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EDITORIAL

The Silent Burden of Communication Disorders: Artificial Intelligence for Transforming Recognition, Informed Decisions and Outcomes

Humaira Shamim Kiyani

Communication is a fundamental human right and the cornerstone of learning, employment, social participation and overall well-being. Communication disorders including speech, language, voice, fluency, hearing, and cognitive abilities, affect hundreds of millions of people worldwide. The World Health Organization (WHO) reported that more than 430 million people have hearing loss that is incapacitating, while millions more experience acquired and developmental communication disorders associated with autism spectrum disorder, stroke, traumatic brain injury, Parkinson's disease, dementia and other neurological conditions.[1]

Due to a lack of epidemiological data, underreporting, insufficient screening programs and a shortage of speech-language pathologists the actual prevalence in Pakistan is still unknown. Consequently, many people remain undiagnosed or only receive intervention after the most effective period for recovery has been lost. Communication disorders represent a silent burden, making them one of the most overlooked health challenges.[2] In contrast to physical disabilities, communication difficulties such as problems with speaking, understanding, reading, writing, or social communication often go unrecognized which can result in delayed diagnosis, poor academic performance, unemployment, social isolation, psychological distress, and a lower quality of life. The nature of these impairments can make early recognition difficult, complicating clinical assessment and delaying informed decision-making.[3]

Artificial intelligence (AI) offers an unprecedented opportunity to make invisible burden visible by identifying early or subtle changes in speech language, and communication abilities that may not be readily observed during traditional clinical assessment. Progress in speech analytics, natural language processing, machine learning, and digital biomarkers can generate objective data to supplement traditional evaluation methods and support more accurate and timely clinical decision making.[4]

In particularly AI driven technologies will make an impact in resource-constrained settings where access to specialist services by speech-language pathologists may be limited. Automated speech-language screening and assessment tools powered by AI could enable earlier detection of communication disorders, objective measurement of speech and language abilities, tracking of progress over time and flagging/referral prioritization of those in need of specialist services. Technologies with built-in intelligence may also bring rehabilitation outside of traditional clinical settings via telepractice or remote monitoring and personalized therapy powered by machine learning that can adapt to the learner's performance and provide just-in-time feedback. Clinical decision-support systems may help streamline and improve management by integrating assessment information with up-to-date evidence to guide more consistent, efficient and evidence-informed practice.[5]

However even with these advancements technologies are meant to augment clinical practice not replace clinicians. The ethical integration of these technologies into practice includes interpretable algorithms, equity across demographic groups, patient privacy, clinical validity, and accounting for variability in language and culture. It is also important to note the clinical knowledge, reasoning, and sensitivity of speech-language pathologists. Building therapeutic relationships understanding each individual's unique circumstances and delivering person-centered care remain fundamentally human responsibilities that cannot be replicated by technology alone.[6]

Addressing the hidden burden of communication disorders will require a collaborative approach in which technological innovation and clinical expertise work together. When implemented ethically and

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integrated thoughtfully into speech-language pathology practice, AI can support earlier recognition of communication difficulties, strengthen clinical decision-making, expand access to high-quality rehabilitation and ultimately improve participation, independence, and long-term quality of life for individuals living with communication disorders.[7]

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ORIGINAL ARTICLE

Evaluation of Neonatal Factors on 17 Alpha Hydroxyprogesterone Level in Neonates- An Experience in Tertiary Care Setting

Humaira Javed[1], Ghazanfar Abbas[2], Muhammad Aamir[3], Muhammad Omar[4], Masooma Fatima[5]

ABSTRACT

Objective: This study aimed to determine the effect of various neonatal factors on the serum 17 alpha hydroxyprogesterone (17α-OHP) levels in neonates.

Study Design: An observational analytical study.

Place and Duration of Study: This study was conducted at the Department of Chemical Pathology of Shifa International Hospital Islamabad, Pakistan from 29th January 2026 to 30th June 2026.

Materials and Methods: The study was conducted on 132 study participants that included healthy neonates as controls and sick neonates as cases. The sample size was calculated using the WHO sample size calculator. Non-probability consecutive sampling techniques were employed. Clinical data of all neonates were collected using a structured data sheet and their serum 17 alpha hydroxyprogesterone levels were measured using a chemiluminescence-based auto analyzer (Maglumi X3, Snibe Diagnostics, China). Statistical analysis was performed on IBM SPSS Software version 26, with the Mann–Whitney U test applied for comparison of continuous variables to determine statistical significance.

