No	51	7	
Multi-organ failure			
Yes	39	22	0.002
No	48	6	

DISCUSSION

Acute kidney injury was documented in 11.1% septic neonates in our study. The incidence of AKI was reported to be 4.24% in one study[14] from India, while another study[15] AKI was reported to be in 26% of the septic neonates. Studies from different regions of the world have reported different incidences of AKI that may be attributed to demographic differences. Furthermore the variability can also be explained keeping in view the different definitions used to define AKI in different studies.

Acute kidney injury in septic neonates was more common in males as compared to female neonates in our study. Similar results are reported in studies from Iran[16], Pakistan[17], and Western India[14] where AKI was predominantly documented in male neonates. However, Momtaz *et al*[8] have documented a female preponderance in their study. History of delayed cry at birth was documented in nearly half of the study population in our study. Shalaby MA *et al*[18] have also reported birth asphyxia as a significant risk factor for AKI in sick neonates in a study from Saudi Arabia. Low apgar scores and asphyxia at birth are postulated to be significant risk factors of AKI in neonates irrespective of sepsis in a large number of studies[14,19].

The majority of our study population presented with acute kidney injury secondary to early onset sepsis. Nearly three quarter of the neonates included in our study were full term. Mwamanenge *et al*[20] in a study on critically ill neonates in Tanzania, have reported similar results with nearly 81% of neonates with AKI presenting in the first 7 days of life. Also this study documented AKI predominantly in full term neonates.[20] Another study from Iran[8] showed that full term neonates were primarily affected by acute kidney injury. We found that majority of the patients who presented with AKI secondary to neonatal sepsis were born through vaginal delivery. Lee CC *et al*[21] also documented similar results in their study where caesarean section was presumed to be protective against neonatal AKI.

Nearly a quarter of patients enrolled in our study expired (presuming those who left against medical advice as non survivors). Ali *et al*[17] reported a mortality of 14.9% in neonates with acute kidney injury in a study conducted in Multan. Mortazavi *et al*[15] reported a mortality rate of 20.5%, while another study from Turkey[22] reported a mortality of 23.8% in sick neonates with AKI. Our results are generally in agreement with these studies.

CONCLUSION

Acute kidney injury is quite prevalent in neonates with sepsis and is a significant cause of mortality and morbidity. Asphyxiated newborns with sepsis and AKI are more prone to adverse outcomes. Early diagnosis and meticulous management is required to improve outcomes in these patients.

LIMITATIONS OF THE STUDY

Due to an overburdened neonatology unit and paucity of resources in our setting we could not use the newer definition of acute kidney injury according to Kidney Disease Improving Global Outcomes criteria. Further studies are recommended for evaluating outcomes in patients with different stages of acute kidney injury.

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ORIGINAL ARTICLE

ASSOCIATION BETWEEN SITTING TIME AND NECK-SHOULDER PAIN AMONG OFFICE WORKERS: A CROSS-SECTIONAL STUDY

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Background: Much of literature is available on aetiological factors and prevalence of neck-shoulder pain in office workers but limited evidence is available on association between sitting time and Neck-Shoulder Pain (NSP) among office workers. The objective of this study was to determine the association between sitting time and NSP among office workers. **Methods:** This was a cross-sectional study, the data were collected using non-probability convenient sampling technique. Selection criteria included participants having work related aches, working duration more than 3 hours in a day and 20 hours per week, and between 20–60 years of age. Data were analysed on SPSS-21. **Results:** The working hours per week with intensity of neck and shoulder pain was grouped as no pain, mild pain, moderate pain and severe pain. The sitting was further classified as low, moderate or high sitting. The results were found statistically significant with age, gender, smoking and pain intensity within the last month. **Conclusion:** Total sitting time per week at office was significantly associated with neck-shoulder pain among office workers. Further, high total sitting time per week was associated with increased NSP intensity compared to moderate sitting or low sitting with age, gender, smoking status or pain intensity.

Keywords: Neck-shoulder pain, Sitting, Pain intensity, Office workers, Sedentary, Exercise, Posture

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INTRODUCTION

Neck shoulder pain (NSP) is a frequent and very common in general population.[1] Neck shoulder pain is work related musculoskeletal issue with an obscure and undefined mechanism but still some of its major causes can be defined as an alterations in physical action, correction of posture and autonomic nervous system control which can be the pathogenesis of unending neck and shoulder pain.[2] Neck and shoulder pain which occurs deliberately is contemporary to health issues which annihilated millions of workers round the clock which shows that three foremost reasons for the pain that is constant sitting, continuous motion of hand in the same manner attention and application of the mind.[3]

Biomechanical risks which are commonly accepted aspects for neck shoulder pain may comprise of restricted functioning postures, high force demand, working with arms raised and repetitive movements. Reasonable evidence suggests that neck pain and tenderness is linked to workplace exposures.[4] Ergonomics is the science behind workplace design and human interaction and its effects. Ergonomics in Pakistan is still not regarded as an important element of most of the big projects and businesses, contrary to the fact that physical exposure of upper limb during work may cause body pain, lack of sensation, tingling of various body parts.[5]

However, risk factors linked with neck pain in workers includes geriatric, smoking, previous musculoskeletal pain, increasing working hours, low social support, job dearth and insecurity, low physical potential, poor workstation design, poor postures, recurring and precise work and prolonged sitting.[6–8]

A systematic analysis conducted in Miami in 2019 concluded that the increased proliferation of innovations necessitates better ergonomic interventions. The survey population of people who use computers in their office settings showed signs of decreasing physical activity and worsening posture. Their work required them to sit and use computers for long durations. Neck shoulder pain was reported by the majority of the clients. 60% of staff with musculoskeletal disorders saw a drop in productivity as a result of poor posture during the workday.[9]

The etiological studies which consisted of complaints related to neck, shoulder and hands problems among computer workers are unclear and difficult to understand because their overall mental or physical stress actually count in.[10] Many root causes of neck, shoulder and hands problems are acknowledged which include physical exposure during work and conditions such as psychosocial conditions. Physical exposures include motionless neck and arms postures and doing monotonous and repetitive tasks.[11–13]

The epitomizing purpose of this research is to basically determine the aetiology, the association and the associated factors contributing and enabling the Neck-Shoulder pain among office workers in Pakistan. It was seen long sitting without support usually ends with neck shoulder pain. The objective of the study was to find association between sitting hours and the pain in neck and shoulders.

