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Editorial

Maternal Malnutrition: Burden on Maternal and Neonatal Health in Pakistan

Amna Iqbal Butt

Maternal malnutrition contributes to adverse outcomes of pregnancy and chronic health issues in the neonate.[1] Pakistan is facing many public health threats including increased prevalence of maternal malnutrition. This health issue in women of childbearing age group imparts a burden on maternal and neonatal health, resulting in increased rates of maternal and neonatal mortality.[2] Maternal malnutrition can be characterized by undernutrition, overweight and obesity, and micronutrient deficiencies. The National Nutrition Survey 2018 reported that one in seven women of reproductive age (14.4 percent) are underweight, an improvement from 18 percent in 2011.[3]

Current Situation in Pakistan

Early maternal nutritional status can be assessed by calculating body mass index and mid-arm circumference. There is an association found between poor maternal nutrition and adverse pregnancy outcomes, particularly in low-resource settings.[2] A study conducted in Kashmir found that 39% of pregnant women were malnourished according to their body mass index. The factors associated with the increased frequency of maternal malnutrition include low socio-economic status, area of residence, level of education, food scarcity, and insufficient care during the antenatal period.[4]

Another study conducted in Sindh reported that pregnant women in their first trimester have inadequate dietary habits including low intake of major food groups such as fruits, vegetables, and dairy. The dietary inadequacies may adversely affect both fetal development and the well-being of neonates and mothers.[5] Aims of sustainable development goals (SDGs) 2 and 3 are to eliminate hunger and all types of malnutrition, and by 2030, reduce the global maternal mortality ratio to less than 70/100,000 live births. One of the targets is to end preventable deaths of newborns and reduce neonatal mortality to 12 per 1,000 live births. These goals can be achieved by implementing strategies to improve maternal and neonatal health in developing countries, like Pakistan, where malnutrition is a barrier to achieving these goals.[3]

Health Outcomes in Mothers and Neonates

The risk of complicated pregnancies and deliveries is related to the maternal malnutrition. Mothers with inadequate nutrition and chronic diseases posed an increased risk of chronic illnesses in the neonates resulting in early onset and creating a cycle of ongoing health issues. Maternal undernutrition and micronutrient deficiencies affect over 42% of women in Pakistan. It increases the risk of intrauterine growth restrictions, low birth weight, and preterm babies.[1] This resulted in high maternal and neonatal mortality rates that act as a barrier to economic growth and an adverse impact on human capital.[3]

Operational Work Plan for Control

To tackle this dual challenge, a comprehensive approach is essential. The key points of this approach are outlined below:

- **Dietary Education and Supplementation:**

Raise awareness about balanced nutritional requirements during pregnancy and provide necessary supplements and fortifications, such as iron, folic acid, and micronutrients in high-risk pregnancies. Additionally, addressing maternal wasting and food insecurity with balanced protein and energy supplementation is vital.[4,6]

- **Holistic Health Services:**

Given the importance of early maternal nutrition on pregnancy outcomes, screening should be recommended based on reliable indicators such as dietary diversity scores or a combination of tools. An extensive antenatal care & counseling should be implemented to address screening and management of nutritional deficiencies counseling.[2,5]

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- **Community Participation:**

Engage health workers to educate and assist women and the community in acquiring healthy lifestyles and utilizing healthcare services.[3]

- **Policy Implementation:**

Implement and keep surveillance of national initiatives like the Pakistan Maternal Nutrition Strategy 2022–27 to prevent undernutrition in women.[3]

- **Research and Data Collection:**

Carry out continual research to track trends, identify high-risk groups, and assess the effectiveness of interventions.[3]

- **Aaghosh Program Initiative:**

Pregnant women, and mothers of children under 2 years are entitled to the Aaghosh program in 13 districts of Punjab. To improve the community perception and promote the healthcare-seeking behaviour of mothers, a program is launched for community mobilization and conducting awareness campaigns. The target population of this program is pregnant women, lactating mothers with children under two, and young children. The elements of the program consist of frequent medical check-ups, birth registration, immunization for children under two, nutritional education, and family planning. The program partners are the Punjab Social Protection Authority, the Health and Population Department, and the School Education Department. The duration of the program is from 2020 to 2026.[7]

CONCLUSION

In Pakistan, maternal malnutrition poses a threat to maternal and neonatal well-being. These health issues impart a major burden on the community and healthcare and need to be addressed by implementing holistic health services, promoting nutritional education, program execution, improving community participation, and enhancing research platforms. The Aaghosh program initiative should be extended to other provinces to reduce the regional inequalities in maternal health outcomes and improve neonatal development. Such initiative supports Pakistan's national health strategies and Sustainable Development Goals, helping to achieve the targets and reduce the prevalence of malnutrition by prioritizing the overall health and well-being of women of childbearing age.

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Original Article

Outcomes of Percutaneous Coronary Intervention in Chronic Total Occlusion among Women

Muhammad Waleed Hassan, Asif Manzoor, Azhar-ul-Hassan Qureshi, Muhammad Wajahat Alam Lodhi, Waqas Mohiuddin, Muwahhid Rasheed, Sohail Aziz

ABSTRACT

Objective: To determine the outcomes in female patients who underwent percutaneous coronary intervention (PCI) for chronic total occlusion.

Methodology: This longitudinal descriptive study was conducted at the Department of Cardiology, Fauji Foundation Hospital, Rawalpindi from July 2024 to March 2025 after taking approval from the ethical committee. A total of 56 female patients with a diagnosed chronic total occlusion (CTO) who had persistent angina symptoms despite optimal medical therapy were included using non-probability convenience sampling. All these patients were eligible to undergo CTO-PCI with normal kidney function and gave informed consent. The patients underwent CTO-PCI by interventional cardiologists. All the patients had a monthly follow-up for 3 months post-procedure. The outcomes assessed were rate of procedural success, in-hospital major adverse cardiovascular events (MACE) and cardiac mortality & symptom-free at 3 months. Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 25.

Results: The procedural success rate was found in 37(66.1%) patients. In-hospital MACE occurred in 5(8.9%) patients. Two (3.6%) patients died whereas, 40(71.4%) patients were symptom-free at 3 months. In-hospital MACE was significantly less in the successful CTO-PCI group (p-value=0.04) and the majority [34(94.4%)] of the patients in the successful CTO-PCI group were symptom-free at 3 months (p-value=0.001). However, there was no significant difference in mortality at 3 months between the two groups with a p-value of 0.53. The procedural failure was significantly associated with the elderly age group >60 years (p-value=0.02) and diabetes mellitus (p-value=0.004).

Conclusion: The CTO-PCI has a procedural success rate of 66.1% and the majority of the patients were symptom-free after the procedure. The statistically significant outcomes were reduction in the frequency of in-hospital MACE and symptom-free at 3 months in the successful CTO-PCI group. The procedural failure was significantly associated with the elderly age group and diabetes mellitus.

Keywords: *Chronic total occlusion. Percutaneous coronary intervention. Myocardial infarction.*

INTRODUCTION

Coronary chronic total occlusion is a severe form of coronary artery disease (CAD) that manifests as complete blockage of a coronary artery for >3 months and thrombolysis in myocardial infarction (TIMI) grade 0 flow. It is one of the most challenging situations for interventional cardiologists due to the complexity of the lesions.[1,2] Around 15-35% of the patients with significant CAD who undergo diagnostic intervention have CTO. It is one of the major reasons for referring patients for coronary artery bypass grafting (CABG). Literature has reported that around 30% of the chronic total occlusions cannot even be bypassed by CABG. Percutaneous coronary intervention is still performed for CTO lesions. However, the success rate of CTO-PCI is low with complex procedures and a high rate of complications. The success rate is mainly determined by the complexity of the lesion, the technical expertise of the cardiologist and the co-morbidities of the patients.[3,4] The procedure-related complications of CTO-PCI are coronary dissection, cardiac tamponade, perforation of the coronary artery, myocardial infarction, stroke, major bleeding, side branch occlusion and referral for urgent CABG.[5] The procedural complications if not recognized, prevented or managed properly can lead to drastic effects.[4]

Fortunately, due to the recent advancements in PCI equipment and techniques, the success rates of PCI have increased. Previously, the success rate of PCI for CTO was 60% but now the success rate of 80-90% has been reported in dedicated CTO-PCI centers.[4] In addition to improvement in the success rate, there has been a marked decrease in the complications related to the procedure in recent years owing to higher clinical expertise and dedicated devices.[6] The benefit of successful PCI in CTO lesions is the relief of patient symptoms such as angina, shortness of breath and improvement in the exercise capacity owing to decrease in the extent of ischemia.[7] Lower frequency of major adverse cardiovascular events (MACE) with the successful procedure has also been reported but the role of the procedure in improving long-term outcomes such as increased survival has not been well established.[8]

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Being present in one-third of the patients with CAD, it is mandatory for cardiologists to know the optimal evaluation and management of CTO lesions. The decision to perform PCI for CTO lesions depends on whether the anticipated benefits exceed the risks of the procedure. Although the PCI of CTO lesions is complex, the dramatic improvement in the success rate of the procedure in dedicated CTO centers has drawn significant attention and interest of interventional cardiologists. The number of CTO-PCI procedures in Pakistan is limited owing to expensive PCI equipment and a lack of dedicated CTO operators. Due to this, the data is limited in our setup and our results may vary from the studies conducted in developed countries. This study was designed to determine the outcomes of patients who underwent CTO-PCI in our setup. As most of the patients presenting in Fauji Foundation Hospital are females, this study was conducted specifically in the female population.

METHODOLOGY

This longitudinal descriptive study was conducted at the Department of Cardiology, Fauji Foundation Hospital, Rawalpindi over a 9 months period from July 2024 to March 2025 after taking approval from the ethical committee (Letter No. 857/RC/FFH/RWP, 28-06-2024). Coronary chronic total occlusion was defined as total occlusion with grade 0 TIMI flow for more than 3 months. A sample size of 50 was calculated at 90% confidence interval, 7% margin of error and proportion of successful PCI at 90.1% using the WHO sample size calculator.[9] A total of 56 female patients with diagnosed CTO who had persistent angina symptoms despite optimal medical therapy were included in the study using a non-probability convenience sampling technique. All these patients were eligible to undergo CTO-PCI with normal kidney function. The exclusion criteria were patients with non-CTO lesions and patients with complex CTO lesions managed either conservatively or referred for CABG. Informed written consent was obtained from all the patients or their attendants.

The percutaneous coronary intervention was performed in patients with chronic total occlusion by interventional cardiologists. The procedure was performed through femoral access. The demographic & clinical data of the patients and their baseline angiograms were reviewed to assess the feasibility of the procedure. The procedural details and outcomes were recorded by two other experienced operators. All the patients had a monthly follow-up for 3 months post-procedure. The outcomes assessed were rate of procedural success, in-hospital MACE and cardiac mortality & symptom-free at 3 months. Based on procedural success, patients were divided into two groups: successful and unsuccessful CTO-PCI groups. In-hospital MACE, cardiac mortality & symptom-free at 3 months were compared between the two groups. Procedural success was labeled as recanalization of a CTO lesion with <50% stenosis and grade 3 TIMI flow. The in-hospital MACE included cardiovascular mortality, myocardial infarction (MI), coronary artery perforation, cardiac tamponade, cardiogenic shock, major bleeding and stroke. All deaths due to cardiac causes such as myocardial infarction (MI), heart failure, arrhythmia, or deaths that were unwitnessed or procedure-related were defined as cardiac mortality.[10]

STATISTICAL ANALYSIS

Data entry and analysis were carried out using the Statistical Package for the Social Sciences (SPSS) version 25. The numerical and categorical variables were expressed using mean±standard deviation and frequency & percentage, respectively. The association between categorical variables was determined using the Chi-square test. Fisher's exact test was applied where the expected frequency was less than 5 in any of the cells. The significant p-value was taken as ≤0.05.

RESULTS

The mean age of the female patients with coronary chronic total occlusion was 59±2.5 years. Out of 56 patients, 16(28.6%) were diabetic, 14(25%) were hypertensive, 22(39.3%) had dyslipidemia, and 40(71.4%) had a history of CAD.

The procedural success was found in 37(66.1%) patients achieving successful revascularization, whereas, it was unsuccessful in 19(33.9%) patients. In-hospital MACE occurred in 5(8.9%) of the total patients. Forty (71.4%) were symptom-free while 2(3.6%) patients died at 3 months follow-up.

The outcomes were compared between patients with successful and unsuccessful CTO-PCI groups. In-hospital MACE was significantly less in the successful CTO-PCI group (p-value=0.04) and the majority [34(94.4%)] of the patients in the successful CTO-PCI group were symptom-free at 3 months with statistical significance (p-value=0.001). Six (15%) symptom-free patients belonged to the unsuccessful CTO-PCI group and this was attributed to partial restoration equivalent to TIMI grade 2 flow. However, there was no significant difference in mortality at 3 months between the two groups with a p-value of 0.53 (Table 1).

Our results showed that procedural success was associated with age and diabetes mellitus. The procedure was unsuccessful in most of the elderly above 60 years (p-value=0.02) and diabetic patients (p-value=0.004) (Table 2).

DISCUSSION

One of the most challenging interventions in the field of interventional cardiology is CTO-PCI. In CTO-PCI, there is prolonged procedural time that leads to increased exposure to radiation. It requires complex interventional tools and is much more complicated as compared to non-CTO-PCI.[11] There has been a significant improvement in the success rate of CTO-PCI in recent years resulting from better operative expertise and advanced equipment. The procedural success improves the function of the left ventricle, hence improving the patient's symptoms, increasing their physical activity and satisfaction.[12]

Table 1: Comparison of Outcomes of the Patients between Successful and Unsuccessful CTO-PCI Groups

Outcomes		Successful CTO-PCI Group Frequency & Percentage	Unsuccessful CTO-PCI Group Frequency & Percentage	Total	p-value
In-Hospital MACE	**No**	36(97.3%)	15(78.9%)	51(91.1%)	0.04*
	Yes	1(2.7%)	4(21.1%)	5(8.9%)	
	Total	37(66.1%)	19(33.9%)	56(100%)	
Cardiovascular Mortality		0(0%)	2(10.5%)	2(3.6%)	
Myocardial Infarction		1(2.7%)	0(0%)	1(1.8%)	
Perforation		0(0%)	1 (5.3%)	1(1.8%)	
Cardiogenic Shock		0(0%)	1(5.3%)	1(1.8%)	
Cardiovascular Mortality at 3 Months	**Yes**	1(2.7%)	1(5.9%)	2(3.6)	0.53
	No	36(97.3%)	16(94.1%)	52(92.9%)	
	Total	37(66.1%)	17(30.4%)	54(96.4%)	
Symptom-Free at 3 Months	**Yes**	34(94.4%)	6(37.5%)	40(71.4%)	0.001*
	No	2(5.6%)	10(62.5%)	12(21.5%)	
	Total	36(64.3%)	16(28.6%)	52(92.9%)	

**Significant p-value*

Table 2: Association of Procedural Success with Age and Co-Morbidities

Parameters		Successful CTO-PCI Group (n=37)	Unsuccessful CTO-PCI Group (n=19)	Total	p-value
Age Groups (Years)	**≤50**	4(10.8%)	1(5.3%)	5(8.9%)	0.02*
	51-60	19(51.4%)	4(21.1%)	23(41.1%)	
	61-70	10(27%)	6(31.5%)	16(28.6%)	
	>70	4(10.8%)	8(42.1%)	12(21.4%)	
	Total	37(66.1%)	19(33.9%)	56(100%)	
Diabetes Mellitus	**Diabetic**	6(16.2%)	10(52.6%)	16(28.6%)	0.004*
	Non-Diabetic	31(83.8%)	9(47.4%)	40(71.4%)	
	Total	37(66.1%)	19(33.9%)	56(100%)	
Hypertension	**Hypertensive**	10(27%)	4(21.1%)	14(25%)	0.75
	Non-Hypertensive	27(73%)	15(78.9%)	42(75%)	
	Total	37(66.1%)	19(33.9%)	56(100%)	
Lipid Profile	**Deranged (Dyslipidemia)**	14(37.8%)	8(42.1%)	22(39.3%)	0.75
	Normal	23(62.2%)	11(57.9%)	34(60.7%)	
	Total	37(66.1%)	19(33.9%)	56(100%)	
History of CAD	**Present**	29(78.4%)	11(57.9%)	40(71.4%)	0.108
	Absent	8(21.6%)	8(42.1%)	16(28.6%)	
	Total	37(66.1%)	19(33.9%)	56(100%)	

**Significant p-value*

The mean age of the patients with coronary chronic total occlusion was 59±2.5 years in our study. Out of 56 patients in our study, 16(28.6%) were diabetic, 14(25%) were hypertensive, 22(39.3%) had dyslipidemia and 40(71.4%) had a history of CAD. In line with our results, another study reported a mean age of the patient of 59.49 ± 9.16 years. In that study, 19.61% of the patients were diabetic, 24.18% had hypertension, 43.46% had dyslipidemia, and 66.99% had a history of myocardial infarction (MI).[13] In contrast to our results, the mean age of the patients was 63±10 years in a study conducted by Gilpin et al. in the UK.[9]

Our results revealed a procedural success rate of 66.1%. In-hospital MACE occurred in 8.9% of the patients. At 3 months follow-up, 2(3.6%) patients died due to cardiovascular causes whereas, 71.4% of the patients were symptom-free. In a meta-analysis, outcomes of CTO-PCI compared to medical treatment or CABG were observed. The procedure was successful in 75.4% of the patients and was linked with a decrease in cardiac mortality and MACE at long-term follow-up. It was also revealed that patients who underwent CTO-PCI developed a marked improvement in their left ventricular ejection fraction. However, in contrast to other studies, the procedure did not decrease long-term MACE.[14] According to another meta-analysis, a significant decrease in the frequency of angina with CTO-PCI was observed.[15]

Gilpin et al. reported in-hospital MACE in only one (0.5%) patient. They found that cardiac mortality occurred in 4(2.4%) and MI in 14(8.3%) patients at follow-up.[9] In another study, the procedural success was significantly lower in CTO-PCI as compared to non-CTO-PCI. The CTO-PCI was linked with a higher incidence of cardiac tamponade and coronary artery perforation as compared to non-CTO-PCI. It was also reported that MACE occurred more frequently in CTO-PCI cases than in non-CTO-PCI. However, there was no difference in cardiac mortality between the two groups.[16] Another study compared CTO-PCI with non-CTO-PCI where the procedural success was lower in CTO-PCI (76%) than non-CTO-PCI (95.7%). The incidence of in-hospital MACE was higher in CTO versus non-CTO-PCI with a higher frequency of MI, bleeding, cardiogenic shock and in-hospital mortality. However, there was no difference in long-term MACE between CTO and non-CTO-PCI.[17] Another study reported a success rate of 58.7% with CTO-PCI.[18] In a study conducted in India, the CTO-PCI had a success rate of 74.6%. In-hospital mortality occurred in 0.9% of the patients. Cardiac tamponade occurred in 0.6% and coronary artery perforations in 1.3% of patients.[19] The results of a meta-analysis revealed that MACE was significantly less in patients who underwent PCI for CTO compared to patients managed with medical therapy alone.[20] A study conducted in Pakistan at the Peshawar Institute of Cardiology found that the success rate of CTO-PCI was 77.45% and 13.73% of the patients experienced in-hospital MACE.[13]

Our results showed that the frequency of MACE was significantly less in patients with successful CTO-PCI. Two other studies reported better prognosis in patients with procedural success and a decrease in MACE.[21,22] Our results showed that the procedure was significantly unsuccessful in most of the elderly above 60 (p-value=0.02) and diabetic patients (p-value=0.004). Similar to our findings, a study reported high procedural success in younger patients as compared to older patients.[23] In contrast, a systematic review reported no difference in procedural success between younger and older patients.[24] In a study, diabetes mellitus was related to a high rate of procedural failure while the association with other variables such as age, hypertension, smoking, gender, dyslipidemia and history of CAD was not significant.[13]

CONCLUSION

The CTO-PCI has a procedural success rate of 66.1% and the majority of the patients were symptom-free after the procedure. The statistically significant outcomes were reduction in the frequency of in-hospital MACE and symptom-free at 3 months in the successful CTO-PCI group. The procedural failure was significantly associated with the elderly age group above 60 and diabetes mellitus.

