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EDITORIAL

WRITING IS AN IMPORTANT BUT NEGLECTED COMMUNICATION SKILL FOR MEDICAL GRADUATES

Tehseen Iqbal
DG Khan Medical College, Dera Ghazi Khan, Pakistan

Medical students are required to communicate knowledge, understanding, interpretation, inferences, arguments, deductions and predictions by the appropriate use of clear and concise written English. The doctor who knows English is better aware of current trends in medicine, can get or continue medical education abroad, can participate in medical conferences abroad, and can work in a team with foreign specialists. English allows the doctor to have an appointment with foreign patients in clinics. Academic writing is extensively acknowledged as a key skill for students to boost their educational performance at higher education level. Assessment through essay write-up answers should be encouraged as guessing by students is eliminated in essay examination. There is no option to select from the given possible choices and they have to provide the answer rather than selecting/ticking the good response.

Keywords: Communication skill, Writing Skill, Essay questions, Medical Writing
Pak J Physiol 2021;17(2):1–2

Biomedical Admissions Test (BMAT) of Cambridge Assessment Admissions Testing (For assessments from August 2020 to July 2021) requires medical students to communicate knowledge, understanding, interpretation, inferences, arguments, deductions and predictions by the appropriate use of clear and concise written English.[1] Medical Schools Council (UK) describes in its 'Statement on the core values and attributes needed to study medicine' the core competence of students to have effective communication skills in all four areas of communication, i.e., reading, writing, listening and speaking.[2] English Fluency is vital for communication in the healthcare field. When compared with other fundamental skills such as listening, speaking and reading, writing is the most difficult skill. The English language may become very important when it comes to communicating with co-workers, bosses, and patients in a hospital or other medical setting.[3] The doctor who knows English is better aware of current trends in medicine, can get or continue medical education abroad, can participate in medical conferences abroad, and can work in a team with foreign specialists. English allows the doctor to have an appointment with foreign patients in private clinics.[4] In Pakistan, India and Singapore, English is used as a functional first language in the medical profession. English language is expanding as an instrument of international communication and education in medicine. Domestic and internationally distributed journals of medicine are published in English. The ability to write accurately in English will be a growing need but recognition of these linguistic needs must be developed in the early stages of medical education.[5]

The process of writing requires writers to have a clear understanding of the subject matter and make use of cognitive abilities. Specifically, writing helps students develop higher-order thinking skills that involve three cognitive processes-analysis, evaluation, and creation. These higher-order thinking skills are needed for medical students to grow as successful medical professionals.[6] In Pakistan the writing skills of the students are alarmingly weak and substandard. (Figure-1) Writing is a cognitive process that tests memory, thinking ability and verbal command to successfully express the ideas, because proficient composition of a text indicates successful learning.[7]

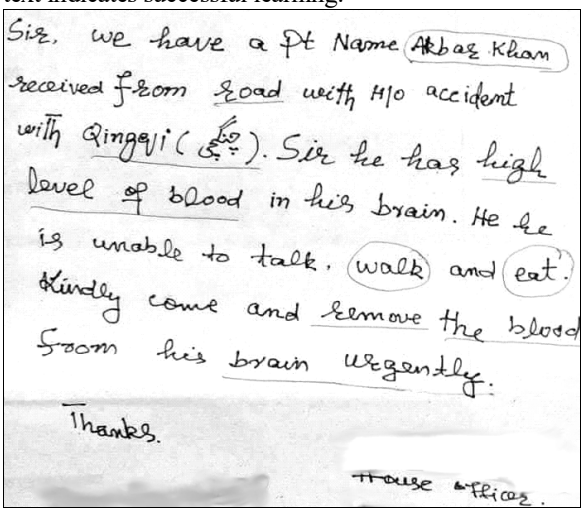
Sir, we have a Pt Name Akbaz Khan received from Road with H/o accident with Qingqi (کنگی). Sir he has high level of blood in his brain. He he is unable to talk, walk and eat. Kindly come and remove the blood from his brain urgently.
Thanks.
House offica.

Figure-1: A sample of writing by a House Officer

Throughout his training and medical practice, a doctor has to write down i) a good and elaborate history of patient's illness, ii) investigation orders, iii) call letters to other departments/doctors, iv) referral letters and v) prescription instructions. For acquiring post-graduation, doctors write thesis or dissertation. For promotion, they have to write many research papers. Almost all postgraduate students are anticipated to have developed English language proficiency, especially, in academic writing to show mastery over the application of linguistic and communicative capability with sound

grammatical knowledge and academic vocabulary in order to produce quality texts likely to be published in an impact factor research journal.[7] For all these endeavors, good writing skill is needed and we all know that good writing skill needs practice.

Academic writing is extensively acknowledged as a key skill for students to boost their educational performance at higher education level.[6] During undergraduate studies, students are taught these skills through assignments and attempting SEQs or SAQs during examinations. Examination is the most powerful motivation for students to learn writing skills. Some medical universities in Pakistan are offering MCQs-only examinations to their medical graduates. This type of examination requires that students just tick the right answer and they will not be asked to write down anything. This will be disastrous for medical profession and will drastically undermine writing skills of our doctors. Our doctors will become non-competent for international medical world and this will adversely affect the flow of foreign remittances to Pakistan.

The examination system does not encourage learners' creative writing; it does not encourage our students to be analytical or critical.[8] Most of our medical universities offer examinations which contain MCQs and SEQs or SAQs. Generally, MCQs assess superficial knowledge of the students. Therefore, assessment through essay write-up answers should be encouraged. Guessing by students is eliminated as in essay examination, there is no option to select from the given possible choices and they have to provide the answer rather than selecting the good response. It is expected that the primary role of long essay write-up would create synthesis skills in students. Therefore, medical educationists should at least revise the pattern of assessment proportion or develop alternate tool to develop the writing skills in students.[9]

Physiology paper of University of Health Sciences (UHS), Lahore has 45 MCQs and 9 SEQs. To prevent the irreparable loss to our doctors' writing abilities, we should add a long essay type question, in place of two MCQs, from Cardiovascular System or Respiratory System in First Professional paper and form Nervous System or the Kidneys in Second Professional paper to inculcate the habit of good writing in medical students.

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ORIGINAL ARTICLE

COMPARISON OF PLATELET INDICES IN HYPOPRODUCTIVE AND HYPERDESTRUCTIVE THROMBOCYTOPENIA

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Background: Thrombocytopenia is one of the most common haematological disorders and is also a life-threatening condition. The two types of thrombocytopenia, hypoproductive and hyperdestructive can best be distinguished by bone marrow examination; but it is an invasive, time-consuming and expensive process. The aim of this study was to determine the role of platelets indices in distinguishing between the two types which is a cost-effective and non-invasive modality of investigations. **Methods:** It was a cross-sectional study, conducted in the Pathology Laboratory, Rehman Medical Institute, Peshawar, conducted from 1st July 2019 to 30th June 2020. Non-probability convenience sampling technique was used. Sample size was calculated using WHO formula, and a total of 74 thrombocytopenic patients referred for bone marrow aspirate and trephine biopsy were included in the study. Clinical record, complete blood count (CBC) and bone marrow trephine biopsy were obtained and computed. **Results:** Mean Platelet Volume was 10.57±1.33 fl in hypoproductive group and 11.637±1.98 fl in hyperdestructive group. The difference between the groups was statistically significant (p=0.017). Platelet Distribution Width was 12.68±3.16 fl in hypoproductive group and 14.811±3.61 fl in hyperdestructive group (p=0.014). Platelet Large Cell Ratio was 30.81±9.23% in hypoproductive group and 36.993±10.25% in hyperdestructive group (p=0.010). **Conclusion:** Platelet indices can be used as a reliable tool for distinguishing between hypoproductive and hyperdestructive thrombocytopenia.

Keywords: Thrombocytopenia, Platelet, Platelet indices, Mean Platelet Volume, Platelet Distribution Width, Platelet Large Cell Ratio

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INTRODUCTION

Platelets, also called thrombocytes are derived from megakaryocytes in the bone marrow. In circulation, their main function is to stop bleeding by forming primary platelet plug at the site of vessel injury.[1] The normal platelet count is 150,000 to 450,000 per µL of blood. In a healthy person, platelets live for about 10 days in circulation.[2] Platelet count of less than 150,000 is considered as thrombocytopenia.[2,3] The MPV ranges from 6.8–10.4 fL, PDW ranges from 9–14 fL and PLCR ranges between 15 and 35% in normal individuals.[4]

Thrombocytopenia is one of the most common haematological disorders and can become life-threatening in case of severe disease. The various mechanisms involved in thrombocytopenia are decreased platelet production (hypoproductive thrombocytopenia) or increased platelet breakdown (hyperdestructive thrombocytopenia).[4] The causes of hypoproductive thrombocytopenia are drugs, chemicals, radiotherapy, leukaemia, lymphoma, chemotherapeutic agents, aplastic anaemia, infections like HIV and megaloblastic anaemia. The causes for hyperdestructive thrombocytopenia are either idiopathic also known as immune thrombocytopenic purpura, secondary to systemic lupus erythematous, infections (HIV, hepatitis, malaria), drug induced, e.g., heparin, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura and enlarged spleen.[5] For proper management of patients, it is important to know whether thrombocytopenia is due to decreased production or increased breakdown of platelets. For differentiating between them, bone marrow examination is gold standard but it is an invasive, expensive and time consuming procedure.[4]

Due to recent advances in automated blood cell analysers, it is possible to measure various parameters. These include platelet indices like platelet distribution width (PDW), mean platelet volume (MPV) and platelet large cell ratio (PLCR).[6]

A study conducted in India showed that Platelet distribution width (PDW) is higher in hyperdestructive thrombocytopenia with a mean value of 16.07±0.17, while in hypoproductive thrombocytopenia its value was low with a mean PDW 12.15±0.25.[4] Another study in Iraq also showed that platelet distribution width and mean platelet volume were higher in hyperdestructive thrombocytopenia and vice versa in hypoproductive cases. Mean value of PDW in patients with hyperdestruction was 15.61±0.73 while in hypoproduction it was 13.83±1.75 and mean value of MPV was 12.33±0.46 and 10.08±1.81 respectively.[7] These platelet indices provide some

important information but are not accepted for routine clinical use. If these indices are really informative regarding platelet kinetics, they might become very useful laboratory measures for thrombocytopenia.[8] Thus, by using a cost-effective, non-invasive, non-ionizing modality of investigation, thrombocytopenic purpura can be detected at earlier stage.

The objective of this study was to compare the platelet indices (PDW, MPV, PLCR) in hypoproductive and hyperdestructive thrombo-cytopenia and to determine the role of platelet indices for differentiating hypoproductive from hyperdestructive thrombocytopenia.

MATERIAL AND METHODS

It was a cross-sectional, analytical study, conducted in the Pathology Laboratory, Rehman Medical Institute (RMI), Peshawar, from 1st July 2019 to 30th June 2020. The study was approved from RMI Ethical and Research Board. A total of 74 thrombocytopenic patients were included. The sample size was calculated using WHO formula with prevalence of thrombocytopenia taken as 2.3% in Pakistan[9] and margin of error kept at 3.5%. Non-probability convenience sampling technique was used. Patients of all age groups and both genders visiting the RMI Laboratory for bone marrow biopsy for haematological diseases, and with platelet counts less than 100×10^9/L, confirmed after peripheral blood film review were included in the study. Patients having artefacttual thrombocytopenia, pregnant women or those taking drugs like heparin, quinine, quinidine etc. were excluded from the study.

After informed consent, a detailed personal and medical history was taken and recorded on hospital notes. Bone marrow aspirate and trephine biopsy were done from posterior iliac crest. Bone marrow aspirate slides were stained with Giemsa stain. Bone marrow trephine biopsy was performed in standardized manner according to the protocol[3] and stained with Hematoxylin and Eosin.

A sample of 3 ml venous blood was drawn in EDTA vacutainer from all patients and complete blood count was performed by Haematology Analyser (Sysmex, XN-1000) to determine platelet count and platelet indices. Total leukocyte count (TLC) and haemoglobin (Hb) estimation was also done. A peripheral blood film was examined to estimate platelet count and to rule out pseudo-thrombocytopenia. Data were collected on a structured proforma which elicited information regarding patients' demographics, platelet indices and bone marrow findings.

The data was entered and analysed using SPSS-22. Mean and standard deviation were calculated for numerical variables and statistical comparison was performed using Student's *t*-test keeping $p\leq0.05$.

RESULTS

Out of 74 thrombocytopenic patients recruited in the study, 47 (63.51%) were grouped as hypoproductive and 27 (36.49%) were grouped as hyperdestructive thrombocytopenia patients on the basis of bone marrow examination. The mean age was 33.81±22.1 years. MPV was 10.57±1.33 fL in hypoproductive group and 11.637±1.98 fL in hyperdestructive group. The difference between the groups was statistically significant (p=0.017). PDW was 12.68±3.16 fL in hypoproductive group and 14.811±3.61 fL in hyperdestructive group (p=0.014). PLCR was 30.81±9.23% in hypoproductive group and 36.993±10.25% in hyperdestructive group (p=0.01) (Table-1).

Other compared blood parameters included TLC, Hb and Platelet count. Mean TLC was $47.94\pm100.35\times10^3$/µL in hypoproductive group and $7.38\pm4.73\times10^3$/µL in hyperdestructive group (p=0.040). Mean Hb was 8.52±2.35 gm/dL in hypoproductive group and 11.6±2.74 gm/dL in hyperdestructive group (p<0.001). The mean platelet count was $60.74\pm37.3\times10^3$/µL in hypoproductive group and $58.88\pm36.6\times10^3$/µL in hyperdestructive group, with insignificant statistical difference (Table-2). In hypoproductive thrombocytopenia patients the major cause was leukaemia followed by aplastic anaemia. All hyperdestructive thrombocytopenia patients showed normo-cellular bone marrow with increased megakaryopoiesis (Table-3).

Table-1: Platelet indices in hypoproductive and hyperdestructive thrombocytopenia

Parameter	Hypoproductive thrombocytopenia (n=47)	Hyperdestructive thrombocytopenia (n=27)	*p*
MPV (fL)	10.57±1.33	11.637±1.98	0.017
PDW (fL)	12.68±3.16	14.811±3.61	0.014
PLCR (%)	30.81±9.23	36.993±10.25	0.010

Table-2: Blood parameters in hypoproductive and hyperdestructive thrombocytopenia

Parameter	Hypoproductive thrombocytopenia (n=47)	Hyperdestructive thrombocytopenia (n=27)	*p*
TLC ($\times10^3$/µL)	47.94±100.35	7.38±4.73	0.040
Hb(gm/dL)	8.52±2.35	11.6±2.74	<0.001
Platelets ($\times10^3$/µL)	60.74±37.3	58.88±36.6	0.835

Table-3: Bone marrow findings of thrombocytopenia patients [n=74, n (%)]

Thrombocytopenia	Bone marrow findings	Frequency
Hypoproductive thrombocytopenia	ALL	17 (23)
	AML	15 (20)
	Multiple myeloma	4 (5.5)
	Aplastic anaemia	4 (5.5)
	CLL	4 (5.5)
	Megaloblastic anaemia	3 (4.5)
Hyperdestructive thrombocytopenia	Normo-cellular bone marrow with increased megakaryopoiesis	27 (36)

DISCUSSION

In the present study MPV was significantly higher in hyperdestructive thrombocytopenic patients than hypoproductive group. A study conducted by Khairkar *et al*[10] compared similar groups and concluded that MPV was significantly higher in hyperdestructive as compared to hypoproductive thrombocytopenia patients. Similar studies comparing MPV in these two groups also reported MPV to be significantly higher in hyperdestructive than hypoproductive thrombocytopenic patients.[4,11–17] The cause of this is that newly formed platelets are larger in size than the circulating platelets and with increase in age of the platelets, their size decreases. As there is an active production of platelets by the bone marrow in case of hyperdestructive thrombocytopenia, this leads to a higher MPV in these patients.[5,18] In spite of this fact certain researchers like Khanna *et al*, and Xu *et al*, found MPV to have low sensitivity and specificity to predict the bone marrow involvement in thrombocytopenia.[19,20] Vinholt *et al* reported that MPV along with other platelet indices can be helpful in distinguishing the type of thrombocytopenia.[21]

In the present study PDW was significantly higher in hyperdestructive thrombocytopenic patients compared to hypoproductive thrombocytopenic patients. The findings of the present study were consistent with other studies[11,13,17,22] but Elsewefy *et al*[2] reported this increase to be insignificant, the reason might be the use of different analyzer (Beckman Clouter) than ours.

In the present study, PLCR was significantly higher in hyperdestructive group as compared to hypoproductive thrombocytopenic patients. This is in accordance with similar studies reporting significant increase in PLCR in hyperdestructive thrombocytopenic patients.[2,11,12] Some researchers like Babu and Basu[23] and Borkataky *et al*[22] reported this increase to be insignificant between the two groups but still concluded that PLCR can be a good tool in differential diagnosis of patients with abnormal platelet count.

Another study in which platelet indices were studied along with platelet antibodies in thrombocytopenic patients, the platelet indices were reported to be significantly higher in hyperdestructive thrombocytopenic patients as compared to hypoproductive thrombocytopenic patients and it was endorsed that these indices may be considered as a reliable diagnostic tool for determining the type of thrombocytopenia.[24]

CONCLUSION

Platelet indices can be used as a reliable tool to distinguish between hypoproductive and hyperdestructive thrombocytopenia. This diagnostic tool can be beneficial for thrombocytopenia patients in terms of cost, time, and invasion. Further studies with larger sample size are recommended to validate the findings of this study.

LIMITATIONS OF THE STUDY

Sample technique was non-random convenience sampling so there can be a potential bias.

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UR: Introduction, data entry, write up, literature review

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ORIGINAL ARTICLE

CORRELATION BETWEEN ANAEMIA AND THROMBOCYTOPENIA IN YOUNGER PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Thrombocytopenia needs careful attention of the physician for immediate management to avoid complications. Limited local data is available on low platelet count among patients of all age group on diagnosis of acute lymphoblastic leukaemia (ALL). The aim of this study was to assess the frequency and correlation of anaemia and liver function tests with thrombocytopenia among patients diagnosed with ALL. **Methods:** A prospective study was conducted on patients diagnosed with ALL at Hayatabad Medical Complex, Peshawar. Age, gender, presence of anaemia and deranged liver function tests were correlated with low platelet count in patients with ALL. **Results** A total of 90 patients diagnosed with ALL at the department during the study period were included in the study. Mean age of the patients was 15.43±4.756 years. Among these, 64 (71.1%) were male and 26 (28.9%) patients were female. Low platelet count was observed in 41 (45.5%) of the patients while 49 (54.5%) had platelet count within the range. Low haemoglobin levels were strongly related to presence of low platelet count in our target population (p<0.001). Deranged liver functions were not significantly correlated to thrombocytopenia in patients with ALL. **Conclusion:** Low platelet count was a common finding at the time of diagnosis among patients diagnosed with acute lymphoblastic leukaemia. Presence of anaemia was statistically significantly correlated with presence of low platelet count among the patients at the time of diagnosis of ALL.

Keywords: Acute lymphoblastic leukaemia, ALL, Diagnosis, Platelet, Haematology, Blood, Anaemia

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INTRODUCTION

Haematological malignancies have been diagnosed at much high frequency in last two decades as compared to remote past because of advancements in diagnostic procedures.[1] These malignancies are basically a diverse group of disorders with wide range of symptoms, management plans and prognostic factors.[2] Almost all age groups have been affected by leukaemias or lymphomas, but younger age group has been mostly affected by acute lymphoblastic leukaemia (ALL) as compared to older age group.[3]

Normal haematological parameters are necessary for the overall homeostasis. Platelets are one of the most important components of blood and play the main role in haemostasis on exposure to trauma and other conditions of injury.[4] A lot of haematological malignancies may involve this cell line directly or indirectly.[5] Malignancies which indirectly involve platelets warrant more careful eye of the treating physician to diagnose the abnormality in time for better management and to avoid further complications.[6]

Studies have been performed globally regarding low platelet count in ALL either at time of diagnosis or with response to standard treatment offered for this condition. As per 10 years of study performed by Jaime-Pérez *et al*[7], anaemia and thrombocytopenia at time of diagnosis of ALL were found in 83% of the cases, leukocytosis in 36.6% and leucopoenia in 36.1% of cases. Grunnan *et al*[8] found same phenomenon in patients of ALL after induction therapy and revealed that platelet counts after induction treatment may improve treatment stratification for patients with childhood ALL. Bayhan *et al*[9] reported the incidence of thrombocytopenia purpura, though rare, but the phenomenon cannot be completely ruled out among patients suffering from ALL. Liver functions are not usually affected in initial phases of ALL disease, however, due to systemic spread, diffuse infiltration of tumour cells may result in hepatomegaly in addition to splenomegaly. Recently, different childhood ALL cases with hepatic dysfunction have been reported which may be related to comorbid viral infections, autoimmune hepatitis and obstruction of hepatic arteries by leukemic cells resulting in hypoxia.[10,11]

Haematological malignancies have been commonly encountered malignancies in our part of the world and pose a great burden on our health care budget. Leukaemias if complicated with other problems may lead to increased mortality and morbidity and make things complex both for the patient and the treating team. Limited local data is available low platelet count among patients of all age group at diagnosis of ALL. Sultan *et al*[12] reported that low platelet count was a common finding among adult patients in Pakistan. The present study was planned with the rationale to find the frequency and factors related to low platelet counts especially anaemia and hepatic dysfunction among patients diagnosed with ALL at Oncology Department, Hayatabad Medical Complex, Peshawar.