METHODOLOGY

This cross-sectional study was carried out to evaluate the association between sitting time and neck-shoulder pain among office workers. Information regarding neck shoulder pain in office workers were collected through a Questionnaire.[14] Data was collected after ethical approval of the Institute. The data was collected from different office setups such as Descon Power Solutions, Rasheed Associates, and the Pesticide Company Multan from April to November 2019 using convenience non-probability sampling technique. Inclusion criteria were age between 20 and 60 years, working at least for 3 hours in a day and 20 hours in a week. Exclusion criteria were pregnancy, absence due to sickness at the day of testing, any traumatic injury to the neck or shoulder region, refusal to sign consent, or working less than 20 hours in a week. The data was managed and analysed using SPSS-21 and Microsoft Excel 2013. To find out any association between variables, Chi-square test was applied, and $p<0.05$ was considered significant.

RESULTS

A total of 200 office workers (80% response rate) participated in this study. The mean age of participants was 32±10.11 years (Range: 21–60 years). Cross-tabulation of working hours per week with intensity of neck shoulder pain was done and was grouped into no pain, mild pain, moderate pain and severe pain.

A total of 60 participants were falling in age group of 21–30 and the Chi-square showed non-significant association ($p=0.11$). In age group of 31–40 years, non-significant association was found on Chi-square test ($p=0.13$). Forty-three office workers were in age group 41–50 years. Results of this age group were significant. In age group 51–60 years, non-significant association was found ($p=0.53$) (Table-1).

Association of neck shoulder pain and total working hours per week has been stratified with gender where there are 138 males and 62 females (Table-2).

The association of working hours per week and neck shoulder pain with smoking is shown below. The total smokers in the study were 49 and non-smokers were 151 (Table-3).

The association of working hours per week and neck-shoulder pain with symptoms which affected productivity at work within the last month. This was classified as no effect, mild effect, moderate effect and severe effect (Table-4).

Table-1: Association between working hours per week/total sitting time and neck shoulder pain stratified by age [n (%)]

Age (Years)	Working hours per week/total sitting time	Neck shoulder pain				Total (n=200)	*p*
		No pain	Mild	Moderate	Severe		
21–30	Low Sitting	0	6 (10)	2 (3.33)	0 (0)	8	0.11
	Moderate Sitting	3 (5)	11 (18.33)	19 (31.67)	6 (10)	39	
	High Sitting	1 (1.67)	2 (3.33)	6 (10)	4 (6.67)	13	
	Total	4	19	27	10	60	
31–40	Low Sitting	2 (3.13)	4 (6.25)	1 (1.56)	1 (1.56)	8	0.13
	Moderate Sitting	2 (3.13)	11 (17.19)	18 (28.13)	7 (10.94)	38	
	High Sitting	1 (1.5)	2 (3.13)	11 (17.19)	4 (6.25)	18	
	Total	5	17	30	12	64	
41–50	Low Sitting	2 (4.65)	6 (13.95)	1 (2.33)	0 (0)	9	0.051
	Moderate Sitting	1 (2.33)	8 (18.60)	11 (25.58)	2 (4.65)	22	
	High Sitting	0	2 (4.65)	8 (18.60)	2 (4.65)	12	
	Total	3	16	20	4	43	
51–60	Low Sitting	0	1 (3.03)	1 (3.03)	0 (0)	2	0.53
	Moderate Sitting	2 (6.06)	3 (9.09)	10 (30.30)	6 (18.18)	21	
	High Sitting	0	4 (12.12)	5 (15.15)	1 (3.03)	10	
	Total	2	8	16	7	33	

Table-2: Association between working hours per week/total sitting time and NSP, stratified by gender [n (%)]

Gender	Working hours per week/total sitting time	Neck shoulder pain				Total	*p*
		No pain	Mild	Moderate	Severe		
Male	Low Sitting	1 (0.72)	3 (2.17)	0 (0)	0 (0)	4	0.02
	Moderate Sitting	5 (3.62)	17 (12.32)	43 (31.16)	20 (14.49)	85	
	High Sitting	1 (0.72)	7 (5.07)	30 (21.74)	11 (7.97)	49	
	Total	7	27	73	31	138	
Female	Low Sitting	3 (4.84)	14 (22.58)	5 (8.06)	1 (1.61)	23	0.49
	Moderate Sitting	3 (4.84)	16 (25.81)	15 (24.19)	1 (1.61)	35	
	High Sitting	1 (1.61)	3 (4.84)	0 (0)	0 (0)	4	
	Total	7	33	20	2	62	

Table-3: Association between working hours per week/total sitting time and NSP, stratified by smoking [n (%)]

Smoking Status	Working hours per week/total sitting time	Neck shoulder pain				Total	*p*
		No pain	Mild	Moderate	Severe pain		
Smoker	Low Sitting	2 (4.08)	0 (0)	0 (0)	0 (0)	2	0.00
	Moderate Sitting	0 (0)	6 (12.24)	11 (22.45)	4 (8.16)	21	
	High Sitting	0 (0)	2 (4.08)	16 (32.65)	8 (16.33)	26	
	Total	2	8	27	12	49	
Non-Smoker	Low Sitting	2 (1.32)	17 (11.26)	5 (3.31)	1 (0.66)	25	0.01
	Moderate Sitting	8 (5.30)	27 (17.88)	47 (31.13)	17 (11.26)	99	
	High Sitting	2 (1.32)	8 (5.30)	14 (9.27)	3 (1.99)	27	
	Total	12	52	66	21	151	

Table-4: Association between working hours per week/total sitting time and NSP, stratified by pain intensity within the last months [n (%)]