LIMITATIONS & RECOMMENDATIONS

It was a single-centered study with a small sample size and included only female patients. The patients did not have long-term follow-up. The study did not find the association of patient outcomes with various CTO scores. Multi-centered trials should be conducted in Pakistan with a large sample size including patients from both genders. Percutaneous coronary intervention can be safely opted for CTO cases and efforts should be put in further improving equipment and technical expertise for the procedure.

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Authors' Contributions:
M.W.H: Conceptualized the study, drafted the manuscript, and coordinated the research.

A.M: Supervised the study and reviewed the manuscript.
A.H.Q: Managed methodology, ethical approval, and patient recruitment.
M.W.A.L: Performed statistical analysis and interpreted results.
W.M: Assisted in data collection and literature review.
M.R: Maintained the database and helped in proofreading.
S.A: Critically reviewed the manuscript and approved the final version.

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Original Article

Comparison of Soft Tissue Healing in Immediate Implant Placement in Fresh Extraction Socket with and without the Use of Platelet-Rich Fibrin

Tehmina Maryam, Babar Pasha, Muhammad Afzal, Muhammad Nazir Khan, Aiema Nawaz, Noman Tahir

ABSTRACT

Objective: To compare soft tissue healing in immediate implant placement in fresh extraction socket with and without the use of platelet-rich fibrin (PRF) by evaluating cover screw exposure on 7th post-operative day and pink esthetic score on 3rd and 6th post-operative month.

Methodology: This quasi-experimental study was conducted at the Department of Oral & Maxillofacial Surgery, Armed Forces Institute of Dentistry, Rawalpindi over 10 months from June 2022 to March 2023 after ethical approval. Total 60 patients aged 18-60 years with maxillary or mandibular teeth indicated for extraction without active infection and with adequate gingival architecture and bone volume were included. Thirty participants were included in intervention and control groups. Written informed consent and demographic details were acquired from patients and recorded. Participants were non-randomly allocated via non-probability convenience sampling to receive immediate implant placement with or without PRF based on the availability of intervention material and clinical scheduling. Follow-up evaluations were done on the 7th post-operative day for presence or absence of cover screw exposure and at 3 and 6 months for mean pink esthetic scores (PES). Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 27.

Results: The mean age of the participants was 37.97±13.37 years. The sample consisted of 27(45%) males and 33(55%) females. Among the PRF intervention group (n=30), 4(13.3%) had cover screw exposure on the 7th post-operative day, while 26(86.7%) did not. While for the control group, the presence and absence of cover screw exposure was 15(50%). Participants who received PRF treatment demonstrated significantly higher mean PES scores at both the 3rd month (11.73 ± 1.143) and the 6th month (10.93 ± 0.907), compared to 9.17 ± 0.747 and 8.90 ± 0.845, respectively, in those without PRF. A statistically significant difference between the mean PES of the groups ($p < 0.001$) was observed between study groups.

Conclusion: The mean PES of the intervention (PRF) group in post extraction immediate implant placement was significantly higher than the control group at 3rd & 6th month follow-up, indicating better soft tissue healing with PRF use. Platelet-rich fibrin-treated group also demonstrated significantly lesser occurrence of cover screw exposure on 7th post-operative day highlighting the beneficial impacts of PRF adjunct in immediate implant placement.

Keywords: *Immediate dental implant loading. Platelet-rich fibrin. Bone regeneration. Tooth extraction.*

INTRODUCTION

In recent years, immediate implant loading following tooth extraction has gained considerable attention in dental implantology due to its potential advantages in reducing treatment time and preserving alveolar bone dimensions.[1] This approach involves placing a dental implant into the extraction socket immediately after tooth removal, which can streamline the treatment process and enhance patient satisfaction.[2] However, the success of immediate implant placement depends significantly on the preservation of soft tissue integrity and optimal healing around the implant site.[3]

Platelet-rich fibrin (PRF), a second-generation platelet concentrate, has emerged as a promising adjunct in various dental procedures, including immediate implant placement.[4] Platelet-rich fibrin is derived from the patient's own blood and contains a high concentration of platelets, leukocytes, and growth factors that promote wound healing and tissue regeneration.[5] Its application in immediate implant procedures aims to enhance soft tissue healing, stabilize the blood clot, and facilitate faster integration of the implant into the surrounding bone.[6]

The effectiveness of PRF in enhancing soft tissue healing around immediately placed implants has been a subject of growing interest among researchers and clinicians. Several studies have investigated its impact on parameters such as gingival biotype preservation, reduction in post-operative complications, and improvement in aesthetic outcomes. The pink esthetic score (PES) is a widely accepted tool to evaluate the soft tissue parameters around an implant, including the morphology and anatomy of the papilla, the level of attachment of facial mucosa and its curvature along with the color & texture of soft tissue.[7] Immediate implant placement in the anterior maxilla is challenging due to aesthetic demands and the risk of soft and hard tissue loss. The aesthetic success of implant-

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supported prostheses depends on factors like implant positioning, soft tissue manipulation, prosthesis type, and individual patient characteristics such as bone & soft tissue quality. Bone loss after extraction, particularly involving the buccal wall, often leads to gingival structure compromise, thereby reducing the aesthetic outcome. Studies indicate up to a 50% reduction in alveolar width within six months post-extraction, with a greater loss if the buccal wall is involved.[8] Cover screws can be spontaneously exposed post-implant due to overlying gingival tissue perforation and may lead to bone loss. Its early detection can prevent future complications.[9] Platelet-rich fibrin accelerates the healing of bone and gum tissues, promotes tissue regeneration, supports vascularization, hence reducing the need for bone grafts, thereby making it a valuable adjunct in immediate implant placements.[10]

This study aims to evaluate the efficacy of PRF in enhancing soft tissue healing in immediate implant placement, addressing the need for improved aesthetic outcomes in the anterior maxilla. It seeks to provide new insights into PRF's benefits in minimizing bone and soft tissue loss, particularly in the Pakistani population where such data is limited. This research will fill existing gaps by offering evidence-based recommendations, contributing to the global understanding of PRF's role in dental implantology, and potentially setting new standards for clinical practice in Pakistan.

METHODOLOGY

This quasi-experimental study was conducted at the Department of Oral & Maxillofacial Surgery, Armed Forces Institute of Dentistry, Rawalpindi over a duration of 10 months from June 2022 to March 2023. The ethical approval of the study was obtained from the Institutional Review Board (Letter No. 918/Trg, 13-05-2022). The sample size calculation was done using 95% confidence level, 80% power, and the difference between two means (PES) of 1.17 units with a standard deviation of 0.52.[11] A minimum of 30 participants were enrolled in both groups (control and intervention). The patients were recruited using non-probability convenience sampling technique. Informed written consent was obtained from all participants. Patients aged between 18 and 60 years, with maxillary or mandibular teeth indicated for extraction (without active infection), adequate gingival architecture, and sufficient bone volume were included. Edentulous patients, those undergoing chemotherapy or radiotherapy, patients with systemic diseases such as diabetes, smokers, or with poor oral hygiene were excluded from the study. Patients were non-randomly assigned to one of two groups based on the availability of intervention materials and clinical scheduling. The control group (n=30) received immediate implant placement following minimally traumatic extraction without the placement of platelet-rich fibrin. The intervention group (n=30) received immediate implant placement following minimally traumatic extraction with PRF placed in situ.

Clinical outcomes assessed were presence or absence of cover screw exposure, and mean PES. A 0-1-2 scoring system was used to assign PES, where 0 indicated the lowest and 2 was the highest score for each of the following seven parameters: distal papilla, mesial papilla, level of soft tissue at mid-labial area, alveolar process deficiency and soft tissue color, contour and texture. The maximum possible score was 14.[12]. Follow-up evaluations were performed on the 7th post-operative day for presence or absence of cover screw exposure, and at 3rd and 6th months post-operatively for mean PES.

STATISTICAL ANALYSIS

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 27. Quantitative variables, such as age and PES were expressed as mean±standard deviation. Qualitative variables such as gender and cover screw exposure were expressed as frequencies and percentages. Fisher's exact test was used to compare presence or absence of cover screw exposure between the groups. Independent sample t-test was used to compare the mean PES between control and intervention groups. Effect modifiers, like age were stratified and post-stratification independent sample t-test was applied. A p-value of ≤0.05 was considered significant.

RESULTS

The mean age of the participants was 37.97±13.37 years. The sample consisted of 27(45%) males and 33(55%) females. Table 1 summarizes the descriptive statistics regarding cover screw exposure on the 7th post-operative day for both groups. Only 4(13.3%) patients in the intervention group experienced cover screw exposure on the 7th day post-operative day as compared to 15(50%) in the control group. These results were statistically significant (p=0.005).

Table 2 presents the comparison of mean pink esthetic scores at 3rd and 6th month follow-up between the two groups. Participants treated with PRF demonstrated significantly higher scores at both the 3rd month (11.73±1.143) and 6th month follow-up (10.93±0.907) as compared to those without PRF

(9.17±0.747 and 8.90±0.845 at 3rd and 6th months respectively) (p=0.001).

Table 3 compares the mean PES at 3rd and 6th month follow-up between two age groups. In both age groups, participants treated with PRF showed significantly higher PES compared to those without PRF treatment at 3rd and 6th months (p=0.001).

DISCUSSION

Soft tissue healing in immediate implant placement within fresh extraction sockets is crucial for successful outcomes. Utilizing PRF enhances this process by promoting faster regeneration and improved tissue quality compared to procedures without PRF. This approach not only supports rapid healing but also enhances long-term stability and integration of the implant in the surrounding tissues, highlighting PRF's role as a valuable adjunct in contemporary implant dentistry.[13]

Our study revealed a mean age of participants of 37.97±13.37 years with 27(45%) males & 33(55%) females. In contrast to our findings, Zaidi et al. observed a mean age of 51.1±12.5 years among patients in Karachi. The study demonstrated a statistically significant prevalence of dental issues among participants older than 61 years, including 40% females and 60% males. Comparing these demographics underscores the broad demographic variability in dental health studies, highlighting both the consistency and variability in age and gender distribution across different research contexts.[14]

Our results showed that the frequency of cover screw exposure on 7th post-operative day was significantly more in the control group (50%) without PRF as compared to the PRF intervention group (13.3%). Similar to our findings, Ali et al. reported that 30% patients had cover screw exposure at 3 months follow-up in the control group, whereas it was not seen in any patient in the PRF-treated group.[15]

Table 1: Comparison of Cover Screw Exposure between Control and Intervention Groups

Cover Screw Exposure On 7th Post-Operative Day	Intervention Group (n=30)	Control group (n=30)	Total	p-value
Yes	4(13.3%)	15(50%)	19(31.7%)	0.005*
No	26 (86.7%)	15(50%)	41(68.3%)	
Total	30(100%)	30(100%)	60(100%)	

**Significant p-value*

Table 2: Comparison of Mean Pink Esthetic Score between Two Groups

Pink Esthetic Score (PES)	Intervention Group Mean±SD	Control Group Mean±SD	p-value
3rd Month Follow-Up	11.73±1.143	9.17±0.747	0.001*
6th Month Follow-Up	10.93±0.907	8.90±0.845	0.001*

**Significant p-value*

Table 3: Comparison of Mean Pink Esthetic Scores between Two Age Groups

Age Groups (Years)	Pink Esthetic Score	Groups	(n)	Mean±SD	p-value
<40	**3rd Month Follow-up**	**Intervention Group**	14	11.93±0.997	0.001*
		Control Group	13	9.00±0.816	
	6th Month Follow-up	**Intervention Group**	14	11.00±0.877	0.001*
		Control Group	13	8.92±0.862	
≥40	**3rd Month Follow-up**	**Intervention Group**	16	11.56±1.263	0.001*
		Control Group	17	9.29±0.686	
	6th Month Follow-up	**Intervention Group**	16	10.88±0.957	0.001*
		Control Group	17	8.88±0.857	

**Significant p-value*

Cover screw exposure can occur due to many reasons including the type of screw, the patient's anatomy, and the technique of the implantologist. Early cover screw exposure can lead to bone loss. Guney et al. reported that cover screw morphology influences epithelial maturation and inflammatory response of the peri-implant soft tissue.[16] Fang et al. reported that PRF enhances soft and hard tissue healing even in infected post-extraction implant sockets.[9] Sharma et al. also compared soft and hard tissue changes around dental implants with and without PRF. It was observed that radiographic crestal bone loss was significantly lesser in the PRF group as compared to the control group.[17] The findings of a meta-analysis also revealed that PRF-treated patients exhibited superior bone healing outcomes post-dental implantation.[18]

Our study revealed that patients treated with PRF had significantly higher PES at both 3rd (11.73±1.143) and 6th month follow-ups (10.93±0.907) as compared to those without PRF (9.17±0.747 and 8.90±0.845 at 3rd and 6th months, respectively). Aldosari et al. reported that the pink and white esthetic scores offer standardized methods for assessing peri-implant soft tissue and the use of platelet-rich fibrin in immediate implant procedures increases pink esthetic score.[19] Sharafuddin et al. conducted a randomized controlled trial (RCT) on patients undergoing immediate dental implant placements at various sites, with and without PRF & connective tissue graft (CTG). They reported that CTG and PRF augmentation enhanced tissue biotype and peri-implant keratinized tissue width. At 3rd month post-operation, the test group, with CTG and PRF had higher pink esthetic scores (12.17±1.72) than the control group (10.67±1.37). These results align closely with our findings, suggesting consistent trends in the aesthetic outcomes of PRF use in dental implant surgeries over time.[20] Kamal et al. compared PRF and subepithelial CTG for soft tissue augmentation around dental implants in an RCT in Egypt. Contrary to our findings, they found that mean PES at 6th month follow-up was significantly higher in the subepithelial CTG group (12.50±1.05) as compared to the PRF group (11.33±0.52).[11] Ahmed El Komi et al. emphasized the stability benefits of injectable PRF over platelet-rich plasma. They observed increased PRF's efficacy in enhancing soft tissue quality post-implantation.[21] Additionally, Ali et al. observed improved soft tissue healing in test groups receiving PRF as compared to control. Platelet-rich fibrin enhances the release of growth factors, supports angiogenesis and decreased chance of implant exposure to micro-organisms of the oral cavity.[15] Chandana et al. studied the impact of PRF on rebound soft tissue in immediate implant placement. Their results also showed significantly better soft tissue thickness and healing in PRF as compared to control.[22]

CONCLUSION

The mean PES of the intervention (PRF) group in post-extraction immediate implant placement was significantly higher than the control group at 3rd & 6th month follow-ups, indicating better soft tissue healing with PRF use. Platelet-rich fibrin-treated group also demonstrated significantly lesser occurrence of cover screw exposure on 7th post-operative day, highlighting the beneficial impacts of PRF adjunct in immediate implant placement.

LIMITATIONS & RECOMMENDATIONS

This study was limited by its single-center design and relatively small sample size. Future research should explore larger cohorts and multicenter studies to further validate these findings. It is recommended that platelet-rich fibrin should be considered a valuable therapeutic adjunct in enhancing soft tissue healing and esthetic results in dental implantology.

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Authors' Contributions:
T.M: Data acquisition and analysis.
B.P: Conceptualization, study design, and drafting of the manuscript.
M.A: Data analysis, interpretation, and critical revision of the article.
M.N.K: Data analysis and revision for intellectual content.
A.N: Data acquisition and manuscript drafting.
N.T: Final revision for approval and contribution to data analysis.

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Original Article

Diagnostic Accuracy of Alanine Aminotransferase in Intrahepatic Cholestasis of Pregnancy and its Association with Maternal & Fetal/Neonatal Complications

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ABSTRACT

Objective: To estimate the accuracy of alanine aminotransferase (ALT), using bile acid levels as the gold standard in the diagnosis of intrahepatic cholestasis of pregnancy (ICP) and to assess the maternal & fetal/neonatal complications between ICP cases and healthy pregnant females as well as cases with high and very high ALT levels.

Methodology: After approval from the ethical committee, this cross-sectional study was carried out at the Department of Obstetrics and Gynecology, Mohi-ud-Din Islamic Medical College, Mirpur during six months from September 2024 to February 2025. Using non-probability convenience sampling, 2500 pregnant women were included after taking their informed consent. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of ALT were evaluated using bile acid levels as a gold standard. The frequencies of maternal and fetal/neonatal complications were compared between cases of ICP and healthy pregnant females and cases with high ALT (41-100 IU/L) and very high ALT levels (>100 IU/L). Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.

Results: The frequency of ICP was 2.4%. Serum ALT has a sensitivity of 83.3%, specificity of 99%, and diagnostic accuracy of 98.6%. A receiver operating characteristic (ROC) curve showed an area under the curve of 0.911 indicating excellent diagnostic accuracy. Preterm delivery, induction of labor, low birth weight, and respiratory distress were more significant in patients with ALT levels >100 IU/L.

Conclusion: Serum ALT is a reliable marker for the diagnosis of ICP with high sensitivity, specificity, and diagnostic accuracy. The maternal and fetal/neonatal complications were significantly higher in patients with ICP particularly those with very high ALT levels.

Keywords: *Alanine aminotransferase. Bile acids. Intrahepatic cholestasis.*

INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) frequently occurs in pregnancy with an incidence ranging from 0.1% to 15.6%. The frequency of ICP varies in various ethnic groups and geographical regions with genetic, hormonal, and environmental factors playing their role in the disease causation.[1,2] The disease is prevalent in South Asia.[3] It mostly manifests in the second and third trimester of pregnancy with itching in palms and soles. In 14-25% of pregnant women, jaundice is also present.[1] The disease is more common in increased maternal age, multiple gestations, twin pregnancies, positive hepatitis C serology, gall stones, and positive family history.[2] It is characterized by a compromised flow of bile. Hormones released during pregnancy impair the transport of bile acids into the bile ducts. Normally, bile acids from the fetus are transported into the mother. In ICP, the reciprocal process occurs with bile acids being transported into the fetus.[4] High bile acid levels lead to the secretion of cytokines in the liver with resultant inflammation and liver damage. In addition, it also causes liver damage by inducing oxidative stress and mitochondrial damage. High levels of bile acids are also toxic to the fetus and cause leukocyte migration and inflammation of the placenta.[5]

The diagnosis is made based on clinical manifestations, liver function tests and exclusion of other causes of itching and liver disease in pregnancy.[5] The bile acids are elevated in serum in >90% of the cases with the greatest sensitivity.[6] Serum alanine aminotransferase (ALT) has the greatest sensitivity among the liver enzymes for diagnosis of ICP with 2-10 times higher levels.[7]

The disease is associated with both maternal and fetal complications. The maternal complications include higher chances of developing gestational hypertension, diabetes mellitus, premature rupture of membranes (PROM) and postpartum hemorrhage (PPH). The fetal/neonatal complications include preterm delivery, stillbirth, fetal distress, neonatal asphyxia, and meconium staining of amniotic fluid.[8]

Intrahepatic cholestasis of pregnancy has profound effects on maternal and child health. This study was designed to estimate the diagnostic accuracy of ALT in the diagnosis of ICP keeping bile acid levels as gold standard. In the literature, the data on the diagnostic accuracy of ALT in ICP is limited. Serum bile acids are highly sensitive and specific marker of ICP but they are expensive and not routinely performed. On the other hand, ALT is inexpensive and is routinely done investigation. The results of

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the study would help in using serum ALT as a diagnostic marker in ICP if its sensitivity and specificity are found to be high. The study also determined the maternal and fetal/neonatal complications in these patients compared to normal healthy pregnant females and with respect to the rise in levels of ALT. Knowing the frequency and complications of the disease in our setup will guide us in early diagnosis and management of the condition to combat adverse outcomes.