PATIENTS AND METHODS

This cross-sectional study was conducted at the Oncology Department, Hayatabad Medical Complex, Peshawar, from August 2018 to July 2019. All patients of ALL diagnosed by consultant oncologist/ haematologist on the basis of National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines[13] were included in this study using non-probability consecutive sampling technique. Sample size was calculated by WHO sample size calculator using population prevalence proportion of cytopenia in ALL as 6%.[14] Inclusion criteria included paediatric and young patients between the age of 1 and 25 years diagnosed with ALL waiting for induction therapy. The patients already put on treatment, and those suffering from other conditions such as autoimmune disorders, chronic liver disease, pregnant women, and undiagnosed thrombocytopenia were excluded from the study.

The ethical approval from the Ethical Review Board, and written informed consent from the patients or their caregivers were included in the study. The diagnosis of thrombocytopenia was made with platelet count of less than 150,000/µl[15], estimated through Chemistry Analyser Machine®. Anaemia was described as haemoglobin values of <11 g/dl.[16] Abnormal liver function included either of liver enzymes or bilirubin levels more than normal range.[17] Correlation of age, gender, presence of anaemia and deranged liver function tests was evaluated with low platelet count.

Characteristics of patients and the distribution of the platelet count were described by using the descriptive statistics. Chi-square test was used to determine difference between group variances. Binary logistic regression analysis was done to evaluate the relationship of age, gender, presence of anaemia and deranged liver function tests, and were correlated with low platelet count. All statistical analysis was performed using SPSS-24, and $p \leq 0.05$ was considered significant.

RESULTS

A total of 90 patients diagnosed as acute lymphoblastic leukaemia at our department during the study period were included in analysis. Mean age of the patients was 15.43±4.756 years; 64 (71.1%) were male, and 26 (28.9%) were female. Low platelet count was observed in 41 (45.5%) of the patients while 49 (54.5%) had platelet count within the range. Pearson chi-square analysis (Table-1) showed that low haemoglobin levels were strongly related to presence of low platelet count in our target population ($p<0.001$). Binary logistic regression analysis confirmed this association and patients with low levels of haemoglobin had clearly more chances of having low platelet count as well ($p<0.001$) (Table-2). Odds Ratio with 95% CI was 6.227 (2.402–16.142). Deranged liver functions were not found to be significantly associated with thrombocytopenia in patients with ALL ($p>0.05$).

Table-1: Characteristics of the study group and platelet count in the ALL patients [n (%)]

Patient Demographics		Normal Platelet count	Low Platelet count	*p*
Age	≤12 year	24 (48.9)	14 (34.1)	0.154
	>12 year	25 (51.1)	27 (65.9)	
Gender	Male	37 (75.5)	27 (65.8)	0.315
	Female	12 (24.5)	14 (34.2)	
Deranged liver function	No	24 (48.9)	17 (41.5)	0.475
	Yes	25 (51.1)	24 (58.5)	
Presence of anaemia	No	38 (77.5)	15 (36.6)	<0.001
	Yes	11 (22.5)	26 (63.4)	

Table-2: The correlated factors relating with low platelet count: the binary logistic regression

Demographics	*p*	Odds Ratio (95% CI)
Age (ref. is <12 years)	0.315	1.659(0.619–4.447)
Gender (ref. is male)	0.515	0.698(0.237–2.058)
Deranged Liver functions (ref. is normal liver functions)	0.266	1.722 (0.661–4.486)
Presence of anaemia (ref. is no anaemia)	0.000	6.227 (2.402–16.142)

DISCUSSION

Cancers are one of the leading causes of mortality and morbidity among all age groups all around the world.[1] Variety of laboratory and clinical findings may be part of spectrum of acute lymphoblastic leukaemia. Jawaid *et al*[18] investigated haematological causes of thrombocytopenia in children at Aga Khan University Hospital, Karachi, and concluded that haematological malignancies were one of the commonest causes of low platelet count in the age group. Our study supports their findings as around 40% of patients diagnosed with ALL in our study had low platelet count but no significant correlation was found between low platelet count and paediatric patients with ALL. Abnormal liver function tests were not found to be correlated with thrombocytopenia in patients with ALL.

Adly *et al*[19] published a study in 2015 regarding evaluation of immature platelet count in distinguishing thrombocytopenia in paediatric ALL from immune thrombocytopenia. They found that children diagnosed with acute lymphocytic leukaemia (ALL) had considerably elevated immature platelet fraction (median 10%, $p<0.01$), suggesting that thrombopoiesis is stimulated despite virtual absence of bone marrow progenitors. We did not study immature platelet count or included patients of ITP but found that low platelet count was a consistent finding among patients of ALL. Another study by Bhushan *et al*[20] found the same phenomenon. Considering the high prevalence of thrombocytopenia in patients with haematological malignancies, they tried to establish the presentation of acute leukaemia with normal platelet count at diagnosis. They found that patient with ALL may have normal platelet count with anaemia and

leukopenia. Our findings support their results in general as 65.8% of our male patients had low platelets. Presence of anaemia was a strong predictor of low platelet count in our target population.

Kakaje *et al*[21] from Syria concluded that most patients diagnosed with ALL had either abnormal platelet count (89.3%) or low haemoglobin level (88.8%) when presenting, and only (2.0%) having normal levels for both. They suggested that having normal haemoglobin and platelet count can be used for quick screening in crisis time like in Syria for prioritizing the patients.[21] Both parameters they found deranged in their study participants were also found abnormal in our study subjects, i.e., 26 (63.4%) patients of ALL with low platelet count presented anaemia ($p<0.05$). This shows that anaemia and low platelet count should not be missed before starting the treatment of ALL.

The major limitation of our study was the lack of generalizability as patients from single centre were included in the study with smaller sample size. Patients were not evaluated before the diagnosis of ALL to look for baseline platelet count. Future studies with better design may generate better and generalizable results.

CONCLUSION

Low platelet count was a common finding at the time of diagnosis among patients with ALL but was not significantly correlated to ALL. Presence of anaemia was statistically significantly correlated with presence of low platelet count among these patients at the time of diagnosis of ALL. A large prospective study is recommended for comprehensive correlation between thrombocytopenia and ALL.

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ORIGINAL ARTICLE

CORRELATION OF SERUM VISFATIN WITH ANTHROPOMETRIC AND GLYCAEMIC PARAMETERS IN NON-DIABETIC SUBJECTS WITH AND WITHOUT PARENTAL HISTORY OF TYPE II DIABETES MELLITUS

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Background: Visfatin has insulin like metabolic effects and has a key role in insulin secretion in response to glucose stimulus. This existed link of visfatin with obesity and glucose metabolism is still to be explored and debatable. We aimed to find out the correlation between visfatin and selected anthropometric and biochemical parameters in non-diabetic subjects with type II diabetic parents and with non-diabetic parents. **Methods:** This cross-sectional analytic study was conducted at the Diabetes Clinic of Lahore General Hospital and Department of Physiology, Postgraduate Medical Institute, Lahore. It comprised of 40 on-diabetic subjects with non-diabetic parents (Group I) aged 30–50 years, and 40 age and sex matched non-diabetic subjects with type II diabetic parents (Group II). Blood pressure, BMI and waist hip ratio, fasting levels of serum visfatin, insulin and glucose were measured and indices of insulin resistance (HOMA-IR), sensitivity (HOMA-%S) and beta cell function (HOMA-%β) were calculated. **Results:** Serum visfatin did not correlate with any of the anthropometric and glycaemic parameters assessed in group I and II. However, in combined analysis of female non-diabetic subjects, a statistically significant negative correlation between serum visfatin and waist circumference/waist hip ratio, and a positive correlation of serum visfatin with insulin sensitivity index (HOMA-%S) was found. **Conclusion:** A decline in visfatin production is seen with increasing visceral obesity in non-diabetic female subjects. The fall in visfatin levels seems to play a part in lowering insulin sensitivity in them.

Keywords: Visfatin, diabetics, Type II diabetes mellitus, T2DM, Insulin resistance, Insulin sensitivity, Beta cell function

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INTRODUCTION

Obesity is a matter of great concern globally as its prevalence has nearly tripled between 1975 and 2016. Approximately 11% of adult men and 15% of women were found obese worldwide in year 2016.[1] Non-communicable and preventable diseases are showing increasing trend in Pakistan. In Pakistan 4.8% of the population is obese and 20.8% is overweight as per 2016 WHO findings.[2] Abdominal obesity is an integral component of the metabolic syndrome, associated with many chronic ailments such as type 2 diabetes mellitus and cardiovascular disease. It has been generally accepted that adipose tissue not only stores energy, act as thermal insulator but it has immune and endocrine function as well. It secretes a large number of hormones and cytokines involved in autocrine, paracrine, and endocrine signalling. In obesity, adipose tissue function is impaired and this dysfunction has a strong linkage with insulin resistance.[3]

Visfatin is a multifunctional protein found in all living beings with highly conserved amino acid sequences. Adipose tissue, hepatocytes, and circulating leucocytes such as granulocytes and monocytes are reported as its preferential sources.[4–6] Its adipokine function was first brought into notice by Fukuhara *et al*[5]. They showed that this protein is predominantly being produced by the visceral fat of humans and mice and functions like insulin in the body. Visfatin facilitates glucose entry in adipocytes and myocytes, encourages triglyceride accumulation in preadipocytes by inducing the expression of PPAR-γ and many others.[5] Visfatin's downregulation reduces the sensitivity of rat hepatocytes towards insulin.[7] Earlier, visfatin was named as pre-B cell colony-enhancing factor (PBEF) as it found to be involved in the growth of B lymphocyte precursors in conjunction with interleukin 7 and stem cell factors.[8] It also works as an enzyme (nicotinamide phosphoribosyl transferase/Nampt) regulating the synthesis of nicotinamide adenine dinucleotide (NAD) molecules within the beta cells of the pancreas and hence improves insulin secretion in response to glucose challenge.[9]

The relationship between visfatin, obesity and glucose metabolism has been studied a lot in the past years but it is still indecisive whether visfatin is a marker of obesity or a new key player in the pathogenesis of diabetes. The objective of this study was to find out whether circulating visfatin levels show any correlation with the selected anthropometric and glycaemic parameters in them.

METHODOLOGY

It was a cross-sectional study, conducted in 2018 at the Diabetes Clinic of Lahore General Hospital (LGH) and Department of Physiology, Postgraduate Medical Institute, Lahore. Forty non-diabetic subjects with non-diabetic parents (group I) and 40 non-diabetic subjects with type II diabetic parents (group II) were enrolled in the study. Individuals with history of any acute illness for the past 2 weeks or with chronic inflammatory disease, diabetes or any systemic disease, grade I hypertension, taking any kind of medication, smokers and morbid obese with BMI ≥30[10] were excluded. Pregnant women or with history of menstrual irregularities, acne and hirsutism were also excluded.

Approval of the study was given by Ethical Committee of Postgraduate Medical Institute Lahore. Written consent along with clinical history was sought from each subject. General physical examination was also carried out for each subject. Blood pressure was recorded twice in sitting posture at the left arm after having a rest for 15 minutes using a mercury sphygmomanometer. Weight was taken in minimal clothing and without shoes, height by a height chart, and BMI was calculated. Waist circumference was taken at a point midway between the lowest palpable rib and the uppermost lateral border of iliac crests. Hip circumference was measured at the widest part of the hips by a tape measure keeping the subject in upright position with feet together and without clothes. Waist hip ratio was obtained by dividing waist circumference (WC) by hip circumference (HC). WHR= WC/HC[11]

All the samples were drawn between 8 am to 9 am after overnight fasting under aseptic measures. Gel activated vacutainer tubes were used for the blood collection and sera was extracted after centrifugation. Serum glucose was determined and remaining amount of sera was shifted in properly labelled Eppendorf tubes and frozen at -20 °C for further analysis. Serum visfatin levels were determined by direct ELISA method, catalogue #11560, Glory science company, USA. Assay range of the kit was 1–20 µg/l, Intra-assay precision <9%, inter-assay precision <15% and sensitivity of the assay ≥1 µg/l. Serum Insulin levels were determined by direct Human ELISA kit, Diametra Italy, Ref # DK0076 using an analyzer STAT FAX 303 reader. Serum glucose was analyzed using GOD-PAP enzymatic colorimetric method of Human Diagnostics, Germany kit ref # 10260.

Homeostasis Model of Assessment was used for the calculation of insulin resistance, sensitivity and beta cell function indices.

HOMA-IR= fasting serum glucose (mg/dl)×fasting serum insulin (µIU/ml)/405

HOMA-%S= 1/HOMA-IR×100

HOMA-%β= 360×fasting serum insulin (µIU/ml)/ fasting serum glucose (mg/dl) –63.[12]

Data were analysed on IBM-SPSS-26. Normality of the distribution of the study variables was checked by Shapiro-Wilk's statistics. Mean±SD was given for normally distributed quantitative variables while median with interquartile range (IQR) for non-normally distributed quantitative variables. Independent-samples *t*-test and Mann Whitney U Test were applied for observing mean and median (IQR) difference of the two groups. Pearson's correlation was used to explore the correlation between serum visfatin and selected anthropometric and glycaemic parameters; $p<0.05$ was considered as statistically significant. Simple linear regression analysis was applied to assess the linear relationship between serum visfatin and other quantitative variables.

RESULTS

Demographic, anthropometric and biochemical parameters of the study population are shown in Table-1 and 2. Group I and II were of similar age and gender (20 males, 20 females in each group). There was no significant difference in BMI of males and females of group I and II respectively. However, the waist circumference and waist hip ratios of group II females were significantly higher than that of group I females. No significant difference was observed in the above-mentioned parameters of the males of group I and group II respectively. Group II had significantly lower serum visfatin than that of group I. Insulin resistance (HOMA-IR) was significantly higher while insulin sensitivity (HOMA-%S) was significantly lower in group II in comparison to group I respectively. Beta cell function (HOMA-%β) of group II was also significantly higher than group I.

No significant correlation was achieved between serum visfatin and BMI, waist, hip circumferences or waist-hip ratio in either of the group ($p>0.05$). Moreover, serum visfatin didn't show any significant correlation with serum glucose, insulin and indices of insulin resistance (HOMA-IR), insulin sensitivity (HOMA-%S), or percentage of beta-cell function (HOMA-%β) in group I ($p>0.05$) and II ($p>0.05$) as shown in Table-3. In the collective analysis of female subjects of group I and II, a significant but weak negative correlation was obtained between serum visfatin and waist circumference and waist-hip ratio and positive correlation with the insulin sensitivity index (HOMA-%S) was obtained. No significant correlation between serum visfatin and anthropometric or glycaemic parameters was found in the combined analysis of male subjects of groups I and II (Table-4). On applying simple linear regression analysis, it was found that among female non-diabetic subjects, a unit increase in the waist circumference decreases serum visfatin level 0.16 times. Waist circumference contributed 28% of variability in the fasting levels of visfatin (Table-5).

Table-1: Comparison of demographic and anthropometric parameters of group I and II

Parameters (unit)		Group I (n=40)	Group II (n=40)	*p*
Age (Years)° Median (IQR)		32.00(31.00–36.00)	34.00(32.00–38.00)	0.11
Systolic blood pressure (mmHg)° Median (IQR)		120.00(110.00–125.75)	120(110.00–120.00)	0.80
Diastolic blood pressure (mmHg)° Median (IQR)		80(70.50–85.00)	80.00(78.50–85.00)	0.93
BMI (Kg/m^2)° Median (IQR)	Males	23.96 (21.58–26.14)	25.66(22.07–27.74)	0.31
	Females	23.78(21.11–27.32)	26.36(22.69–27.64)	0.13
Waist circumference (Cm)▪ Mean±SD	Males	89.24±9.62	93.91±9.99	0.14
	Females	81.24±9.03	88.43±7.54	0.01*
Hip circumference (Cm)▪ Mean±SD	Males	97.87±7.92	100.77±8.79	0.28
	Females	97.57±6.13	101.53±7.86	0.08
Waist Hip Ratio▪ Mean±SD	Males	0.91±0.07	0.92±0.07	0.58
	Females	0.83±0.06	0.88±0.06	0.02*

*Statistically significant (p<0.05); Results are expressed as Median (IQR) and Mean±SD; compared by °Mann-Whitney U test and ▪Independent-samples t test respectively. Group I (non-diabetic subjects with non-diabetic parents) & group II (non-diabetic subjects with type II diabetic parents)

Table-2: Comparison of biochemical parameters of group I and II

Biochemical Parameters (unit)	Group I (n=40)	Group II (n=40)	*p*
Fasting serum glucose (mg/dl)▪ Mean±SD	81.67±10.24	85.72±11.17	0.10
Fasting serum insulin (µIU/ml)° Median (IQR)	4.25(3.43–5.47)	13.45 (11.40–15.88)	0.00*
HOMA-IR° Median (IQR)	0.90 (0.64–1.03)	2.73 (2.21–3.79)	0.00*
HOMA-%S° Median (IQR)	111.11 (97.09–156.25)	36.63 (26.37–45.29)	0.00*
HOMA-%B° Median (IQR)	70.90 (48.64–117.75)	234.01 (147.48–334.50)	0.00*
Fasting serum visfatin (ng/ml)° Median (IQR)	11.25 (9.50–13.00)	9.00 (8.00–10.88)	0.00*

*Statistically significant (p<0.05)

Results are expressed as Median (IQR) and Mean±SD; compared by Mann-Whitney U-test and Independent-samples *t*-test respectively

Table-3: Correlation of serum visfatin with anthropometric and glycaemic parameters in group I and II using Pearson correlation

Pearson's correlation of serum Visfatin with	Group I (n=40)		Group II (n=40)	
	p	*R*	*p*	*R*
BMI	0.09	-0.27	0.11	0.25
Waist circumference	0.07	-0.29	0.35	0.15
Hip circumference	0.09	-0.28	0.19	0.21
Waist hip ratio	0.25	-0.19	0.85	0.03
Serum glucose	0.54	-0.10	0.87	0.03
Serum insulin	0.89	-0.02	0.59	-0.09
HOMA-IR	0.96	-0.01	0.57	-0.10
HOMA-%S	0.98	-0.01	0.42	0.13
HOMA-%β	0.95	-0.01	0.76	-0.05

*Statistically significant (p<0.05)

Table-4: Correlation of serum visfatin with anthropometric and glycemic parameters in combined analysis of group-I & II males and females

Pearson's correlation of serum Visfatin with	Group I and II non-diabetic male subjects (n=40)		Group I and II non-diabetic female subjects (n=40)	
	p	*r*	*p*	*r*
BMI	0.93	0.02	0.05	-0.32
Waist circumference	0.80	0.04	0.00*	-0.53
Hip circumference	0.88	0.03	0.06	-0.30
Waist hip ratio	0.61	0.08	0.00*	-0.48
Serum glucose	0.67	0.07	0.14	-0.24
Serum insulin	0.16	-0.23	0.04*	-0.33
HOMA-IR	0.19	-0.21	0.05	-0.34
HOMA-%S	0.30	0.17	0.04*	0.36
HOMA-%β	0.24	-0.19	0.23	-0.21

*Statistically significant (p<0.05)

Table-5: Simple linear regression of visfatin with waist circumference in combined analysis of group I and II female non-diabetic subjects (n=40)

Strata	Variable	Constant	Co-efficient	R	R^2
Female	Waist Circumference	23.99	-0.16	0.53	0.28