Symptoms affected productivity	Working hours per week/total sitting time	Neck shoulder pain				Total	*p*
		No pain	Mild	Moderate	Severe		
No Effect	Low Sitting	1 (4.76)	2 (9.52)	0 (0)	0(0)	3	0.78
	Moderate Sitting	7 (33.33)	6 (28.57)	1 (4.76)	0	14	
	High Sitting	1 (4.76)	3 (14.3)	0 (0)	0(0)	4	
	Total	9	11	1	0	21	
Mild Effect	Low Sitting	3 (3.57)	7 (8.33)	5 (5.95)	1 (1.19)	16	0.00
	Moderate Sitting	1 (1.19)	22 (26.19)	24 (28.57)	1 (1.19)	48	
	High Sitting	1 (1.19)	1 (1.19)	14 (16.7)	4 (4.76)	20	
	Total	5	30	43	6	84	
Moderate Effect	Low Sitting	0 (0)	7 (9.72)	0 (0)	0 (0)	7	0.00
	Moderate Sitting	0 (0)	4 (5.55)	26 (36.1)	12 (16.66)	42	
	High Sitting	0 (0)	5 (6.94)	13 (18.05)	5 (6.94)	23	
	Total	0	16	39	17	72	
Severe Effect	Low Sitting	0 (0)	1 (4.34)	0 (0)	0 (0)	1	0.11
	Moderate Sitting	0 (0)	1 (4.34)	7 (30.43)	8 (34.78)	16	
	High Sitting	0 (0)	1 (4.34)	3 (13.04)	2 (8.69)	6	
	Total	0	3	10	10	23	

DISCUSSION

The study showed that neck-shoulder integrity is affected by total sitting time in office during working hours and during leisure time and office workers are prone to develop neck-shoulder symptoms due to prolong sitting.

A study done by Hallman DM *et al*[14] has explained association between sitting and neck shoulder pain. A linear association was impacted from the association between neck shoulder pain and total sitting time in a day. The results have clearly shown that participants with moderate sitting time were having less neck shoulder pain intensity while those having high shoulder pain intensity were those with more total sitting time daily in office. A non-significant association was found among males that implied less neck shoulder pain intensity associated with less sitting.[14] On the contrary, the present study shows that moderate sitting showed linear association between total sitting time per day and neck-shoulder pain in office workers. When stratifying by gender, a significant association was found among males in moderate sitting time which was associated with moderate and severe pain in some individuals. High sitting time was also associated with moderate pain among males. A non-linear association was found among females as no moderate or severe pain was observed in high sitting in them, only moderate and mild pain was seen in some of the females in moderate and high sitting.

Hallman DM *et al*[15] demonstrated negative and positive association of less and moderate sitting times with neck shoulder pain respectively but there was no association found between high sitting time and NSP. We found that less/low sitting time showed non-significant association between total sitting time and neck-shoulder pain among office workers. But a significant association was found between moderate and high sitting times and NSP.

In a study[16] the results found were statistically significant for sitting and smoking. In another study[17] sitting was referred as 'new smoking'. The present study demonstrated no difference of neck shoulder pain in smokers and non-smokers.

Yue P *et al*, have found neck shoulder pain association with long time standing, prolonged sitting, physical exercise and type of back support used.[18] The present study also confirms that prolonged sitting is associated with neck shoulder pain. Increase in physical exercise/work, light or strenuous activities was significantly associated with neck shoulder pain intensity. A review by Mayer J *et al*[19] in 2012 elaborated association of neck shoulder pain with physical work in office set ups which confirms our findings.

Streud T *et al*[20] have found some major causative factors to neck shoulder pain such as jobs with

high demands and working with flexed neck posture with uncomfortable lifting. In support of this along with prolonged sitting, we found another causative factor for NSP which is high force demand or level of physical demand needed to perform work at office which is significantly associated with NSP.

We suggest that by using proper back supported chairs and by taking small breaks during working hours, the intensity of NSP can be minimized because if not managed properly, it might be really harmful for causing NSP and other musculoskeletal (MSK) problems. This idea is in support of another systematic review which claimed that MSK symptoms can be reduced by using supported chairs for those individuals who work for longer periods of time.[12]

CONCLUSION

The total sitting time per week at office was significantly associated with neck-shoulder pain among office workers. High total sitting time per week was associated with increased NSP intensity compared to moderate sitting or brief sitting. Increased age can increase the pain associated with increased sitting time. Gender and smoking have no such effect on pain in neck or shoulder region among office workers. Use of proper back support during sitting and small intervals to stretch and relax during long sitting hours can reduce incidence of NSP. Further work on larger scale is recommended.

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ORIGINAL ARTICLE

COMPARISON OF BONE MINERAL DENSITY BETWEEN TYPE 2 DIABETIC AND NON-DIABETIC PATIENTS AND ITS CORRELATION WITH SERUM INSULIN, HbA1c AND DURATION OF ILLNESS IN TYPE 2 DIABETIC PATIENTS

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Background: Relationship between osteoporosis and type II diabetes mellitus (T2DM) is complex. Although many studies have been conducted, still it remains a controversial subject. Osteoporosis is diagnosed by measuring bone mineral density and the current gold standard is quantitative computed tomography (QCT). This study aimed to compare the bone mineral density (BMD) in type 2 diabetics and non-diabetics using QCT, and to correlate BMD with duration of disease, Glycated Haemoglobin A (HbA1c) level, and Serum Insulin level. **Methods:** This cross-sectional study was conducted between Aug 2016 and Dec 2019 at Radiology Department, Mayo Hospital Lahore. One hundred type 2 DM and 100 healthy individuals were included. BMD, HbA1c, Fasting Blood Sugar and serum insulin levels were measured in all. BMD, HbA1c and Serum Insulin were compared between the cases and controls. Moreover, the correlation between bone mineral densities, duration of disease, HbA1c and serum Insulin level was assessed. **Results:** A significant difference between HbA1c, fasting blood sugar levels and serum insulin levels of the two groups was noted. However, no significant difference was observed in the QCT scoring of the groups. Osteoporosis was diagnosed in 19 diabetics and 12 healthy individuals. BMD changes significantly correlated with the duration of illness and HbA1c. There was no significant correlation and between BMD and Serum Insulin Levels. **Conclusion:** BMD shows a significant correlation with duration of diabetes and HbA1c. These factors play a negative impact on BMD in T2DM. There was no significant correlation between serum insulin and BMD.