METHODOLOGY

After approval from the ethical committee (Letter No. 1-2/24-MIMC/ERB/0024, 20-09-2024), this cross-sectional study was carried out at the Department of Obstetrics & Gynecology, Mohi-ud-Din Islamic Medical College, Mirpur during six months from September 2024 to February 2025. A sample size of 2500 pregnant females was calculated using 95% confidence interval, 10% margin of error, 89.7% sensitivity of ALT, and prevalence of ICP among pregnant females at 1.5%.[9] The pregnant women who presented in the Outpatient Department (OPD) for routine antenatal checkups or any complaints of pruritus during the second or third trimester of pregnancy were included after taking their informed consent using non-probability convenience sampling technique. The exclusion criteria were other causes of liver disease such as any type of hepatitis, acute fatty liver of pregnancy, eclampsia), and skin diseases that cause pruritus such as scabies. Pregnant women with pruritus, deranged liver function tests [with alanine aminotransferase (ALT) >40 IU/L, aspartate aminotransferase (AST) >40 IU/L and raised bile acids (>10 µmol/L)] presenting in the second or third trimester of pregnancy with the exclusion of other causes of liver disease were labeled as having ICP.[2] The demographic variables and co-morbidities of the participants were noted. The diagnostic accuracy parameters of ALT were determined by taking bile acids as the gold standard for ICP. True positive (TP) were the patients who are positive on both ALT and bile acid testing while true negative (TN) refers to those negative on both diagnostic modalities. Patients positive on ALT but negative on bile acids are labeled as false positive (FP) & false negative (FN) indicates patients positive on bile acids but negative on ALT. The ALT levels 41-100 IU/L were labeled as high and >100 IU/L were categorized as very high levels. All the participants were followed up and the maternal & neonatal complications were compared between ICP cases and healthy pregnant females & cases with high and very high ALT levels. The maternal complications included preterm delivery, induction of labor, postpartum hemorrhage, placental abruption, and cesarean section. The fetal/neonatal complications were low birth weight, respiratory distress, meconium aspiration, Apgar score (appearance, pulse, grimace, activity and respiration) & intrauterine death (IUD)/stillbirth.

STATISTICAL ANALYSIS

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) version 26. Descriptive statistics (mean and standard deviation) and frequency (percentage) were used for numeric and categorical variables, respectively. The numeric and categorical variables were compared using an independent t-test and a Chi-square test, respectively. A p-value of ≤ 0.05 was statistically significant. A 2x2 table was made by taking bile acids as a gold standard. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR-), and diagnostic accuracy of ALT were calculated. A ROC curve was made to determine the area under the curve (AUC) and the diagnostic accuracy of ALT. An AUC equal to or greater than 0.6 is considered meaningful. Values between ≥ 0.6 and <0.7 indicate poor diagnostic accuracy, ≥ 0.7 to <0.8 suggest fair accuracy, ≥ 0.8 to <0.9 reflect good accuracy while values above 0.9 represent excellent diagnostic accuracy.[10]

RESULTS

In our study, ICP was present in 60(2.4%) out of 2500 patients. The ICP cases and healthy pregnant females did not differ significantly in their demographic variables and co-morbidities (Table 1).

Sixty (2.4%) patients had ICP with high levels of bile acids. Out of these 60, 50(83.3%) patients had ALT levels above the normal range while 10(16.7%) patients had ALT within the normal range. Out of 2440 patients without disease, 25(1.03%) patients had high ALT showing false positive results. The relation of ALT with ICP taking bile acids as the gold standard was statistically significant (Table 2).

Serum ALT has a sensitivity of 83.3%, specificity of 99%, PPV of 66.7%, NPV of 99.5%, LR+ of 83.3, LR- of 0.17, and diagnostic accuracy of 98.6%. A ROC curve showed an area under the curve of 0.911 which indicates the ALT has excellent diagnostic accuracy in the ICP (Figure 1).

Preterm delivery, induction of labor and rate of cesarean section were more common in patients with

ICP as compared to healthy pregnant females with statistical significance (p-values=0.0007, 0.02, and 0.019, respectively). However, the frequency of postpartum hemorrhage and placental abruption were not statistically different. The neonatal complications were significantly more frequent in ICP versus healthy pregnant females except for IUD/stillbirth (Table 3).

Preterm delivery (p-value=0.02) and induction of labor (p-value=0.007) were significantly more common in patients with very high ALT levels. Among fetal/neonatal complications, low birth weight and respiratory distress occurred more frequently in patients with very high ALT levels. These results were statistically significant (Table 4).

Table 1: Demographic Variables and Co-morbidities of the Study Participants

Variables			ICP Cases (n=60)	Healthy Pregnant Females (n=2440)	p-value
Age (Years)		**Mean±SD**	29.1±4.5	28.5±3.8	0.22
BMI (kg/m²)			31.5±5.6	30.4±5.1	0.09
Parity	**Primigravida**	**Frequency (Percentage)**	17(28.3%)	830(34%)	0.35
	Multigravida		43(71.7%)	1610(66%)	
Gestational Diabetes			16(26.7%)	438(18%)	0.08
Gestational Hypertension			7(11.7%)	147(6%)	0.72

Table 2: Relation of ALT with Serum Bile Acids

Serum ALT levels	Gold Standard Test (Serum Bile Acid Levels)		Total	p-value
	ICP Cases (High)	Healthy Pregnant Females (Normal)		
High	50(83.3%) (TP)	25(1.03%) (FP)	75(3%)	0.00001*
Normal	10(16.7%) (FN)	2415(98.97%) (TN)	2425(97%)	
Total	60(2.4%)	2440(97.6%)	2500(100%)	

**Significant p-value*

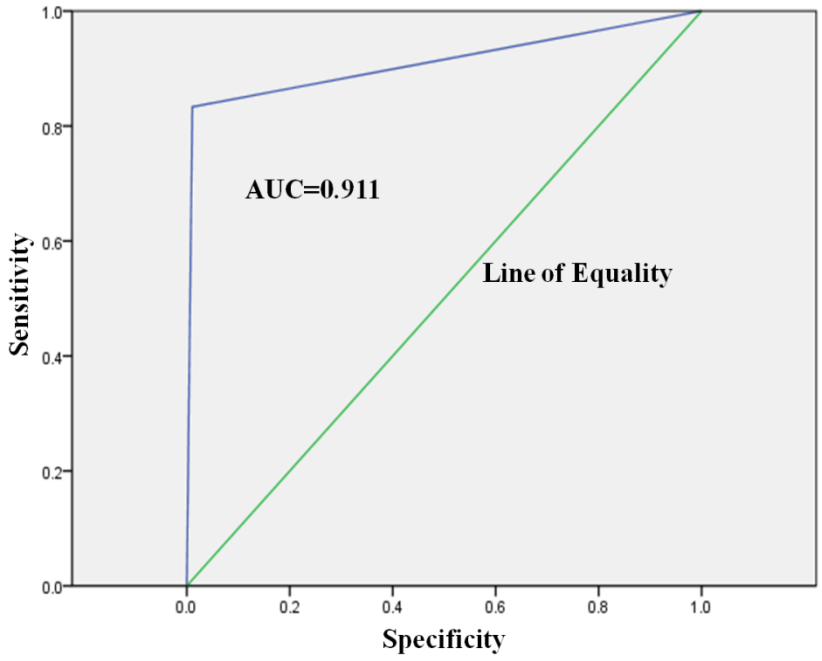

Figure 1: ROC Curve Showing the Diagnostic Accuracy of Serum ALT in ICP

Table 3: Maternal and Fetal/Neonatal Complications in ICP Cases versus Healthy Pregnant Females

Complications		ICP Cases (n=60)	Healthy Pregnant Females (n=2440)	p-value
Maternal	Preterm Delivery	14(23.3%)	244(10%)	0.0007*
	Induction of Labor	26(43.3%)	730(30%)	0.02*
	Cesarean Section	23(38.3%)	611(25%)	0.019*
	PPH	5(8.3%)	172(7.05%)	0.70
	Placental Abruption	4(6.7%)	73(3%)	0.10
Fetal/Neonatal	Low Birth Weight	12(20%)	195(8%)	0.008*
	Respiratory Distress	10(16.7%)	171(7%)	0.004*
	Meconium Aspiration	11(18.3%)	122(5%)	<0.0001*
	Apgar Score <7	10(16.7%)	171(7%)	0.004*
	IUD/Stillbirth	7(11.7%)	171(7%)	0.16

**Significant p-value*

Table 4: Maternal and Fetal/Neonatal Complications in Cases with High and Very High ALT Levels

Complications		ICP Cases with High ALT Levels (n=50)		p-value
		High ALT Levels (41-100 IU/L) (n = 30)	Very High ALT Levels (>100 IU/L) (n=20)	
Maternal	Preterm Delivery	5(16.7%)	9(45%)	0.02*
	Induction of Labor	11(36.7%)	15(75%)	0.007*
	Cesarean Section	11(36.7%)	12(60%)	0.10
	PPH	2(6.7%)	3(15%)	0.33
	Placental Abruption	2(6.7%)	2(10%)	0.67
Fetal/Neonatal	Low Birth Weight	4(13.3%)	8(40%)	0.03*
	Respiratory Distress	3(10%)	7(35%)	0.03*
	Meconium Aspiration	4(13.3%)	7(35%)	0.07
	Apgar Score <7	4(13.3%)	6(30%)	0.14
	IUD/Stillbirth	3(10%)	4(20%)	0.31

**Significant p-value*

DISCUSSION

Intrahepatic cholestasis of pregnancy causes liver dysfunction in pregnancy and its incidence varies from one geographical region to another. Only a few markers have been evaluated for its workup. Most of the studies have evaluated bile acids for the diagnosis of ICP and its relation with maternal and fetal/neonatal complications.[11]

The frequency of ICP was 2.4% in our study. The prevalence of the disease was 1% in the UAE and 3.73% in India.[12,13] The studies conducted in China showed the disease prevalence of 1.73% and 3.81%.[14,15] This indicates that the prevalence of ICP can vary within the same country and from one country to another as well. Our study found no significant difference in age, gestational age, body mass index (BMI), diabetes, and hypertension between cases and healthy pregnant females. Another study also reported no difference in age and gestational age between these two groups. [16]

In our study, serum ALT had a sensitivity of 83.3% and specificity of 99% at the cut-off value of 40 IU/L. The ROC curve showed an AUC of 0.911 indicating excellent diagnostic accuracy. Tasin et al. reported that serum bile acids and ALT were significantly higher in ICP as compared to non-ICP pregnant females. The sensitivity and specificity of ALT were 88% and 87% at 62 IU/L cut-off value and AUC of 0.89 showing good diagnostic accuracy.[16]

Our study results revealed a significantly higher frequency of complications in ICP patients with very high ALT levels. A study conducted in UAE compared complication rate between patients with bile acids <40 μmol/L and >40 μmol/L. The frequency of complications was statistically high in patients with bile acid levels >40 μmol/L such as premature birth, respiratory distress, low birth weight, poor Apgar score, and admission in neonatal intensive care unit (NICU).[12] A meta-analysis conducted by Zhou et al. also showed that severe ICP diagnosed by high bile acid levels was significantly related to poor maternal and neonatal outcomes such as premature rupture of membranes, preterm birth, admission to NICU and meconium-stained liquor.[17] A study conducted in Pakistan

revealed that ICP was responsible for PPH (10%), premature rupture of membranes (73%) and instrumental deliveries (75.5%). The disease caused meconium aspiration in 23.75%, preterm birth in 25%, low birth weight in 18.75%, fetal distress in 20%, NICU admission in 18.75% and stillbirth in 2.5% of the patients.[18] But the study did not compare the frequency of complications in non-ICP pregnant females.

A study conducted by Feng et al. reported that preterm birth, low birth weight, meconium aspiration, low Apgar score, and NICU admission were more common in ICP patients with higher levels of bile acids.[19] Another study reported significant differences in the frequencies of preterm delivery (18.60% in ICP versus 4.87% in non-ICP) and cesarean sections (51.31% in ICP versus 31.60% in non-ICP). Placental abruption and PPH were almost the same between the two groups. The birth weight was significantly less in ICP patients (9.30% versus 3.35%). However, fetal distress and stillbirth were not different between the groups.[14] A study from India revealed higher frequencies of induced labor (64.6% versus 18.8%) and cesarean sections (34% versus 24%) in ICP patients as compared to non-ICP participants. All other maternal and fetal complications were not significantly different between the two groups.[13] Wu et al. found a higher incidence of preterm delivery (22.24% vs 9.26%), PROM (6.02% vs 4.02%), cesarean section (56.14% vs 39.92%), low birth weight (4.63% vs 1.10%), and admission in NICU (27.41% vs 15.13%) in ICP patients as compared to non-ICP pregnant females.[15]

CONCLUSION

Serum ALT is a reliable marker for the diagnosis of intrahepatic cholestasis of pregnancy with a high sensitivity (83.3%), specificity (99%), and diagnostic accuracy (98.6%). The maternal and fetal/neonatal complications were significantly higher in patients with ICP as compared to non-ICP pregnant females. These complications were also significantly high in ICP cases with very high ALT levels (>100 IU/L).

LIMITATIONS & RECOMMENDATIONS

It was a single-centered cross-sectional study. The sensitivity and specificity of serum ALT were not estimated at various cut-off values in our study. Multi-centered studies with analytical designs are recommended. The sensitivity and specificity of serum ALT should be estimated at various cut-off values for accurate diagnosis and management. Our study recommends the use of ALT for the diagnosis of disease and monitoring of patients for maternal and fetal/neonatal complications.

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Authors' Contributions:
A.Y: Conceived the study, drafted the manuscript, and coordinated data collection.
N.S: Supervised the research and reviewed the manuscript.
R.K: Managed patient enrollment and clinical data verification.
F.H: Performed statistical analysis and contributed to results interpretation.
A.F: Assisted in literature review and data organization.
H.Z: Proofread the manuscript and ensured reference accuracy.

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Original Article

Association of Serum Cortisol, Interleukin-6, and Serotonin with Depression

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ABSTRACT

Objective: To explore the relation between serum cortisol, interleukin-6 (IL-6), and serotonin (5-HT) levels in patients with melancholic depression, atypical depression, and healthy controls.

Methodology: This cross-sectional comparative study was conducted from August 2023 to July 2024 at the Combined Military Hospital, Muzaffarabad. After ethical approval of the study, a total of 105 participants were enrolled using a non-probability convenience sampling method, including 35 healthy individuals, 35 with melancholic depression, and 35 with atypical depression. Serum levels of cortisol, IL-6, and serotonin were measured using enzyme-linked immunosorbent assay. The Statistical Package for the Social Sciences (SPSS) version 25 was used to analyze the data and determine associations between these biomarkers across different depression groups.

Results: Elevated serum IL-6 levels were found in both depression groups compared to healthy controls ($p < 0.001$). Serum cortisol levels were significantly higher in both depression groups as compared to controls, with a notable difference between the two depression types ($p < 0.001$). Serum serotonin levels were lower in both depression groups as compared to controls with no significant variation between melancholic and atypical depression ($p < 0.001$). Positive correlations were observed between serum IL-6 and cortisol ($p = 0.001$; $r = 0.629$) in melancholic depression, while IL-6 exhibited a negative correlation with serotonin ($p = 0.014$; $r = -0.411$) in atypical depression. Serum cortisol also displayed a negative correlation with serotonin in melancholic depression but the results were statistically insignificant ($r = -0.33$, $p = 0.05$).

Conclusion: This study revealed elevated cortisol & interleukin-6 levels and decreased serotonin levels in depression groups as compared to healthy controls. The melancholic & atypical depression subtypes did not significantly differ for interleukin-6 and serotonin levels. However, a significant elevation in serum cortisol levels was observed in patients with melancholic depression versus those with atypical depression.

Keywords: *Depression. Cortisol. Interleukin-6. Serotonin. Biomarkers.*

INTRODUCTION

Major depressive disorder (MDD) is commonly referred to as clinical depression and it is among the most prevalent psychiatric disorders, impacting around 280 million individuals globally.[1] The condition is more than just occasional sadness or mood fluctuations; it is a serious illness that can severely impair an individual's ability to carry out daily tasks, leading to poor quality of life. Depression often disrupts personal relationships and professional life, which can result in social isolation and the deterioration of self-esteem.[2] Importantly, depression is the primary psychiatric condition linked to suicide, with patients exhibiting the highest suicidal tendencies among those with mental health disorders.[3] Furthermore, untreated depression is associated with the early onset of various systemic diseases, including cardiovascular diseases, and can reduce life expectancy by up to seven years.[4]

It is identified by a persistent low mood lasting a minimum of two weeks, along with symptoms such as anhedonia (loss of interest or pleasure), low self-esteem, feelings of guilt, changes in appetite, sleep disturbances, and persistent fatigue.[5] It typically occurs in an individual's thirties, with a second peak in the fifties. The disorder also exhibits varying frequencies among different socio-economic and gender groups, with women and those in low socioeconomic strata more likely to experience depressive episodes.[6]

Melancholic and atypical depression are two subtypes of major depressive disorder. Melancholic depression is often marked by a loss of interest in activities that were once enjoyable, psychomotor retardation, weight loss, and anhedonia. Suicidal thoughts are common among individuals with this subtype.[7] Atypical depression is characterized by emotional reactivity, excessive sleep, weight gain, and leaden paralysis (feelings of heaviness in the limbs).[8]

The pathophysiology of depression encompasses a complex interplay among environmental, genetic, and neurobiological factors. Empirical evidence indicates a correlation between depression and the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, immune responses, and the serotonergic

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system.[9] Elevated interleukin-6 levels and disturbances in serotonin metabolism have been shown to contribute specifically to the pathogenesis of depression. Interleukin-6 is believed to affect serotonin production by activating the indoleamine 2,3-dioxygenase pathway, which results in decreased serotonin levels.[10] Additionally, the HPA axis, responsible for regulating the body's stress response, is frequently overactive in individuals with depression. This leads to increased cortisol levels, which may further worsen depressive symptoms.[11]

This study planned to evaluate the levels of serum cortisol, IL-6, and serotonin in patients with melancholic depression, atypical depression, and healthy controls. Previous studies did not analyze these markers collectively so, we measured these biochemical markers in two distinct subtypes of MDD, i.e. melancholic and atypical depression. Identifying these biomarkers could offer insights into the pathophysiology of depression and contribute to more effective & personalized treatment strategies for the diverse population of Pakistan.