Dependent variable; fasting serum visfatin

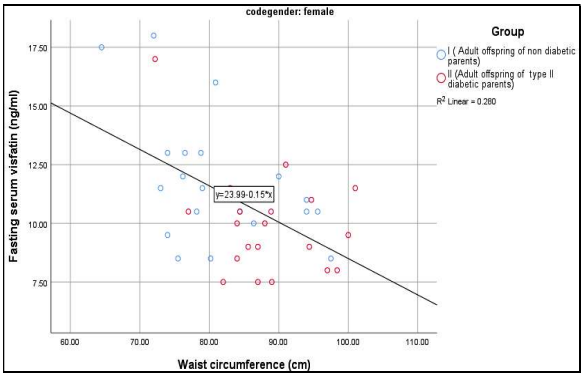

Figure-1: Scatter plot showing negative correlation between waist circumference and serum visfatin in combined analysis of group I and II female non-diabetic subjects (n=40) by Pearson's correlation

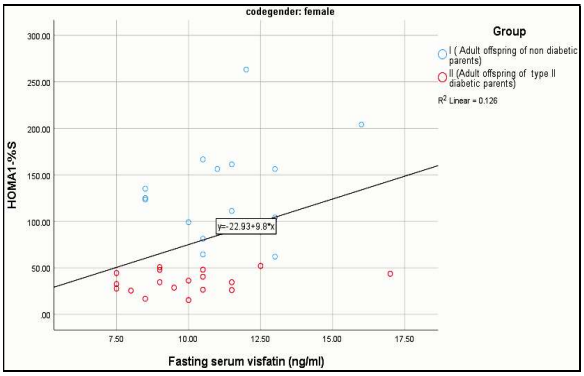

Figure-2: Scatter plot showing positive correlation between serum visfatin and HOMA-%S in combined analysis of group I and II female non-diabetic subjects (n=40) by Pearson's correlation

DISCUSSION

The relationship of visfatin with obesity and abnormal glucose metabolism is a complex one. Both positive and negative correlation of serum visfatin with anthropometric and glycaemic parameters has been reported in the past literature in non-diabetics.[13,14]

Results of our study support the inverse relation of visfatin with waist circumference and waist hip ratio and positive with insulin sensitivity in non-diabetic female subjects. Previously in a study, a significant negative correlation was reported between visfatin/insulin ratio and the body mass index and waist circumference of obese women with metabolic syndrome. The researcher proposed that the release of adipokines such as visfatin becomes greatly reduced as person gains fat especially in the abdominal area. This decline in visfatin/insulin ratio influence the development and progression of insulin resistance.[14] This fact is further supported by a study where glucose uptake was diminished in mouse adipocytes after/following visfatin down regulation.[15] In another study on obese females, visfatin levels were lowest in those who had highest BMI and waist circumference among others; however, no significant correlation between serum visfatin and above-mentioned obesity markers was documented.[16] Kaminska *et al*, found high levels of visfatin in obese subjects and a strong negative correlation of visfatin with the waist hip ratio and a nearly significant positive correlation with hip circumference. As majority of candidates were female, they proposed that raised visfatin levels in obese female subjects were probably associated with pear shaped rather than abdominal obesity.[17] Marked improvement in insulin sensitivity was observed after caloric restriction in a group of Spanish obese non-diabetic women and was associated with increase in their visfatin levels.[18]

In obese males, a positive correlation between visfatin level and their waist hip ratio was reported by Jian *et al*., emphasizing the direct relation of visfatin with visceral adiposity in them.[19] Studies' documenting the direct relationship between circulating visfatin levels with obesity markers suggest that with an increase in adiposity there is increase release of pro-inflammatory adipokines including visfatin. These cytokines are involved in promoting insulin resistance both locally and systemically.[20]

It may, therefore, be hypothesized that different associations between visfatin's circulating level and various anthropometric parameters could be due to the difference in visfatin's genetic expression in adipose tissue in both genders or it is related more with grades of obesity and abnormal carbohydrate metabolism.

Limitation of the study were, its small sample size, cross-sectional design and methods of assessment of insulin resistance and beta cell function.

CONCLUSION

Visfatin secretion decreases with increasing obesity in non-diabetic female subjects and has a role in insulin sensitivity reduction.

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ORIGINAL ARTICLE

OBSTRUCTIVE SLEEP APNOEA AND ROLE OF INTERLEUKIN-6 AS A CIRCULATORY BIOMARKER: A CROSS-SECTION STUDY

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Background: Despite reported link between OSA and systemic inflammation, results are indecisive so far for different inflammatory markers to evaluate the casual relation with OSA and its co-morbidities. Objective of this study was to assess the relationship of inflammatory cytokine Interleukin-6 with obstructive sleep apnoea (OSA) and its severity among Pakistani population. **Methodology:** A total of 180 subjects (55 without apnoea, 33 mild, 34 moderate, and 58 with severe apnoea) were analysed in this cross-sectional study, between Dec 2018 and Jan 2020 in Dow University Hospital, Karachi. Apnoea was confirmed and subjects were grouped on the basis of Apnoea Hypopnea Index (AHI) by overnight Polysomnography. Plasma IL-6 was analysed using enzyme-linked immunosorbent assay (ELISA). One-way ANOVA was used for comparison. Potential effect of age and BMI was controlled using ANCOVA and effect size was reported as partial eta squared. **Results:** Mean IL-6 levels were associated with severity of apnoea with 4.72±1.50 pg/ml, 21.08±6.83 pg/ml, 25.41±7.97 pg/ml and 26.96±7.39 pg/ml in no OSA, mild, moderate and severe OSA respectively, with statistically significant difference between all groups except between moderate and severe OSA. After controlling the potential effect of age and BMI still yielded a significant positive association between severity of OSA and IL-6 levels (effect size (partial η^2) of 59% (p<0.001)). **Conclusion:** Higher Interleukin-6 levels were observed in OSA and associated with its severity. It could provide a feasible method to improve timely diagnosis of OSA and can point toward presence of a more severe clinical phenotype.

Keywords: Sleep, Obstructive Sleep Apnoea, Interleukin-6, Inflammation

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INTRODUCTION

Obstructive Sleep Apnoea (OSA) is a condition associated with repeated episodes of partial or complete obstruction of upper airway, intermittent hypoxia during sleep, sleep fragmentation and excessive daytime sleepiness (EDS).[1,2] When there is complete obstruction of airway the event is called as apnoea, while in case of partial obstruction it is hypopnea. Number of apnoea-hypopnea episodes per hour of sleep is called as Apnoea Hypopnea Index (AHI) and indicates the severity of apnoea. On the basis of AHI, apnoea can be divided as mild (AHI= 5–10), moderate (AHI= 15–30) and severe apnoea (AHI ≤30) as mentioned by the American Academy of Sleep Medicine (AASM) revised scoring criteria.[2] Polysomnography is the gold standard diagnostic test for this condition which can not only measure AHI but also monitor time spent in each sleep phase, EEG, The ECG and many other sleep related parameters.[3]

Its prevalence is continuously increasing as a recent research described it as 9 to 38%.[1] The OSA has been observed to be associated with many adverse health outcomes and comorbidities like excessive day time sleepiness (EDS), impaired quality of life (QoL), increase occupational and motor vehicle accidents, oxidative stress, metabolic disorders, cognitive deficits, hypertension, coronary artery disease, diabetes, stroke, congestive heart failure, atrial fibrillation, cardiovascular events and mortality.[4–7] Common risk factors are advanced age, obesity, male gender, postmenopausal women and craniofacial dysmorphisms.[4] Continuously increasing prevalence, numerous adverse health outcomes and sudden death are the reason to increase OSA related research studies in past few decades to investigate about its aetiology, exact pathophysiology and other contributory factors, but results are still indecisive.

According to studies OSA has been found to be associated with increased systemic inflammatory responses and many of its related outcome can be explained by underlying inflammatory process that may contribute to a higher risk for end-organ morbidity.[3,8] Studies mentioned higher levels of proinflammatory mediator such as Interleukin-6 (IL-6), CRP and TNF-α in OSA and support the hypothesis for the relationship of OSA and inflammation.[9] There is a need to assess the casual relationship of OSA and systemic inflammatory pathways and to evaluate the circulatory biomarkers which can point toward the presence of OSA, its severity and related comorbidities.[8]

Interleukin-6 is one of the most significant inflammatory markers and an excellent proxy for the inflammatory/immune system.[10] It belongs to the category of four-helical cytokines and synthesised and secreted by many cells in body.[11] Physiologically it

involves in homeostasis, intracellular signalling, haematopoiesis, bone metabolism, liver metabolism, immune system coordination, proliferation and differentiation of B and T lymphocytes and neural development and survival.[10–12] Literature explains the complex biology of IL-6, as how one cytokine can have extremely different biological effects on different cells in different biological states.[11] It behaves as a cytokines as well as a myokine. It pays a role in different autoimmune diseases, diabetes, atherosclerosis, cancers, encephalitis, rheumatoid arthritis and reinforce different inflammatory states.[12] Studies also described its role in normal sleep physiology and higher circulatory levels with alterations in sleep duration and low quality of sleep.[13] Due to its vital role in haematopoiesis, immunomodulation, homeostasis and inflammatory processes, it's up-regulation or over-expression can disturb functions of multiple organ system.[14] Under normal condition, its levels are 1–5 pg/ml but during inflammation it can go through immense amplification and levels can rise up to 1,000 folds and extreme conditions can leads to sepsis IL-6 levels in µg/ml.[11] Its prognostic levels may varies from 5 to 100,000 pg/ml, depending on the nature and severity of the disease. Its multifunctional role describe the significance of its quantitative detection in assessing the severity of different diseased conditions.[14] We can conclude that elevated levels of IL-6 can be consider as the major alarm signal for the body.[11]

Studies described the elevated levels of IL-6 in OSA subjects.[10] It has been suggested that repetitive hypoxia, re-oxygenation and oxidative stress during OSA may lead to activation of inflammatory cells which increase the production of cytokines IL-6. IL-6 is a well-established risk factor for cardiovascular disorders and could be related to OSA related cardio-metabolic comorbidities. Studies described the elevated levels of inflammatory mediators IL-6 as well as concomitant reduction of anti-inflammatory mediators IL-10 so tilting the balance toward more proinflammatory status.[2,3,15] Studies also described the role of genetic variation as different genetic background in different population may account for variability in the results for IL-6 levels in different countries, which emphasizes for the need of current study in our population.[15]

Despite multiple related co-morbidities and complications, OSA is still an underdiagnosed and underestimated disease in our community due to lack of information not only in general public but also in our medical practitioners. We have only few OSA related research studies for our local population, which examined relationship of OSA with metabolic syndrome, disturbance in lipid profile, derangements in glucose metabolism and relationship with physical activity.[6,7,16,17] There is a dire need to evaluate a biomarkers like IL-6, which can point toward the presence of OSA, its severity and related comorbidities. It could provide a feasible and accessible method to improve the timely diagnosis of OSA and to identify patients who are at high risk of CVS complications and required immediate intervention. The objective of this study was to assess the relationship of inflammatory cytokine Interleukin-6 with obstructive sleep apnoea and its severity among Pakistani population.

MATERIAL AND METHODS

This cross-sectional study was conducted in Dow University of Health Sciences, Karachi, from Dec 2018 to Jan 2020. Study approval was taken from Ethical Review Board of the University. Study population were subjects coming to Sleep Laboratory, referred from many hospitals of Karachi as well as from many cities of Pakistan for the diagnosis of their OSA.

Inclusion criteria were subjects with snoring, witnessed apnoea or EDS (ESS Score >9), having indication for Polysomnography. The Epworth Sleepiness Scale (ESS) is a questionnaire which can tell us about chances of having EDS when score is >9.[18] Subjects with recent surgery, pregnancy or current serious illness were excluded.

Each subject arrived at 9 PM on their respective night booked for Polysomnography. Oral and written consent was taken with verbal explanation of procedure while approved performa was used to record anthropometric measurements, medical and personal history and medical examination. All subjects went through split-night polysomnography for their AHI and OSA diagnosis. Test was performed by using a multichannel polysomnographic machine (Philips Respironic Alice 5 and Alice 6). Polysomnographic data gave us recording for chest and abdominal wall movement, ECG, air flow and volumes, nasal pressure, Arterial oxygen saturation (Sa_{O2}), pulse waveform, the bilateral electro-oculogram (EOG), electroencephalo-gram (EEG), chin and anterior tibial electromyograms (EMG).[3] The OSA subjects were categorized on the basis of their AHI as mild (32 subjects), moderate (35 subjects) and severe apnoea (58 subjects) while a comparison group without apnoea was comprised of 55 subjects. Control group included age and gender matched subjects with ESS score <9, no sign and symptoms of apnoea and no EDS.

After OSA diagnosis they were analysed for IL-6 levels by taking blood sample in the morning, in a vacutainer tube containing EDTA. Centrifugation was performed to separate plasma and stored it at -80 °C until further processing for IL-6 analysis.

Enzyme-link immune-sorbent assay (ELISA) kits (DIA source, SA Belgium) was used with standard automated procedures, in Biochemistry Laboratory. Sample handling, temperature control and working procedures were managed according to manufacturer's

protocol. Dichromatic readings were taken by calculating the mean of duplicate determinations.

SPSS-20 was used for data analysis. Quantitative variables, e.g., age, weight, height, BMI and IL-6 were stated as Mean±SD, whereas qualitative variables were mentioned as frequency. Mean levels of IL-6 were compared by using independent sample *t*-test for two groups, while one way ANOVA was used to make comparison between sub-groups; $p \leq 0.05$ was considered as significant. Potential effect of age and BMI was controlled using ANCOVA and effect size was reported as partial eta squared.

RESULTS

Mean age of study group was 48.92±11.28 years (No OSA: 46.04±12.6, mild OSA: 47.88±10.41, moderate OSA: 51.80±12.39, severe OSA: 50.48±9.10). There were 110 (61%) males subjects and 70 (39%) females subjects while no statistically significant difference was present in the IL-6 levels between genders (p=0.77). Mean AHI score (apnoea score) for mild, moderate and severe apnoea groups and ESS score for all groups are mentioned in Table-1. Mean plasma IL-6 level for whole study group was 18.82±11.41 pg/mL. IL-6 levels for no apnoea group was 4.72±1.50 while mean levels for all three apnoea groups was 25.02±7.74 (p<0.0001).

Table-1 shows the means and standard deviations of IL-6 levels, age, and BMI of different groups. Mean IL-6 levels showed increasing trend with severity of apnoea with 4.72±1.50 pg/ml, 21.08±6.83 pg/ml, 25.41±7.97 pg/ml and 26.96±7.39 pg/ml in no OSA, mild, moderate and severe OSA respectively with statistically significant difference between all groups except between moderate and severe OSA. (Table-2 shows the mean differences and respective *p*-values in different groups). Despite there was non-significant difference of mean age between groups and statistically significant difference in the mean BMI scores between groups as both are showing an increasing trend according to severity of OSA, controlling for these two variables still yielded a significant positive association between severity of OSA and IL-6 levels. After controlling for the possible effect of BMI and age, the association between OSA severity and IL-6 was statistically significant with and effect size (partial η^2) of 59% (p<0.001).

Table-1: Comparison of IL-6 levels, age, ESS scores, AHI scores and BMI in different groups of obstructive sleep apnoea (Mean±SD)

Severity of OSA	IL-6 levels	Age	ESS Score	Apnoea score	BMI
No OSA (n=55)	4.72±1.50	46.04±12.6	3.38±2.24	0.00±0.00	24.97±5.68
Mild OSA (n=33)	21.08±6.83	47.88±10.41	11.09±3.45	10.25±3.72	32.98±5.93
Moderate OSA (n=34)	25.41±7.97	51.80±12.39	13.68±3.34	24.67±6.45	36.15±9.18
Severe OSA (n=58)	26.96±7.39	50.48±9.10	16.71±4.27	50.28±15.16	37.91±8.05

Table-2: Mean differences of IL-6 in different groups of obstructive sleep apnea

Severity of OSA	Severity of OSA	Mean Difference	*p*	95% Confidence Interval	
				Lower Bound	Upper Bound
No OSA	Mild OSA	-16.3570	<0.001	-19.062	-13.651
	Moderate OSA	-20.8309	<0.001	-23.511	-18.150
	Severe OSA	-22.2421	<0.001	-24.555	-19.930
Mild OSA	No OSA	16.3570	<0.001	13.651	19.062
	Moderate OSA	-4.4739	0.004	-7.476	-1.471
	Severe OSA	-5.8851	<0.001	-8.564	-3.206
Moderate OSA	No OSA	20.8309	<0.001	18.150	23.511
	Mild OSA	4.4739	0.004	1.471	7.476
	Severe OSA	-1.4111	0.295	-4.065	1.243
Severe OSA	No OSA	22.2421	<0.001	19.930	24.555
	Mild OSA	5.8851	<0.001	3.206	8.564
	Moderate OSA	1.4111	0.295	-1.243	4.065

DISCUSSION

Our findings are suggesting an association of plasma IL-6 levels with OSA and its severity in our population. Results are still significant even after controlling the potential effect of age and BMI.

Literature review reflects the variability in this association in different study populations. Gozal *et al*[3], examined a small group of polysomnographicaly diagnosed OSA children and found significantly higher levels of IL-6, independent to obesity. After treatment with tonsillectomy and adenoidectomy (T and A) IL-6 levels were decrease as those of controls. Huang *et al*[9] conducted their research in Taiwan and failed to get any significant difference with respect to IL-6 levels (p=0.104) in age and BMI matched case-control groups. A multicentre study[15] examined Spanish children and found significantly higher levels of IL-6 in OSA children as compare to age and BMI matched controls (p=0.009) and suggested a role of IL-6 in OSA related comorbidities. Motamedi *et al*[2], evaluated a young adult cohort with moderate to severe apnoea and determined higher IL-6 levels. As study population consist of young individuals and hypertension/CVDs were infrequent so they concluded

that higher levels of IL-6 in their study was most likely due to presence of oxygen desaturation. They proposed that repetitive hypoxia may induced vascular injuries, aggravation of existing inflammatory process and may lead to development of cardiovascular and cerebrovascular issues and cognitive deficits in OSA. A recently published meta-analysis[10] examined 63 studies (57 studies with adult population and 6 studies with children) and concluded that plasma/serum levels of IL-6 were significantly higher in majority of studies. Van Eyck *et al*[19], examined over-weight and obese children and couldn't get any association between interleukin-6 levels with severity of OSA. Study population of Tam *et al*[20] consisted on 44 mild apnoeic children. They evaluated multiple parameter of inflammation in OSA group with comparison to controls and failed to find any significant correlation between majorities of inflammatory mediators including IL-6 with OSA. Their negative findings could be due to the initial and mild form of apnoea in those children.

Studies reinforced that OSA induces a reversible low-grade systemic inflammatory response and increase inflammatory mediators such as IL-6 which may leads to further activation of multiple downstream pathways and further proceed for different end-organ morbidities, identified for OSA. Intermittent hypoxia, hypercapnia and sleep fragmentation are reported for activation of pro-inflammatory pathways along with down-regulation of anti-inflammatory markers, as exemplified by decline in IL-10 levels in some study.[3] IL-6 is considered as a well-recognized risk factor for CVDs and could be considered as an important link between OSA and CVDs. Studies explained correlation of IL-6 levels with the degree of hypoxia and sleep duration independent of obesity.[15]

The CVDs remains a highly prevalent cause of morbidity and mortality worldwide.[21] OSA can be considered as a modifiable risk factor for prevention of CVDs, related complications and to reduce huge economic burden on health care system. There is need to focus on all the contributing factors and related biomarkers which could have a possible role in the aetiology or treatment of OSA.

One important strength of current study is the analysis of our results after adjustment of age and BMI which could play a role as confounders. Future longitudinal studies are advisable to determine temporal relationship. Blood IL-6 levels could be a feasible and accessible method to improve the timely diagnosis of OSA and related morbidities and to decrease the burden of more serious complications.[2] Plasma IL-6 measurements may be used in OSA treatment follow-ups, when polysomnography is not available.

CONCLUSION

Higher Interleukin-6 levels were observed in OSA and associated with the severity of disease. Measurement of IL-6 levels could provide a feasible method to improve the timely diagnosis of OSA and can point toward the presence of a more severe clinical phenotype and required immediate intervention. It may also facilitate the implementation of better treatment options for OSA patients in our country. This study further supports the hypothesis that elevated levels of IL-6 in OSA could be the reason of inflammatory stress and related co-morbidities in these patients.