Results: A total of 132 neonates were included in the study, of which 92 (69.7%) were full-term and 40 (30.3%) were preterm. Among them, 44 (33.3%) were sick and 88 (66.7%) were healthy. 17α-OHP levels were significantly higher in preterm compared to term neonates ($p < 0.05$), in sick compared to healthy ($p < 0.05$).

Conclusion: The present study supports existing evidence that several neonatal factors are associated with elevated 17α-OHP levels. Therefore, these neonatal factors should be taken into account when interpreting 17α-OHP levels in the neonates.

Key Words: *17 Alpha Hydroxyprogesterone, Birth Weight, Congenital Adrenal Hyperplasia, Gestational Age.*

Introduction

In the biosynthesis of glucocorticoids and mineralocorticoids in the adrenal glands, a complex cascade of intermediate compounds is involved in the production of cortisol. Among these intermediates, a steroid hormone 17α-OHP, produced in the adrenal cortex, serves as a key precursor in cortisol synthesis. It undergoes hydroxylation by the enzyme 21-hydroxylase, leading to its conversion into 11-deoxycortisol.[1] A genetic defect in the *CYP21A2* gene, encoding the 21-hydroxylase enzyme, results in impaired enzymatic activity, giving rise to a clinical condition known as Congenital Adrenal Hyperplasia (CAH).[2] Although other enzymes in the cortisol biosynthetic pathway may also be involved in causing CAH, 90-95% cases of these cases result from genetic mutation for 21-hydroxylase enzyme only, making 17α-OHP as the sentinel test for biochemical diagnosis of CAH.[2,3] CAH cases are divided into three subgroups based on the severity of *CYP21A2* gene mutation and age at presentation: (a) Salt wasting CAH, in which there is <1% enzyme activity presenting in infancy. (b)Simple virilizing CAH, with 1-2% enzymatic activity presenting in childhood. (c) Non-classical congenital adrenal hyperplasia (NC-CAH) with 20-50% enzymatic activity usually manifested during puberty or later.[4] The classic CAH incidence is around 1: 14,000.[5] The basal serum 17α-OHP concentrations >10 ng/ml (>30 nmol/L) indicate CAH, while lower basal concentrations are usually present in NC-CAH.[6]

Globally, various studies have shown that 17α-OHP levels are not only elevated in CAH but other neonatal factors such as gestational age, birth

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weight, and the presence of illness may significantly raise 17α-OHP concentrations.[7,8,9] Studies done in Korea and Iowa, USA showed that preterm and low-birth-weight infants, in particular, tend to exhibit higher 17α-OHP levels. Similarly, a study conducted in India showed that neonatal stress due to hypoxia, sepsis, or respiratory distress can also elevate 17α-OHP level.[10] An equivocal interpretation of raised 17α-OHP in neonates can lead to unnecessary investigations, parental anxiety, or delayed recognition of true pathology.[11] Given these challenges, there is a clear need to systematically evaluate the impact of neonatal variables on 17α-OHP levels. A better understanding of these associations would help refine interpretation in early life, reduce diagnostic uncertainty, and support more accurate clinical assessment.[12,13] In developing countries such as Pakistan, there is a scarcity of data regarding the impact of these neonatal factors on 17α-OHP levels, primarily due to the absence of a national neonatal screening program. Consequently, the diagnosis of CAH often relies heavily on lab assay of 17α-OHP levels ignoring the complex interplay of other neonatal factors leading to over diagnosis of CAH resulting in unnecessary follow up investigations, parental anxiety, increased health care costs and potential overtreatment.[14] Understanding the influence of neonatal factors, including gender, birth weight, gestational age, and health status on 17α-OHP levels may help improve the accuracy of neonatal screening and the interpretation of results. Apropos, a need was felt to evaluate the effect of these neonatal factors on serum 17α-OHP levels. An observational analytical study was therefore, undertaken to evaluate the effect of these factors on 17α-OHP levels, in order to minimize false positive results and reduce unnecessary parental anxiety.