METHODOLOGY

This cross-sectional comparative study was conducted from August 2023 to July 2024 at the Combined Military Hospital, Muzaffarabad. The biochemical assays and data analysis were performed at the Biochemistry Department, University of Health Sciences, Lahore after the approval of the project from advanced studies and research board (Letter No. UHS/REG-23/ERC/3142, 20-06-2023). The required sample size was calculated by the World Health Organization (WHO) sample size calculator, ensuring a study power of 96% and a significance level of 5%. The anticipated mean±standard deviation (SD) cortisol levels for group 1 (cases) and group 2 (healthy controls) were 11.92±4.2 ng/mL and 8.90±3.4 ng/mL, respectively.[4] Based on these parameters, the minimum sample size for each group was calculated to be 35, resulting in a total sample size of 105. The non-probability convenience sampling technique was used to enroll participants and they were divided into 3 groups with 35 each: melancholic depression, atypical depression and healthy controls. Those aged 18 years or above, patients meeting the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-5-TR) for melancholic & atypical depression were included in the study.[12] Pregnant females, subjects with organic mental illness or comorbid severe systemic illness were excluded from the study. A structured proforma was used to collect data after taking written informed consent. Healthy hospital controls were enrolled from the attendants of the patients visiting the hospital outpatient department (OPD). Patients visiting psychiatry OPD for depression were interviewed and labeled as having melancholic and atypical depression based on the criteria given in the DSM-5-TR along with psychometric assessments for the severity of depression based on the Beck Depression Inventory-II (BDI-II). According to the DSM-5-TR, early morning awakening, lack of mood reactivity, weight loss, psychomotor changes, and profound anhedonia were the symptoms of melancholic depression. In atypical depression, the patients present with increased appetite, weight gain, hypersomnia, leaden paralysis, mood reactivity, and sensitivity to rejection. Reliable and validated BDI-II has 21 items and each item scored 0-3 with a total score range from 0-63. This scoring shows the severity of depression as follows:

- **0-13:** Minimal depression
- **14-19:** Mild depression
- **20-28:** Moderate depression
- **29-63:** Severe depression[13]

Using aseptic techniques, 5 ml of blood sample was collected in a gel vacutainer between 8:00 AM to 10:00 AM. The sample was centrifugated at 6000 rpm for 10 minutes and serum was separated. The serum was preserved at -80°C before analysis. The levels of serum cortisol, interleukin-6, and serotonin were determined using enzyme-linked immunosorbent assay (ELISA). The Cortisol ELISA kit (Cal-biotech Inc., China, Lot #113324) was used to measure serum cortisol, with the assay based on competitive binding, where color intensity is inversely correlated with cortisol concentration. The IL-6 and serotonin ELISA kit (BT LAB China, Lot#113326 and 113319), used a capture antibody to bind IL-6/ anti-serotonin antibody, followed by horseradish peroxidase (HRP) conjugation and chromogenic substrate reaction. Absorbance readings were taken using a microplate reader (Biorad-450), and concentrations were calculated from standard curves, with cortisol and IL-6 in ng/ml and serotonin in pg/ml.

STATISTICAL ANALYSIS

The data was analyzed using Statistical Package for the Social Sciences (SPSS) version 25. Descriptive statistics were used for continuous variables, while categorical variables were presented as frequencies and percentages. The Chi-square test was applied for the comparison of categorical variables. For

normally distributed data, Analysis of Variance (ANOVA) was used to compare means of three groups and the Kruskal-Wallis test was utilized for non-normally distributed data. The correlation between serum cortisol, IL-6, and serotonin levels was examined using Pearson's correlation tests. A p-value of ≤0.05 was taken as significant.

RESULTS

A total of 105 participants were recruited in this study, comprising 35 in each group of melancholic, atypical depression, and healthy controls. The comparison of presenting symptoms, disease duration, symptom severity, and family history between melancholic and atypical depression groups showed notable trends. Somatic symptoms were more prevalent in both depression groups, 54.3% of patients with melancholic and 65.7% of patients with atypical depression reported these symptoms. In the atypical depression group, only 11.4% of patients presented with cognitive symptoms, whereas, in the melancholic group, 12.9% of patients had cognitive symptoms. Regarding disease duration, 57.1% patients in the melancholic group and 68.6% in the atypical group had been suffering for less than 6 months. Melancholic depression was associated with a greater proportion of severe cases (40%), while atypical depression was primarily characterized by mild symptoms (68.6%). A family history of psychiatric illness was seen in 2.9% patients of melancholic depression and 8.6% patients of atypical depression. There was no statistically significant difference observed between the groups for these variables (p >0.05).

Biomarker analysis revealed significant differences in serum levels of cortisol, serotonin, and IL-6 between the depression groups and healthy controls. Cortisol levels were significantly higher in melancholic depression (194.8±81.3 ng/ml) compared to atypical depression (155.3±61.7 ng/ml) and healthy controls (82.9±25.4 ng/ml) (p-value <0.001). Serotonin levels were significantly lower in both the depression groups (melancholic: 341.6±56.9 pg/ml, atypical: 347.2±83.6 pg/ml) as compared to healthy controls (741.2±89.0 pg/ml) with a p-value of <0.001. Interleukin-6 levels were statistically higher in both depression groups (melancholic: 122.4±66.4 ng/ml, atypical: 118.4±57.0 ng/ml) as compared to healthy controls (66.5±35.5 ng/ml) (p <0.001). A comparison of biomarkers between melancholic, atypical depression, and healthy controls is shown in Table 1.

Serum cortisol and IL-6 levels showed a statistically significant positive correlation in melancholic depression (r=0.629, p=0.001) and healthy controls (r=0.658, p=0.001), whereas no significant correlation was observed in atypical depression (r=0.318, p=0.063). In atypical depression, there was a significant negative correlation between IL-6 and serotonin (r=-0.411, p=0.014) versus melancholic depression or healthy control group. Serum cortisol and serotonin levels showed a significant negative correlation in patients with melancholic depression, whereas, no significant correlations in any other group (Table 2).

DISCUSSION

This study is conducted to determine three key factors considered to contribute to the development of depression: cytokines, stress hormones, and neurotransmitters. We found significantly higher serum cortisol and IL-6 levels & low serotonin levels in melancholic & atypical depression versus the healthy control group (p <0.001). These findings align with previous studies showing elevated IL-6 levels in MDD, reinforcing the concept that inflammation has a contributing role in the disorder's pathophysiology.[14]

Table 1: Comparison of Biomarkers between Melancholic, Atypical Depression, and Healthy Controls

Biochemical Markers	Melancholic Depression	Atypical Depression	Healthy Controls	p-value
	Mean±SD			
Serum Cortisol Levels (ng/ml)	194.8±81.3	155.3±61.7	82.9±25.4	<0.001*
Serum Serotonin Levels (pg/ml)	341.6±56.9	347.2±83.6	741.2±89.0	<0.001*
Serum IL-6 Levels (ng/ml)	122.4±66.4	118.4±57.0	66.5±35.5	<0.001*

**Significant p-value*

Table 2: Correlation Analysis of Serum Biomarkers in Different Groups

Groups	Parameters	Correlation Coefficient	p-value
Melancholic Depression	**Serum IL-6 Levels Serum Cortisol Levels**	0.629	0.001*
Atypical Depression	**Serum IL-6 Levels Serum Cortisol Levels**	0.318	0.063
Healthy Controls	**Serum IL-6 Levels Serum Cortisol Levels**	0.658	0.001*
Melancholic Depression	**Serum IL-6 Levels Serum Serotonin Levels**	-0.155	0.374
Atypical Depression	**Serum IL-6 Levels Serum Serotonin Levels**	-0.411	0.014*
Healthy Controls	**Serum IL-6 Levels Serum Serotonin Levels**	0.041	0.814
Melancholic Depression	**Serum Cortisol Levels Serum Serotonin Levels**	-0.33	0.05
Atypical Depression	**Serum Cortisol Levels Serum Serotonin Levels**	0.295	0.086
Healthy Controls	**Serum Cortisol Levels Serum Serotonin Levels**	0.008	0.962

**Significant p-value*

In depression, the innate immune system remains chronically activated, producing elevated levels of IL-6 and similar inflammatory mediators, which can interfere with neurotransmitter function, induce glucocorticoid resistance, and trigger maladaptive behaviors.[2,15] On the contrary, another study involving 4756 women found no significant relationship between IL-6 levels and depression incidence over 6-18 years, suggesting variability in findings depending on study design and population.[16]

Despite the significant elevation of IL-6 in both depression subtypes, our study found no distinction between melancholic and atypical depression regarding IL-6 levels. Another study observed no significant differences in IL-6 levels between depression subtypes.[17]

In this study, we observed a significant increase in serum cortisol levels in both melancholic (194.8±81.3 ng/mL) and atypical depression (155.3±61.7 ng/mL) as compared to controls (82.9±25.4 ng/mL) with a significant difference between the two depression subtypes ($p < 0.001$). Elevated cortisol levels in depression have also been depicted by Levi et al. and Patil et al.[18,19] Cortisol dysregulation is often linked to the HPA axis' impaired response to stress, and prolonged cortisol elevation can contribute to the maladaptive stress responses seen in depression. Moreover, melancholic depression may be characterized by more pronounced HPA axis hyperactivity.[19] In our study, melancholic depression exhibited significantly higher cortisol levels compared to atypical depression. Another study found that cortisol levels were significantly elevated in the depression group compared to healthy controls ($p=0.008$). However, no significant differences were observed between the group with depression and the psychiatric diseases group Additionally, cortisol levels were notably higher in individuals with depression ($p=0.004$) and in those with two or more suicide attempts ($p < 0.001$).[20]

The current study also measured serum serotonin levels. The mean serum serotonin (5-HT) levels were higher in healthy controls (741.2±89.0 pg/mL) compared to diseased patients with melancholic (341.6±56.9 pg/mL) and atypical depression (347.2±83.6 pg/mL) ($p < 0.001$). Our findings are in line with the theory that serotonin depletion in depression may be linked to immune system activation and the inflammatory response, which can suppress serotonin synthesis.[21] This is consistent with previous research by Colle et al. showing that individuals with depression have significantly lower serotonin levels compared to those without depression.[22] Obermanns et al. reported that depressed patients have lower levels of serotonin in both platelets and serum.[21] Cytokines like IL-6 are implicated in the suppression of serotonin synthesis through the activation of the enzyme indoleamine 2,3-dioxygenase, which diverts serotonin precursors to kynurenine instead of serotonin.[23] This process helps explain the lower serotonin levels observed in depression, as cytokines also modulate serotonin reuptake and metabolism.[24]

In this study, we found that interleukin-6 levels were statistically higher in both depression groups (melancholic: 122.4±66.4 ng/ml, atypical: 118.4±57.0 ng/ml) as compared to healthy controls (66.5±35.5 ng/ml) ($p < 0.001$). These findings align with those reported in recent studies. Koutentaki et al. and Li et al. reported a significant positive correlation between IL-6 and cortisol in patients with depression, supporting the idea that immune system activation via cytokines, such as IL-6, can stimulate the release of cortisol.[25,26]

Regarding serotonin, we found a significant negative correlation between IL-6 and serotonin in the atypical depression group ($r=-0.411$; $p=0.014$), similar to the study conducted by Falcicchia et al. which suggested that cytokines, including IL-6, may reduce serotonin production through the activation of the indoleamine 2,3 dioxygenase enzyme, which diverts tryptophan metabolism towards kynurenine production instead of serotonin.[27]

This study contributes valuable insights into the role of peripheral serotonin, cortisol, and interleukin-6 levels in the pathophysiology of melancholic and atypical depression. These findings may pave the way for more precise diagnostic approaches and tailored therapeutic strategies, ultimately alleviating the burden of this debilitating condition.

CONCLUSION

This study revealed elevated cortisol & interleukin-6 levels and decreased levels of serotonin in individuals with melancholic & atypical depression versus healthy control group. Serum interleukin-6 and serotonin levels did not change significantly in both melancholic & atypical depression subtypes. However, serum cortisol levels were significantly higher in the melancholic depression group as compared to atypical depression. A significant positive correlation was found between cortisol and interleukin-6 levels in melancholic depression and a significant negative correlation between IL-6 and serotonin in the atypical depression group and serum cortisol and serotonin levels in the melancholic depression group.

LIMITATIONS & RECOMMENDATIONS

The notable limitations include the cross-sectional study design, small sample size, and the limited cytokine analysis, focusing solely on IL-6. Furthermore, the reliance on morning cortisol samples may not fully reflect the chronic dysregulation of the HPA axis in depression. Future studies with larger sample size, longitudinal design, and a broader range of inflammatory markers are necessary to enhance our understanding of the complex mechanisms underlying MDD and improve clinical outcomes.

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Authors' Contributions:
A.T: Conduct experimental work, sample collection, and manuscript writing.
F.B: Study planning and manuscript review.
M.H.K: Patient selection and manuscript review.
M.A.S: Manuscript writing and critical analysis of findings.
N.W: Methodology drafting and implementation of study techniques.
A.S: Proofreading, grammar check, and manuscript review
S.H: Manuscript writing and statistical analysis.

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Original Article

Hepatoprotective Role of Silybum Marianum against Azithromycin-Induced Histological and Biochemical Changes in Albino Wistar Rats

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ABSTRACT

Objective: To observe the harmful effects of azithromycin on the liver of albino Wistar rats and analyze the protective effect of silybum marianum against azithromycin-induced histological and biochemical changes in their liver.

Methodology: This experimental study was conducted at the Baqai Medical University, Karachi from December 2023 to March 2024. After ethical approval, 30 male albino Wistar rats were categorized into three groups with 10 rats each. Group A was kept as a control, group B was given azithromycin 200 mg/kg/day for 7 days orally, group C received azithromycin 200 mg/kg/day for 7 days, and silybum marianum (seed extract) 100 mg/kg/day for 21 days orally. After the experiment, all the rats were sacrificed and 3 ml blood was drawn out by cardiac puncture for estimation of liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST) & alkaline phosphatase (ALP) and superoxide dismutase (SOD). The liver of the sacrificed rats was removed and stained with hematoxylin and eosin (H&E). Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 24.

Results: The mean levels of liver enzymes were significantly raised and SOD levels were significantly reduced in group B as compared to group A and C rats. The liver parenchyma of group B rats exhibited significant histopathological changes such as inflammation, hemorrhage, and steatosis, whereas, all group A and most of the group C rats showed no histopathological changes with statistical significance.

Conclusion: Azithromycin-treated rats showed significant biochemical and histopathological changes in the liver parenchyma as compared to control group rats. In azithromycin and silybum marianum-treated rats, the biochemical and histopathological changes were significantly reduced as compared to azithromycin-treated rats alone, indicating the hepatoprotective effects of silybum marianum.

Keywords: *Silybum marianum. Azithromycin. Superoxide dismutase. Aspartate aminotransferase. Oxidative stress.*

INTRODUCTION

Hepatotoxicity is an injury to the liver caused by various xenobiotics such as food additives, antifungal medicines, radioactive elements, environmental toxins, and drugs. It can be hepatocellular, cholestatic, and mixed, resulting in raised levels of liver enzymes including alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase.[1] Drugs that cause hepatic injury include chlorpromazine, amoxicillin, macrolides, tetracycline, metoclopramide, antiepileptic drugs, anti-tuberculous drugs, and chemotherapeutic drugs.[2]

Azithromycin is an effective, semisynthetic broad-spectrum macrolide, a derivative of erythromycin, that has been used to treat infections over the last 50 years.[3] It is used in the treatment of pulmonary infections, enteric fever, gastrointestinal diseases, infections of the genitourinary system, pharyngitis, and tonsillitis.[4] Azithromycin acts by preventing the growth of bacteria. It binds with the 23S rRNA of the 50S bacterial ribosomal subunit and inhibits their protein synthesis.[5] It is adequately absorbed orally and remains stable in gastric acid. It penetrates almost all body tissues and has the largest volume of distribution. Azithromycin is concentrated and excreted in the bile as an active drug and eliminated from the body through feces, and partially it is excreted in the urine.[6]

Hepatocellular injury induced by azithromycin appears in 1-3 weeks after commencement of treatment in 1-2% of cases. This results in the release of free radicals, these free radicals produce oxidative stress causing increased levels of liver enzymes and infiltration of neutrophils and phagocytes (Kupffer's cells). It results in cholestatic jaundice, hepatic failure, and even death. The histopathological changes that appear in the liver are inflammation, steatosis, congestion, and hemorrhage.[7]

Silymarin is a natural compound derived from the silybum marianum (milk thistle) family, Asteraceae Compositae. It inhibits the activity of free radicals because of its antioxidant properties. The extract of its fruit and seeds is used worldwide to treat liver diseases such as cirrhosis, alcoholic liver disease, viral hepatitis, and drug-induced hepatotoxicity.[8] Its anticancer, anti-fibrinolytic, anti-inflammatory,

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antiviral, and antibacterial effects are proven. It promotes protein synthesis and facilitates the reconstruction of damaged liver tissue.[9] Silymarin is quickly absorbed after oral administration and has good tissue distribution. It is excreted partly through the biliary system and partly by the kidneys.[10]

Post-COVID-19 pandemic, azithromycin has become the most widely used drug globally. Recently, complications caused by azithromycin have been reported more frequently. This drug can induce oxidative stress on the liver, potentially leading to hepatotoxicity. The current study aims to determine the antioxidative effects of silymarin seed extract along with their possible protective effects against azithromycin-induced hepatic injury, by utilizing histological and biochemical parameters.

METHODOLOGY

This experimental study was conducted from December 2023 to March 2024 in the animal house of Baqai Medical University, Karachi after approval from the Board of Advanced Study and Research, Baqai Medical University, and the ethical committee (Letter No. BMU-EC/02-2022, 25-04-2022). A sample size of 30 was collected through the Resource Equation method.[11] A total of 30 healthy, adult, male albino rats aged 10-12 weeks, and weighing 150- 200 grams were included. Any sick rats were excluded from the study. The rats were marked using a permanent marker and separated randomly into three groups (10 per group): A, B, & C. Group A was kept as a control group. Group B was given azithromycin 200 mg/kg/day for 7 days.[12] Group C was given silybum marianum seed extract 100 mg/kg/day orally in the morning for 21 days, and azithromycin 200 mg/kg/day for 7 days. After the weight measurement, the animals were kept in plastic cages in natural 12-hour day or light and 12-hour night or dark cycles. The room temperature was kept constant at 21-24°C, with humidity ranging from 60-70%. They were given a laboratory pellet diet and water ad libitum. Acclimatization was conducted one week before the study to assess their physical condition based on their behaviour, weight changes, and activities.

To produce hepatocellular injury, Zyto (HIGH-Q) tablets (Generic Name Azithromycin) were bought from a local drugstore. The seeds of silybum marianum were ordered from the Shaheen Chemist in Islamabad. The Department of Pharmacognosy, University of Karachi, verified its botanical identification, and a voucher number (SMS-07-22) was generated. Seed extract of silybum marianum was prepared for the study after mixing it with ethanol. The 1000 g seeds of silymarin were crushed into fine powder and soaked in 1.5 litres of 99.8% ethanol. After three days, filtered through filter paper, the extract was evaporated to dryness in a rotary evaporator and again re-soaked in ethanol and filtered, and dried. The 1000 g of silymarin seed powder yielded 46 g of ethanol extract, which is 4.6% of the dry weight of silymarin seeds. Silybum marianum seed extract 100 mg/kg/day was given orally to rats for 21 days in group C.[13] Rats of groups A, B, & C were sedated and sacrificed on the 22nd day of the study. The blood was drawn by cardiac puncture and stored in ethylenediaminetetraacetic acid (EDTA) tubes and gel-containing tubes for liver function tests and superoxide dismutase (SOD). The livers were dissected out and fixed in 10% formalin. Then it was processed through fixation, embedding, dehydration, and clearing into tissue blocks. The tissue was cut into 5-micron-thick slices with the help of a rotary microtome and then stained with Hematoxylin and Eosin (H&E). A light microscope was used to detect the histopathological changes, i.e., inflammation, hemorrhage, and steatosis.

The grading system ranged from 0 to 3, with each value representing a specific degree of histopathological change. The grades were determined as a percentage to measure the extent of the detected histopathological changes. Grade 0 (zero) signified a negative outcome, while grades 1(0-30%), 2(31-50%), and 3(51-100%) denoted mild, moderate, and severe changes, respectively.

STATISTICAL ANALYSIS

The Statistical Package for the Social Sciences (SPSS) version 24 was used to analyze the data. The normality of the data was assessed using Shapiro-Wilk. The data was found to be normally distributed ($p > 0.05$). The biochemical parameters i.e. lipid profile (ALP, AST, and ALT) and SOD were expressed as mean±standard deviation (SD) and all groups were compared using a one-way ANOVA and Post-Hoc Tukey's test. For the analysis of histopathological parameters, Fisher's exact test was used. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The results revealed that the mean levels of hepatic enzymes were significantly raised in group B as compared to groups A and C. The mean SOD levels were significantly decreased in group B rats as compared to groups A and C (Table 1).