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ORIGINAL ARTICLE

NIGELLA SATIVA: ROLE IN IRON OVERLOAD

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Background: In thalassemia patients, iron is overloaded because of multiple blood transfusions. This overloaded iron can damage the organs badly which is a major cause of death in these patients. Iron overload in thalassemia major can only be treated by removing the excessive iron through different chelating agents. Commercially available chelating agents are costly and associated with multiple adverse effects. Effectiveness of naturally present chelators is under study. This study was conducted to explore the chelating effect of one of the commonly used herb *Nigella sativa*. **Methods:** This experimental, randomized controlled trial was conducted in Zoology Department, Government College University, Lahore in its multidisciplinary laboratory. A total of 36 male albino mice were divided into three groups, 12 in each group. Iron was overloaded in groups 2 and 3 by intravenous injections of iron dextran (0.1 ml/g body weight) for 15 days on daily basis. After 15 days, iron dextran injection was discontinued, and mice were allowed to feed on *Nigella sativa* (200 mg/Kg body weight) for further 15 days. Blood sampling was done at baseline, 15, and 30 days to analyse iron concentration in serum, heart, kidney, and liver. Data was analysed using Student's *t*-test. **Results:** The artificially administered iron in mice increased the iron levels in serum, liver, heart and kidneys to a significant level while administration of *N. sativa* significantly decreased these levels. **Conclusion:** We propose clinical trials of *Nigella sativa* in thalassemia major patients as an adjunct therapy to explore its efficacy and safety.

Keywords: Thalassemia, Iron overload, *Nigella sativa*, Mice, Clinical trial

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INTRODUCTION

Thalassemias are genetic disorders that are prevalent worldwide with highest death rates in West and South Asia. The current management of thalassemia patients is blood transfusion along with chelating agents to prevent iron overload.[1] Almost 200–250 mg of iron is present in a unit of transfused blood leading to damage of important organs such as liver, kidney, and heart as a result of iron overload.[2] In the absence of active remedies this iron accumulation can cause death due to cardiac failure or arrhythmias in the patients of thalassemia major.[3]

Most effective remedy for removal of this overloaded iron is the chelation therapy by using chelating agents. These agents remove iron by forming the unwavering resolvable multiplexes and are then removed from the body in urine or faeces. Desferrioxamine is one of the commonly used chelators but its cost, availability, and association with multiple adverse effects makes the management of iron overload difficult.[4] In developing countries, all these factors lead to high mortality due to iron induced organ damage.[5]

Because of these limitations of chelating agents, investigators are focusing on beneficial plants and herbs for chelating therapy. *Nigella sativa* is a wonder herb with a wide range of pharmacological effects and remarkable religious background. A number of research studies have proven the efficacy of *Nigella sativa* seeds in various diseases like cough, bronchitis, and influenza.[5] The activity of an essential element of *Nigella sativa*, thymol, has been investigated by Kishwar *et al*. They concluded that thymol could reduce the toxicity of iron and other metals by forming the complexes with these metals.[6]

This experimental study was aimed to determine the *Nigella sativa* seed's protective effects against organ damage caused by iron overload and in elimination of excessive iron from the body as a possible treatment option for iron overload in patients of thalassemia major.

MATERIAL AND METHODS

This experimental, randomized controlled trial was carried out in the Zoology Department, Government College University, Lahore, in collaboration with its multidisciplinary laboratory.

For this experimental study, two months old, healthy male albino mice weighing 25–50 grams were obtained and kept in the animal house of Government College University Lahore. Fresh water and commercial pelleted diet were provided daily. Mice cages were placed in a well-ventilated room with constant temperature of 24 °C, humidity 50–70% and 12-hour light/dark cycle. *Nigella sativa* seeds were obtained and certified by Botany Department, Government College University, Lahore, through proper taxonomical rules. Seeds were crushed into powder form and calculated form of the powder was mixed with mice feed.

Mice were divided into three groups; Group 1 was normal control group while groups 2 and 3 were experimental groups each comprising of twelve mice. Group 1 mice were fed on normal diet throughout the experiment and injected with the salt solution; concentration of solution was like that present in iron dextran injection. Groups 2 and 3 were fed on normal diet and injected through tail vein with iron dextran injection 0.1 ml/g body weight for 15 days on daily basis. Then two mice were sacrificed with an interval of five days to determine whether the iron was being overloaded or not. After confirmation (by blood tests) group 3 mice were allowed to be fed on *Nigella sativa* (200 mg/Kg body weight) mixed feed for 15 days.

Blood samples from all mice were taken three times, at days 0, 15, and 30. Intra cardiac technique was used for blood sampling under chloroform anaesthesia. This blood was than centrifuged at 6,000 rpm and obtained serum was collected through micropipette and stored at -8 ºC. After 30 days mice were sacrificed, and organs (heart, kidney, and liver) were removed. Every organ was divided into three equal parts. One was preserved in 10% formalin for microtome while two were stored at -86 ºC for iron analysis and protein carbonyl content determination. For iron analysis, serum and frozen organs were first digested in aqua regia. Aqua regia (1 ml/organ) was added to organs and serum in separate test tubes and was left overnight at room temperature. Serum and organs were dissolved in aqua regia. That mixture was then allowed to boil for 2–3 minutes. After boiling, 1 ml distilled water was added, and mixture was filtered to remove any impurities. Then optical density was measured using atomic absorption spectrophotometer to determine the amount of accumulated iron. Student's *t*-test was applied to compare the individual parameters in different groups.

Samples were assigned different numbers for identification, described, and placed in small plastic cassettes. After fixation and embedding, eosin and haematoxylin stain was used for histological sections. Slides were examined under ×10 and ×40 power of light microscope. The microscopic qualitative parameters including necrosis of cells and size of nucleus were observed.

RESULTS

Artificially administered iron in mice led to significantly increased levels of iron in serum, liver, heart, and kidneys as compared to normal control group (Table-1).

After 15 days of *N. sativa* treatment of iron overloaded mice, concentration of iron in serum, liver, kidney, and heart was decreased (Table-2, Figures 1–4).

Histopathological analysis reinforced the above findings. Iron overload induced organ damage was evident under the microscope in the form of necrosed cells, increased size of nucleus, absent or scanty cytoplasm in groups 2 and 3 (Figures 1b, 2b, and 3b). In group 3 after herb treatment significant improvement in cells of kidney, liver and heart was observed (Figures 1c, 2c and 3c).

Table-1: Comparison of iron levels in serum, liver, kidney, and heart between control and iron overloaded groups (Mean±SEM, mg/ml)

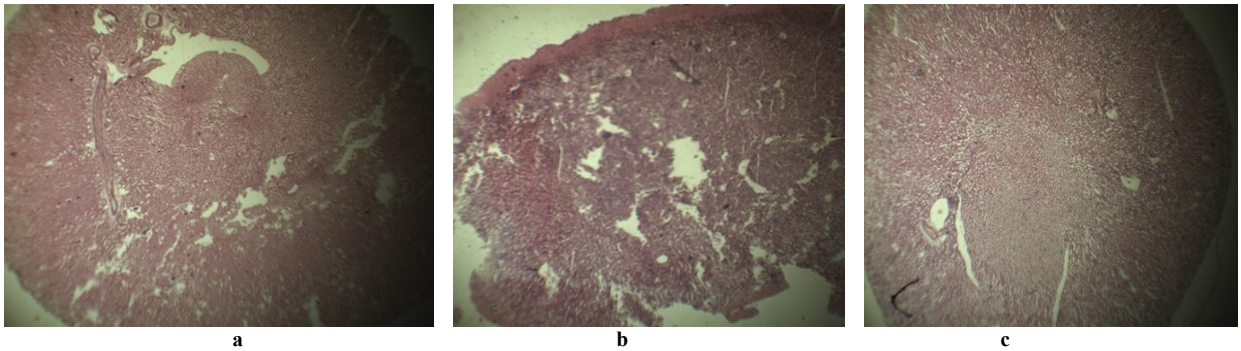
Groups	Control	Iron overloaded	*p*
Serum iron concentration	0.003295 ±0.002145	0.017641 ±0.001141	0.003*
Liver iron concentration	0.00260 ±0.00078	0.02287 ±0.00143	0.05*
Kidney iron concentration	0.00269 ±0.00069	0.01896 ±0.00043	0.05*
Heart iron concentration	0.00317 ±0.00113	0.03010 ±0.00111	0.03*

*Significant

Table-2: Comparison of serum iron concentration in herb treated group with control group and iron overloaded control group after 30 days (Mean±SEM, mg/ml)

Groups	Iron overloaded	Iron overloaded +herb treated	*p*
Serum iron concentration	0.0256920 ±0.000121	0.0042744 ±0.0061561	0.05*
Liver iron concentration	0.0210143 ±0.0000111	0.0056160 ±0.0004352	0.04*
Kidney iron concentration	0.0351089 ±0,0096451	0.0048415 ±0.0000321	0.03*
Heart iron concentration	0.0339128 ±0.0023141	0.0032690 ±0.0010061	0.01*

*Significant



a b c

Figure-1: Comparison of kidney tissues (a) control kidney having low iron level (b) iron overloaded kidney, cells damaged by iron (c) iron overloaded+herb treated kidney, cells are repaired

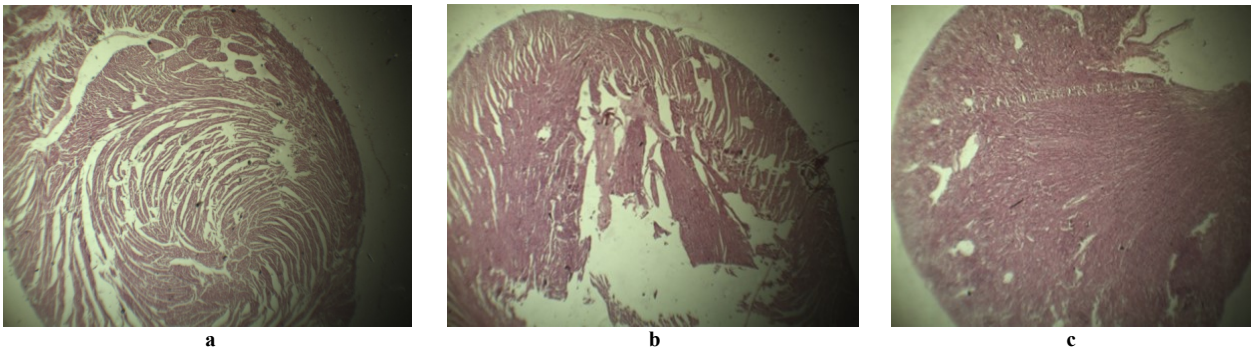


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Figure-2: Comparison of heart tissues (a) control heart having low iron level (b) iron overloaded heart, cells damaged by iron (c) iron overloaded+herb treated heart, cells are repaired

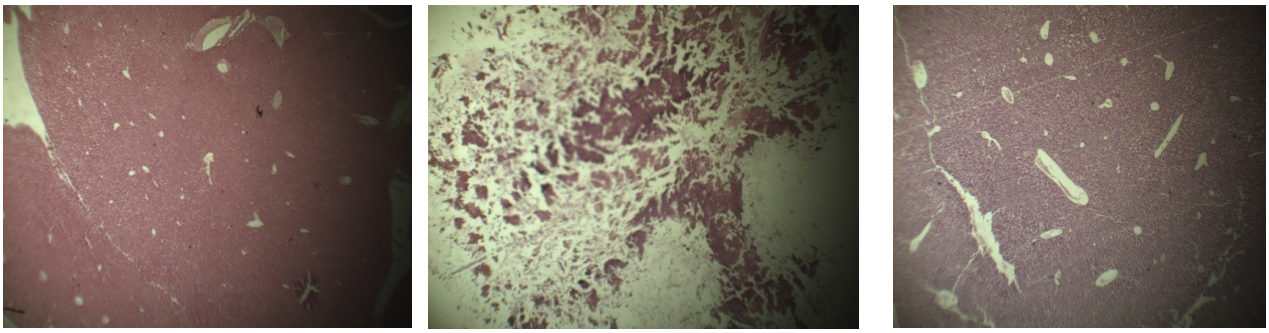

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Figure-3: Comparison of liver tissues (a) control liver having low iron level (b) iron overloaded liver, cells damaged by iron (c) iron overloaded+herb treated liver, cells are repaired

DISCUSSION

The present study was conducted to observe the effect of *N. sativa* on artificially iron overloaded mice. After 15 days iron overload was confirmed by comparing with the control group. In a previous study serum iron concentration in normal men and women was compared with that of iron overloaded patients. Serum iron level in normal men and women was reported as 6–186 ng/ml and 3–162 ng/ml respectively while in iron overloaded patients the iron concentration was increased to a range of 940–4,240 ng/ml.[7] Das *et al*[8] also conducted a study on mice with iron overload and serum iron concentration was increased to double the amount of iron in the serum of normal mice. The serum iron level in iron overloaded+herb treated mice was very close to the control value, i.e., 0.0033 mg/ml. Another study proved that *Medicago sativa* and *Allium porrum* extracts reduced the iron concentration to significant levels.[9]

Extraneously overloaded iron was also accumulated in liver, kidneys, and heart. Liver iron concentration in control mice was 0.0021 mg/ml which was increased to 0.0228 mg/ml after giving iron injections. Das *et al*[8] also reported the increased liver iron concentration (685.79%) in iron overloaded mice. *N. sativa* treatment significantly decreased the iron level of liver. Another study conducted by Danladi *et al*[10] observed the effect of *N. sativa* on the liver antioxidant enzyme activities which were decreased by CCl4 treatment and subsequently increased by *N. sativa*.

After overloading iron in mice, a rapid increase in the kidney iron concentration was also observed. High concentration of iron in kidney was decreased by the *N. sativa* treatment. It was observed from the present study that iron level was decreased to 0.0047 mg/ml after treating the iron overloaded mice with *N. sativa*. Chelating ability of another plant extract known as *Tetracarpidium conophorum* was also reported in a previous study. Chelating ability of *T. conophorum* for ferrous iron was observed as more than 70%.[11] Iron level in heart was increased to 0.0301 mg/ml in iron overloaded mice after 15 days of iron accumulation while the iron concentration in control group was 0.0031 mg/ml. Wongjaikam *et al*[12] demonstrated iron overloading in rats and found significantly ($p<0.05$) increased cardiac iron concentration as compared to the control rats.

N. sativa treated mice had a decreased iron concentration of 0.0036 mg/ml in heart which is very low as compared to iron overloaded control group, i.e., 0.0301 mg/ml. Zaoui[13] reported that *N. sativa* oil (1 ml/Kg/day) oral administration in rats for 12 weeks could significantly reduced serum cholesterol, glucose, triglyceride levels, platelet counts and leukocytes by 15–30% as compared to control group.

Histopathological analysis of liver, kidney and heart in the current study reported that there was a clear difference between the tissues of control group, iron overloaded group and iron overloaded+herb treated group. Tissues of the control group mice were very healthy whereas iron overload showed liver, kidney, and heart defects as necrosis of the cells. The size of the nucleus was also increased as compared to normal mice. Cytoplasm was absent in the cells of iron overloaded mice. In a previous histopathological study, it was demonstrated that iron overload damaged the tissues and hepatic necrosis and other morphological changes were observed in iron overloaded rats.[14]

CONCLUSION

Our study demonstrated beneficial effect of *N. Sativa* in iron overloaded mice. Future studies are recommended to replicate these in thalassemia major patients receiving blood transfusions.

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ORIGINAL ARTICLE

SPECTRUM OF BIOCHEMICAL ALTERATIONS IN PATIENTS WITH COVID-19

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Background: The COVID-19 pandemic has claimed millions of lives around the globe. In addition to respiratory involvement, multi organ failure has also been noted in these patients. We tried to assess the biochemical abnormalities in these patients to have a better understanding of this disease and its complications. **Methods:** Adult patients (n=107) who tested positive for COVID-19 by RT-PCR were included in this study. Blood was analysed for Urea, Creatinine, Ferritin, Lactate Dehydrogenase (LDH), Calcium, Magnesium and Phosphorus in Cobas C501 (Roche Diagnostics) using spectrophotometric technology. Sodium, Chloride, Potassium and Bicarbonate were analysed on NOVA electrolyte analyser using ion-selective electrodes. **Results:** Urea and creatinine were elevated in 33.6% and 22.4% patients respectively. Ferritin and LDH were high in 88.8% and 93.5% patients respectively. Reduced levels of electrolytes was observed , i.e., Sodium in 44.9%, Potassium in 22.4%, Bicarbonate in 53.3%, Calcium in 48.6%, and Phosphorus in 23.4% patients. There were no significant differences in abnormalities in the different age groups (p>0.05). **Conclusion:** COVID-19 patients suffer from pulmonary disease as well as multi-organ involvement as seen by the biochemical alterations, and this should be kept in mind while treating these patients.

Keywords: COVID-19, Pandemic, Biochemical, Multi-organ failure, Electrolytes

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INTRODUCTION

Since December 2019, Severe Acute Respiratory Syndrome Corona Virus-2 (SARS COV-2) started spreading from Wuhan, China, and on 11th March 2020, World Health Organization[1] declared COVID-19 disease. Many of the patients are asymptomatic or have mild disease with upper respiratory tract infection, but diffuse alveolar damage and acute respiratory failure is also seen.[1,2] Multiple organ failure has been seen to cause the morbidity and mortality in COVID-19 patients.[3]

The SARS COV-2 virus interacts with angiotensin-converting enzyme 2 (ACE2) via its spike protein. It invades the human cell by binding with (ACE2) on the cell membrane. Therefore, cells with ACE2 expression such as type II alveolar cells (AT2) in the lung may act as target cells and be susceptible to COVID-19 infection.[4] ACE2 protein has been proved to have an abundant expression in many cells, such as intestinal epithelial cells, renal tubular epithelial cells, alveolar epithelial cells, heart, arterial smooth muscle cells, and gastrointestinal system.[5] That is why we can assume that SARS COV-2 may invade the lung, upper respiratory tract, ileum, heart and kidney, causing Acute Kidney Injury (AKI), acute heart injury, dyspnoea and diarrhoea especially in patients with a high viral load.[6,7]

The binding of SARS COV-2 to ACE2 can cause angiotensin dysregulation, activation of innate and adaptive immunity, and a hypercoagulable state leading to AKI.[8] The hypercoagulability could cause acute tubular necrosis, progressing to cortical necrosis and irreversible kidney failure.[8]

The ACE2 is main counter regulator of the Renin-Angiotensin System (RAS), which is critical for blood pressure and electrolyte control. SARS COV-2 binds with ACE2 and causes its degradation and so reduces the counteraction of ACE2 on RAS. This causes increased reabsorption of sodium and water, excretion of potassium and increased blood pressure. Severe hypokalemia can cause ventricular arrhythmias and respiratory muscle dysfunction which can cause severe morbidity and mortality.[9] Very few studies with small sample sizes have been conducted regarding the biochemical abnormalities associated with COVID-19 disease, especially in Pakistan. We aim to observe the biochemical parameters to assess the presence of multi organ derangement in these patients.

METHODOLOGY

This cross-sectional study was conducted in the Department of Chemical Pathology, Liaquat National Hospital, Karachi. A total of 107 adult patients who tested positive for COVID-19 by RT-PCR from 1st January 2021 to 28th February 2021 were included in the study after approval by the Ethical Review Committee. Non-probability consecutive sampling was done. Patients who were less than 18 years were not included in the study.

Blood was collected in yellow top tube (BD Vacutainer) containing gel and allowed to clot. Serum was separated and stored at -20 °C. Dilutions and aliquots were prepared where needed. Blood was analysed for urea, creatinine, ferritin, lactate dehydrogenase (LDH), calcium, magnesium and

phosphorus in Cobas C501 (Roche Diagnostics) using spectrophotometric technology. Sodium, chloride, potassium and bicarbonate were analysed on NOVA electrolyte analyser using ion-selective electrodes. The reference intervals used for urea and creatinine were <50 mg/dL and 0.5–1.5 mg/dL respectively. As regards Ferritin and LDH the gender based reference intervals were, Ferritin M: 30–400 ng/ml, F: 15–150 ng/ml, LDH M: 135–225 U/L, F: 135–214 U/L respectively. For chloride, sodium, potassium and bicarbonate, the reference intervals were 94–110 mmol/L, 136–145 mmol/L, 3.5–5.3 mmol/L and 22–34 mmol/L respectively. The values were 8.6–10.2 mg/dl, 1.7–2.55 mg/dl and 2.5–4.5 mg/dl respectively for calcium, magnesium and phophorus.