Materials and Methods

This observational analytical study was conducted in the department of chemical pathology of Shifa International Hospital Islamabad, over a period of 5 months after the ethical approval from the Institutional Review Board and Ethics Committee (IRB & EC) of Shifa International Hospital (IRB# 525-25). The sample size of this study was estimated using WHO sample size calculator that included level of Significance: 5%, power of the test: 80%, population Standard Deviation: 3.67, anticipated Population mean: 1.8.[10] The sample size calculated was 132 that included both healthy and sick neonates within the first week of life delivered at the tertiary care hospital and neonates age greater than 30 days and diagnosed CAH cases were excluded. Out of 132 total subjects, 44 sick neonates were selected as cases and 88 healthy neonates were selected as control subjects, keeping a ratio of 1: 2 for cases and controls respectively.

Cases were sick neonates admitted to the NICU for management of conditions such as respiratory distress syndrome, birth asphyxia, and sepsis, while controls were healthy neonates without any clinical complications and who did not require NICU admission. Each study group was further assigned into one of the two groups; Pre-Term and Term neonates which were classified according to World Health Organization (WHO) guidelines.[15] The Pre-Term as well as Term neonates included both cases and control subjects finally making the distribution of study subjects into four groups; Pre-Term Sick and Term sick and Pre-term healthy and Term Healthy.

A structured data collection form was used to record information regarding neonates that included demographics, maternal gestational status(preterm/term), clinical condition (sick/healthy) and birth weight. Three ml of venous blood was collected in serum separator tubes (Greiner-Bio-One) for estimation of serum 17α-OHP in accordance with standard laboratory protocols. Serum was separated by centrifugation at 4000 rpm for 10 minutes within two hours of collection and stored at -20^{0}Celsius till analysis. Serum specimens were thawed at room temperature prior to analysis and run in batches for determination of 17α-OHP levels by chemiluminescent immunoassay (CLIA) method, using Maglumi X3, fully automated immunoassay analyzer by Snibe Diagnostics, Shenzhen New Industries Biomedical Engineering Co., Ltd., Shenzhen, China. The assay was calibrated using low and high calibrators, with assigned values traceable to Snibe internal reference standard. The assay performance was verified using two levels of controls; 17-a-OHP QC1 and 17-a-OHP QC2.

Data was entered in IBM SPSS Software version 26. The descriptive statistics used for qualitative variables such as preterm/term, sick/healthy in the

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study were frequency and percentages and for quantitative variable such as 17 OHP median and interquartile ranges were used.

Non-parametric test such as Mann Whitney-U test was used to compare 17α-OHP levels among the two gender, birth weight (<2.5kg and >2.5kg), Pre-term and term, sick and healthy. Kruskal Wallis test was used to find the difference between four groups including Preterm Sick, Preterm Healthy, Term Sick and Term Healthy. Additionally, Pearson's correlation analysis was performed to determine association between different variables and 17α-OHP levels.

P value of ≤0.05 was considered statistically significant for all data analysis.

Results

This study included 132 neonates, comprising of 44 sick neonates (33.3%) and 88 healthy neonates (66.7%) (Figure 1). Sick neonates showed higher 17α-OHP levels compared to in healthy neonates ($p < 0.05$). There was no statistically significant difference between the male and female groups ($p = 0.729$). However, neonates with birth weight <2.5 kg had higher 17α-OHP level compared to those with birth weight ≥2.5 kg ($p < 0.001$). Preterm neonates had higher 17α-OHP compared to term neonates ($p < 0.001$). (Table I)

Further subgroup analysis showed that the preterm group included preterm healthy (PH) neonates (n=15 ;11.4%), preterm sick (PS) neonates (n=25; 18.9%), while the term group comprised term healthy (TH) neonates (n=73; 55.3%) and term sick (TS) neonates (n=19; 14.4%). A Kruskal–Walli's test was conducted to compare 17α-OHP levels across these four groups. The mean rank distribution indicated that the PS group had the highest 17α-OHP levels (mean rank = 104.08), followed by the TS group (96.21), while the PH (66.17) and TH (45.97) groups showed comparatively lower levels (Figure 2)

Pearson correlation analysis using log-transformed 17α-OHP levels demonstrated a significant moderate negative correlation with gestational age ($r = -0.465$, $p < 0.001$) and birth weight ($r = -0.443$, $p < 0.001$), indicating that 17α-OHP levels decrease with increasing gestational age and birth weight. Additionally, health status showed a strong positive co-relation with log-transformed 17α-OHP levels ($r = 0.658$, $p < 0.001$), while no significant correlation was observed with newborn gender ($r = -0.017$, $p = 0.848$) (Table II).