The comparison among the groups exhibited that the levels of hepatic biomarkers in the group B rats were remarkably raised ($p < 0.05$) than the mean AST, ALT, and ALP levels of the group A and group C rats. The mean AST, ALT, and ALP levels of the group C rats were not notably different ($p > 0.05$) from those of the group A rats. The mean serum SOD level of the group B rats was significantly lower ($p < 0.05$) than the mean serum SOD level of the group A and group C rats as displayed in Table 2. The microscopic examination of H&E-stained liver sections of groups A & C showed normal architecture of the liver. It is shown in Figures 1 and 2, respectively. The liver parenchyma of group B rats exhibited significant histopathological changes such as inflammation, hemorrhage, and steatosis as shown in Figures 3, 4, & 5.

All rats in group A showed no histopathological changes. In azithromycin-treated group B, 10(100%) rats exhibited moderate inflammation. Mild steatosis was seen in 4(40%) and moderate steatosis in 4(40%) of group B rats. Five (50%) showed mild congestion and hemorrhage was observed in 5(50%) and moderate in 4(40%) of the rats in group B. Absence of inflammation, steatosis, congestion & hemorrhage was observed in 6(60%), 8(80%) and 7(70%) of group C rats, respectively. The histopathological changes of group C rats were mostly of mild severity. All these results were statistically significant (Table 3).

Table 1: Comparison of Biomarkers (ALT, AST, ALP & SOD) among the Groups (one-way ANOVA)

Variables	Group A (Mean±SD)	Group B (Mean±SD)	Group C (Mean±SD)	p-value
AST Levels (IU/L)	86.2±4.2	113±5.8	91.6±9.7	<0.05*
ALT Levels (IU/L)	50.7±4.0	174.7±5.7	52±9.1	
ALP Levels (IU/L)	42.3±3.6	169±5.6	45.5±4.0	
SOD Levels (U/mL)	3.16±0.9	0.54±0.4	3.03±1.1	

**Significant p-value*

Table 2: Differences in Mean Levels of AST, ALT, ALP & SOD between Different Groups (Post-Hoc Tukey's Test)

Groups Comparison	AST (IU/L)		ALT (IU/L)		ALP (IU/L)		SOD (U/mL)	
	Differences of Mean	p-value	Differences of Mean	p-value	Differences of Mean	p-value	Differences of Mean	p-value
A & B	26.80000	0.000*	124.00000	0.000*	126.70000	0.000*	-2.60000	0.001*
B & C	21.40000	0.000*	122.70000	0.000*	123.50000	0.000*	-2.49600	0.001*
A & C	5.40000	0.125	1.30000	0.990	3.20000	0.992	-0.12900	0.997

**Significant p-value*

Table 3: Association of Histopathological Changes in the Liver among Groups A, B, & C Rats

Parameters		Groups (Frequency & Percentage)			p-value
		A (n=10)	B (n=10)	C (n=10)	
Inflammation	**None**	10(100%)	0(0%)	6(60%)	0.001*
	Mild	0(0%)	0(0%)	3(30%)	
	Moderate	0(0%)	10(100%)	1(10%)	
Steatosis	**None**	10(100%)	2(20%)	8(80%)	0.001*
	Mild	0(0%)	4(40%)	2(20%)	
	Moderate	0(0%)	4(40%)	0(0%)	
Congestion & Hemorrhage	**None**	10(100%)	1(10%)	7(70%)	0.001*
	Mild	0(0%)	5(50%)	3(30%)	
	Moderate	0(0%)	4(40%)	0(0%)	

**Significant p-value*

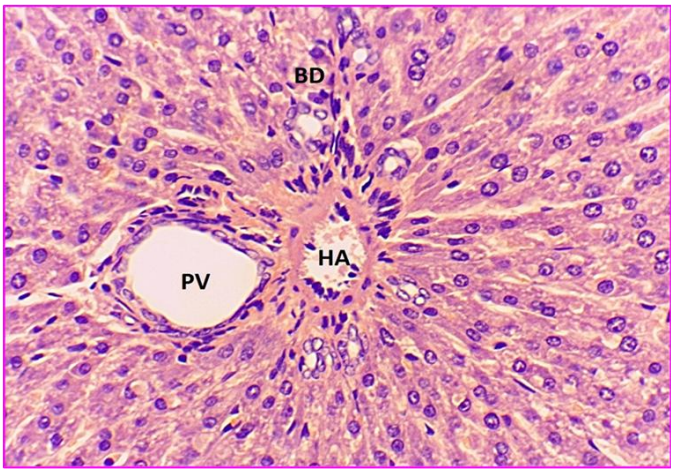

Figure 1: Liver Section of All Group A Rats shows the Normal Portal Area with Bile Duct (BD), a Branch of the Hepatic Artery (HA), and Portal Vein (PV), Radiating Sinusoids (H&E stain, 400X magnification)

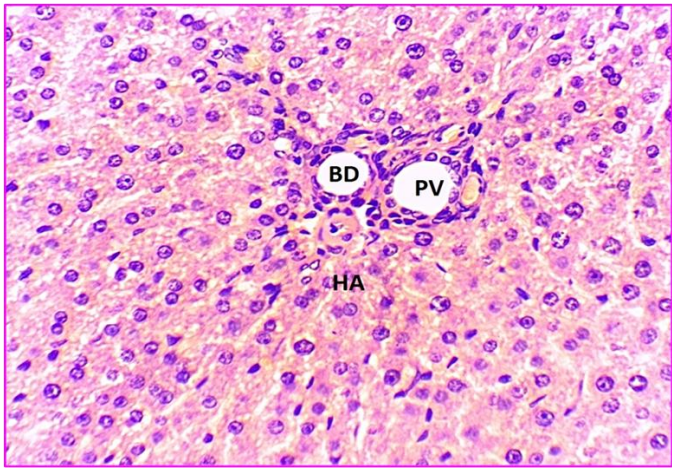

Figure 2: Microscopic Picture of a Section of the Liver of Group C (silymarin-treated) Rats showing a Normal Portal Area, no Inflammatory Cells Seen. Bile Duct (BD), and a Branch of the Hepatic Artery (HA), the Portal Vein (PV) (H&E stain, 400X magnification)

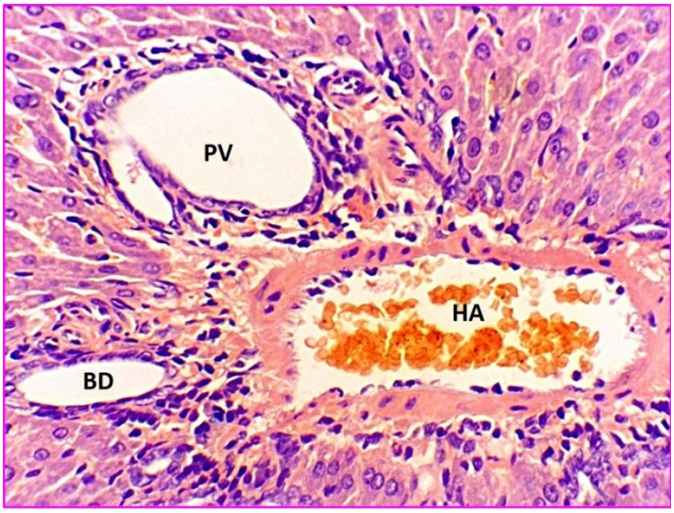

Figure 3: Microscopic Image of the Liver of Group B Rat Displaying Marked Infiltration in the Portal Area by Inflammatory Cells, Portal Vein (PV), Congested Hepatic Artery (HA), and Bile Duct (BD) (H&E stain, 400X magnification)

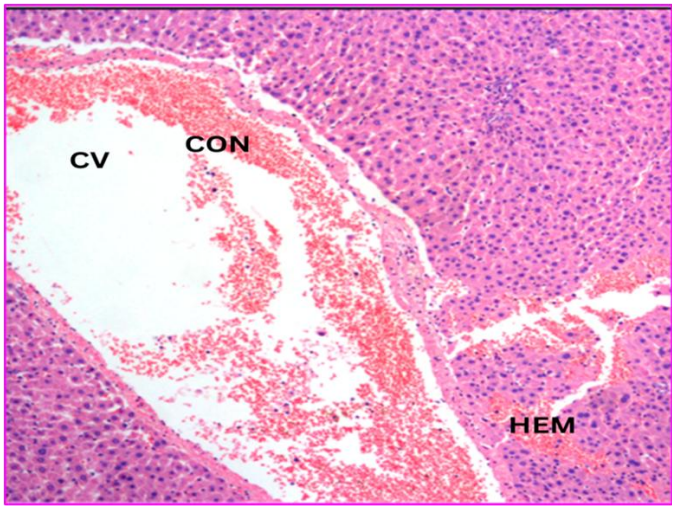

Figure 4: Photomicrograph from a Section of Hepatic Tissue of Group B Rats showing Congestion (CON) in the Central Vein (CV), and Hemorrhage (HEM) between the Hepatocytes (H&E stain, 100X magnification)

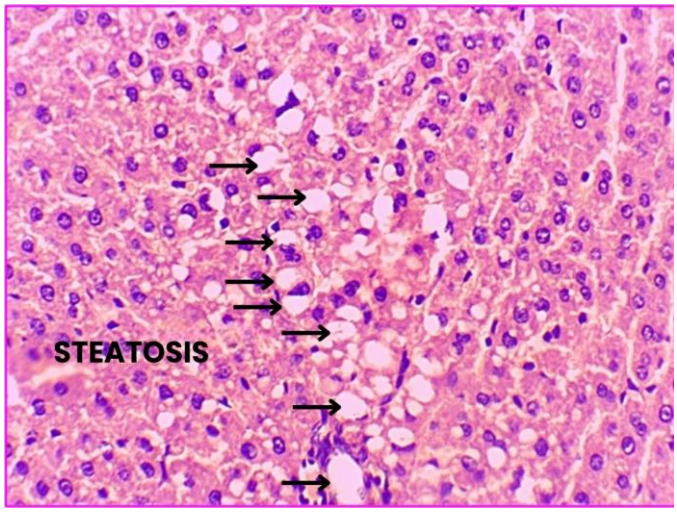

Figure 5: Photomicrograph of a Section of Group B Rat Liver showing Mild Steatosis (Arrow) (H&E stain, 400X magnification)

DISCUSSION

Our results showed significantly increased levels of liver enzymes (ALT, AST and ALP) and decreased SOD levels in group B azithromycin-treated rats as compared to group A control group. Similar to our results, Omara et al. showed that azithromycin-treated rats had a significant increased ALT & AST and decreased SOD levels. This is attributed to damage to liver cells caused by azithromycin resulting in cellular leakage, dysfunction, and liberation of free radicals.[14] Ali et al. also reported that azithromycin-induced hepatotoxicity is indicated by a significant increase in hepatic enzymes and decreased SOD levels. The levels of ALT, AST, and ALP were increased by 131.8%, 75.2%, and 153.6%, respectively in azithromycin-treated rats as compared to controls. The SOD levels were decreased by 607.4% in azithromycin-treated rats. The study linked these findings to oxidative stress, mitochondrial dysfunction, and bile transport inhibition leading to cell death due to azithromycin.[15] In another study conducted by Dadoub et al. in 2022, ALT and AST levels of the control group (21.5±2.5, 41.87±2.98) were significantly lesser as compared to those of azithromycin-treated rats (ALT: 76.29±3.6, AST: 80.7±1.9). The antioxidant SOD levels were also significantly reduced in azithromycin-treated rats. The antioxidative enzyme superoxide dismutase controls oxidative stress by preventing the liberation of free radicals. Cells rely on SOD as their primary intracellular defense mechanism against oxidative stress caused by free radicals. Superoxide dismutase acts as a catalyst in the conversion of superoxide radicals ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2) and oxygen (O_2).[16] In a study conducted in 2022, Hamza et al. also observed decreased level of SOD in hepatotoxicity.[17] These results are consistent with our findings, in which decreased SOD levels were observed in group B compared to group A.

The liver enzymes in group C rats of our study were significantly decreased as compared to group B. The levels of SOD were also increased due to the antioxidant effects of silymarin. Silymarin reduces oxidative stress and inhibits the liberation of free radicals, thus enhancing the healing process. A review done by Karimian et al. showed that silymarin is the preferred natural chemical for the treatment of liver diseases in rats due to its antioxidant and free radical scavenging effects.[18] Radwan et al. also showed a significant increase in liver enzymes in azithromycin-treated rats as compared to controls. The ALT, AST & ALP levels (99.17±3.9, 136.17±4.7 and 395.8±13.7, respectively) were significantly reduced in azithromycin-treated rats as compared to silymarin and azithromycin-treated rats (ALT: 59.83±2.7, AST: 104.50±4, ALP: 148.6±4.9). A significant increase in SOD levels was also observed in silymarin and azithromycin-treated rats.[19]

In this study, microscopic examination of the liver sections of rats of all three groups revealed that the rats of group B underwent significant histological changes as compared to the other two groups. We observed a marked distortion in the hepatic parenchyma. Inflammation of hepatocytes indicated by swelling and ballooning with the disturbance in the arrangement of the hepatic cords were seen. In addition, steatosis of microvacuoles with small droplets of fat showed hyperchromatic nuclei in the cytoplasm of the hepatocytes. A similar study was conducted by Ortiz et al. in 2021, which showed similar findings of steatosis and ballooning of hepatocytes due to the liberation of free radicals and oxidative stress. The study also observed dilated portal vein, mononuclear cell infiltration, congestion, and hemorrhage in the periportal area.[20] Fotouh et al. in 2023 described that after administering Azithromycin, areas of coagulative necrosis were observed. These changes were characterized by intense sinusoidal congestion and the appearance of new blood vessels. Researchers linked these changes to mitochondrial dysfunction resulting from raised reactive oxygen species and reduction of adenosine triphosphate.[21]

Dilatation of the central vein and hepatic sinusoids, along with hepatocellular infiltration around the central vein with lymphocytes and eosinophils and infiltration of neutrophils was also observed in our study. Similar findings were also described by Shiri Aghbash et al., who observed that the infiltration of neutrophils and Kupffer's cells occurred due to oxidative stress and liberation of free radicals in azithromycin-induced hepatotoxicity.[22] Other studies demonstrated that silymarin reduces liver damage by neutralizing free radicals and inhibiting lipid peroxidation in membrane-bound fatty acids after exposure to hepatotoxic substances.[23,24]

A randomized controlled trial conducted by Jin et al. demonstrated that silymarin supplementation significantly decreased liver stiffness and improved liver function in patients with metabolic dysfunction-associated steatotic liver disease, highlighting its potential in managing liver conditions associated with metabolic dysfunction.[25] Similarly, another clinical trial evaluated silymarin's efficacy in preventing anti-tuberculosis drug-induced liver injury, finding that patients receiving silymarin

exhibited lower levels of liver enzymes and a reduced incidence of hepatotoxicity compared to the control group.[26]

CONCLUSION

Azithromycin-treated rats showed significant biochemical and histopathological changes in the liver parenchyma as compared to control group rats. In azithromycin and silybum marianum-treated rats, the biochemical and histopathological changes were significantly reduced as compared to azithromycin-treated rats alone, indicating the hepatoprotective effects of silybum marianum.

LIMITATIONS & RECOMMENDATIONS

The study determined exclusively the effects of acute exposure to azithromycin, potentially neglecting essential effects related to chronic use or drug accumulation. Further studies should be conducted to observe the long-term effects of azithromycin on the liver by administering azithromycin for weeks or months. It is also suggested that a human trial is recommended to suggest the hepatoprotective effect of silymarin with the administration of azithromycin.

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Authors' Contributions:
S.A: Conception, design, data collection, analysis, and interpretation
M.T: Statistical analysis and interpretation of data
U.B: Analysis of histological slides
S.I.A: Critical revision and final approval of the article
T.K: Drafting of the article
M.M.H: Contribution to the methodology (silymarin seeds extract formation)

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Original Article

Cardiac Defects in Neonates Born to Mothers with Gestational Diabetes Mellitus and their Association with Maternal Treatment Regime

Amna Siddique, Muhammad Umar Rasool, Riffat Naeem, Arooj Mirza, Salwa Naeem, Shahid Hamid

ABSTRACT

Objective: To determine the type of cardiac anomalies on echocardiography in neonates delivered to mothers diagnosed with gestational diabetes mellitus (GDM) and evaluate the association of neonatal cardiac defects with maternal treatment during pregnancy.

Methodology: This cross-sectional study was conducted at the Department of Pediatrics, Ittefaq Trust Hospital, Lahore from August 2024 to January 2025 after ethical approval. A total of 108 neonates delivered to mothers with gestational diabetes were included using non-probability consecutive sampling technique. After taking informed consent from parents, they were screened by using echocardiography and neonatal cardiac anomalies were noted, if present. All the neonates with cardiac anomalies were followed-up in outpatient department (OPD) for spontaneous closure of defect for 3 months. The data was analyzed by the Statistical Package for the Social Sciences (SPSS) version 25.

Results: The mean age of neonates at presentation was 2.00±0.72 days. Gestational diabetes was diagnosed in the second trimester in 72(66.7%) and the third trimester in 36(33.3%) mothers. Out of 108 females, 12(11.1%) were only on insulin, 76(70.4%) were prescribed metformin only, 16(14.8%) were taking combination of metformin with insulin and 4(3.7%) were on diet control. On echocardiography, patent ductus arteriosus (PDA) was detected in 56(70%) cases, ventricular septal defect (VSD) in 8(10%) neonates, septal hypertrophy with PDA in 8(10%) cases, VSD with PDA in 8(10%) cases while 28(25.9%) had normal cardiac functioning. At follow-up, spontaneous closure of defect occurred in 68(85%) neonates with congenital cardiac defects. On comparison, PDA was significantly more common in neonates of diabetic mothers on metformin ($p < 0.05$).

Conclusion: Neonates born to diabetic mothers had a high frequency of cardiac abnormalities (74%). The risk of cardiac anomalies was significantly high in neonates born to females with GDM who were treated with metformin alone.

Keywords: *Gestational diabetes. Echocardiography. Patent ductus arteriosus, Ventricular septal defect.*

INTRODUCTION

The World Health Organization defines gestational diabetes as the detection of blood glucose levels of more than 180 mg/dl at 2 hours post-prandial, after 20th week of gestation on oral glucose tolerance test (OGTT). Gestational diabetes affects around 14% of pregnancies worldwide. Its incidence varies depending on risk factors, screening and diagnosis methods. The prevalence of the disease is increasing in association with the growing rates of type 2 diabetes and obesity.[1,2] Consequently, the prevalence rate of GDM in Pakistani research ranged from 4.41% to 57.90%.[3]

During early pregnancy, sensitivity to insulin increases with higher uptake of glucose to cope up with the energy needs during pregnancy. During the second or third trimester, various hormones produced during pregnancy, for example, progesterone, placental growth hormone, lactogen, estrogen and cortisol cause resistance to insulin. This decreases the transport of glucose into the cells.[4] Developing novel diabetes treatments may depend on our ability to comprehend how placental signals improve beta cell (β-cell) secretion and insulin production.[5,6]

In GDM, β-cell dysfunction arises from reduced incretin hormones (glucose-dependent insulin tropic polypeptide, glucagon-like peptide-1), cytokine-induced insulin signaling disruption (via interleukin-6, tumor necrosis factor-α), and cellular stressors like endoplasmic reticulum stress, mitochondrial dysfunction, and decreased pancreatic duodenal homeobox-1 expression - leading to impaired insulin secretion and glucose uptake.[7] In addition to promoting hyperinsulinemia, reduced glucose uptake, glycogen synthesis, and a diminished ability of insulin to regulate hepatic gluconeogenesis, human placental growth hormone and pituitary growth hormone have diabetogenic actions.[8]

There are various predisposing factors of gestational diabetes such as increased age, obesity and family history or previous history of diabetes. Gestational diabetes is linked with poor maternal and perinatal outcomes such as cesarean sections, polyhydramnios, preeclampsia, premature rupture of membrane, preterm delivery, stillbirth, macrosomia and neonatal hyperbilirubinemia.[9]

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Hyperglycemia is also a known contributing factor to the development of congenital defects, or structural or functional anomalies that occur during intrauterine life. This can be detected throughout pregnancy or at any point during 6 weeks of delivery.[10,11] Poor maternal glycemic management is linked to a high prevalence of congenital abnormalities. This also demonstrated a nearly universal lack of preconception care.[2]

Through literature, it has been observed that the risk of cardiac anomalies is high among neonates delivered to diabetic mothers and needs careful screening and management. However, in routine, neonates are not screened at the time of delivery and develop symptoms later, which leads to severe hazardous consequences. This study was planned to determine the frequency and type of cardiac anomalies on echocardiography in neonates delivered to mothers diagnosed with gestational diabetes mellitus and evaluate their association with treatment modalities among mothers. On the basis of findings, the early screening of neonates for cardiac anomalies, especially among diabetic mothers and those who were taking hypoglycemic medications can be recommended. The local data regarding association of cardiac defects with treatment modalities of mothers with GDM was sparse, so our results will also add knowledge in this regard as well.