Keywords: Bone density, osteoporosis, T2DM, glycated haemoglobin A, HbA1c, Insulin, QCT

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INTRODUCTION

Diabetes Mellitus (DM) is the most common metabolic disorder of the bone with 11.77% prevalence in Pakistan. It is considered an independent risk factor for fractures which is not related to increase BMI or classical osteoporosis risk factors.[1] Nearly 60% of patients with Typ2 2 Diabetes Mellitus (T2DM) have low Bone Mineral Density (BMD).[2]

There are multiple mechanisms through which bone is affected in type 2 diabetics which include insulin deficiency or resistance, hyperglycaemia and atherosclerosis. However, the exact mechanism is still unknown.[3] Diabetes is a chronic metabolic disorder which can cause damage to various organ system. A complex pathophysiological interaction exists between T2DM and bone health. T2DM directly affects bone metabolism and strength, and the indirect effect of anti-diabetic medicine induced altered bone metabolism is also observed.[4] Quite a lot of evidence shows that increased blood sugar levels cause impairment in bone matrix and biochemical formulation.[5]

Assessment of osteoporosis is done by the measurement of BMD which is heritable and varies according to race, age and sex.[6] There are different method of measuring the bone mineral density, however, most commonly used is dual-energy X-ray absorptiometry (DXA) and This study aimed to compare the BMD, HbA1c and serum insulin between type 2 diabetic and non-diabetic patients of same age group and to correlate the BMD using quantitative computed tomography (QCT) with the duration of disease, HbA1c levels, serum insulin levels in the diabetic group. (QCT).[7] DXA due to its low radiation dose and cost is most commonly used.[8] However, QCT provides a similar and more sensitive method for detecting bone mineral loss when compared to DXA.[9]

The main advantage of the QCT over DXA is the ability to separate the mineral density of trabecular and cortical bone.[10] As trabecular bone is metabolically more active than cortical bone, the changes in trabecular bone are considered to be the most sensitive predictor of early bone loss and vertebral fracture risk.[11] QCT provides the true volumetric density of trabecular bone separately from the cortical bone in units of g/Cm^3 whereas DXA estimates areal density measured in g/Cm^2.[12] There are errors due to spinal degenerative changes and aortic calcification in DXA value. QCT is

independent of BMI whereas increase BMI causes DXA BMD values to be high.[13]

This study aimed to compare the BMD, HbA1c and serum insulin between type 2 diabetic and non-diabetic patients of same age group and to correlate the BMD using quantitative computed tomography (QCT) with the duration of disease, HbA1c levels, serum insulin levels in the diabetic group.

MATERIAL AND METHODS

It was an analytic, cross-sectional study, conducted at Department of Diagnostic Radiology and Medical Imaging, Mayo Hospital Lahore, from 22nd August 2016 to 30th December 2019. The study was approved by the Advance Board of Research and Studies at King Edward Medical University and written informed consent was taken from all participants. The sample size was estimated using the formula:

$$n = \frac{z^2_{1-\frac{\alpha}{2}}(2\sigma^2)}{d^2}$$

With 95% confidence interval, 10% margin of error, and expected prevalence of osteoporosis in diabetes mellitus being 60%, the calculated sample size was 57, but 100 subjects were taken in each group. According to the inclusion and exclusion criteria, 100 diagnosed cases of T2DM aged 40–60 years and 100 healthy controls were selected.[14] The minimum duration of insulin therapy for the subjects was one year. Both groups were matched for age and gender. Non-probability purposive sampling technique was used for data collection. Diabetic patients who were on oral hypoglycaemics, suffering from any other endocrine abnormality, immobilized for more than three weeks due to any trauma, fracture or fixation by plaster or quiet rest in bed due to any illness (e.g., prolonged tuberculosis or paralysis), had a disease affecting bone metabolism (e.g., rickets, osteomalacia, osteogenesis imperfecta, fibrous dysplasia), past medication affecting bone (e.g., steroids, cyclosporine, anti-seizure drugs, depo provera, anti-cancer drugs, antihypertensives etc.) were excluded from the study.

A detailed clinical history was taken from a total of 200 individuals fulfilling the criteria regarding previous prolonged illness, metabolic and endocrine disease. HbA1c and serum insulin levels were estimated using CERA STAT 1000 and Siemens Immulite-2000. Fasting blood sugar was estimated with Accu-Chek® glucometer. Both groups underwent QCT for measurement of BMD on CT Scanner (Toshiba Xvision EX). Lateral scout image of the patient's lumbar spine was obtained including the first three lumbar vertebrae.[15] Three separate slices with a thickness of 10 mm were selected. After obtaining the necessary axial slices, selection of a region of interest (ROI) was selected inside the cancellous part of the vertebral body, not including the cortical bone. Fractured vertebrae and vertebrae with obvious pathology like deformity, haemangioma and metastasis were excluded. The CT density of ROI (Figure-1) was estimated by the software automatically and plotted onto the regression line. Using the regression line, BMD of the selected ROI was calculated by the software and was shown in units of mg/Cm^3. The procedure was repeated for each of the three vertebral slices obtained and the software took out an average BMD value obtained from the three slices. Using Felsenberg classification recommended by the American College of Radiology, BMD values were classified into osteoporosis, osteopenia and normal.[16]

Data analysis was carried out through SPSS-20. The Spearman correlation coefficient was applied to see the correlation and interdependency of BMD, duration of diabetes (Only for cases), while HbA1c level and serum insulin level in cases and controls. Shapiro Wilk test was used to confirm the normality of data. Based on distribution of data, Mann Whitney U test was applied to compare the BMD, HbA1c and serum insulin levels, and $p<0.05$ was taken as significant.