Data was analysed using SPSS-25. Frequency and percentage of the abnormalities of the different parameters was calculated. The frequency of the abnormalities of these parameters in the different age groups were also calculated using Chi-square and $p<0.05$ was taken as significant.

RESULTS

Out of a total of 107 patients of age 18–90 (Mean 56.6) years, 69 (64.5%) were males and 38 (35.5%) were females. There were 13, 20, 18 and 56 patients in the age groups of ≤40 years, 41–50, 51–60 and >60 years respectively. The frequency of abnormalities of the different parameters in the patients is shown in Table-1.

There were high levels of urea and creatinine signifying the presence of renal disease in COVID patients. Ferritin and LDH are also increased as they are acute phase reactants. There were greater percentage of low levels of electrolytes in these patients that is low sodium, potassium, bicarbonate, chloride, calcium, magnesium and phosphorus. We looked at the abnormalities of the parameters according to the age groups (Table-2). It shows that when the patients are divided in different age groups, the analyte abnormalities are still the same and there are no significant differences in abnormalities in the different age groups ($p>0.05$). Low bicarbonate only was seen with $p=0.036$ which was significance.

The frequency of abnormalities of the different analytes is shown in Table-3. Significant differences are seen in case of sodium and calcium ($p=0.029$ and 0.025 respectively). In the rest of the parameters there were no significant gender differences seen.

Table-1: Frequency of patients with abnormalities of different parameters

Analyte	Abnormality	Frequency	%
Urea	**High**	36	33.6
Creatinine	**High**	24	22.4
Ferritin	**High**	95	88.8
Lactate Dehydrogenase	**High**	100	93.5
Chloride	**Low**	9	8.4
	High	4	3.7
Sodium	**Low**	48	44.9
	High	3	2.8
Potassium	**Low**	24	22.4
	High	2	1.9
Bicarbonate	**Low**	57	53.3
Calcium	**Low**	52	48.6
	High	1	0.9
Magnesium	**Low**	12	11.2
	High	3	2.8
Phosphate	**Low**	25	23.4
	High	9	8.4

Table-2: Frequency of patients with abnormalities of the analytes in the different age groups [n (%)]

Analyte	Abnormality	≤40 Years	41–50 Years	51–60 Years	>60 Years	*p*
Urea	**High**	5 (35.7)	6 (30.0)	5 (27.8)	20 (36.4)	0.905
Creatinine	**High**	1 (7.1)	3 (15)	3 (16.7)	17 (30.9)	0.211
Ferritin	**High**	12 (85.7)	17 (85)	18 (100)	48 (87.3)	0.384
LDH	**High**	13 (92.9)	18 (90)	17 (94.4)	52 (94.5)	0.934
Chloride	**Low**	2 (14.3)	0 (0)	2 (11.1)	5 (9.1)	0.4
Sodium	**Low**	7 (50)	8 (40)	9 (50)	24 (43.6)	0.687
Potassium	**Low**	1 (7.1)	5 (25)	5 (27.8)	13 (23.6)	0.575
Bicarbonate	**Low**	5 (35.7)	13 (65)	14 (77.8)	25 (45.5)	0.036
Calcium	**Low**	9 (64.3)	10 (50)	8 (44.4)	25 (45.5)	0.817
Magnesium	**Low**	4 (28.6)	1 (5)	2 (11.1)	5 (9.1)	0.135
Phosphate	**Low**	4 (28.6)	4 (20)	6 (33.3)	11 (20)	0.907

Table-3: Frequency of patients with abnormalities of the analytes in the different genders [n (%)]

Analyte	Abnormality	Male	Female	*p*
Urea	**High**	25 (36.2)	11 (28.9)	0.445
Creatinine	**High**	19 (27.5)	5 (13.2)	0.088
Ferritin	**High**	62 (89.9)	33 (86.8)	0.751
LDH	**High**	64 (92.8)	36 (94.7)	1.000
Chloride	**Low**	6 (8.7)	3 (7.9)	1.000
Sodium	**Low**	36 (52.2)	12 (31.6)	0.029
Potassium	**Low**	13 (18.8)	11 (28.9)	0.328
Bicarbonate	**Low**	41 (59.4)	16 (42.1)	0.086
Calcium	**Low**	39 (56.5)	13 (34.2)	0.025
Magnesium	**Low**	6 (8.7)	6 (15.8)	0.577
Phosphate	**Low**	14 (20.3)	11 (28.9)	0.548

DISCUSSION

In our study males were 65.5% as compared to 35.5% females. A study showed that more males were affected by the disease as compared to females probably because males were exposed more because they had to leave their homes.[10] In a study in Iran[11], 35% of patients with COVID-19 had impaired urea and creatinine levels suggesting that renal dysfunction is quite a common complication of this disease. Some researchers have found coronavirus COVID-19 particles in the urine of these patients showing that these viral particles

may have been present in the kidney, filtered in the glomerulus and passed into the urine.[11] Other studies stated that patients with COVID-19 pneumonia showed a rapid increase in urea and creatinine levels showing acute renal injury which could be due to muscle breakdown in these patients as there was associated hyperuricaemia and hypoalbuminaemia.[12,13] These findings are similar to our study where elevated urea and creatinine levels in 33.6% and 22.4% of patients respectively is reported, and the levels of these parameters are more in patients aged ≥60 years.

A study observed increased LDH and ferritin levels in COVID-19 patients.[14] A meta analysis showed elevated ferritin and LDH levels in COVID-19 patients suggesting use of serum ferritin level to monitor prognosis in these patients.[15] These findings are similar to our study. Another study pointed out a positive association between renal injury and acute renal failure with the risk of death.[16] A study from Iran reported a significant decrease in sodium levels in severe disease. It noted that for potassium levels the decrease was not significant.[17] A similar study showed the decline in serum sodium was non-significant with increase in disease severity, but there was a decline in serum potassium with disease severity.[10] We found hyponatremia and hypokalaemia in 44.9% and 22.4% patients respectively. Another study compared the electrolyte levels in severe and non-severe COVID-19 patients and found significantly low levels of sodium, potassium, and calcium in severe as compared to non-severe patients.[18] They say that COVID-19 patients especially with underlying heart or lung disease may have acute respiratory distress syndrome and acute cardiac injury which can be exacerbated by the prevalent hypokalemia.[18]

CONCLUSION

COVID-19 infection in addition to causing pulmonary disease also has multi-organ involvement evident by the biochemical alterations. Further research is required on the long term implications of these imbalances to counteract the complications of this disease.

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ORIGINAL ARTICLE

PREVALENCE OF OSTEOPENIA AND OSTEOPOROSIS IN WOMEN USING DUAL ENERGY X-RAY ABSORPTIOMETRY SCAN

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Background: There is lack of official scientific data on prevalence osteopenia and osteoporosis for all age groups of females around the globe which keeps the health authorities from taking adequate measures to prevent the community from microfracture and reporting to the emergency department with fractures of femur neck, spine and radius. Our objective was to calculate the prevalence of osteopenia and osteoporosis in pre-menopausal and postmenopausal women of Pakistan using Dual Energy X-ray Absorptiometry (DXA) Scan. **Methodology:** This cross-sectional study was conducted at Dow University of Health Sciences, Karachi. Women aged 25–85 years were randomly selected and divided into pre-menopausal (Group I) and postmenopausal (Group II) using proformas filled from patients' history. Each group was subdivided into normal (pre A, post A), osteopenic (pre B, post B) and osteoporotic (pre C, post C) groups by DXA Scan. Number of females in each subgroup were divided by total number of females in each group and multiplied by hundred to get the point prevalence in percentage. **Results:** In pre-menopausal Group I, prevalence of osteopenia and osteoporosis was 12.9% and 43.5% respectively, while 43.5% women were normal. In postmenopausal Group II prevalence of osteopenia was 42.6% and osteoporosis was 29.2%, while 28% were normal. **Conclusion:** Osteopenia is equally prevalent in women of Pakistan irrespective of menopausal status whereas osteoporosis is found to be more prevalent in postmenopausal compared to pre-menopausal women.

Keywords: Osteopenia, Osteoporosis, DXA Scan, Pre-menopausal, Postmenopausal, Menopause

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INTRODUCTION

Prevalence of a disease is a measure of number of cases present in a given time and location which directly affects the measures taken for the prevention, cure, diagnosis, treatment and rehabilitation strategies.[1] Osteoporosis is a systemic skeletal disorder which is characterized by decrease in bone strength thereby increasing risk of fragility fractures[2] which result specifically on minimal trauma where a normal person will not break a bone leading to pain, disability and dependence on others.[3]

Osteopenia and osteoporosis being major public health problems worldwide show low bone mass and density thereby increasing morbidity and mortality rate in underdeveloped as well as developed countries.[4] Osteoporotic fractures represent a significant economic burden on not only the individuals but the healthcare system. Osteoporosis is a multi-factorial disease with modifiable and non-modifiable risk factors. Factors that are generally non-modifiable are age, sex, genetic background, and other under lying illnesses. Modifiable factors are dependent on lifestyle.[5] Though studies show low prevalence of smoking in Pakistani females, alternatives of tobacco intake are very common.[6,7] Therefore, improving mass education may significantly influence personal habits thereby reducing suffering and alleviating the economic burden on healthcare system.[5]

Data regarding screening, diagnosis and treatment of osteoporosis and fragility fractures is not only sparse in our part of the world but also in developed countries where osteoporosis is well recognized which needs more vigilant approach towards this problem.[3,8] The gold standard for diagnosis of Osteopenia and Osteoporosis is Bone Mineral Density, measured by Dual Energy X-ray Absorptiometry (DXA) scan. Its high cost limits its use for screening, diagnosis and research studies leaving a vacuum for reliable data.[3,8]

World over osteoporosis is less common in Blacks and more in Whites and Asians. Nearly 1.5 million Osteoporotic fragility fractures occur annually leading to disability or death. In population over 50 years of age, it is estimated that fractures related to osteoporosis are so common as one in every eight men and one in every two women.[9] Even though there is high prevalence of osteopenia and osteoporosis in Pakistan, not much information is available regarding the prevalence of the disease.[3] The epidemiological data is lacking owing to the paucity of official and published data. Not only this, DXA Scan machines availability only in big cities limits the facility.[3] The objective of this study was to calculate the prevalence of osteopenia and osteoporosis in pre-menopausal and postmenopausal women of Pakistan using Dual Energy X-ray Absorptiometry (DXA) Scan.

METHODOLOGY

This cross-sectional study (Project No. RF 90) was conducted at Dow University of Health Sciences Karachi Pakistan after seeking approval from Institutional Review Board and Ethical Committee. The sample size of 174 subjects was calculated by OPEN EPI sample size calculator with 5% margin of error and 95% confidence interval. Consecutive sampling technique was used. Total numbers of female subjects randomly selected were 174. Female subjects of 25–85 years age were included in the study. Patients visiting Dow Radiology, their attendants, volunteers, patients from dental and orthopaedic OPD, Ojha Campus and patients from dental OPD Patel Hospital participated in the study. Women with endocrine disorders, menorrhagia, oligomenorrhea and polymenorrhea were not included. Pregnant and lactating women, patients on oral contraceptive pills and hormone replacement therapy, people addicted to beetle nut and pan chewing were excluded from the study.

All subjects interested to participate were asked to sign a consent form and information sheet. Simultaneously, a proforma regarding subject's history was filled. Patients were divided into pre-menopausal Group I and postmenopausal Group II. Group I included 85 women while Group II included 89 women. DXA Scan was then performed on the basis of which the women were subdivided into normal (pre A, post A), osteopenic (pre B, post B) and osteoporotic (pre C, post C) groups where pre A, B, C were pre-menopausal and post A, B, C were postmenopausal. DXA Scan uses the T-Scoring system label subjects as normal, osteopenic and osteoporotic. T-Score is the comparison of bone mineral density of a subject to that of young adult reference population. T-score -2.5 or below was defined as osteoporotic, T-score -1.0 or greater was normal and T-core between -1.0 and -2.5 was considered as osteopenia according to World Health Organization.[10]

Group pre A and pre B include 37 women each while pre C included 11 subjects. Group post A included 25, post B included 38 and post C included 26 women. The number of subjects in each subgroup were divided by total number of subjects in each group and multiplied by 100 to get the point prevalence in percentage. In order to calculate the prevalence, sample was randomly selected from the entire population. The random selection method increased the chances of the sample to be representative of the population. For the prevalence to be calculated the number of people in each group were divided by the total number of people in the sample. Point prevalence was then calculated in percentage which was the proportion of a population with osteopenia and osteoporosis at a specific point in time.

RESULTS

Table-1 shows the overall prevalence of osteopenia and osteoporosis in our subjects. Total female participants of the study were 174, out of whom 62 were normal, 75 were osteopenic, and 37 were osteoporotic. Prevalence of normal bone, osteopenia and osteoporosis was found to be 35.6%, 43.1%, and 21.2% respectively.

Subjects in normal (Pre A) group being 37 in number gave a prevalence of 43.5% normal females in this group. Osteopenic (Pre B) group included 37 females and prevalence of osteopenia came out to be 43.5% in this group. Whereas prevalence of osteoporosis came out to be 12.9% as osteoporotic (Pre C) group included 11 females.

Total numbers of females included in postmenopausal Group II were 89. Females in normal (Post A) group being 25 in number gave a prevalence of 28.1% normal females in this group. Osteopenic (Post B) group included 38 women and prevalence of osteopenia was 42.7% in this group. Prevalence of osteoporosis came out to be 29.2% as osteoporotic (Pre C) group included 26 females.

Wilcoxin Signed Rank Test was applied to test the differences between the groups. In pre-menopausal group, 37 (43.5%) subjects were normal while 25 (28.1%) were normal in postmenopausal group (p<0.001). The differences between prevalence of osteopenia in pre-menopausal and postmenopausal groups were not significant (p=0.317). In osteoporotic subgroups (Pre C *vs* Post C) the differences were significant (p<0.01).

Table-1: Comparison of DXA Scan status between Pre- and postmenopausal women [n (%)]

Parameter	Pre-menopausal	Postmenopausal	Z	*p*
Normal	37 (43.5)	25 (28.1)	-3.46	<0.001*
Osteopenia	37 (43.5)	38 (42.7)	-1.0	0.317
Osteoporosis	11 (12.9)	26 (29.2)	-3.87	<0.01*
Total	85	89		

*Significant

DISCUSSION

In several parts of the world the prevalence of osteoporosis has not yet been documented primarily because of scarcely available facility for DXA Scan which is the gold standard for measuring bone mineral density (BMD).[5,6] Even in Pakistan no such data is available which can truly portray the actual prevalence of osteopenia and osteoporosis in pre-menopausal and postmenopausal women of Pakistan which can be very helpful in controlling and preventing the disease.[6]

Highlighting the significance of DXA Scan Saima *et al*, concluded that the use of quantitative heel ultrasound (QUS) in their study limits the reliability of results as it does not measures the bone mineral density accurately and just gives a rough idea about bone health.[6] QUS is a technique that is cost-effective and estimates calcaneal BMD. The patients eventually have to undergo a DXA Scan before any medical intervention.[8]

It is estimated that almost 30% of postmenopausal women in the USA and Europe[11] are found to be osteoporotic, whereas, number of people with osteoporosis is estimated to be 15 million in Asia[12]. Worldwide, about 200 million people are affected with

osteoporosis. Osteoporotic fragility fracture are seen in around 40% of women and 20% of men with osteoporosis.[13]

Despite the grave situation, both osteopenia and osteoporosis are one of the ignored public health care problems. Mass education related to these problems is also suboptimal in Pakistan. Proper knowledge of the burden of the disease through research will help policy makers and healthcare providers to design better strategies. During the past years some hospital based studies showed prevalence of osteoporosis in which heel ultra sound is used though there is shortfall of data on bone mineral density using DXA Scan.[13]

Data available from studies from Pakistan depict 5.6–17.8% cases of osteoporosis in pre-menopausal women and 20–49.3% cases of osteoporosis in postmenopausal women.[6,14–23] Another study calculated the risk estimation of osteoporosis to be 75.3% and showed that the risk increases with increasing age. It was found to be 97% in women of 75–84 years of age and 55% in women of 45–54 years of age.[24]

Data on prevalence of osteopenia and osteoporosis is lacking in Pakistan and invariably all used heel ultrasound with the exception of one study in which DXA Scan was used and another study in which the BMD testing tool was not mentioned. The available data is from Karachi, Lahore, Peshawar, Quetta, Faisalabad, and Peshawar. There is no data available from rural areas so the burden of the disease in Pakistan is underestimated.[3] As there is lack of awareness, education, and healthcare facilities in the rural areas, it is presumed that the burden of osteopenia and osteoporosis is actually much higher than it is published.

The only study comparable to ours was the one conducted by Naeem *et al*[19] using DXA Scan in the city of Karachi, Pakistan. They focused on only postmenopausal women whereas our study considered both pre-menopausal and postmenopausal women. The current study found that in postmenopausal women, osteopenia is 42.6% in comparison to 44.8% reported by Naeem *et al*[19]. Prevalence of osteoporosis in postmenopausal women was 29.2% in our study as compared to Naeem *et al* which was 28.6%. Naeem *et al* found osteopenia more and osteoporosis less prevalent compared to our study. With small differences our results are nearly the same reported by Naeem *et al*[19].

Saima *et al*[6] worked on premenopausal and postmenopausal female subjects using Quantitative Heel Scan. They found that the prevalence of osteopenia in premenopausal women was 63.8% while osteoporosis was higher (49.3%) in postmenopausal women. Our study found the same results of osteopenia being more common in pre-menopausal women and osteoporosis being more common in postmenopausal females but prevalence of osteopenia in pre-menopausal women was 43.5% while that of osteoporosis in postmenopausal females came out to be 29.2%. This comparison of Quantitative Heel Scan verses DXA Scan results showed a difference of approximately 20%. Causes of alarmingly high prevalence of osteopenia in pre-menopausal women needs to be explored through surveys and necessary investigations to elaborate upon contributing risk factors though they may be modifiable or non-modifiable like Calcium and Vitamin D levels.

No study has worked out the significance between subgroups of pre-menopausal and postmenopausal groups. Our study suggests that prevalence in osteopenia in pre- and postmenopausal groups is insignificant which directs us to conclude that osteopenia is common in female population of Pakistan irrespective of their menopausal status.

Life expectancy in Pakistan is expected to increase from 46.6 years in 1960 to 72.4 years in 2023.[25] This is expected to increase the overall burden of osteoporosis in Pakistani population. If the prevalence of osteopenia and osteoporosis continues to increase, it may cause undesirable effects on the economy of our country through expenses on health of people vulnerable to bone fractures. Socioeconomic changes have resulted in sedentary lifestyle and modified eating habits. It is high time that the policy makers develop strategies to provide a cure to younger osteopenic women in turn reducing the burden of osteoporosis in older female population.

CONCLUSION

Only 43.5% of pre-menopausal and 28.1% postmenopausal women had normal bone density. Osteopenia was observed in majority of both pre- and postmenopausal women. Osteoporosis was more common in postmenopausal as compared to premenopausal women.

LIMITATIONS OF THE STUDY

As the data was collected from only one city (Karachi), it cannot be generalized to the whole female population of Pakistan. Also men were not included in the study. More studies are needed to portray a true picture of prevalence of osteopenia and osteoporosis in Pakistan.

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ORIGINAL ARTICLE

EFFECT OF TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION IN PAIN REDUCTION AND VENOUS BLOOD FLOW AUGMENTATION IN PATIENTS DIAGNOSED WITH VARICOSE VEINS

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Background: Patients with varicose veins experience pain in affected leg which is due to venous blood stasis in the lower limb therefore it is essential to determine the severity of pain along with venous blood flow in the affected area. For this purpose the study was conducted to observe the effect of Transcutaneous Electrical Nerve Stimulation for pain reduction and venous blood flow augmentation. **Methods:** In this study, 80 subjects were included which were divided into Group A (Healthy controls) n=40 and Group B (Varicose vein patients) n=40 which was further subdivided into Group 1 (Varicose vein controls) and Group 2 (Transcutaneous Electrical Nerve Stimulation application). Numeric Rating Scale was used to measure pain score and Duplex Ultrasound was done to measure popliteal vein peak flow velocity (Cm/Sec) and blood flow (mL/min) in both Group A, Group 1 and Group 2 (before and after experiment). **Results:** The Mean±SD of Numeric Rating Scale (NRS) in Group 2 (5.56±0.651) was statistically significantly reduced (p=0.004) as compared to Mean±SD in Group 1 (7.36±0.757). Similarly the Mean±SD of popliteal vein peak flow velocity (15.20±1.42) and popliteal vein blood flow (93.08±5.049) was statistically significantly raised (p=0.001) as compared to mean±SD of popliteal vein peak flow velocity (10.06±1.31) and popliteal vein blood flow (71.04±2.894) in Group 1. **Conclusion:** TENS can be used as an effective adjunctive treatment modality in patients suffering from varicose veins.