METHODOLOGY

This cross-sectional study was conducted at the Department of Pediatrics, Ittefaq Trust Hospital, Lahore from August 2024 to January 2025 after approval from the institutional ethical review board (Letter No. IHT/Adm/30, 18-04-2024). A sample size of 108 neonates was estimated by keeping 95% confidence level, 7% absolute precision and the percentage of abnormal fetal echocardiographies in neonates delivered to mothers with gestational diabetes mellitus as 15.8%.[3] The parents gave written informed consent for inclusion in the study. A total of 108 neonates were enrolled by non-probability consecutive sampling technique from delivery wards and neonatal intensive care unit (NICU). The inclusion criteria were neonates of age 3-28 days of life, who were delivered at term (>37 weeks of gestation), and whose mothers had confirmed gestational diabetes. Gestational diabetes mellitus was confirmed after 20th weeks of gestation using 75-gram OGTT. According to the International Association of Diabetes and Pregnancy Study Groups, diagnosis of GDM was based on 1-hour postprandial glucose levels $\geq$153mg/dl, 2-hours postprandial glucose levels $\geq$180 mg/dL or fasting plasma glucose $\geq$92 mg/dL.[2] Neonates delivered to mothers with chronic diabetes, mothers with heart disease, family history of congenital heart disease, hypertensive disorder, and history of exposure to cardiac teratogens were excluded from the study. Demographic data like age of neonate at presentation, gravidity, mode of delivery, weight of neonate at birth, any other non-cardiac problem, and record of mother on anti-glycemic treatment regimen were noted on the proforma. At the time of presentation, after initial clinical examination, all the neonates were screened by using echocardiography and neonatal cardiac anomalies were noted, if present. All the neonates with cardiac anomalies were followed-up in outpatient department (OPD) for spontaneous closure of defect for 3 months.

STATISTICAL ANALYSIS

The data was analyzed by the Statistical Package for the Social Sciences (SPSS) version 25. Mean±SD were used for numerical data and categorical data was reported as frequency and percentage. Fisher's exact test was applied to compare cardiac anomalies and various treatment modalities in mothers. A p-value $\leq$0.05 was taken as significant.

RESULTS

In this study, we enrolled a total 108 neonates delivered to mothers with gestational diabetes for echocardiographic assessment. The mean weight of neonates at presentation was 3.00±0.59 kg. Table 1 shows the clinical data of the study participants.

On 1st echocardiography (done after 72 hours), 80(74.1%) neonates had cardiac anomalies while 28(25.9%) had normal cardiac functioning. Out of 80 neonates diagnosed with cardiac anomalies, patent ductus arteriosus (PDA) was detected in 56(70%), ventricular septal defect (VSD) in 8(10%), septal hypertrophy with PDA in 8(10%) and VSD with PDA in 8(10%) cases (Figure 1).

On comparison, PDA (63.2%) was more common in neonates of diabetic females taking metformin as compared to other congenital cardiac defects (p=0.001). Among neonates born to diabetic mothers on insulin, PDA and VSD with PDA were observed in 33.3% of the neonates each. At follow-up, defect was spontaneously closed in 68(85%) neonates. Spontaneous closure of defect (88.2%) was also high in neonates of diabetic mothers on metformin (p=0.001) (Table 2).

Table 1: Clinical Data of Mothers enrolled in the Study (n=108)

Characteristics		Frequency & Percentage
Gravidity	**Primigravida**	24(22.2%)
	Multigravida (Gravida 2-4)	76(70.4%)
	Grand Multigravida (Gravida ≥5)	8(7.4%)
Mode of Delivery	**Vaginal Delivery**	8(7.4%)
	Cesarean Section	100(92.6%)
Time of GDM Diagnosis	**2nd Trimester**	72(66.7%)
	3rd Trimester	36(33.3%)
Treatment Modalities of Diabetic Mothers	**Insulin only**	12(11.1%)
	Metformin only	76(70.4%)
	Insulin with Metformin	16(14.8%)
	Diet Control	4(3.7%)

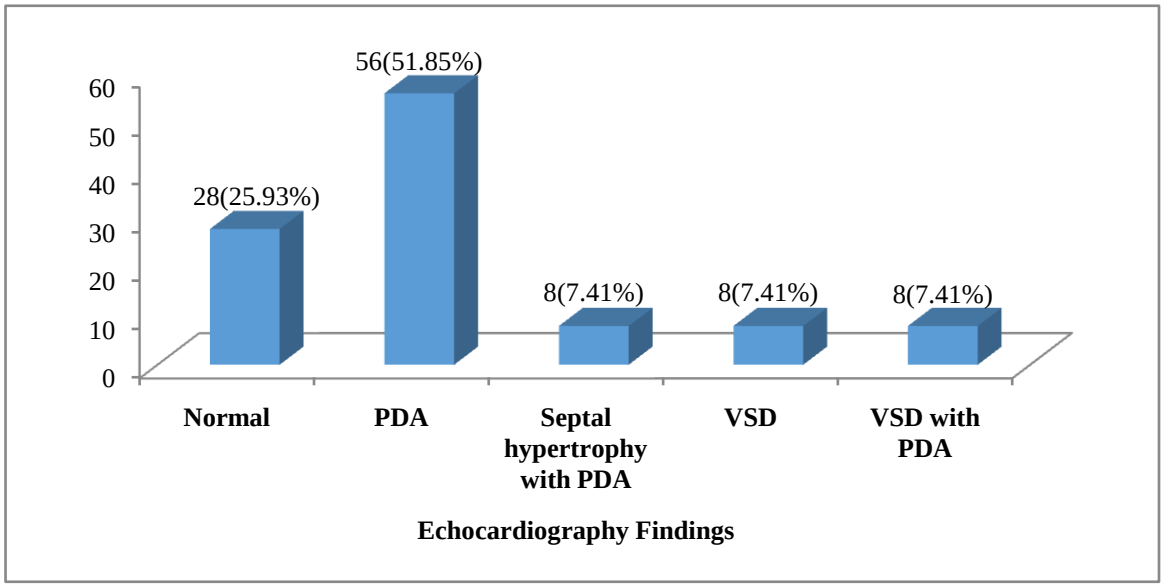

Figure 1: Distribution of Echocardiography Findings of Neonates

Table 2: Association of Neonatal Echocardiography Findings and Treatment Modalities of Diabetic Mothers

Echocardiography Findings	Treatment Modalities				p-value
	Insulin (n=12)	Metformin (n=76)	Combination of Insulin with Metformin (n=16)	Diet Control (n=4)	
Normal	4(33.3%)	8(10.5%)	12(75.0%)	4(100%)	0.001*
PDA	4(33.3%)	48(63.2%)	4(25.0%)	0(0%)	
Septal Hypertrophy with PDA	0(0%)	8(10.5%)	0(0%)	0(0%)	
VSD	0(0%)	8(10.5%)	0(0%)	0(0%)	
VSD with PDA	4(33.3%)	4(5.3%)	0(0%)	0(0%)	

*****Significant p-value*

DISCUSSION

Pregnancy-related hyperglycemia is a hallmark of gestational diabetes. Gestational and pre-gestational diabetes have a negative impact on pregnancy and the postpartum period. The fetus develops hyperglycemia and hyperinsulinemia due to maternal hyperglycemia. This promotes anabolism, which in turn promotes the growth of connective tissue, muscle, and fat. When hyperglycemia and hyperinsulinemia coexist, fetal fat and protein accumulation increases, leading to macrosomia.[12] Paauw et al. also reported that hyperinsulinism had hyperglycemia, which can result leading to development of cardiac hypertrophy in neonates.[13] A

clinical review on diabetes during pregnancy found that both pre-gestational and gestational diabetes were associated with increased risk of congenital defects and cardiac anomalies were the most commonly observed defects.[14] Bayoumy et al. used tissue Doppler imaging to study cardiac function and found that fetal cardiac function at 30 weeks of gestation was compromised in diabetic pregnant females as compared to normal pregnant females.[15]

In this study, we observed that 80(74.1%) neonates had congenital cardiac defects. Patent ductus arteriosus was the most common congenital cardiac anomaly (70%), followed by VSD in 10% neonates, septal hypertrophy with PDA in 10% and VSD with PDA in 10% of the cases. Afridi et al. performed comparable research in Karachi and found that 67(45.57%) of the 147 newborns of mothers with GDM had a congenital cardiac abnormality. Unlike our study, patent foramen ovale (PFO) was the most prevalent cardiac abnormality (23%), followed by PDA (14.9%), VSD (5.4%), tetralogy of Fallot (TOF) (2.7%), hypertrophic cardiomyopathy (HCM) (2.7%), atrial septal defect (ASD) (2.7%), and transposition of the great arteries (TGA) (1.36%).[16]

A study done by Arjmandnia et al. in Iran also reported that 49% neonates born to diabetic mothers had congenital cardiac defects. Contrary to our results, TOF (69.5%) was most prevalent in neonates followed by PDA (41%).[17] Similar to our results, Sadiq et al. from Peshawar found that on echocardiographic assessment of neonates, PDA (32%) was the most common congenital heart defect, followed by PFO (29.33%), HCM (14%), ASD (10%), VSD (6.66%), TGA (4.66%) and TOF (3.33%).[18] According to a study conducted in Faisalabad, Pakistan, 47.3% of the children born to a diabetic mother had abnormal echocardiogram results. The most common abnormalities were PDA (32.6%), VSD (25.3%), ASD (14.5%), TGA (7.9%), and hypertrophic obstructive cardiomyopathy (5.7%).[19] Various approaches to control diabetes, different sampling populations and methods for detecting cardiac abnormalities might all contribute to the variation in regional, national and international frequencies.

Our statistically significant results showed that all congenital anomalies were more common among neonates of diabetic females taking metformin as hypoglycemic treatment. The most prevalent cardiac defect in neonates of GDM mothers on metformin was PDA (63.2%), followed by septal hypertrophy with PDA (10.5%), VSD (10.5%) and VSD with PDA (5.3%). This difference might be due to variation in glucose levels in mothers taking different regimes. Mothers taking insulin might have better glycemic control resulting in decreased frequency of anomalies in these patients. Similarly, a cohort study reported that children born to mothers with pre-existing diabetes who were exposed to second-line anti-diabetic medications during pregnancy had a higher incidence of major congenital anomalies or cardiac malformations compared to those exposed to insulin.[20] A study done in Saudi Arabia also reported that PDA (71.5%), followed by hypertrophic cardiomyopathy (36.5%) and VSD (11%) were the most common congenital cardiac anomalies among infants of diabetic mothers. Out of 293 diabetic mothers included in their study, 43.7% were on diet control, 33.1% were on insulin, 16.4% were on medications, and 6.8% had a combined treatment regimen. However, no significant association ($p=0.313$) was found between different treatment modalities and the frequency of congenital cardiac defects.[21]

During 3 months follow-up, we also observed that spontaneous closure of defect was high in neonates of mothers treated with metformin (78.9%), compared to those treated with insulin (33.3%), or with combination treatment (25%) ($p <0.05$). Patent ductus arteriosus is a congenital cardiac condition in which the ductus arteriosus of a newborn does not shut after birth. Usually, the PDA closes 48 hours after delivery.[22] In literature, the rate of spontaneous closure of PDA was about 66% within the first year of life that increased to 80% during the first five years of life.[23]

Another study reported that out of all pediatric cardiomyopathies, 25-40% of neonates had cardiac hypertrophy. The ventricular function after delivery had been associated with elevated maternal lipid and glucose indices. These results pointed to a possible link between future cardiovascular health and in-utero cardiac development.[24]

CONCLUSION

Neonates born to diabetic mothers had a high frequency of cardiac abnormalities (74%). The risk of cardiac anomalies was significantly high in neonates born to females with GDM who were treated with metformin alone.

LIMITATIONS & RECOMMENDATIONS

The study had a few limitations including single-centered and cross-sectional study design. The data on glycemic control of mothers during pregnancy is lacking. The risk of cardiac defects in mothers taking metformin was high which needs to be investigated in relation to glucose levels and

glycemic control of mothers. We recommend screening of the neonates of diabetic mothers for congenital heart disease. Furthermore, it is also recommended to conduct a multi-centered trial on different methods for the prevention of congenital heart disease in newborn and plan strategies for early cure.

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Authors' Contributions:
A.S: Topic selection, study design, and methodology.
M.U.R: Data collection and entry.
R.N: Literature review, referencing, and quality assurance.
A.M: Statistical analysis and result interpretation.
S.N: Plagiarism check and clearance.
S.H: Proofreading and final approval.

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Original Article

Attitudes of Medical Faculty Enrolled in Masters in Health Professions Education towards Professionalism in a Private Pakistani University: A Mixed-Methods Study

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ABSTRACT

Objective: To examine the attitudes of medical faculty enrolled in the Masters in Health Professions Education (MHPE) program towards professionalism at Shifa Tameer-e-Millat University, Islamabad.

Methodology: The study was conducted at Shifa Tameer-e-Millat University, Islamabad from August 2024 to January 2025 after ethical approval. After taking informed consent, universal sampling technique was used to enroll all 40 MHPE faculty members. Using a convergent parallel mixed-methods design, both quantitative and qualitative data were gathered to explore dimensions of professionalism including excellence, altruism, honor & integrity, accountability, duty and respect. A 36-item validated Likert-scale questionnaire assessed attitude of MHPE faculty, complemented by four in-depth semi-structured interviews. Quantitative data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 23. For the analysis of qualitative data, Notta software was used.

Results: Quantitative analysis revealed most of the participants were females [28(70%)] and belonged to MBBS background [21(52.5%)]. Our participants exhibited poor professional attitudes indicated by low mean scores of professionalism (2.07±0.45) and its six domains. A significant positive correlation was present between professionalism and variables such as excellence (r=0.77), duty (r=0.68), and altruism (r=0.68). Regression analysis confirmed these as key predictors. Qualitative themes highlighted challenges in curriculum integration, resource gaps, and the influence of institutional culture.

Conclusion: Our results showed poor attitudes of MHPE enrolled medical faculty towards professionalism indicated by low mean scores of professionalism and its six domains of altruism, respect, honor & integrity, accountability, duty, and excellence. Our thematic analysis identified professionalism as one of the fundamentals of medical education. Time & resource constraints and role ambiguity were major challenges towards integration of professionalism into medical courses and the role of institutional support was also highlighted.

Keywords: *Professionalism. Faculty. Attitude.*

INTRODUCTION

Professionalism is a cornerstone in medical education, essential for cultivating healthcare professionals who are not only skilled and compassionate but also have ethical integrity. Medical faculty plays a pivotal role in this process, serving as educators and role models who shape the professional identities of future medical professionals.[1] Their attitudes and behaviors significantly influence the learning environment and the development of medical students. The attitude of medical faculty towards professionalism reflects their personal and professional beliefs about the importance of ethical behavior and responsibility in medical practice. This attitude influences how they interact with students, colleagues, and patients, and how they prioritize professional values in their teaching.[2] In the modern era, there is a rapid advancement in the use of technology for delivering better patient care, however, the development of global guidelines for digital professionalism should also be emphasized.[3]

Recent research highlights professionalism as a dynamic construct influenced by socio-cultural, institutional, and individual factors. Cornett et al. emphasize the role of identity formation, while explicit integration in curricula is also considered critical.[4] Studies in Pakistan underscore contextual challenges such as hierarchical norms, inconsistent training, and resource disparities.[5] Faculty development, curriculum reforms, and ethical modeling are recurrent themes across global literature.[6]

Masters in Health Professions Education (MHPE) program serves dual roles as clinicians and academic mentors. Their understanding and practice of professionalism shape the next generation of healthcare professionals. However, despite its recognized importance, professionalism is often inconsistently embedded in medical training, especially in private institutions. This study aims to evaluate how medical faculty pursuing MHPE perceive professionalism and its constructs. By analyzing both quantitative metrics and narrative insights, the research aimed to provide a comprehensive understanding of the prevailing

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attitudes and identify areas requiring targeted interventions.

METHODOLOGY

The study was conducted at Shifa Tameer-e-Millat University, Islamabad from August 2024 to January 2025. A convergent parallel mixed-methods design was used. Ethical approval was obtained from the institutional review board (Letter No. 0279-23, 22-07-2023). After taking informed consent, universal sampling technique was used to enroll all 40 MHPE enrolled faculty members of Shifa Tameer-e-Millat University, Islamabad. The quantitative data was collected by using a 36-item validated questionnaire measuring six domains of professionalism: honor and integrity, excellence, duty, altruism, respect and accountability.[7] A 5-point Likert scale (1=strongly disagree to 5=strongly agree) assessed responses. The questionnaire was developed following the seven-step process outlined in AMEE Guide No. 87, as exemplified in Akbar et al.'s 2023 BMC Medical Education study, and underwent pilot testing to ensure reliability (Cronbach's α=0.8).[8] The mean and standard deviation for each item, domain, and total score were calculated. Qualitative data came from four semi-structured, audio-recorded interviews. This is followed by four in-depth semi-structured interviews. To increase the validity of the results, data triangulation was done using information from several sources, such as observational data and interviews. This method cross-checked data from many perspectives to ensure a thorough comprehension. The authenticity of the data was determined after cross-matching the recordings with transcripts and scribed notes of individual interviews. Responses from each interview were examined and analyzed in detail.

STATISTICAL ANALYSIS

Quantitative data was analyzed in the Statistical Package for the Social Sciences (SPSS) version 23, including descriptive statistics, Pearson correlation, and multiple linear regression to identify predictors of professionalism. The p-value of $\leq$0.05 was considered as significant. Thematic analysis was conducted using open coding. Notta software was applied to transcribe the audio recordings from the four interviews conducted.

RESULTS

Demographics of the 40 participants showed 28(70%) were females and 12(30%) were males. Most of the participants [32(80%)] were single, 23(57.5%) identified as Punjabi, 24(60%) were in the 2nd year of MHPE and 21(52.5%) had MBBS background. The poor professionalism was indicated by a low mean score for professionalism (2.07±0.45). The mean scores for six domains of professionalism are given in Table 1. Significant and strong correlations were found between professionalism and all the individual domains: excellence (r=0.77), duty (r=0.68), altruism (r=0.68), honor & integrity (r=0.62), accountability (r=0.53), and respect (r=0.53) with a p-value of 0.001. These results are shown in Table 2. Regression analysis revealed excellence (β=0.66, p=0.000) and altruism (β=0.43, p=0.025) as significant predictors of professionalism.

Qualitative analysis identified three themes: (1) Professionalism as foundation, (2) Challenges in curriculum integration, (3) Need for institutional support. These themes represent recurring patterns that reflect the perceptions and experiences of medical faculty regarding professionalism (Table 3 and Figure 1).