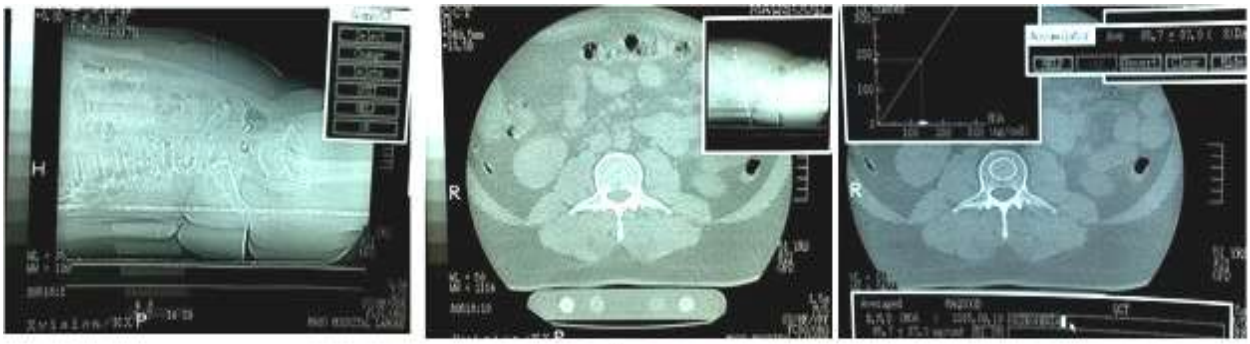

Figure-1: QCT in 56 years old diabetic female done on Toshiba Xvision/EX. (a) scanogram (b) ROI taken along with the level of vertebrae taken and position of the phantom (c) the BMD taken from 3 vertebrae is shown as Averaged BMD

RESULTS

The study included 100 subjects suffering from T2DM and 100 healthy controls. There were 57% males and 43% females in the group with T2DM. Control group included 52% males and 48% females. No significant difference was observed between the ages of the two groups (p=0.771). The duration of diabetes among cases was 6.15±2.92 ranged from 2 to 14 years. (Table-1).

There was a significant difference between height, BMI, HbA1c, fasting blood sugar and serum insulin levels of the 2 groups with p-values of 0.024, 0.001, 0.001, 0.001, and 0.001 respectively. However, no significant difference was noted in the weight and QCT scoring of the two groups (p=0.430, p=0.477).

Among cases QCT findings showed that osteoporosis was diagnosed in 19 cases, osteopenia in 67 cases and 14 cases were normal. While in controls QCT findings showed that osteoporosis was diagnosed in 12 controls, osteopenia in 79 and 9 controls had normal findings on QCT.

The correlation of BMD with the duration of diabetes in cases is illustrated in Figure-2, Table-2. Correlation of BMD with HbA1c and serum insulin level in cases and controls is shown in Figure-3 and 4.

Table-1: Comparison of study parameters between cases and controls

Parameter		Mean±SD	Median (IQR)	p
Age (years)	Cases	48.26±6.16	48.0 (9.00)	0.771
	Controls	48.12±4.97	48.0 (6.00)	
BMD	Cases	98.09±17.61	100.0 (23.75)	0.477
	Controls	99.8±15.39	102.0 (21.50)	
HbA1c	Cases	9.75±1.27	9.8 (1.00)	0.001*
	Controls	6.37±0.41	6.4 (0.70)	
Serum insulin	Cases	27.91±8.10	27.0 (5.00)	0.001*
	Controls	20.25±2.59	21.0 (3.00)	
Duration of illness	Cases	6.15±2.92	6.0 (4.00)	

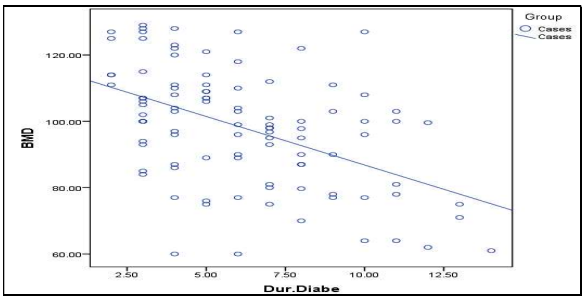

Figure-2: Correlation of BMD with duration of diabetes in cases

Spearman correlation test: Correlations Coefficient= -0.470, p= 0.001

Table-2: Correlation between study parameters in cases and controls

	Type 2 diabetics			Healthy controls	
BMD	**HbA1c**	**Serum insulin**	**Duration**	**HbA1c**	**Serum insulin**
r	0.360	0.158	0.47	0.112	0.353
p	0.001	0.116	0.001	0.228	0.001

All values generated by Spearman correlation test

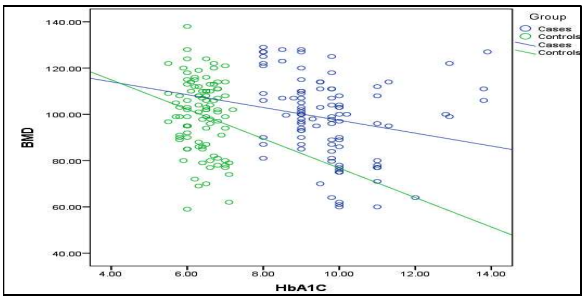

Figure-3: Correlation of BMD with HbA1c in cases and controls

Cases: Type 2 Diabetic Patients, **Controls:** Non-diabetic
Correlations Coefficient: Cases= -0.360, p=0.001, **Controls=** -0.112, p=0.228

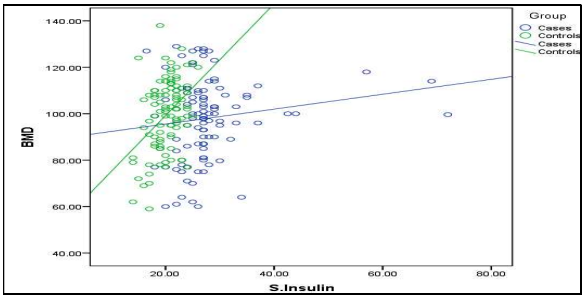

Figure-4: Correlation of BMD with serum insulin level in cases and controls (Spearman correlation)