Keywords: Varicose veins, Transcutaneous Electrical Nerve Stimulation

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INTRODUCTION

Varicose veins are present in the sub-cutaneous tissue of lower limbs.[1] The prevalence of varicose veins worldwide ranges from 25–55% in women and 10–30% in men while in Pakistan its prevalence is 15%.[2] There are various predisposing factors that contribute to the development of Varicose veins which include advancing age, prolonged standing, family history, sedentary lifestyle, smoking, obesity, lower extremity trauma, increased estrogen levels, pregnancy, history of venous thrombosis in the past.[1,3] The major challenge which prevents the development of chronic venous disease is pumping of blood upward against gravity (CVD).[4] The competent one way valves located in the veins and the calf muscle pump (CMP) in the lower legs are recognized as the main mechanism for pumping blood upward.[5] The development of weakness in venous walls and calf muscles leads to the incompetence of one-way venous valves and calf pump failure which results in the reflux and stasis of blood in the lower extremity veins.[6,7] Varicose vein patients present with throbbing pain, night cramps, swelling, itching, cosmetic disfigurement and heaviness in the lower limbs.[8,9]

The most pragmatic diagnostic technique for Varicose veins is Duplex ultrasound which determines valvular incompetence in the sephanopopliteal and sephanofemoral junction, vein diameter, venous blood velocity and blood flow in lower limb veins.[10] As the patients of Varicose veins often present with pain in the legs therefore to appraise and evaluate the intensity of pain, a Numeric Rating Scale (NRS) is used. NRS is a horizontal line on which numbers from 0–10 are arranged which serve as the pain score of the patients.[11] Treatment options for Varicose veins is conservative and surgical treatment. Conservative treatment includes leg elevation, weight reduction, quitting cigarette smoking, performing regular exercise and use of Compression stockings.[5] Surgical treatment includes Stripping and ligation, Sclerotherapy, Endovascular heat ablation and Sub-fascial Endoscopic Perforator Surgery.[12]

Transcutaneous electrical nerve stimulation (TENS) is used to curtail pain and revamp muscle performance.[13] American Physical Therapy Association has described Transcutaneous electrical nerve stimulation (TENS) as a noninvasive and safe method used for alleviating acute and chronic pain.[13] The application of Transcutaneous electrical nerve stimulation (TENS) to the skin aids in mitigating post-operative, migraine, labor, musculoskeletal, neuropathic and osteoarthritic pain.[14] In addition to curtailing pain, it also enhances muscle performance and improves the blood flow.[15,16] Limited data is

available on the use of TENS in decreasing pain and augmenting blood flow in Varicose vein patients. Therefore, the current study is carried out to determine the effects of TENS in mitigating pain and enhancing blood flow in Varicose vein patients.

MATERIAL AND METHOD

Current study was carried out in the Department of Physiology of Islamic International Medical College, Rawalpindi in collaboration with the Department of Surgery, Physiotherapy and Radiology of the District Head Quarter and Railway General Hospital, Rawalpindi. This study was conducted from 1st October 2018 to 30th September 2019 which was a Quasi Experimental study and it was approved by the Ethical Review Committee of Islamic International Medical College, Riphah International University.

Non-probability convenience sampling was used for data collection during the study. A total of 80 subjects were included in the study The inclusion criteria included both males and females (25–60 years of age) who were diagnosed patients of Varicose veins Grade II (only varicosities in veins) and Grade III (varicosities in veins with oedema) according to Clinical, Etiological, Anatomical and Pathological (CEAP) classification[10] and normal healthy individuals with no physical disability or any other chronic disease.

Written informed consent was taken from all subjects and they were divided into Group A and Group B. Group A included 40 healthy subjects (Controls) on which no intervention was done and Group B included 40 subjects who were diagnosed cases of varicose veins Grade II and Grade III. Group B was further subdivided into Group 1 (Varicose vein controls) n=20 in which no treatment modality was used and Group 2 (n=20) which included subjects of varicose veins on which application of Transcutaneous Electrical Nerve Stimulation (TENS) was done. Detailed medical history was taken by asking the subjects to fill out a proforma which included their name, age, sex, occupation, disease symptoms, duration of disease, aggravating and relieving factors, past history, family history and any previous treatment.

Physical examination was performed by exposing lower limb till the middle of thigh. The location of the varicosities was noted and palpation was done for any skin induration or pitting oedema. The subjects of Group A and Group B were assessed through Numeric Rating Scale (NRS) for measurement of pain score which is an eleven point scale (0–10) with end points showing the extremes of pain.[11] Subjects were asked to rate their pain score by pointing the number on the NRS scale from 0–10 which represented their pain score.

The Duplex scan (Sonoscape-S11 4D Ultrasound Machine, Guangzhou Medical Equipment China) of lower extremity veins was performed to measure the popliteal vein peak flow velocity (Cm/Sec) and popliteal vein blood flow (ml/min).[10] The subjects were placed in the lying left lateral position on the couch. In case of Group A, any of the two lower limbs was exposed up to the knee joint while in case of Group B the affected leg with varicose veins was exposed up to the knee joint. Gel was applied on the probe of Duplex ultrasound machine and the probe was placed on the popliteal fossa of the exposed leg. The position of popliteal vein was located and the values of the popliteal vein peak flow velocity (Cm/Sec) and popliteal vein blood flow (mL/min) were recorded.

After performing this, the subjects of Group 2 (n=20) were exposed to Transcutaneous Electrical Nerve Stimulation (Classic TENS of Body clock company, Germany) intervention. After explaining the procedure, the pre-gelled two channel electrodes of TENS device were placed on the affected leg exposed till the knee joint in prone position. One electrode was placed below the proximal end of calf muscle heads, and second electrode was placed above the Achilles tendon. The patients were then placed in the supine position. The stimulator was set on a frequency of 4–5 Hz, pulse width of 100–200 microseconds with continuous mode of stimulation set for 30 minutes duration. The intensity was set according to patient's comfort which was 3–4 mA.[17] Calf muscle twitching was observed during this 30 minutes time.

These patients were given five sessions of 30 minutes duration a week for three weeks. After three weeks of the sessions, the Numeric Rating Scale (NRS) and Duplex ultrasound was done again to assess the pain score, the popliteal vein peak flow velocity (Cm/Sec) and popliteal vein blood flow (mL/min) to see any improvement in the pain score and popliteal vein blood flow of Group 2 subjects after the TENS application.

Data was analysed using SPSS-22. Results were expressed as Mean±SD. Independent *t*-test for comparison between the groups was applied and $p<0.05$ was regarded as statistically significant.

RESULTS

The Mean±SD of NRS, Popliteal vein peak velocity (Cm/Sec) and popliteal vein blood flow (mL/min) between Group A and Group B were compared by using independent sample *t*-test and is shown in Table-1. The Mean±SD of NRS in Group 1 (7.36±0.757) and Group 2 (5.56±0.651) were compared by using independent sample *t*-test and is shown in the Figure-1. The Mean±SD of Popliteal vein peak velocity (Cm/sec) in Group 1 (10.06±1.31) and Group 2 (15.20±1.42) were compared by using independent sample *t*-test and is shown in the Figure-2. The Mean±SD of Popliteal vein blood flow (ml/min) in Group 1 (71.04±2.894) and Group 2 (93.08±5.049) were compared by using independent sample *t*-test and is shown in the Figure-3.

Table-1: Comparison of Mean±SD of NRS, popliteal vein peak velocity (Cm/Sec) and popliteal vein blood flow (mL/min) between Group A and Group B

Study Parameters	Group A Control group (n=40)	Group B Experimental group (n=40)
NRS	0.00	7.60±0.22**[a]
Popliteal Vein Peak Velocity (Cm/Sec)	17.70±0.47	10.65±0.39**[b]
Popliteal Vein Blood Flow (mL/min)	117.0±1.47	73.40±.077**[c]

**p=0.001, statistically significant, **[a]=Comparison of NRS of Group A *vs* Group B, **[b]=Comparison of popliteal vein peak velocity (Cm/Sec) GroupA *vs* B, **[c]=Comparison of popliteal vein blood flow (mL/min) Group A *vs* B

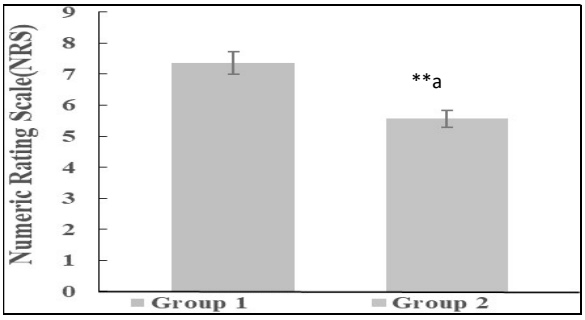

Figure-1: Comparison of Mean±SD of NRS in Group 1 and Group 2 of varicose vein patients

**p=0.004 is considered statistically significant
**a= Comparison of Mean±SD of NRS in Group 1 and Group 2

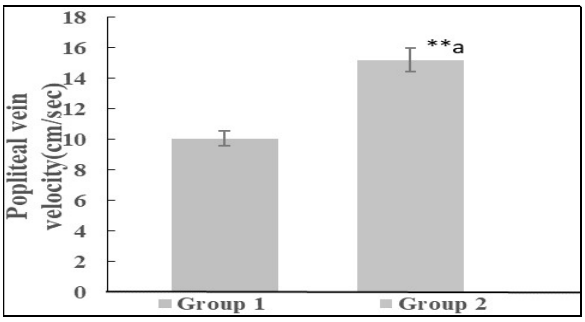

Figure-2: Comparison of Mean±SD of popliteal vein peak velocity in Group 1 and Group 2 of varicose vein patients

**p=0.001, statistically significant, **a=Comparison of Mean±SD of Popliteal vein peak velocity (Cm/Sec) in Group 1 and Group 2

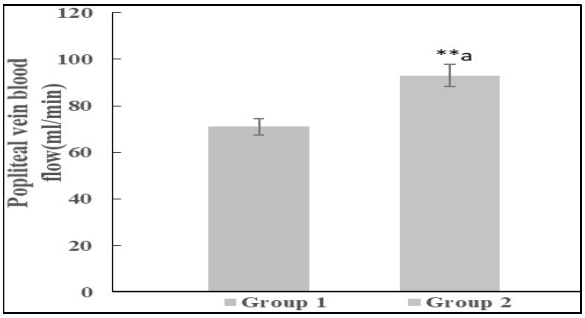

Figure-3: Comparison of Mean±SD of popliteal vein blood flow (mL/min) in Group 1 and Group 2 of varicose vein patients

**p=0.003, statistically significant, **a=Comparison of Mean±SD of Popliteal vein blood flow (mL/min) in Group 1 and Group 2

DISCUSSION

In the current study it was observed that when TENS is used on the Calf muscle of Varicose vein patients (C2 and C3 Varicosities with oedema) for half an hour for a period of 3 weeks, it reduced the pain score of the patients measured with the help of NRS, and augmented the popliteal vein peak flow velocity, and blood flow easured by Duplex scan. This observation is supported by the findings in a study by Shimoura *et al*[18] who carried out a randomized control trial on patients with knee osteoarthritis (n=50) and randomly divided them into TENS group (25) and sham-TENS group (n=25). They measured the pain score of the patients with the help of Visual Analogue Scale (VAS) after the application of TENS under the patella for 30 minutes in both groups and concluded that use of TENS can improve the pain score of the patients. But in contrast to our study they did not measure the peak flow velocity and blood flow in popliteal veins of knee osteoarthritis patients.

Another study was carried out by Santana *et al*, in which they recruited forty-six, primigravida patients with a gestational age of more than 37 weeks and a cervical dilation of 4 Cm.[19] TENS was applied to the primigravida patients at the beginning of the active phase of labor for 30 minutes and pain intensity was assessed by Visual Analogue Scale (VAS) after the intervention period. It was concluded that the use of TENS produces a momentous decrease in pain and adjourns the use of pharmacological analgesia. But in contrast to our study they did not measure the blood flow in popliteal veins in these patients.

Another study was carried out by Choi, and Lee[20], who recruited 11 patients with lower extremity lymphedema and randomly divided them into experimental group (TENS group n=6) and a control group (n=5) who received drug treatment prescribed by the doctor. TENS application was performed on the experimental group on the site of oedema, 3 times per week for a period of 3 weeks. Surface tape measurement of the lower extremity was used to measure the reduction in oedema in the lower extremity. They concluded that TENS is very effective in reducing oedema by augmenting venous blood flow in lower extremity.

Another study was carried out by Broderick BJ *et al*[21], in which they studied the effect of electrical stimulation on calf muscle by comparing the blood flow changes in lower limb while the subjects are at bed rest with the changes when the calf muscles have been electrically stimulated. They concluded that electrical stimulation can increase the blood flow in lower limbs and can alleviate the debilitating effects of venous stasis.

CONCLUSION

Application of TENS has proven to be a beneficial treatment modality for varicose veins. The use of TENS has not only reduced the pain score of patients but also improved the venous blood flow which leads to a reduction in the venous stasis, oedema formation and other complications.

FUTURE RECOMMENDATIONS

- In future a large sample size should be taken to observe the effects of TENS in Varicose vein patients.
- Longer duration follow-up studies should be conducted to observe the effects of TENS in Varicose vein patients.
- Studies should be carried out to see the combined effect of TENS with Compression stockings in varicose vein patients.

STUDY LIMITATIONS

The study limitations include a small sample size due to financial position. The follow-up time was also short due to time constraint.

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ORIGINAL ARTICLE

DERMATOGLYPHICS ASSOCIATION WITH CRIMINAL INTENT

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Background: The researches carried out on various type of human behaviour showed that it has been impacted upon by genetic as well as environment components. The aggressive behaviour having intent of criminality is also governed by both environmental and genetic makeup. Here we have analysed and explored environmental factors. **Methods:** Samples from 100 convicted criminals and 100 from general population were taken with simple convenient sampling, after obtaining informed consent and maintaining strict confidentiality. Fingerprints of both left and right thumb were taken on ten print cards. The environmental element was checked by studying association of any specific fingerprint pattern with the criminal intent. Right thumb of offenders was compared with that of normal population. **Results:** It became cleared that number of loop pattern was high in normal population, i.e., 60% as compared to 44% of offenders. The arch pattern was 9% in general population and 2% in offenders. The whorls were high in offenders 54%, as compared to 32% in general population. The statistical analysis of right hand was performed by chi square test and the p=0.002, statistically significant. Left thumb showed no significant differences between the two groups. **Conclusion:** This study reveals the association of dermatoglyphics with criminal intent. The right hand can be a significant tool in scrutinizing criminals on large scale.

Keywords: Finger printing, criminals, fingerprint patterns, dermatoglyphics, criminal intent

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INTRODUCTION

Crime is an unlawful act made punishable by law. Intent is the description of an act to be performed. Criminal intent is defined as a state of mind that must accompany certain crimes to constitute a violation.[1] The criminal intent is the result of associated with criminal behaviour, aggression is of prime importance.

Numerous studies have described increased prevalence of psychological and personality disorders in detainees, as compared to normal population[2] and multiple epidemiological studies performed in different territories showed that the incidence of increasing mental disorder is 5 to 10 times more than abnormal population.[3] Previously conducted studies demonstrated significance between aggression and premeditated murder correlations[4], and between mental problems and committing a crime among prisoners[5]. In a follow-up study, it was concluded that pervasive anger persists in whole life of an offender and this is one of the four primary motivations among rapists.[6]

The scientific study of fingerprints (FP) is called dermatoglyphics (derma: skin, glyph: carvings).[7] Fingerprints are characterised by alternating strips of raised friction ridges (minutia) and grooves that form unique pattern. The FP pattern remains the same throughout life but they increase only in size till puberty. That is why FP are used widely as a means of identification worldwide in the field of forensic medicine, anthropology, ethnology and population genetics. No two individuals, even the identical twins who share same DNA have same FP.[8] There is 1 in 64,000 million chance of FP being identical in two persons.[9]

Various studies have been carried out on dermatoglyphic variations in people globally.[10–13] These studies showed that there is a great variation in FP patterns indifferent regions of the world, yet loop is the most common, followed by whorl and arch. In the present study we look for any specific variation in the FP pattern of convicted criminals using three pattern system and check if there is any correlation with their criminal intent.

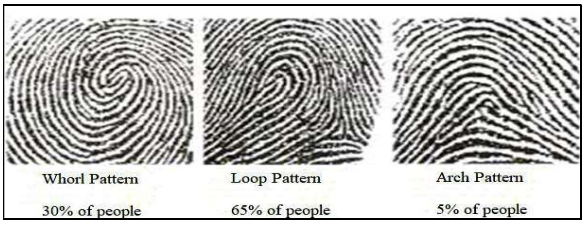

Figure-1: Classification of Fingerprints
(Source: Holt SB. Br Med Bull 1961;17:247–50)

METHODOLOGY

This was a comparative study. The samples were acquired after taking informed consent, and confidentiality was maintained. Fingerprints of both left and right thumb were taken on 10 print cards. Samples were analysed at Department of Forensic Sciences, University of Health Sciences, Lahore. Sample size was calculated by adopting WHO recommended formula. Simple convenient sampling was done from two

different groups, each having 100 samples. Group 1 included convicted offenders of major crimes (murder, sexual assault and kidnapping), and Group 2 included general population without having a history of crime or psychiatric problem.

The fingerprints were observed through magnifying glass and were characterized in three main groups, i.e., loops, whorls and arches. Statistical analysis was performed on SPSS-20. The differences between the FP of general population and convicted offenders were evaluated with Chi-square test and Fisher Extract test. Distribution of pattern of samples was depicted on distribution curve, and $p<0.05$ was considered significant.

RESULTS

In total subjects combined, arch was seen in 18 (4.5%), loops in 227 (56.8%) and whorl in 155 (38.8%) subjects, (Table-1).

There was association between these patterns and the criminal intent. Thumb impressions of both right and left thumb were taken from convicted offenders and general population. On comparison of the right thumb of convicted criminals with that of normal population, it appeared that number of loops was high in non-convicted people. Contrary to loops, which were higher in general population, whorls were more in number in the right hand of convicted offenders. Chi-square test was applied for both right and left hand fingerprint patterns of general total population and total offenders. Chi-square test revealed significant differences when fingerprints of both hands taken together were analysed (p=0.012) (Table-2).

Table-1: Combined frequency and percentage of FP of both groups

Fingerprint	Frequency	%
Arch	18	4.5
Loop	227	56.8
Whorl	155	38.8

Table-2: Group fingerprint hand cross tabulation

Group		Fingerprint			Total	*p*
		Arch	Loop	Whorl		
Right hand						
General population	**n**	9	59	32	100	0.002* [a]
	%	9	59	32	100	
Offenders	**n**	2	44	54	100	
	%	2	44	54	100	
Total	**n**	11	103	86	200	
	%	5.5	51.5	43	100	
Left hand						
General population	**n**	4	63	33	100	0.812 [b]
	%	4	63	33	100	
Offenders	**n**	3	61	36	100	
	%	3	61	36	100	
Total	**n**	7	124	69	200	
	%	3.5	62	34.5	100	
Total						
General population	**n**	13	122	65	200	0.012* [a]
	%	6.5	61	32.5	100	
Offenders	**n**	5	105	90	200	
	%	2.5	52.5	45	100	
Total	**n**	18	227	155	400	
	%	4.5	56.8	38.8	100	

a=Chi-square, b=Fisher's Exact test, *Significant

DISCUSSION

This study reveals the association of dermatoglyphics with criminal intent. There are more than ten million prisoners all around the world and nearly one million prisoners[14], in each decade are added to the world's prison population.[2] One out of seven people being incarcerated worldwide suffers from severe mental ailments.[15] Prisoners mostly suffer from poor physical and mental health during their detention.[16] On comparison of the right thumb of convicted criminals with that of normal population, it became clear that number of loops were high in normal.