Table 2: Descriptive Statistics and Correlation of Professionalism Score with Score of Individual Domains

Professionalism Domains	Mean±Standard Deviation	r-value	p-value
Honor and integrity	2.46±0.67	0.62	0.001*
Excellence	2.38±0.48	0.77	0.001*
Duty	2.36±0.70	0.68	0.001*
Altruism	2.36±0.70	0.68	0.001*
Accountability	2.43±0.57	0.53	0.001*
Respect	2.24±0.78	0.53	0.001*

**Significant p-value*

Table 3: Thematic Analysis of Qualitative Data

Theme	Subthemes	Comments Verbatim
Professionalism as Foundation	Identity formation, ethics in practice, continuous learning	• "Professionalism weaves itself through our curriculum... it shapes the healthcare provider of integrity" • "Professionalism drives the essence of lifelong learning – it is the basis that we strive to progress, adopt and seek better ways to serve our patients" • "Professionalism cannot be taught in isolation, it silently defines who we eventually become as teachers and clinicians shaping our identity with our communication, interaction, and decision-making"
Challenges in Curriculum Integration	Time constraints, role ambiguity, cultural barriers	• "Balancing multiple obligations makes professionalism hard to integrate explicitly" • "Our overburdened routine and excessive responsibilities are the main challenges to explicitly incorporate professionalism. It often gets ignored despite its crucial significance, cultural barriers, and ill-defined roles make it difficult to understand how to teach and practice professionalism consistency"
Need for Institutional Support	Mentorship, faculty training, inclusive environment	• "Faculty need structured support and development to truly model professionalism" • "Having a mentor would help us understand how to handle real-life professionalism • A supportive and open environment makes it easier to talk about and practice professionalism"

Figure 1: Word Cloud Depicting key Themes Emerging from Faculty Perspectives on Medical Professionalism

DISCUSSION

The quantitative aspect of our study examined the influence of key attitudinal dimensions; respect, honor and integrity, excellence, duty, altruism, and accountability on professional conduct. Our results revealed that most of the participants were females (70%) and had an MBBS background (52.5%). The mean professionalism score (2.07±0.45) and scores of its individual domains were not up to the mark in MHPE students. Fahmirauf et al. conducted a study on clinical medical students to measure the level of medical professionalism and factors associated with professionalism scores in Malaysia. Most of their study participants were females (74.8%) and had non-medical backgrounds (85.8%) before enrolling for MBBS. Their professionalism scores reflected good attitudes in contrast to our results.[9] The other existing literature also emphasized the multifaceted nature of medical professionalism. A study done by Ghaznavi et al. to assess professionalism among undergraduate medical students reported that overall professionalism and its six tenets declined from 1st year to final year. This decline was attributed to lack of formal activities and courses teaching professionalism in the later years of medical college.[10] However, a study in India revealed that final year medical students were more aware of the importance of key professional attributes like communication, responsiveness and accountability of a doctor.[11] A two phase study done in Saudi Arabia showed advancement in professionalism from the second to third year in MBBS following an exclusive course on professionalism.[12]

In our results, honor & integrity had the highest scores (2.46±0.67) and respect had the lowest score (2.24±0.78). Rasul et al. also assessed medical professionalism among students and faculty members of a medical college in Lahore. Among their faculty members, honor & integrity were the highest rated (3.84±1.23) attributes of professionalism, whereas, the poorest scored domain was excellence (2.17±1.26).[13] Ahmed et al. also conducted a comparative study to evaluate medical professionalism among faculty of public and private medical and dental colleges in Karachi. They observed that excellence was the highest scored attribute of professionalism among faculty of private medical colleges, whereas, duty, honor & respect had the highest scores among faculty of public medical colleges.[14]

The present study offers a comprehensive analysis of medical faculty professionalism. The qualitative findings revealed three major themes: the foundational role of professionalism in shaping ethical identity, challenges in integrating professionalism into overloaded faculty roles & curriculum, and the pressing need for institutional support. A study on young medical doctors' perspectives on professionalism by Jalil et al. in Pakistan reported that young doctors believed the integration of professionalism and ethics into the curriculum was not necessary. Other factors identified as predictors of bad professionalism were false pride, lack of willingness to work in interprofessional collaborations and normalization of anger among seniors.[15] Another study done in Iran also emphasized the role of institutional support in mitigating the effect of personal constraints on professionalism. They recognized that factors like family issues, mental & physical health status, and poor communication skills acted as barriers towards professionalism.[16] Another qualitative study on perspectives about professionalism among undergraduate students in a medical college in India also revealed that ethics, accountability and integrity were recognized as important attributes of professionalism. They emphasized that professionalism should be explicitly taught in the medical curriculum.[11]

The challenges for integration of professionalism into the curriculum were time constraints, role ambiguity and cultural barriers in our study. Bhardwaj reported that the significant drivers of unprofessional behaviors were uncooperative leadership, poor organizational culture & work environment, no recognition for model behaviors, reduced opportunities for professional growth, resource constraints and lack of autonomy & decision-making capabilities. The study also identified a few other domains of professionalism like lifelong commitment, mentoring & coaching, and role modelling in addition to those six described in our research. Mentorship and structured support systems were consistently emphasized as necessary for cultivating a culture of professionalism.[17] A study reported that most of the medical students perceive their teachers as positive role models and the most important qualities of positive role models highlighted by the students were respect and empathy.[18]

Our study underscored the centrality of items like excellence and altruism as strong predictors of professional attitudes. The significantly high correlation between excellence and professionalism illustrated that faculty members who strived superior academic, clinical, and ethical standards were more likely to uphold professional values in their roles. Altruism also emerged as a strong driver of professionalism, highlighting the enduring relevance of selflessness, compassion, and service orientation in health professions education. The American Board of Internal Medicine foregrounds altruism, honor & integrity and excellence as foundational pillars of professionalism.[19]

CONCLUSION

Our results showed poor attitudes of MHPE enrolled medical faculty towards professionalism indicated by low mean scores of professionalism and its six domains of altruism, respect, honor & integrity, accountability, duty, and excellence. Excellence, duty, and altruism were identified as strong predictors of professionalism. There was a significant & strong correlation between excellence and professionalism scores. Our thematic analysis identified professionalism as one of the fundamentals of medical education. Time & resource constraints, role ambiguity, and cultural barriers were major challenges towards the integration of professionalism into medical courses and the role of institutional support was also highlighted.

LIMITATIONS & RECOMMENDATIONS

This study had several limitations. Firstly, it was confined to a single private university, limiting the generalizability of the results. Secondly, the sample size (n=40), though appropriate for this study, was relatively small for nationwide conclusions. Lastly, while the interviews yielded valuable insights, the presence of social desirability bias could not be ruled

out. Future studies should consider multi-site research and larger sample sizes. Longitudinal modules on professionalism should be implemented within MHPE curricula. The faculty development workshops focusing on ethical reasoning, mentoring, and reflective practice should be designed. Institutional policies to foster a culture of equity and respect must be strengthened.

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Authors' Contributions:
Z.Q: Study design, concept, acquisition of data, and manuscript drafting
M.N.A.K: Final approval and statistical analysis
H.Z: Final approval and statistical analysis
L.Q: Data analysis and critical review
Z.A: Critical review
L.Y: Critical review

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Original Article

Hypoalbuminemia and its Relation with Acute Respiratory Distress Syndrome and In-Hospital Mortality in Intensive Care Unit Patients

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ABSTRACT

Objective: To evaluate the association of hypoalbuminemia with acute respiratory distress syndrome (ARDS), in-hospital mortality and length of hospital stay in intensive care unit (ICU) patients and to determine the relation of ARDS with demographics and worst clinical outcomes.

Methodology: This cross-sectional study was done at the Federal Government Polyclinic Hospital, Islamabad after ethical approval. The study duration was 8 months from March 2024 to October 2024. After taking informed consent from patients or their attendants, 350 patients admitted to the medical ICU were included using non-probability convenience sampling technique. The demographic details of the patients, their co-morbidities and primary diagnoses were noted. The serum samples of the patients were sent to the laboratory for estimation of serum albumin levels. Patients were allocated to group I having serum albumin <3.5 mg/dl (n=210) and Group II with serum albumin levels >3.5 mg/dl (n=140). The outcomes assessed were the frequency of ARDS, length of ICU stay, and in-hospital mortality. The statistical analysis was done by the Statistical Package for the Social Sciences (SPSS) version 25.

Results: Out of 350 patients, 136(38.9%) developed ARDS; 34.3% had low albumin levels whereas, only 4.6% had normal serum albumin. In-hospital mortality occurred in 30% of the patients, out of which 20.3% and 9.7% of the patients had low and normal serum albumin, respectively. The mean length of ICU stay was 12.2±1.4 days and 8.9±2.5 days in group I and group II, respectively. These findings were statistically significant. Acute respiratory distress syndrome has a significant association with age, risk factors, serum albumin levels, length of ICU stay, and in-hospital mortality.

Conclusion: Acute respiratory distress syndrome, in-hospital mortality, and length of hospital stay have a significant relation with hypoalbuminemia in ICU patients. There was a significant association of acute respiratory distress syndrome with advanced age, risk factors, in-hospital mortality, and length of hospital stay.

Keywords: *Hypoalbuminemia. Respiratory distress syndrome. Hospital mortality.*

INTRODUCTION

Hypoalbuminemia is characterized by albumin below 3.5 mg/dL in the serum. Albumin has many important physiological roles in our body. It helps in the preservation of colloid osmotic pressure and the transport of various substances such as proteins & fatty acids. It modulates inflammatory response due to its antioxidant properties. It also acts as an anticoagulant by preventing the aggregation of platelets and inhibiting the synthesis of clotting factors by the liver.[1,2] Hypoalbuminemia is a very common and consequential abnormality in patients admitted to the intensive care unit. It can occur owing to reduced synthesis by the liver, increased breakdown, and leakage from capillaries due to systemic inflammation.[3] It is linked to unfavorable outcomes including increased morbidity, ARDS, prolonged hospital stay, and mortality.[4]

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Acute respiratory distress syndrome is a serious inflammatory pulmonary disorder characterized by diffuse alveolar damage and non-cardiogenic pulmonary edema. The condition is of serious concern owing to the high mortality rate and extrapulmonary multiorgan involvement with lethal outcomes.[5,6] The disease is a global health challenge with a variable prevalence ranging from 7.2 to 78.9 cases per 100,000 people annually. The disease contributes to around 45% of in-hospital deaths and 30% of deaths at 28 days.[7] Low levels of serum albumin levels aggravate the disease by decreasing plasma oncotic pressure. This facilitates leakage of fluid into the alveolar space, impairing gas exchange.[5,6] The disease has multiple predisposing factors such as sepsis, pneumonia, smoking, inhalation injury, etc.[8] The prevalence of ARDS has increased in the last few years. This may be attributed to the availability of advanced and better diagnostic modalities. It has been found in ICU and critically ill patients requiring mechanical ventilation.[9]

These findings underscore the clinical importance of monitoring albumin levels in ICU patients who are at high risk of developing ARDS. Hypoalbuminemia is emerging as a pathophysiological factor and a prognostic marker in ARDS, warranting further investigation and potential therapeutic

considerations. A study done in Pediatric ICU patients in Pakistan reported that hypoalbuminemia is linked to an increased risk of mortality.[10] So, this study was designed to determine the association of hypoalbuminemia in ICU patients with ARDS, in-hospital mortality and length of ICU stay. In addition, the study also determined the relation of ARDS with demographic variables, predisposing factors, length of hospital stay, and in-hospital mortality. As far as we know, there hasn't been any research done on adult ICU patients in Pakistan investigating the relation between hypoalbuminemia and ARDS, in-hospital mortality & length of hospital stay.

METHODOLOGY

This cross-sectional study was done at the Federal Government Polyclinic Hospital, Islamabad after ethical approval (Letter No. FGPC/1/12/2024/E-Committee, 16-01-2024). The study duration was 8 months from March 2024 to October 2024. The sample size of 295 was estimated using 95% confidence interval, 5% margin of error and 26% frequency of ARDS in ICU patients.[11] Three-hundred and fifty (350) patients admitted to the medical ICU were enrolled using non-probability convenience sampling after taking informed consent from patients or their attendants. The demographic details of the patients, their co-morbidities and primary diagnoses were noted. Blood samples of the patients were taken and their serum was sent to the laboratory for estimation of serum albumin levels. Patients were allocated to group I having serum albumin <3.5 mg/dl (n=210) and group II with serum albumin levels > 3.5 mg/dl (n=140). The primary outcomes assessed were the frequency of ARDS and in-hospital mortality in these patients. The secondary outcome was the length of ICU stay. Acute respiratory distress syndrome was diagnosed based on Berlin's definition.[12] The association of ARDS was also seen with age, risk factors, hypoalbuminemia, in-hospital mortality and length of ICU stay.

STATISTICAL ANALYSIS

The Statistical Package for the Social Sciences (SPSS) version 25 was used for data analysis. For categorical variables, frequency & percentage were used and for numeric variables, mean & standard deviation were used. Independent t-test, Chi-square test, and Fisher's exact test were used to determine the association of hypoalbuminemia and ARDS with categorical and numeric variables, respectively. The p-value of ≤0.05 was statistically significant.

RESULTS

The majority of the patients were 51-60 years old (34.3%) followed by 61-70 years of age (32.8%). Most of the patients were males (55.4%). However, no significant difference was seen in age and gender between the two groups. The majority of the ICU patients (81.4%) had a predisposing factor, whereas 18.6% had no predisposing factor. The most common predisposing factor was sepsis (29.2%) followed by pneumonia (16.6%) and smoking (15.7%), aspiration (11.4%), pancreatitis (3.1%), and multiple transfusions (2%). The other less common risk factors were decompensated chronic liver disease (DCLD) (1.7%) and drug overdose (1.7%).

Out of 350 ICU patients, 136(38.9%) developed ARDS. Most of the patients with ARDS (34.3%) had low albumin levels whereas, only 4.6% had normal serum albumin. In-hospital mortality occurred in 30% of the patients, out of which 20.3% and 9.7% of the patients had low and normal serum albumin, respectively. The mean duration of ICU stay was 12.2±1.4 days in group I and 8.9±2.5 days in group II. There was a significant difference in the frequency of ARDS, in-hospital mortality and length of ICU stay between patients in groups I and II (p-value ≤0.05). The outcomes of the patients are given in Table 1.

The association of ARDS was also seen with various variables. Acute respiratory distress syndrome has a significant association with age, the disease is most common in advanced age. The majority of the patients with ARDS had a predisposing factor, with statistical significance. The most frequent risk factors seen in ARDS patients were sepsis (18.6%) and pneumonia (8.6%) followed by aspiration (4.3%), smoking (3.7%), pancreatitis (1.4%), multiple transfusions (0.6%), drug overdose (0.6%) and DCLD (0.3%). In-hospital mortality occurred in 24% of the ARDS patients, with a significant p-value. The length of ICU stay was 15.3±1.2 days in ARDS and 8.4±2.1 days in patients without ARDS with a p-value <0.0001 (Table 2).

DISCUSSION

Acute respiratory distress syndrome (ARDS) is a life-threatening disorder with a high mortality rate. Hypoalbuminemia is a poor prognostic factor in ARDS and other critically ill patients. It decreases the plasma oncotic pressure further increasing the extravasation of fluid into pulmonary circulation and worsening hypoxia.[13]

Table 1: Comparison of Outcomes between Groups I and II

Outcomes		Group I	Group II	Total	p-value
ARDS (Frequency & Percentage)	**Present**	120(34.3%)	16(4.6%)	136(38.9%)	<0.00001*
	Absent	90(25.7%)	124(35.4%)	214(61.1%)	
In-Hospital Mortality (Frequency & Percentage)	**Present**	71(20.3%)	34(9.7%)	105(30%)	0.05*
	Absent	139(39.7%)	106(30.3%)	245(70%)	
Length of ICU Stay (Days)	**Mean±SD**	12.2±1.4	8.9±2.5	-	<0.0001*

**Significant p-value*

Table 2: Association of ARDS with Various Variables

Variables		ARDS		Total	p-value
		Present	Absent		
Age Groups (Years) (Frequency & Percentage)	**<40**	2(2.3%)	43(10.6%)	45(12.9%)	<0.00001*
	41-50	3(3.4%)	37(8%)	40(11.4%)	
	51-60	62(18.3%)	58(16%)	120(34.3%)	
	61-70	58(14%)	57(18.8%)	115(32.8%)	
	71-80	11(6%)	19(2.6%)	30(8.6%)	
Gender (Frequency & Percentage)	**Male**	74(21.1%)	120(34.3%)	194(55.4%)	0.76
	Female	62(17.7%)	94(26.9%)	156(44.6%)	
Risk Factors for ARDS (Frequency & Percentage)	**Present**	133(38%)	152(43.4%)	285(81.4%)	0.05*
	Absent	3(0.9%)	62(17.7%)	65(18.6%)	
Serum Albumin (Frequency & Percentage)	**Low**	120(34.3%)	90(25.7%)	210(60%)	<0.00001*
	Normal	16(4.6%)	124(35.4%)	140(40%)	
In-Hospital Mortality (Frequency & Percentage)	**Dead**	84(24%)	21(6%)	105(30%)	<0.00001*
	Alive	52(14.9%)	193(55.1%)	245(70%)	
Length of ICU Stay (Days)	**Mean±SD**	15.3±1.2	8.4±2.1	-	<0.0001*

**Significant p-value*

Our study showed that 60% of the patients had low albumin levels with no significant difference in age and gender between those with low and normal serum albumin. Similarly, McNeil et al. reported that 53.1% of patients had low albumin levels and no significant difference was found in age or gender between the groups.[11] Our results showed that 81.4% of the patients had a predisposing factor, sepsis being the most common (29.2%). Other factors were pneumonia (16.6%), smoking (15.7%), aspiration (11.4%), pancreatitis (3.1%), multiple transfusions (2%), DCLD (2%) and drug overdose (2%). Similar findings were reported in another study in which 84% of the patients had at least one risk factor. Their frequency was as follows: sepsis (39%) pneumonia (18%), multiple blood transfusions (10%), aspiration (9%), drug overdose (3%) and pancreatitis (2%).[11]

Our results showed that 38.9% of the ICU patients developed ARDS. Another study revealed that 26% of the patients admitted to the ICU developed ARDS.[11] The albumin levels were much lower in most of the patients with ARDS (34.3%) in our study. Our study revealed a statistically significant association of ARDS with low albumin levels. In another study conducted by Kumar et al., 42.4% and 8.3% of the patients with low and normal albumin levels developed ARDS. The difference was significant with a p-value of <0.001.[14] Another study showed a significant link between low albumin levels and ARDS.[11]

Our study revealed in-hospital mortality in 30% of the patients with 20.3% of the patients having low albumin levels. This demonstrated a significant relation between low albumin levels with in-hospital mortality. In a study by Zhang et al., 28-day mortality occurred in 8.86% of the admitted ICU patients but this study recruited only dialysis patients and they also observed a significant association between low albumin levels and in-hospital mortality.[15] Ozgungor et al. studied albumin levels as prognostic markers in ICU mortality and found that 28-day mortality in ICU patients can be predicted by 48 hours post-admission serum albumin levels.[16] A study by Maemum et al. reported an increased number of deaths in hospitalized patients with low albumin levels.[17] A study determined the association between lactate-to-albumin ratio (LAR) and deaths in ARDS patients. The study reported that the incidence of deaths in hospitalized patients was significantly higher in patients with higher

LAR.[18] Another study determined the significant relation between hypoalbuminemia and 28-day mortality in trauma patients, which was statistically significant.[14] A retrospective cohort study reported that administration of albumin in ARDS patients resulted in reduced mortality at 28 days as compared to those patients who did not receive albumin.[19] The length of hospital stay was significantly greater in patients with low albumin levels (p-value <0.0001). Our results were in accordance with the results of another study.[11]

Our results revealed a significant association of ARDS with age, predisposing factors, hypoalbuminemia, in-hospital mortality and length of ICU stay. The disease was frequent in advanced age, similar to another study.[20] Most of the patients who developed ARDS had a predisposing factor, with sepsis and pneumonia being the most common. The result of a meta-analysis also revealed sepsis, pulmonary infection and pancreatitis as the most common significant risk factors for ARDS.[21] In-hospital mortality and length of ICU stay were significantly greater in patients with ARDS, similar to another study.[22]

CONCLUSION

Our findings showed ARDS in 38.9% of the ICU patients, being significant in patients with hypoalbuminemia (34.3%). In-hospital mortality occurred in 30% of the patients, 20.3% had low serum albumin and 24% of the patients had ARDS with statistical significance. The length of ICU stay was significantly higher in patients with hypoalbuminemia and ARDS. There was a significant association of acute respiratory distress syndrome with advanced age and risk factors.