Cases: Type 2 Diabetic Patients, **Controls:** Non-diabetic
Correlations Coefficient: Cases= -0.158, p=0.116, **Controls=** -0.353, p=0.001

DISCUSSION

Osteoporosis and diabetes are currently the most widely discussed diseases and are believed to be inter-related that is why they are often found to be present simultaneously. However, there is quite a lot of evidence which shows that increased blood sugar level causes impairment in bone matrix and biochemical formulation. Reduced biomechanical competency is often found even when there is normal or increased BMD as evaluated by DXA Scanner13 The current gold standard for the measurement of bone structure is high resolution peripheral quantitative CT (HRQCT). Unfortunately, in the clinical arena, this is limited by radiation exposure and the cost of investigation. Therefore, it is relatively rarely used.[16]

In this study, QCT findings showed that 19 patients had osteoporosis whereas among controls only 12 individuals had osteoporosis. However, 79 controls and 67 cases were diagnosed with osteopenia. QCT findings were normal among 14 cases and among controls normal findings were seen in only 9 participants. There was no significant difference between the BMD of two groups among cases and controls.

The randomized prospective controlled single-blinded study conducted in Turkey included type 2 diabetics in the patient group and healthy individuals

were included in the control group. The bone mineral densities of the cases were found to be significantly low in terms of the lumbar (L1–4) T scores in the type 2 diabetes group. However, there was no significant difference found between the BMD of type 2 diabetics and healthy controls.[17] In line with our study results, a study showed that the type 2 diabetics with low BMD values were observed to have long-term diabetes and menopause, to have poor glucose control, and to have disordered renal functions.[18]

A study conducted by Sosa et al. showed no significant difference in terms of BMD estimated through DEXA and QCT.[19] Studies suggest that type 2 DM patients, individuals using dietary and oral antidiabetic, and individuals taking insulin have lower BMD values.[20]

Many mechanisms have been asserted to contribute to diabetic osteopenia. One of them is that it can lead to diabetic osteopenia due to deficiency in anabolic activation of insulin.[20] Another mechanism noted in diabetic osteopenia is the suppression of osteoblastic bone formation.[21]

Rotterdam study suggests that individuals with type 2 diabetes have increased fracture risk despite higher BMD. Contrary to our study results, diabetic patients had a slightly higher BMD than the non-diabetic group. Poor glycaemic control in type 2 diabetes was associated with fracture risk, high BMD, and thicker femoral cortices in narrower bones. It is proposed that fragility in apparently 'strong' bones among patients with poorly controlled diabetes is due to altered bone repair process resulting in porous cortices and microfractures.[22]

Contrary to our study, a study conducted by Bridges and colleagues showed no significant correlation between HbA1c and BMD in diabetics.[23] A weak negative correlation is found between HbA1c and BMD in the study conducted on south Indian diabetic patients.[24]

A study conducted on East Asian men showed that patients with DM for >5 years had lower mean BMD in the total hip and femoral neck than those with DM for ≤5 years.[25]

CONCLUSION

Type II diabetes mellitus has an impact on BMD and it increases the risk of fracture. Negative effects of the disease are dependent upon the duration of disease and degree of glycaemic control. Insulin resistance in T2DM deteriorates osteoblast proliferation and activity but enhances osteoclast activity, leading to uncoupled bone remodelling. Frequency of osteoporosis was low while osteopenia was high in type 2 diabetic patients. BMD showed a significant correlation for the duration of diabetes and HbA1c hence these factors play a negative impact on bone mineral density in type 2 diabetic patients. However, no significant effect of serum insulin level on BMD was noted. By improving the glycaemic control damage or loss of BMD can be prevented.

Inability to measure bone turnover markers is surely a limitation to explain different correlation findings in both groups. HbA1c level measured at the time when measurements of BMD reflected only short-term glycaemic control. It is purposed to investigate and correlate bone markers, serial HbA1c levels, and BMD simultaneously in future studies.

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Authors' Contribution

SMD: Conception of idea, design, methodology, data acquisition, interpretation and data analysis, and literature review.
BR: methodology, data acquisition, interpretation and data analysis, and literature review.
SR: Review of intellectual contents, study design and ethical issues.
AHAR: Data analysis, work draft, literature review and bibliography.
FM: Methodology, inclusion exclusion criteria.

Conflict of Interest: None to declare
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ORIGINAL ARTICLE

ONLINE VERSUS CONVENTIONAL PAPER-BASED FORMATIVE ASSESSMENT: DO THEY PREDICT SUMMATIVE SCORES?

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Background: Different mechanisms and methods to conduct formative assessments may influence the learning environment and the learning outcomes. Present study aims to compare the effect of online formative assessments with conventional paper-based formative assessments on summative scores of medical students. **Methods:** It was a prospective-observational study conducted from Oct to Dec 2018 at Shifa College of Medicine, Islamabad. A total of 93 undergraduate students participated in this study. Students were assigned two online formative assessments before the summative assessment of one module and two paper-based formative assessments before the summative assessment of another module. ClassMarker☑ software was used for online assessments. Data were analysed on SPSS-21. Continuous variables were expressed in Mean±SD. For qualitative variables, frequencies and percentages were calculated. Comparison of quantitative data was done using paired *t*-test and student's *t*-test. Association between performance in online and paper-based formative assessment to their respective summative scores was performed by Pearson's correlation coefficient and regression analysis, and $p<0.05$ were considered significant. **Results:** Mean summative score (75.17±9.18) of the module with online formative assessments was significantly higher in comparison to the mean summative score (63.66±10.12) of the module with paper-based formative assessments. Students who performed better in online formative assessments had significantly higher scores in the summative assessment in comparison to the other students. There was a significant ($p<0.001$) and positive ($r=0.45$) correlation between scores of online formative tests and summative tests. **Conclusion:** Online formative assessments have a positive effect on the summative scores of medical students in comparison to the conventional paper-based formative assessments.