Of 100 samples of general population, 59 loops were there, whereas in offenders, out of 100 samples, only 44 were loops. The percentage of loops was higher in general population. On counting whorls, 54 were found in offenders' right hand while 32 were found in right hand of general population. Number of whorl pattern was higher in convicted offenders. On analyzing arch, 9 arch patterns were counted in general population group as compared to only 2 presents in group of convicted offenders. Results of Chi-square test show significant differences in FP patterns of rights hand of general population and the convicted offenders. This result shows that right hand can be a significant tool in scrutinizing criminals on large scale.

When we examined the left thumb, it was found that the number of loop pattern was almost equal in the two groups (63 and 61 in normal population and offenders respectively). The number of whorl pattern was also almost equal in both the groups that was 33 in general population as compared to 36 in offenders. No significant differences were found in arch patterns as they were only 4 in normal and 3 in group of convicted offenders. It was assumed to be statistically insignificant. When the Fisher Exact was performed on the frequency and percentages of left hand the differences were non-significant.

After scanning thumbs of both sides separately, they were analysed together. We divided these prints as a whole in two groups *viz* one group of offenders and another of general population. On examination of 400 thumbs, taken together, loop pattern was found in 227 individuals, out of which 105 were found in offenders and 122 were found in general population. Both the groups showed percentages of 61% and 52.5% respectively. In case of whorls, altogether 155 whorls were present. Of these whorls, 65 were present in general population in comparison of 90 in offenders. The percentages were 32.5 and 45 percent in general population and offenders respectively. Arch pattern was collectively 18 in number of which 13 were of general population and 5 were of offenders. When we studied both thumbs together, the loop pattern was 61% and 52.5%, the whorl was 32.5% and 45%, and arch was

6.5% and 2.5% respectively. Statistical analysis of both hands taken together was performed by applying Chi-square test, p=0.012 was obtained, which being less than 0.05 and was again statistically significant. Normal distribution graph has also been drawn which showed that in general population, there were almost equal percentages of these patterns in both right and left hand. Loop were 59 in right hand and 63 were present in left hand of general population. Likewise, whorls were 32 in right hand and 33 in left thumb. In the same way arches were 9 in right thumb and 4 in left thumb. The collective percentages of these patterns were found to be 61%, 32.5%, and 6.5% of the loops, whorls and arches respectively.

OUTCOME AND UTILIZATION

Environment contributes to criminal behaviour. This study may be helpful in reshaping medico legal framework globally by making separate legislation bodies for genetically or socially deviant individuals; this may include lesser punishment, rehabilitation centres and providing medical care for such individuals.

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AI: Data collection/sample collection, write-up
NA: Data analysis
SQ: Sample collection
ZA: Sample collection
RAR: Data analysis

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Conflict of Interest: No conflict of interest is declared

ORIGINAL ARTICLE

SIBLING RELATIONSHIP AND EXPRESSION OF ANGER AMONG THE CHILDREN OF WORKING WOMEN

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Background: Maternal presence or absence influences the quality of relationship between the siblings. The quality of sibling relationship is affected by the quantity of time parents, especially mother, spend with children. This study was aimed to investigate the sibling relationship and expression of anger in children of working women, and to explore an association between the quality of sibling relationship and the time given by a working mother. **Method:** By using cross-sectional study design a sample of 150 participants, who were the children of working women, was drawn through purposive sampling technique with age range 11 to 14 years having one or more than one sibling. The study was conducted in Lahore and completed in one year. Demographic Information Sheet, Sibling Relationship Inventory, and Child Anger Expression Scale were used to collect data. **Results:** Number of working hours of the mothers emerged as the strongest predictors of the conflicted sibling relationship and accounted for 51% of variance in conflicted sibling relationship. The results also indicated that children living in nuclear family had more external anger, violence, and hostility as compare to the children living in joint family system. **Conclusion:** Sibling relationships are badly affected by the absence of the mother. A mother who spends maximum time at work, is unable to settle down the conflicts arose between the siblings, and due to conflicted relationship they may develop anger and violence.

Keywords: Sibling relationship, Hostility, Anger expression

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INTRODUCTION

Almost every child around the world has at least one sibling. The sibling relationship is likely to last longer than any other relationship in one's lifetime and plays an integral part in the lives of families.[1] Researches on sibling relationship, concluded that siblings are dominant and central in the lives of individuals across the life span. Siblings serve as companions, role models, and confidants for children and adolescents.[2]

During childhood years, conflicts can be seen among siblings and it is an unavoidable part during these ages. During this conflicted situation siblings learn to argue their position, learn to take the perspective of the other and provide accommodation to settle differences. These situations play very important role in the lives of siblings.[3] Whether the parents set limits to control conflicts or siblings settle these conflicts on their own, this resolution of the conflicts, prepare the children for dealings with friends, peers and others throughout the life. However, when these conflicts are not resolved and intensified into violent interactions or the mental or physical abuse by a stronger sibling on a weaker sibling, the relationship among siblings becomes maladaptive and can be extremely dangerous.[3]

The children's skills to resolve conflicts in cognitively and culturally sophisticated ways are greatly influenced by maternal intervention and their presence or absence. Though a mother may not pursue active and positive intervention, her presence is an influencing factor in the relationships of her children. The presence of a mother creates a different dynamic between siblings than when they are alone, which shows that the quality of sibling relationship is affected by the quantity and quality of time the mother spends with her children.[4]

For many parents' management of conflict and violent behaviour between siblings is challenging. A study suggested that parental absence was positively associated with the dysfunctional sibling relationship. Parental non-involvement can play a role in the development of conflicted sibling relationships.[5]

There are many factors in the family system that lead to conflict and anger between siblings. We see in daily life that jealousy is a common factor between siblings. One of these factors is the amount of time that a mother spends with her children. Role of mother is very important to resolves or decrease these conflicts. A working mother mostly cannot spend or give enough quality of time to her children.[4] Maternal absence is linked with more common negative interactions between siblings and there exists correlation between quantity of time spent with children and sibling rivalry. The present study has aimed to find association between the quality of sibling relations and expression of anger among the children of working mothers. The objectives of study were:

- To find the sibling relationship and anger expression in children of working women
- To explore relationship between sibling relationship and anger expression with the working hours of the mothers
- To determine the relation between quantity of time with quality of relationship among the siblings

METHODOLOGY

This was a cross-sectional, correlational study. From the population of private school students of Lahore, a sample of 150 children of working women was drawn through purposive sampling with age range 11–14 years having one or more than one sibling. Demographic Information Sheet, Sibling Relationship Inventory[6] and Child Anger Expression Scale[7] were used to collect information about the respondents, quality of sibling relationship and expression of anger. Sibling relationship inventory consists of 13 items and two subscales, Affection and Hostility. Subscale Affection has 8 items and the Hostility has 5 items. Urdu version of this scale was used.

After collecting the required data from the students, scoring was done as suggests in test manual. Raw score was analysed with SPSS-20. Different statistical methods for example correlation and *t*-test were used to make some inferences for the population.

RESULTS

A total of 150 students participated in the study. Majority (80%) of the participants were female. Twenty-eight percent (28%) participants were 11 years old, 19% were 12 years old, 26% were 13 years old, and 27% participants were 14 years old. Forty-seven percent (47%) participants had joint family and 53% had nuclear family. Working time of 5.3% mothers was 6 hours, 56% worked 8 hours, and 38.7% mothers worked 12 hours a day. Thirty percent (30%) participants had two siblings, 35% had three, 20% had four, and 15% had five siblings.

According to the results 17% of the sample had very severe anger expression, 13% of the sample had moderately severe anger expression, and 57% of the sample had moderately anger expression. Sixty-two percent (62%) of the sample had moderate level of externalized anger whereas 67% of the sample had moderate level of internalized anger. Twenty-six percent (26%) of the sample had moderately severe externalize anger whereas 27% of the sample had moderately severe internalize anger. Three percent of the sample had very severe externalize anger whereas 4% of the sample had very severe internalize anger. Sixteen percent (16%) of the sample had very severe feeling of rejection, 23% of the sample had moderately severe feeling of rejection and 61% of the sample had moderate feeling of rejection. Thirty percent (30%) of the participants were with very severe hostility and violence, 10% were with moderately severe hostility and 53% were with moderate hostility and violence. (Table-1).

Table-2 indicates that differences between the mean score of external anger, hostility and violence and hostility of the siblings living in two family systems. The result revealed the significant difference on the mean scores of the anger expression (external anger) between the siblings living with joint and nuclear family system t (148)= -2.07, $p<0.05$. The result indicated that the siblings living in nuclear family had more external anger (Mean±SD 57.90±5.8) as compare to siblings living in joint family system (Mean±SD 46.44±5.2). In the case of Hostility and Violence, significant mean difference was found between the score of siblings living with joint and nuclear family systems t (148)= -2.10, $p<0.05$. The result indicated that the siblings living in nuclear family had more (Mean±SD 19.98±5.9) hostility and violence as compare to siblings living in joint family system (Mean±SD 9.01±4.5). The results also revealed that siblings living with joint family (Mean±SD 10.02±3.30) had significantly less hostility than the siblings living with nuclear family (Mean±SD 17.88±4.37). On the whole, results shown that external anger, hostility and violence and hostility were more in siblings living in nuclear family system than the siblings living in joint family system.

Table-3 shows the inter correlation between the sibling relationship (conflict, affection) and number of working hours of the mothers. Significant positive relationship was found between conflicted sibling relationship and number of working hours of the mothers r=0.735, $p<0.0001$. Conflicted relationship among the sibling increased, as the number of working hours of mothers increased. There was non-significant relationship was found between affection and number of working hours of the mothers.

Multiple regression analysis was performed for predicting conflicted sibling relationship while using gender, numbers of siblings, mother education and number of working hours of mothers as the Predictors. Number of working hours was emerged as the strongest predictor of conflicted sibling relationship that accounted 51% variance for conflicted sibling relationship between the siblings who had working mother, β=0.735, t=13.08, $p<0.0001$. (Table-4).

Table-1: Number of children experiencing different levels of anger for the subscales of Child Anger Expression Scale (CAES) [n (%)]

Anger Expression	Expression of Anger	Externalize Anger	Internalize Anger	Feeling of Rejection	Hostility and violence
Mild	20 (13)	14 (9)	3 (2)	10 (7)	10 (7)
Moderate	86 (57)	92 (62)	100 (67)	85 (61)	80 (53)
Moderately Severe	19 (13)	39 (26)	40 (27)	32 (23)	14 (10)
Very Severe	25 (17)	5 (3)	7 (4)	23 (16)	46 (30)

Table-2: Independent Sample *t*-test on mean scores of subscales of anger expression scale between siblings living with nuclear and joint family system (n=150)

Variables	Mean±SD		*t*	Cohen's *d*
	Joint (n=70)	Nuclear (n=80)		
Externalize anger	46.44±5.2	57.90±5.8	-2.07*	2.08
Hostility & Violence	9.01±4.5	19.98±5.9	-2.10*	2.07
Hostility	10.02±3.30	17.88±4.37	-2.01*	2.02

*$p<0.05$

Table-3: Inter-correlation between sibling relationships (conflict, affection) and number of working hours of mothers (n=150)

Variables	1	2	3
1. Number of working hours	-	0.036	0.735*
2. Affection	-	-	-0.048
3. Conflict	-	-	-

*$p<0.0001$

Tale-4: Predictors of conflicted sibling relationship (n=150)

Variables	Conflicted Sibling Relationship		95% CI
	Model-1 B	Model-2 B	
Constant	14.087	-8.51	[-13.03, 0.98]
Mother education	-0.476	-0.117	[-0.52, -0.29]
Number of siblings	0.029	0.283	[0.038, 0.528]
Gender	-0.393	-0.029	[-1.11, 1.055]
Family system	1.661	0.639	[-0.32, 1.59]
Working hours of mother		2.391	[2.03, 2.75]
R^2	0.060	0.570	
F	2.306	38.23	
ΔR^2		0.511	
ΔF		171.12	

B=Non-standardized coefficient (beta); CI=Confidence Interval

DISCUSSION

This study aimed to investigate expression of anger and quality of sibling relationship among the children of mothers who were working in offices. The results indicated that most of the siblings had externalized expression of anger, violence and hostility. Conflicts or expression of anger arose between siblings when they had lack of time from their parents especially from their mother. Mother's time play a significance role in children development. The working mothers when cannot give proper time to their children, the children experience irritating and aggressive mood as compare to other children of non-working mothers. A study by Bouchard *et al*[5] suggested that parental absence was positively associated with the dysfunctional sibling relationship.

Main part of the research suggested that quantity of time that mother give or spent with her children show the quality of sibling relationship and anger expressions among them. Nuclear and joint family system also have main impact on sibling relationship (affection and hostility) and anger expression (externalize anger, violence, feeling of rejection, and externalize anger) in children. Children living with nuclear family system have more anger expression as compared to children living with joint family system. Parental non-involvement can play a role in the development of conflicted sibling. In consistence with these results, Howe *et al*[4] concluded that the quality of sibling relationship is affected by the quantity of time the mother spends with her children. Parents are the model for children. Time of both for children is very important to shape their behaviour and relationship. Ross and Lazinski also suggested that the parent's mediation empowers sibling conflict resolution. Mostly mother's time for children play important role for their emotional expressions and relationships with their siblings. The children living in joint family have grandparents and other members who can help them to solve the conflicts but the children living with nuclear families are lack of members that provide guidance for children to settle their conflict that's why these children express more externalize anger and hostility in their relationships.[8]

Results of present study showed that there was significant positive relationship between conflicted sibling relationship and number of working hours of mother. The study result has shown that the working mother could not give enough time to their children which cause feeling of rejection, hostility, violence and the externalize anger that could make the conflicted sibling relationship. Conflicted relationship among the sibling increased, as the number of working hours of mothers increased. Mother's number of working hours emerged as the strongest predictor of conflicted sibling relationship and accounted for 51% variance in the conflicted sibling relationship. Consistent with present research, Bouchard and colleagues also suggested that parental absence was positively associated with the dysfunctional sibling relationship. These results emphasize that parental non-involvement can play a role in the development of conflicted sibling relationships.[5] Bendura (1977) theory explained the parent's role for children like model. If they spend limited time with children, they cannot become a good model for their children.[9] Also, children of these parents are aggressive and their relationship with their own sibling is not communicative. Importantly mother shape the behaviour of her children if mother spend limited quantity of time with them that would badly affect the quality of the relationship between the siblings.

CONCLUSION

On the base of results, it is suggested that siblings may develop hostile sibling relationship due to prolonged

and unsettled conflicts between them. For the resolution of conflicts mother's presence play central role. Absence of mother may predict the conflicted sibling relationships. According to the present study conflicted sibling relationships and expression of anger was significantly correlated. It can be said that with conflicts in mind, siblings may exchange hot words and express anger as the results showed that 17% of the sample had very severe anger expression, 13% of the sample had moderately severe anger expression, 57% of the sample had moderate anger expression. Parental, especially maternal involvement and intervention can play important role in resolving and managing the conflicts between their children. Furthermore, grandparents and other members in the joint family can help children to resolve their conflicts, whereas the children of working women, living in nuclear families remain unable to settle their conflicts. For this reason, these children express more externalize anger and hostility in their relationships.

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ORIGINAL ARTICLE

PERCEIVED STIGMA, FAMILY SUPPORT AND QUALITY OF LIFE AMONG CAREGIVERS OF FEMALE PATIENTS WITH SCHIZOPHRENIA DISORDER

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Background: Family caregivers of persons with schizophrenia experience high level of poor quality of life be causes, Schizophrenia is severe mental illness which leads long term decline in person overall functional competence, alters communication patterns in the family, leads to occupational difficulties, and puts a load on the family caregivers. This study was done to examine how stigma and quality of life correlate with each other, and to determine impact of family support on quality of life of schizophrenia patients. **Methods:** Purposive sampling technique served sample recruitment of 200 caregivers of female patients with schizophrenia and correlational research design was used to analyse data. Devaluation of Consumer Family Scale, Perceived Social Support from the Family Scale, and WHO Quality of Life Scale were used as tools for data collection. Descriptive statistics, Pearson product moment co-relational analysis and hierarchal regression analysis were practiced for data analysis. **Results:** Significant negative relationship was found between perceive stigma and quality of life sub scales (Physical, Psychological and Environmental). Significant positive correlation between familial support and Quality of Life sub scales (Physical, Psychological and Environmental) was found among caregivers of women with schizophrenia. No significant differences were found between males and females with reference of quality of life. **Conclusion:** Stigma and quality of life are negatively associated while family support is positively associated with quality of life. Perceived stigma and family support were significant predictors of Quality of Life.

Keywords: Perceived stigma, Family support, Quality of Life, Caregivers, Females with schizophrenia

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INTRODUCTION

Family caregivers take the most important role in caregiving for people with mental illness. Family caretakers are relatives who give every day voluntary help to an individual requiring support for daily living assignments.[1] According to WHO 29 million people globally have schizophrenia, 20% show chronic illness, and 35% patients show mixed pattern of illness as they sometime remain stable and at other instance suffer from relapse. In Western countries 50% patients of schizophrenia live with families after discharge from institutes while in Asian countries 70% patients live under their family supervision. As they depend upon their families for their personal needs, that is why family faces financial burden, avoidance of own mental health, stress, depression and anxiety.[2]

Families of patients with schizophrenia experience severe tasks because of clinical heterogeneity and variety of signs and their intervention. The caregivers of schizophrenia patients face burden to deal with them as they spend money for their treatment and face extra financial burden. The other factors include the time they spend on their care and also face stigmatization from society.[3] When families face stigma, they get negative comments from society and feel guilty to have mentally ill family member(s). They feel sorrow, fright, loneliness, and less social interaction.[4] The caregivers face psychological burden; a study conducted in Latino family caregivers shows 40% care givers are at risk of developing depression.[5] Caregivers of schizophrenia patients were compared with caregivers of other disease like Alzheimer, cancer and stroke in India. The results show that caregivers of schizophrenia disorder patients face sleep disturbance, body pain, headache, anxiety issues, low quality of life and depression.[6] A study in Mayo Hospital Lahore also revealed that caregivers face high level of burden psychologically and spend poor quality of life. The study highlights the need to consider caregivers' mental health as well.[7]

Family support plays an important role in life of schizophrenia patients and caregivers as family does extensive range of activities which reinforce positive familial social networks through social based programs and services.[8] Familial support is a help provided through other family affiliates to increase bodily and emotional relief for individuals exposed to worrying conditions.[9] Familial support permits the family to work efficiently and increases family's wellbeing. In an investigation most of participants who showed contentment on the quality of life were persons who had got moral support from the family.[10]

The family members face psychological issues and their quality of life is a person's individual experience of life in the perspective of personal value systems and culture to which they belong, compelling into account their aims, values, expectations, and interest.[11] Quality of life is deliberated as a sign of general happiness, including pleasure and contentment with life. Health-Related Quality of Life (HrQOL) is a narrow term that encompasses health components that are related to life satisfaction as well as the ability of self-caring, nursing, mobility and communication.[12]

The current study was done to see problematic factors which affect quality of life of caretakers of female schizophrenics, and to provide information and awareness to authorities and policy makers in both social and clinical fields.

MATERIAL AND METHODS

The current study used correlational research design to observe the relationship between perceived stigma, familial support and quality of life. The study was conducted after getting approval from Ethical Research Committee/Institutional Review Board. Sample size was determined through G-Power analysis keeping α=0.05, with medium effect size 0.15. Purposive sampling technique was used to recruit the sample of 200 caregivers of female schizophrenics from Lahore, Pakistan. The participants of the study were assured about their confidentiality through a proper consent form and all ethical considerations were followed.

First degree relatives (parents, sibling, or offspring) and husbands were selected who were actively involved for at least 6 months in the care of 20–40 year old females diagnosed with schizophrenia (duration of illness 1 to 3 years) as per the DSM5 criteria. Data was collected from both private and public hospitals. Caregivers having history of chronic physical illness, drug abuse, and psychological symptom were excluded from the study. Also participants with incomplete forms or partially filled questionnaires were excluded from the study.

The data was collected from different hospitals in Lahore through a questionnaire translated in Urdu language. The Urdu versions of scales were completed through MAPI guidelines (Forward and backward translation).