LIMITATIONS & RECOMMENDATIONS

It was a single-centered study. The patients were assessed for in-hospital mortality. Follow-up was not done for hospital readmissions, 28-day mortality, and long-term mortality. Studies should be conducted assessing these outcomes in the future. Furthermore, multi-centered studies should also be done. Careful monitoring of serum albumin is recommended in ICU patients and its administration should be evaluated in the light of the findings of clinical trials.

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Authors' Contributions:
S.G: Conceptualization of the study, development of methodology, and manuscript drafting.
M.T: Data collection from ICU records and laboratory coordination.
R.A.J: Literature review, referencing, and formatting of the manuscript.
H.A: Statistical analysis and interpretation of findings.
M.N: Ethical approval processing and technical editing.
M.M: Critical revision, proofreading, and final approval of the manuscript.

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Original Article

Association of Clinical and Biochemical Parameters with Renal Resistive Index in Type 2 Diabetic Patients with Normal Albuminuria

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ABSTRACT

Objective: To determine the association between the renal resistive index (RRI) and different biochemical and clinical parameters in type 2 diabetic patients with normal albuminuria.

Methodology: This descriptive cross-sectional study was conducted at the Department of Radiology, Shaikh Zayed Hospital, Lahore with a diabetic clinic collaboration for one year from January to December 2023. After ethical approval, 300 type 2 diabetic patients with normal albuminuria <30 mg/g were included by non-probability convenience sampling. After obtaining informed consent, patient demographic and clinical details were noted on the proforma. All the patients underwent renal Doppler ultrasound and RRI was estimated. Various biochemical parameters of the patients were estimated. The data analysis was done using the Statistical Package for the Social Sciences version 25.

Results: The average RRI was ≥0.70 in 64(21.3%) patients who were labeled as having nephropathy, ≥0.65 to <0.7 in 82(27.4%), and 154(51.3%) had normal RRI. There was a significant association of renal resistive index with duration of diabetes mellitus (DM), serum creatinine, urinary albumin-to-creatinine ratio (ACR), glomerular filtration rate (GFR), fasting blood glucose, glycated hemoglobin (HbA1c), and lipid profile in type 2 diabetic patients with normal albuminuria (p-value ≤0.05).

Conclusion: Renal resistive index had a significant association with duration of diabetes, serum creatinine, urinary ACR, GFR, fasting blood glucose, HbA1c, and lipid profile. It can be used for early detection of diabetic nephropathy in type 2 diabetic patients with normal albuminuria.

Keywords: *Diabetes mellitus. Diabetic nephropathy. Ultrasonography. Renal artery.*

INTRODUCTION

The global prevalence of diabetes mellitus is rising, making it a serious health issue. Around 537 million people are suffering from diabetes mellitus worldwide and it is expected that the disease prevalence will increase to 783 million by 2045. The increasing prevalence of disease is attributed to advanced age, obesity, unhealthy lifestyles such as lack of physical activity, and poor quality of diet. The greater burden of disease is in low- and middle-income countries.[1] In Pakistan, the prevalence of diabetes mellitus is 26.7% affecting 33,000,000 people.[2]

Diabetes mellitus is a state of chronic hyperglycemia resulting from defects in insulin secretion, insulin resistance or both. Almost 90% of the cases of DM are of type 2 diabetes mellitus.[2] Chronic diabetes leads to substantial complications affecting almost every body organ. These complications include coronary heart disease, atherosclerosis, stroke, neuropathy, nephropathy, retinopathy, osteoporosis, arthropathy, and myopathy. The disease is linked with a high mortality rate; a greater proportion of these deaths are attributed to kidney and heart diseases.[3,4]

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Diabetic nephropathy is the leading reason for renal disorders, i.e., chronic kidney disease (CKD) and end-stage renal disease across the world. About 40% of diabetic patients develop diabetic nephropathy after 10 years of disease initiation. To prevent the progression of diabetic nephropathy, early-stage detection and effective disease management are required to improve the well-being of patients. The criteria for diagnosing diabetic nephropathy is decreased GFR (<60 ml/min per 1.73 m²) and increased urinary albumin-to-creatinine ratio (≥30 mg/g). These tests cannot detect the disease at early stages and their normal limit deviates only when some structural or functional abnormality occurs. So, there is a need for a more reliable, sensitive, and safe method for the early detection of diabetic nephropathy.[5]

Renal resistive index is considered a potential marker for the early detection of diabetic nephropathy. It can be estimated by utilizing Doppler ultrasonography, which targets renal arteries to find the resistance in blood flow within these arteries. It evaluates perfusion and other renal complications of the kidney's vascular system.[6] In traditional diagnostic methods, only the function of the glomerulus is studied, but RRI reflects the changes in the vascular system of the renal parenchyma.[7] A literature survey of the renal resistive index as a screening tool revealed that it is a reliable, safe, easy, and more accurate early diagnostic method for diabetic nephropathy. It is a highly efficient method, even when the patient has a normal range of albuminuria levels.[8,9]

In Pakistan, studies have evaluated the role of RRI in the diagnosis of diabetic nephropathy in type 2 DM but its association with various clinical and biochemical parameters has not been evaluated. This study was designed to explore the association of clinical and biochemical parameters such as body mass index (BMI), duration of diabetes, smoking & hypertension, lipid profile, fasting blood glucose, HbA1C, and GFR with RRI in type 2 diabetic patients with a normal range of albuminuria. The outcomes of this study would give a more detailed insight into the pathophysiology of the disease and would be beneficial in the early diagnosis & management of diabetic nephropathy.

METHODOLOGY

This descriptive cross-sectional study was conducted at the Department of Radiology, Shaikh Zayed Hospital, Lahore with a diabetic clinic collaboration for one year from January to December 2023. The ethical approval was obtained from the institutional review board (Letter No. UHS/Reg-20/2829, 26-11-2022). A sample size of 291 was calculated using the expected proportion of higher RRI in 25.3% of type 2 diabetic patients, 5% margin of error and 95% confidence interval.[10] After obtaining written informed consent, 300 type 2 diabetic patients with age >18 years and normal albuminuria (ACR below 30 mg/g) were enrolled by nonprobability convenience sampling. Pregnant females, patients with renal stones, CKD and GFR <60ml/minute/1.73 m^2 were excluded. Patients were diagnosed with type 2 diabetes mellitus if the fasting blood glucose was ≥126 mg/dl (7.0 mmol/l) after an 8 hour fast or blood glucose level >200 mg/dl (11.1 mmol/l) 2 hours after an oral glucose tolerance test or HbA1c ≥6.5%.[11]

The demographic and clinical details of patients including age, gender, BMI, duration of diabetes, smoking, and hypertension status were noted on the proforma. All the patients underwent renal Doppler ultrasound (General Electronics LogiqS7 ultrasound machine with linear 6-10 MHz and curvilinear 2-5 MHz transducers) and RRI was estimated to determine the resistance to blood flow in renal arteries using the following formula:

$$\text{Renal Resistive Index} = \frac{(\text{Peak systolic velocity} - \text{End diastolic velocity})}{\text{Peak systolic velocity}}$$

Readings were taken from three renal arteries for each kidney and then their average was calculated to find RRI. The value of normal RRI was <0.65, ≥0.65 to <0.7 was borderline and ≥0.7 was considered high (nephropathy).[6] The RRI >0.7 is indicative of diabetic nephropathy with 100% probability.[12] Fasting blood samples of patients were collected and sent to the Pathology department for the estimation of different biochemical parameters such as fasting blood glucose, HbA1c, lipid profile, and serum creatinine. In the lipid profile, total cholesterol, triglycerides, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and high density lipoproteins (HDL) were measured. The urine samples of patients were also sent to the laboratory for determining the urinary albumin-to-creatinine ratio. The GFR was calculated using the Cockcroft-Gault equation using age, weight, and serum creatinine.[13] The reference values for various biochemical parameters were taken as follows: urinary ACR <30 mg/g, serum creatinine=0.6-1.1 mg/dl in females and 0.7-1.3 mg/dl in males, GFR >90 ml/min/1.73 m^2, fasting blood glucose <126 mg/dl, HbA1c=4-5.6%, total cholesterol <200 mg/dl, triglycerides <150 mg/dl, LDL <100 mg/dl, VLDL <40 mg/dl, and HDL >40 mg/dl.[3,14,15]

STATISTICAL ANALYSIS

The Statistical Package for the Social Sciences (SPSS) version 25 was used to enter and analyze the data. The biochemical parameters such as fasting blood glucose, serum creatinine, etc. were reported using mean±standard deviation (SD). The qualitative variables such as gender, age groups, smoking, and hypertension status were presented as frequency and percentage. The association of RRI with qualitative and quantitative variables was measured using Chi-square and Analysis of Variance (ANOVA) tests, respectively. A p-value of ≤0.05 was taken as statistically significant.

RESULTS

In our study, there were 177(59%) males and 123(41%) females. Most of the patients were <60 years old with 131(43.7%) patients in each group (<45 and 46-60 years), respectively. The majority of the patients (57.7%) had a normal BMI of <25 kg/m^2 followed by 90(30%) with overweight BMI of 25-29.9 kg/m^2 and 37(12.3%) patients were obese with BMI of ≥ 30 kg/m^2. The duration of DM was <5 years in 180(60%), 5-10 years in 55(18.3%), and more than 10 years in 65(21.7%) patients. Most of the patients were non-smoker (80%) and non-hypertensive (64.7%).

The mean±SD value of urinary ACR was 18.6±0.04 mg/g, serum creatinine was 0.9±0.05 mg/dl, and GFR was 102.6±0.3 ml/min/1.73 m^2. Patients had mean fasting blood glucose of 153.9±0.02 mg/dl and HbA1c of 8.2%. Regarding lipid profile, the average total cholesterol was 208.3±0.2 mg/dl, triglycerides were 148.3±0.03 mg/dl, LDL of 88.3±0.02 mg/dl, VLDL of 24±0.1

mg/dl, and HDL of 51.6±0.05 mg/dl. Most of the parameters were within normal range except fasting blood glucose, HbA1c, and total cholesterol.

The average RRI was ≥0.70 in 64(21.3%) of the patients labeled to have nephropathy, ≥0.65 to < 0.70 in 82(27.4%), and 154(51.3%) had normal RRI. These results are shown in Table 1.

The renal resistive index had a significant association with duration of diabetes (p-value=0.00001) showing that nearly half of the patients with duration more than 10 years had nephropathy and the majority of those with less than 5 years duration were normal. Most of the patients with 5-10 years duration were borderline. Renal resistive index showed no significant association with gender, age, BMI, smoking, and hypertension status (Table 2).

When the association of RRI was seen with various biochemical parameters, a significant relation was found with serum creatinine, urinary ACR, GFR, fasting blood glucose, HbA1c, and lipid profile. The mean of all biochemical parameters except GFR were increased in borderline and nephropathy groups as compared to normal RRI. However, the mean GFR was decreased in both nephropathy and borderline groups (Table 3).

Table 1: Renal Resistive Index in the Arteries of Left and Right Kidneys

Renal Artery	RRI <0.65 (Normal)	RRI ≥0.65 to <0.7 (Borderline)	RRI ≥0.70 (Nephropathy)
Main Renal Artery (R)	191(63.7%)	64(21.3%)	45(15%)
Segmental Artery (R)	182(60.7%)	74(24.7%)	44(14.6%)
Interlobar Artery (R)	186(62%)	74(24.7%)	40(13.3%)
Main Renal Artery (L)	202(67.3%)	59(19.7%)	39(13%)
Segmental Artery (L)	200(66.7%)	69(23%)	31(10.3%)
Interlobar Artery (L)	211(70.3%)	69(23%)	20(6.7%)
Average	154(51.3%)	82(27.4%)	64(21.3%)

Table 2: Association of Renal Resistive Index with Various Demographic & Clinical Parameters

Variables		RRI <0.65 (Normal)	RRI ≥0.65 to <0.7 (Borderline)	RRI ≥0.70 (Nephropathy)	Total	p-value
Gender	**Male**	93(31%)	48(16%)	36(12%)	177(59%)	0.847
	Female	61(20.3%)	34(11.4%)	28(9.3%)	123(41%)	
	Total	154(51.3%)	82(27.4%)	64(21.3%)	300(100%)	
Age (Years)	**≤45**	75(25%)	32(10.7%)	24(8%)	131(43.7%)	0.464
	46-60	60(20%)	40(13.4%)	31(10.3%)	131(43.7%)	
	>60	19(6.3%)	10(3.3%)	9(3%)	38(12.6%)	
	Total	154(51.3%)	82(27.4%)	64(21.3%)	300(100%)	
BMI (kg/m^2)	**<25**	89(29.7%)	50(16.7%)	34(11.3%)	173(57.7%)	0.169
	25-29.9	52(17.3%)	20(6.7%)	18(6%)	90(30%)	
	≥30	13(4.3%)	12(4%)	12(4%)	37(12.3%)	
	Total	154(51.3%)	82(27.4%)	64(21.3%)	300(100%)	
Duration of DM (Years)	**<5**	126(42%)	40(13.3%)	14(4.7%)	180(60%)	0.00001*
	5-10	7(2.3%)	28(9.3%)	20(6.7%)	55(18.3%)	
	>10	21(7%)	14(4.7%)	30(10%)	65(21.7%)	
	Total	154(51.3%)	82(27.4%)	64(21.3%)	300(100%)	
Smoking	**Smoker**	36(12%)	10(3.3%)	14(4.7%)	60(20%)	0.113
	Non-Smoker	118(39.3%)	72(24%)	50(16.6%)	240(80%)	
	Total	154(51.3%)	82(27.4%)	64(21.3%)	300(100%)	
Hypertension	**Hypertensive**	50(16.7%)	32(10.7%)	24(8%)	106(35.3%)	0.556
	Non-Hypertensive	104(34.6%)	50(16.7%)	40(13.3%)	194(64.7%)	
	Total	154(51.3%)	82(27.4%)	64(21.3%)	300(100%)	

**Significant p-value*

Table 3: Association of Renal Resistive Index with Various Biochemical Parameters

Biochemical Parameters (Mean±SD)	RRI			p-value
	<0.65 (Normal)	≥0.65 to <0.7 (Borderline)	≥0.70 (Nephropathy)	
Urinary ACR	7.7±0.04	19.1±0.04	29±0.04	0.001*
Serum Creatinine	0.7±0.05	0.96±0.05	1.2±0.05	0.001*
GFR	116±0.3	100±0.3	92±0.3	0.001*
Fasting Blood Glucose	143.1±0.02	150.6±0.02	168.2±0.02	0.001*
HbA1c	6.9±0.5	8.2±0.5	9.5±0.5	0.001*
Total Cholesterol	175±0.2	200±0.2	250±0.2	0.001*
Triglycerides	115±0.03	140±0.03	190±0.03	0.001*
LDL	80±0.02	95±0.02	90±0.02	0.001*
VLDL	25±0.1	27±0.1	20±0.1	0.001*
HDL	45±0.05	50±0.05	60±0.05	0.001*

**Significant p-value*

DISCUSSION

The renal resistive index is recognized as a sensitive early diagnostic marker for diabetic nephropathy and is routinely included in the diagnostic evaluation of chronic kidney disease to assess kidney damage.[16,17] However, the association of RRI with demographic, clinical, and biochemical parameters has been explored in only a limited number of studies, primarily among patients with elevated albumin-to-creatinine ratio. In contrast, our study assessed this association in patients with normal ACR levels.

In our study, most of the patients (87.4%) were <60 years old. There were 59% males and 41% females. However, RRI groups (normal, borderline, and nephropathy) showed no significant association with the age and gender of the patients. In a study conducted by Kharsa et al., 78.1% were males, majority of the patients were >60 years old and only age showed a significant association with RRI.[18] Another study reported 60% males with age <60 years.[19] Most of the patients (37%) had an age range of 41-50 years with 63% males in a study by Joseph et al.[20] In contrast, study by Khan et al., had a majority (52%) of females with a mean age of 49.15±11.91 years.[21] In our study, the majority of the patients (57.7%) had normal BMI. Most of the patients were non-smoker (80%) and non-hypertensive (64.7%). Smoking, hypertension status, and BMI showed no significant association with RRI. Kharsa et al. reported 43.2% smokers, 33.9% obese, and 95.8% hypertensive patients. Similar to our results, RRI showed no significant association with any of these clinical parameters.[18] Our results revealed that RRI showed a significant association with duration of DM and abnormal RRI was observed in most patients with more than 5 years of duration. Similar to our results, Tahir et al. reported that RRI increased significantly among patients with >5 years duration of diabetes.[16] Joseph et al. reported that patients with increased RRI had a mean duration of diabetes of 7.41±4.9 years. However, these results were not statistically significant.[20] A study by Fatima et al. showed that the patients with illness duration of 5-10 years had a significant correlation with RRI.[22]

Our results showed a significant association of RRI with serum creatinine, urinary ACR, GFR, fasting blood glucose, HbA1c, and lipid profile. In another study, a significant increase in HbA1c was seen in patients with RRI >0.7.[16] A study by Romano et al. determined the prognostic role of RRI in CKD showing that renal function tests deteriorate rapidly in patients with higher RRI. Renal resistive index was considered an independent marker of the progression of CKD and its associated deaths.[23] Another study reported that higher RRI was significantly associated with higher serum creatinine and decreased GFR.[18] Nasir et al. reported a significant positive correlation of RRI with serum creatinine and microalbuminuria. However, results for HbA1c correlation with RRI were not statistically significant.[19] Another study also reported a significant increase in serum creatinine, microalbuminuria, and HbA1c in patients with elevated RRI. The GFR was significantly decreased in patients with high RRI. However in this study, only 12% of the patients had normal albuminuria.[20] In contrast, a study reported a significant positive correlation of RRI with serum creatinine but no significant correlation of RRI with albuminuria & HbA1c was observed. [21] Fatima et al. also showed a positive link between RRI and serum creatinine and albuminuria.[22] In contrast to our results, in which patients with high RRI showed a significant increase in total cholesterol and triglycerides, a study conducted at the University of Udine, Italy, observed lower levels of total cholesterol and non-HDL cholesterol in patients with an elevated RRI.[23]

CONCLUSION

Renal resistive index had a significant association with duration of diabetes, serum creatinine, urinary ACR, GFR, fasting blood glucose, HbA1c, and lipid profile. It can be used for early detection of diabetic nephropathy in type 2 diabetic patients with normal albuminuria.

LIMITATIONS & RECOMMENDATIONS

Our study had a few limitations. It was conducted at a single-center limiting the generalization of the results. The study did not determine the association of RRI with long-term outcomes such as adverse events and mortality. In light of our results, it is recommended that the renal resistive index should be used as a non-invasive screening marker for early detection of diabetic nephropathy in type 2 diabetic patients.

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Authors' Contributions:
A.S: Study design, patient recruitment, and data collection
M.N: Data analysis, result interpretation, and manuscript drafting
S.K.T: Literature review and referencing
M.F: Data verification and tabulation
S.S: Critical revision, statistical supervision, and final approval of the manuscript

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