Keywords: Assessment, feedback, formative, performance, summative

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INTRODUCTION

Educational delivery has experienced remarkable methodological shifts in the past to maximise student achievement.[1] An imperative component of the educational process is knowledge assessment.[2] Formative assessments are one such means of assessment, aimed to ensure a deeper understanding of the syllabus and the application of knowledge in the medical field. Formative assessment comprises of academic activities that can provide information to be used as feedback to modify teaching and learning methodologies.[3]

The main objective of formative assessments is to gather focused feedback about the delivered content with the intent of further clarification of important concepts.[4,5] The purpose of summative assessment is to evaluate students' knowledge, formative assessment provides feedback to students about their knowledge to positively influence their learning.[6] Frequent formative testing results in greater continuous study throughout a course. As a result, summative scores increase.[7] Moreover, by receiving feedback on their quiz answers, formative assessment enables teachers and learners to direct their effort towards their weaknesses.[8] Henceforth, the entire process of conducting formative assessments strengthens self-reflection thereby eventually enhancing the overall learning experience. Evidence also suggests that formative assessments can play an important role in helping students evolve as life-long learners.[9] However, this strategy is believed to be useful only when there is no presumed evaluation stress present in the minds of students.[10]

Conventional paper-based formative assessments are conducted in a classroom setting and have some limitations which include supervision of a large number of students, the extensive time required for one-on-one feedback and post-hoc analysis of validity and reliability of the questions.[11] Recent trends of use of technology are providing innovative solutions for many aspects of medical education and web-based formative assessments can be used to address the above-mentioned challenges. Potential advantages of conducting computer-based formative assessments include personalised and immediate feedback, flexibility in scheduling, comfortable environment and opportunity for interactive and consistent reinforcement.[12–14]

Kibble assessed 350 learners with two online formative quizzes prior to summative examinations. The results showed quiz scores to be significantly correlated

with summative test performance.[15] However, in some instances, online formative assessment has not been associated with improved learning outcomes. For example, in a developmental psychology course, access to computerized formative assessment in preparation for summative assessment resulted in poorer exam performance.[16]

As evident, research comparing the efficacy of online formative assessment with traditional paper-based formative assessments is sparse and divergent in its findings[17] and many institutions have been reluctant to integrate such assessments into the medical curriculum.[18]

With this background, the objective of this study was to investigate and compare the effect of conventional paper-based formative assessment with computer-based online formative assessment on the performance of medical students in summative modular assessments. This study has explored online formative assessments as an optional addition to the curriculum of pre-clinical integrated medical program in order to improve the academic performance of medical students.

METHODOLOGY

The ethical approval for the study was granted by the Institutional Review Board and Ethics Committee of Shifa International Hospitals Ltd. and Shifa Tameer-e-Millat University, Islamabad.

It was a prospective observational study, conducted in our institute from October to December 2018. A total of 93 undergraduate medical students participated in the study. Amongst them 40 (43%) participants were females and 53 (57%) participants were males. This study was conducted in the Essentials of Medicine (EOM) module and Cardiovascular System (CVS) module of 3rd year MBBS. Two separate computer-based online formative assessments were conducted in the EOM module of 4 weeks duration followed by a summative assessment conducted in November 2018. Similarly, two separate paper-based formative assessments of the same group of students were conducted in the CVS module of 5 weeks duration followed by a summative assessment conducted in December 2018. The participants of the study included all those students who had given their informed written consent to be a part of the study. The students who were absent when the assessments were being carried out were excluded.

Final MCQs for online and paper-based formative assessments were reviewed and validated by subject experts before dissemination to students. The software tool employed for computer-based formative assessment was ClassMarker☑ which is used to develop diverse types of questions, i.e., multiple-choice questions (MCQs), extended matching questions, short essay questions, and true/false questions.[8] Results of the paper-based summative assessments were also collected from the Examination department of the college. The computer-based formatives had thirty multiple-choice questions each and automated feedback was provided to students after attempting each question. The paper-based formatives also had 30 multiple choice questions each and feedback was provided to the students after completion of the assessment in a large interactive group session. The average time taken for attempting MCQs, mean scores and Cronbach's alpha for each group of every formative assessment was recorded. The EOM summative had sixty multiple-choice questions while the CVS summative had eighty multiple-choice questions. Each correct answer was given a score of 1 point. There was no negative marking. Summative assessment is prepared by subject experts according to the table of specification or blueprint for all themes taught in a module and vetted by the review committee of the health profession education department. Item analysis of summative MCQs of each module is also done to ensure reliability, validity and discrimination index on a regular basis and reviewed by faculty members involved in paper setting.

The data obtained was analysed on SPSS-21. Continuous variables were expressed in Mean±SD; qualitative data were expressed in frequencies and percentages. Paired *t*-test and Student's *t*-test were used to compare quantitative variables between different groups. The relationship between performance in online and paper-based formative assessment to their respective summative scores was performed by Pearson's correlation coefficient and regression analysis, and $p<0.05$ was considered statistically significant.

RESULTS

The mean time taken to complete the computer-based formative, the mean score in computer-based formative and the associated summative score, and the mean time taken to complete the paper-based formative, the mean score in paper-based formative and the associated summative score are shown in Table-1.

Results reported a shorter mean duration for completion and higher mean scores in computer-based formative assessment as compared to paper-based formative assessment. Moreover, mean summative scores were also higher among students attempting computer-based formative assessment as compared to paper-based formative assessments.

Table-2 shows the data of students who scored more than 60% in the formative assessments as compared to students scoring less than 60%.

The scores of the two online formative assessments and the respective end of the module summative assessment; and the two paper-based formative assessments and the respective end of module

summative assessment were tested for correlation. The Pearson's Correlation Coefficient showed a positive (r=0.45) and significant (p<0.001) correlation between the online formative test score and the end of module summative test scores as shown in Table-3.