Devaluation of Consumer Family Scale (DCFS), originally developed by Struening *et al*[13], in 2001 was used. The items were analysed on 5-point Likert scale (1= strongly disagree, 5= strongly agree). The scale consisted of 7 items (Cronbach's α reliability= 0.86). Perceived Social Support from the Family Scale (PSS-FA), developed by Procidano and Heller[14]. This scale has 20 items consisting of declarative statements to which the individual answered 'Yes', 'No' or 'Do not know'. (Cronbach's α reliability= 0.89). World Health Organization Quality of Life Scale (WHO-QOLS)[15], developed by Naumann and Byrne was also used. This scale has 26 items. This tool used 5-point Likert scale (1= strongly agree, 5= strongly disagree). The Chronbach's α reliability for this scale is 0.80. Data was analysed using SPSS-21.

RESULTS

Demographic characteristics of participant were analysed using descriptive statistics. Majority (54.5%) of the participants were married, 62% were from nuclear family system, 41.5% had unpleasant home environment, and 44% participants were siblings of the patients. (Table-1).

Table-2 shows that perceived social supports has significant negative correlation with Perceived Stigmatization [r (200)= -0.16, p<0.05] and significant positive correlation with overall quality of life [r (200)= 0.33, p<0.01], and its subscales (physical, social and environmental). Findings also reveal that perceived stigmatization has significant negative relationship with Quality of Life [r (200)= -0.34, p<0.01].

Table-3 reveals that there is no significant difference between male and female on quality of life. Results reveal that first model was found to be highly significant predictor F (15, 184)= 2.81 p<0.005, R^2= 0.18 and accounted for 18% of variance in quality of life. Second model also found to be highly significant predictor F (16, 183)= 4.54 p<0.005, R^2= 0.28 and accounted for 28% of variance in quality of life. Third model was also found to be highly significant predictor F (17, 182)= 5.27 p<0.005, R^2= 0.33 and accounted for 33% of variance in quality of life.

Hierarchal regression analysis for independent and dependent variables is tabulated in Table-4.

Table-1: Demographic characteristics of the subjects (n=200)

Variables	Frequency	Percentages (%)
Marital Status		
Single	91	45.5
Married	109	54.5
Family System		
Nuclear	124	62
Joint	76	38
Home Environment		
Unpleasant	83	41.5
Pleasant	50	25
Satisfactory	67	33.5
Relationship with Patients		
Parents	43	21.5
Husband	43	21.5
Siblings	28	44
Children	26	13

Table-2: Descriptive statistics and inter correlations, for study variables (n=200)

Variables	Mean±SD	1	2	3	4	5	6
1. PSS	11.94±4.26	-					
2. DCFS	19.60±3.17	-0.162*	-				
3. QOL	79.47±12.82	0.332**	-0.344**	-			
4. Physical QOL	21.67±3.69	0.256**	-0.326**	0.785**	-		
5. Psychological QOL	18.61±3.54	0.193**	-0.238**	0.758**	0.498**	-	
6. Environmental QAL	23.66±5.03	0.311**	-0.307**	0.872**	0.566**	0.500**	-

**p<0.01 (2-tailed); *p< 0.05, PSS= Perceived Social Support Scale; DCFS=Devaluation of Consumer Family Scale, QOL= Quality of Life Scale

Table-3: Comparison of quality of life between males and females

QoL	n	Mean±SD	t	p	95% CI	
					LL	UL
Male	86	78.99±12.35	-0.461	0.646	-4.46	2.77
Female	114	79.83±13.20				

QoL= quality of life, CI= confidence interval, LL= lower limit, UL= upper limit

Table-4: Hierarchal regression analysis for independent and dependent variables (n=200)

	Quality of Life of Caregivers	
Predictors	ΔR^2	B
Step 1	0.12*	
Age		0.26*
Family System		0.18***
Relationship with Patient		0.29**
Step 2	0.22***	
Perceived Stigmatization		0.29***
Step 3	0.26***	
Family Support		0.22***
Total R^2	33%	

*p<0.05, **p<0.01, ***p<0.001

a. Dependent Variable: Quality Of Life

b. Predictors in the Models: Perceived Stigma, Family Support, Age, Family System, Relationship with Patient

DISCUSSION

Family caregivers face burden due to dealing with schizophrenia patients. The current study focuses the factor of stigma and highlights how family caregivers of mentally ill people face the society reaction. The present study examines how stigma and quality of life correlate with each other and their impact on quality of life of caregivers.

The current study shows high level of stigma among the caregivers which seems consistent with work from India[16]. Perceived social support has negative correlation with perceived stigmatization which is consistent with a previous study from China[17] where people with care giving for long time showed more stigma compared to people with short period of time.

The current work shows that social support is negatively correlated with quality of life and also with subscales including physical, environmental and social factors it shows consistent results with another study from India which concluded that people who care for schizophrenia patients show poor quality of life.[18] On the other hand people who care for mentally ill patients and have less social support show lower quality of life consistent with research conducted in China[17]. Caregivers who had poor family support were five times more likely to have perceived stigma compared to those with strong social support. The current study also shows that social support is a predictor of quality of life among caregivers of schizophrenia patients.

Sharma *et al*[19] observed that stigma is high among the caregivers of patients with schizophrenia. The research revealed that female caregivers show more stress as compared to males. Females face more burdens compared to males while dealing with mentally ill patients.[19] The current study shows no major differences in quality of life among males and females.

CONCLUSION & RECOMMENDATIONS

Stigma and quality of life (Sub scales: Physical, Psychological & Environmental) are negatively associated while family support is positively associated with quality of life. Perceived stigma and family support was significant predictors of quality of life. There were no significant differences among male and female caregivers with reference of quality of life. Further work with indigenously developed questionnaires and a larger sample size will help in better understanding of the problem.

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REVIEW ARTICLE

ROLE OF TOBACCO SMOKING IN CAUSING HYPOMAGNESEMIA AND RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is a type of immune system disorders that badly affect patient's quality of life. The onset of disease constitutes a complex pathological process, and several mechanisms and triggers are proposed in the development of this disease. Magnesium (Mg) is an important trace element that plays a role in normal functioning of immune system. The deficiency of magnesium is found to be related to abnormal T-cells functions leading to secretion of pro-inflammatory chemicals such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α) and histamine. Similarly, hypomagnesaemia also causes an increase in intracellular calcium level that leads to hyperactivation of phagocytes which are the first line of defence of our immune system. Tobacco smoking is a cause of hypomagnesemia in susceptible patients. The association between tobacco smoking and onset of rheumatoid arthritis is statistically significant in those who are smoking, even for less than 10 years.

Keywords: Rheumatoid arthritis, hypomagnesemia, smoking, risk factor

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INTRODUCTION

Immune disorders are classified as a set of clinical abnormalities in host's defence system which are manifested by progressive production of pathological immune reactions against body's own antigens.[1] Rheumatoid arthritis (RA) is one of the most common progressive autoimmune disease resulting in organ complications, failures, and disturbed life. Besides inflammation of joints, rheumatoid arthritis is also associated with other comorbid diseases such as diabetes mellitus and RA-induced psoriasis.[2] The pathophysiological mechanism behind the onset of disease is a complex process and still under investigation. However, several mechanisms and triggers are proposed which at the end, follows a common pathway in the development of disease. The worldwide prevalence of RA varies, and it is about 1% of total disease burden around the globe. There is no specific age for onset of disease in susceptible patients. The disease can develop in persons of all age groups, but the risk increases with increasing age. In South Asia, the prevalence of disease is not much studied. Only a few cohort studies have been conducted to analyse the frequency of disease in India and Pakistan. The prevalence of rheumatoid arthritis in Pakistan and India is a little higher than that in European and other first world countries. This may be due to dietary patterns and environmental factors that may contribute to the onset of disease. According to a meta-analysis study, the ratio of development of rheumatoid arthritis disease in urban population in Pakistan is 8 per 1,000 people with prevalence of 0.5%. Interestingly, the ratio between male and female in developing the disease is 1:1. In comparison, the prevalence of rheumatoid arthritis in Indian population was found to be 0.2–1%.[3]

The relationship between nutrients and normal immune system development is naturally understood phenomenon. Normal and balanced diet promotes the growth and development of and intact immune system. Food rich in vital nutrients such as carbohydrates, proteins, lipids (including essential fatty acids and oils), vitamins and minerals ensures the protection against different diseases including rheumatoid arthritis disease. Magnesium (Mg) is fourth most common micronutrient in our body after sodium (Na), potassium (K), and calcium (Ca). The recommended daily requirement of magnesium is 360–400 mg for an adult person which is completely acquired through exogenous sources, i.e., food.[4] The normal serum Mg level is maintained through three different mechanisms, i.e., intestinal absorption, Mg storage in bones, and renal excretion. Magnesium is involved in different metabolic and cellular activities. The major function of Mg in our body is to act as cofactor for almost 300 enzymes which are required for metabolism of major biomolecules such as carbohydrates, proteins, and lipids. During Mg deficit conditions, Mg from bones is used for constant serum level.[5] The exact role of magnesium deficiency (MgD) in inflammatory responses is still under debate, but several mechanisms are proposed through which MgD leads to generation of inappropriate immune system functions.[6] MgD causes an increase in thymic cellularity that ultimately increases T-cells functions and release of pro-inflammatory chemicals such as IL-1, IL-6, TNF-α and histamine. MgD also increases cytotoxic activity of T-lymphocytes (CTL) through ATP dependent

mechanism.[7] According to Libako *et al*[8], MgD causes an increase in intracellular calcium level which causes hyperactivation of phagocytes which are first line of host defence mechanisms against foreign pathogens. Chavan *et al*[9], also found hypomagnesaemia in RA patients as compared to control subjects which shows that low blood magnesium level is significantly associated with development of RA disease ($p<0.01$).

Smoking is a factor which affects most of the normal physiological functions of the body. As per studies, tobacco smoking is related to increased insulin resistance causing diabetes mellitus.[9] Similarly, smoking has shown the strongest association with onset of rheumatoid arthritis. Different studies around the globe have revealed that smoking increases the risk of development of disease, as well as complicates the course of disease over time.[10] According to the studies, smoking is linked with release of pro-inflammatory chemicals which triggers the abnormal inflammatory responses against common antigens. This may include release of C-reactive proteins (CRP) which are produced in the liver at a high rate because of cytokines release from adipocytes and macrophages.[11] As per study, patients who are smokers have high level of serum CRPs than the non-smokers. Besides the production and increase release of CRPs, it is also found that smoking is linked with production of Rheumatoid Arthritis (RA) factor and anti-citrullinated peptide antibody (ACPA). Smoking also enhances the Human Leukocyte antigen (*HLA*) induced disturbance in autoimmune responses in susceptible patients.[12] Tobacco smoking results in production of tetra-chlorodibenzo-P-dioxin (TCDD), a bye-product of burning of organic substances.[13] The TCDD is a carcinogenic product and is extremely toxic for human body. TCDD results in overproduction of leukotrienes including interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-8 (IL-8) which act as a trigger for onset of inflammatory responses in body such as in rheumatoid arthritis.[14] Tobacco smoking causes loss of magnesium and other important minerals from body such as calcium and zinc. Chronic smoking i.e., more than 10 cigarettes per day, causes significant decrease in serum magnesium level as compared to healthy non-smokers.[15] Due to lack of data, the direct link of tobacco smoking and hypomagnesemia is not known. However, the habit of tobacco smoking is at peak in depressed persons. Chronic stress results in decrease level of serum magnesium through increased renal excretion which produces harmful effects on the body.[16] Tobacco smoking also causes decrease in appetite by causing abnormality in digestive system's ability to absorb bio-elements including magnesium. Based on these facts, it can be assumed that chronic tobacco smoking leads to hypomagnesemia which can be correlated to other clinical disorders such as rheumatoid arthritis.[17] The aim of the current study is to review the role of smoking causing hypomagnesaemia associated with onset of rheumatoid arthritis in susceptible patients.

METHODOLOGY

This was a literature review study and included meta-analysis of Mg deficiency in the body which can lead to abnormal immune responses especially in inflammatory diseases. Online databases were searched for 'effect of smoking on serum magnesium level' including the NCBI, Cochrane, and Amed. The search terms included rheumatoid arthritis, smoking and inflammatory diseases, smoking and rheumatoid arthritis, pathogenesis, classification, nutritional elements and rheumatoid arthritis. The databases were searched for articles and studies conducted between Jan 1970 to Dec 2020. Case control, meta-analysis and related studies were included in the review while individual studies such as case reports and medical reports were excluded from our study. Similarly, studies on patients of rheumatoid arthritis taking anti-rheumatic drugs and patients suffering from other comorbid diseases were also excluded from the study. The titles, abstracts and the content of articles were selected or filtered on the basis of introduction to rheumatoid arthritis, author's name, year of publication of the study, sample size, epidemiology, causes, pathogenesis, information on different factors involved in onset of the disease, immunomodulation and immune responses, relationship between minerals and other food components with immunomodulation, association between nutritional components and rheumatoid arthritis, regulation of blood magnesium level and odds ratio (OR) for risk of development of rheumatoid arthritis type of study. Data from these filtered studies on smoking and hypomagnesaemia related to onset of rheumatoid arthritis were subjected to statistical analysis using SPSS-20.

RESULTS

Characteristics of studies on Smoking and Rheumatoid Arthritis

As per our search, 433 studies were shown in the result in NCBI database, while the other two libraries did not show any study on the effect of smoking on serum magnesium level. Of these studies, 9 studies were case-control studies and 5 were cohort studies. The average age of the population obtained from each study was 52 years and among these, 94% were female and 6% were male. In the case-control studies, there were 4,764 cases, and 13,647 control samples. In 5 cohort studies, 9,121 cases were included among 566,044 study population. We classified the duration of smoking into 3 categories, i.e., left smoking after <20 years as 'past smokers', smoking for up to 20 years as 'current smokers', and >20 years as 'ever smokers'.

Subgroup analysis for male population

The summary of odds ratio (OR) for male chronic or ever smokers for onset of rheumatoid arthritis was found as 1.88 (1.55–2.27). Similarly, for current and past smokers, the odds ratio for onset of rheumatoid arthritis was 1.86 (1.48–2.33) and 1.75 (1.32–2.30) respectively. In 7 case control studies[18–25], the odds ratio for onset of rheumatoid arthritis in ever, current and past smokers were 1.85 (1.52–2.28), 1.88 (1.48–2.41) and 1.78 (1.33–2.37) respectively. The difference in the smoking status of these subjects was not significant. The tobacco smoking is a risk factor for the development of rheumatoid arthritis in male population who were smokers regardless of the duration of smoking, i.e., ever, current or past smokers.

Subgroup Analysis for Female Population

The Odds ratio for female ever, current, and past smokers for onset of rheumatoid arthritis was found to be 1.26 (1.13–1.43), 1.32 (1.11–1.55) and 1.21 (1.07–1.41), respectively. For the 9 case–control studies[18–26], the calculated odds ratio for ever, current, and past smokers for onset of rheumatoid arthritis were 1.26 (1.06–1.55), 1.18 (0.89–1.62) and 1.25 (1.05–1.49) respectively. For these three subgroups of smokers, there was no significant difference between them (p>0.05). For the 5 cohort studies[27–31], the calculated odds ratio for ever, current and past female cigarette smokers for onset of rheumatoid arthritis was 1.26 (1.06–1.51), 1.36 (1.14–1.66), and 1.21 (0.95–1.48) respectively. In these studies, the difference between the three subgroups was not significant (p>0.05). The quality of one cohort study on women smokers[27] was inferior than the others because the study was on determination of risk factors for rheumatoid arthritis including oral contraceptives and cigarette smoking. The use of oral contraceptives reduces the risk of rheumatoid arthritis during tobacco smoking. Even then, our results are strong after the exclusion of the study.

Association between hypomagnesemia and RA

For meta-analysis of role of decreased serum level of magnesium in onset of rheumatoid arthritis in susceptible patients, we performed online databases search as previously mentioned for 'Association between hypomagnesemia and onset of rheumatoid arthritis' including NCBI, Cochrane and Amed. As per our search, 221 studies were shown in the result in NCBI database, while in Cochrane library and Amed library, no such studies were found. The articles were thoroughly screened, and 201 studies were excluded because those studies were not on determination of the role of hypomagnesemia in onset of rheumatoid arthritis disease. Twenty articles were then included in our study. However, 11 articles were also excluded from the study because those studies were not related to determination of association between decrease magnesium intake in rheumatoid arthritis disease. Among the remaining 9 studies, 7 studies[9,32–37] were based on estimation of serum magnesium level in rheumatoid arthritis patients. The odds ratio for decrease magnesium level and onset of rheumatoid arthritis disease in these 7 studies was estimated as 2.8 (95% CI 1.20–6.58, p=0.021).

One study was based on estimation of serum magnesium level in patients suffering from rheumatoid arthritis conducted by Chavan *et al*,[9] while the other study was on determination of effects of magnesium intake on radiographic osteoarthritis which was performed by Zeng *et al*.[38] In those two studies, 1676 newly diagnosed patients were analysed for serum magnesium level. According to the study by Chavan *et al*,[9] decrease magnesium level with dyslipidemia and hyperuricemia is a risk factor for rheumatoid arthritis and cardiovascular diseases. In this study, other dietary factors such as calcium, potassium and phosphorus were also studied in patients suffering from rheumatoid arthritis. Along with this, serum bilirubin levels i.e., both direct and indirect bilirubin levels were also increased. In other study by Zeng *et al*,[38] the study was not directly related to role of hypomagnesemia in rheumatoid arthritis, rather the case-control study was confined to the findings that increase intake of dietary magnesium is inversely associated with radiographic osteoarthritis and other knee joint problems.

DISCUSSION

This systemic review identified risk factors for the onset of rheumatoid arthritis in susceptible patients. This included the previously discussed environmental risk factors such as tobacco smoking, microbial infections, diet, and environmental pollution. Besides this, genetic factors such as different types of polymorphisms involved in onset of the diseases were also illustrated. The results from this literature review showed some proof of a linear association between lifelong cigarette smoking and RA. Our results also indicated that the risk of onset of rheumatoid arthritis in men is two times more than non-smokers, while the risk of onset of the disease in female is approximately 1.3 times more than the non-smoker women. The association between tobacco smoking and onset of rheumatoid arthritis was statistically significant in those who were smoking for less than 10 years. In patients, who were smoking for more than 20 years, risk of the development of rheumatoid arthritis increased two times as compared to those who were non-smokers. However, the women who were smoking for about 20 years, the risk of development of disease is more than the men. Limited number of articles were included in our study i.e., only 14 studies were included which fulfilled inclusion criteria. Among these 14 studies, 9 studies were based on

original research study while 5 studies were cohort studies, and the publication bias did not affect the results of our review study. The results of our review study are same or in line with the results from previous literature review.[39] This shows that tobacco smoking increases the risk of rheumatoid arthritis than in non-smokers, especially in men. This is because that according to experimental study, oestrogen suppresses the onset of rheumatoid arthritis in female mouse model i.e., oestrogen inhibits the release of cytokines produced by T-helper cells. This reduces the risk for development of rheumatoid arthritis.[40] According to two other studies, it is found that the use of oral contraceptives which decrease the release of oestrogen in female body, enhances the risk factor for development of rheumatoid arthritis in women.[41–42] Smoking also induces the production of anti-citrullinated peptide antibodies i.e., RF positive patients of rheumatoid arthritis. The exact mechanism is still unknown but Padyukov *et al* showed that smoking and RF shares a common HLA-DRB1 epitope allele which is a significant risk factor for onset of RA positive rheumatoid arthritis.[24]

The role of decreased serum level of magnesium in onset of rheumatoid arthritis disease in susceptible patients, as per our review, 9 case-control studies were found in which there was decreased serum concentration of magnesium in patients suffering from rheumatoid arthritis and one study was related to inverse association of magnesium intake with osteoarthritis. Both studies were case-control studies in which it was found that the patients suffering from rheumatoid arthritis and osteoarthritis have significant hypomagnesemia ($p<0.01$) and that the magnesium supplementation has a protective role in joint health. However, other dietary components were also studied such as calcium, potassium and phosphorus. The serum level of direct and indirect bilirubin was also found to be elevated in study population. This means that the other dietary factors may act as confounding factors for association of decreased serum magnesium level with onset of rheumatoid arthritis. Similarly, the hyperbilirubinemia may also be considered as a factor causing increase joint inflammation.

CONCLUSION

The risk factor for the development of rheumatoid arthritis is generally equal for both males and females who are smokers than the non-smoker population. Magnesium is involved in regulation of normal immune responses and decrease in serum magnesium level disturbs functions of the normal immune system. Due to lack of evidence, it is very early to conclude that hypomagnesemia is involved in onset of rheumatoid arthritis.

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