Table-1: Computer-based versus paper-based formative: completion times and scores (n=93)

	Computer based online formative assessment (Mean±SD)	Paper based formative assessment (Mean±SD)	*p*
Time for completion of formative (minutes)	18.69±3.71	25.02±3.16	<0.001
Formative score (%)	61.55±21.67	45.04±19.52	<0.001
Summative score (%)	75.17±9.18	63.66±10.12	<0.001

Table-2: Difference in mean summative scores with a cut-off value of 60 percent scores in online and paper-based formative assessments

Variables	No. of students (%) (n=93)	Mean summative score (Mean±SD)	*p*
Students with more than 60 in online formative in the EOM module	60 (64.52)	77.77±7.64	<0.001
Students with less than 60 in online formative in the EOM module	33 (35.48)	70.45±9.95	
Students with more than 60 in paper-based formative in the CVS module	30 (32.26)	61.60±9.90	0.176
Students with less than 60 in paper-based formative in the CVS module	63 (67.74)	64.65±10.14	

Table-3: Correlation between formative and summative assessment scores

Formative assessments	*r*	r^2	Adjusted r^2	β (95% CI)	*p*
Online formative assessment in EOM module	0.45	0.20	0.19	0.189 (0.11–0.27)	<0.001
Paper-based formative assessments in the CVS module	-0.14	0.02	0.01	-0.074 (-0.18–0.03)	0.171

r=standardized coefficient; r^2=standardized coefficient squared, β=non-standardized coefficient

DISCUSSION

Online formative assessments had an overall positive effect on the summative results of the students. Those students who performed well in the two successive online formative assessments in the EOM module scored significantly higher in the summative assessments in comparison to the rest of the students. In contrast, those students who performed well in the two consecutive paper-based conventional assessments in the CVS module had a summative result similar to the other students.

Several factors could contribute to these findings. Immediate automated feedback after attempting every question was provided to the students in the online formative assessment whereas, in case of the paper-based formative assessments, feedback was provided after completion of the whole test. The online formative assessments were conducted on the weekends which provided enough time for the students to strengthen their weak areas. In contrast, the paper-based formative assessments were conducted in a classroom setting after which students did not have enough time to improve their weak areas because of the succeeding scheduled sessions. Providing enough time on weekends to the students to improve their weak areas could be a very strong reason for the improved summative results as high achiever medical students are more likely to study more on the weekends in comparison to the low achiever students.[9]

Receiving feedback on the same screen after attempting every individual MCQs could also be an important factor for the improved summative score after taking the online formative assessments. In contrast, in the paper-based formative assessments, the key was displayed and discussed with the students after the completion of the whole assessments. A recent study showed that students were more likely to retain information on the same computer screen in comparison to the same content which was provided to the same students on the front and back sides of the pages in print.[10]

Preference for teaching through technology could be another factor for the difference in the summative scores. Using online formative assessments might have made the overall learning experience more attractive for the students proficient in the use of technology.[11,12] However, we cannot be certain about this possible explanation as we did not gauge the preference of our students for incorporating more technological tools for delivery of the curriculum and means of assessment.

An important aspect of our study was that all of our formative assessments were time-bound. This aspect further strengthens our findings as a recently conducted study concluded that formative assessments can result in improved performance in summative provided the formative assessments are time-bound.[13]

A possible criticism of our findings could be that the improved summative results after online formative assessments could be the motivation of academically better students to attempt the formatives.[14] This explanation certainly could not be the reason in our study as only those students who performed better in the online formative assessments performed better in the summative assessments whereas those students who performed better in the paper-based assessments had a

score similar to the other students in their respective summative assessment.

The Pearson's Correlation Coefficient for the paper-based formative assessments showed a negative (r= -0.14) and non-significant (p=0.171) correlation between the paper-based assessments and the end of module summative test scores in the CVS module. These results are in agreement with similar studies in which online formative showed a significant correlation with a similar strength to the summative assessments.[6]

Other possible reasons for our findings could be the comfortable environment of home, feasibility and better mental engagement whereas the paper-based formative assessments were conducted in the classroom in a conventional manner which adds a little stress for the students and might affect their performance.[6,7]

Finally, the last possible reason could be the difference in difficulty level of content of the two modules but this does not seem a very plausible reason especially when an extra effort was made to choose questions for the summative and formative assessments with the same difficulty index. An added advantage of the online assessments over conventional paper-based assessments is that they can be customized to give students multiple chances to select the correct option in case the incorrect option is selected in the first attempt by the student.[6,15] Students in our study were allowed only single attempts but the effect of providing students multiple attempts to choose the correct options might have a further positive effect on the long term retention of important concepts.

In our study, the mean score of the EOM module summative assessment was significantly higher in comparison to the mean summative score of the CVS module. Significant difference in scores of the same class in two different modules where the only difference was that formative assessments were conducted differently gives strength to the hypothesis that the online formative assessment must have had a positive effect on strengthening and reinforcing concepts and hence improved the summative scores.[16]

CONCLUSION

The results of our study showed better learning outcomes with the use of online formative assessments. Keeping in mind the usefulness of formative assessment as an efficient learning tool, there is a growing need to evaluate different tools to ensure their maximum effectiveness. Online structured formative assessments on a regular basis may help students focus on the important concepts, help in better engagement and reflect on their learning. With the recent COVID-19 pandemic, there will be even more focus in the near future on online learning and assessments. Health professions educators are, therefore, encouraged to conduct multiple studies in which other technological modes of delivery and marking should be assessed and incorporated into learning and teaching practices. The usefulness of providing students multiple attempts to select the correct answer as a formative tool should also be explored.

FUTURE RECOMMENDATIONS

Further multicentre studies are required to generalize the results of this study and to further evaluate the outcomes of online formative assessment on the educational progress of the students.

LIMITATIONS

This study was conducted on a single class of the institute. Therefore, the results may not be generalized for all medical students.

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