Table I: Distribution of 17α-OHP Level by Health Status, Sex, Birth Weight, and Gestational Age (n = 132)

Variable	Category	n (%)	Median 17α-OHP (ng/mL)	IQR (ng/mL)	p-value
Health status	Sick neonates	44 (33.3)	29.23	14.45–47.40	<0.05
	Healthy neonates	88 (66.7)	9.82	6.98–12.25	
Sex	Female	49 (37.1)	12.25	8.27–19.00	0.729
	Male	83 (62.9)	11.04	8.45–17.67	
Birth weight	<2.5 kg	106 (80.3)	22.50	17.25–46.42	<0.001
	≥2.5 kg	26 (19.7)	10.67	7.64–14.60	
Gestational age	Preterm	40 (30.3)	19.12	10.91–42.27	<0.001
	Term	92 (69.7)	10.52	7.45–14.14	

Table II: Association of Log of Serum 17α-OHP Levels and Neonatal Variables Using Pearson Correlation (n = 132)

Variables	Pearson coefficient (r)	p-value
Gestational age group	-0.465	<0.001
Birth weight (BW)	-0.443	<0.001
Sick or Healthy	0.668	<0.001
Gender	-0.017	0.848

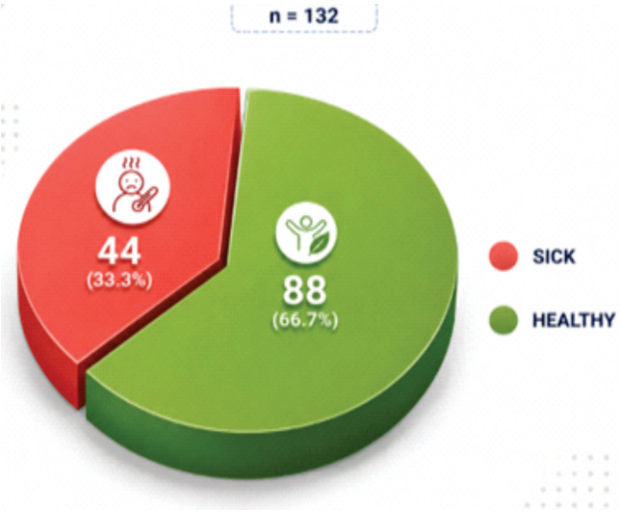

Figure 1: Distribution of Cases and Controls (n=132)

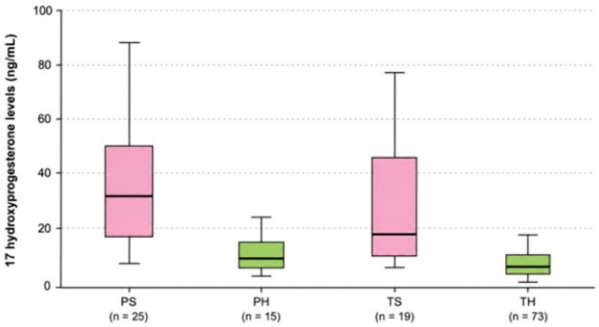

Figure 2: Median and IQR 17 OHP levels Amongst Four Groups: PS, PH, TS, TH.

Discussion

The present study conducted in a tertiary care hospital evaluated the serum 17α-OHP levels in 132 neonates in relation to various neonatal factors. The findings demonstrate that neonatal health status, gestational age which is assigned according to mother, and birth weight significantly influence 17α-OHP concentrations, whereas sex does not appear to have a significant effect.

An important observation from the current study is that serum 17α-OHP levels were significantly higher in sick neonates compared with healthy neonates (median 29.23 ng/mL vs 9.82 ng/mL). Furthermore, the preterm sick subgroup demonstrated the highest levels of 17α-OHP levels with a maximum level of 86.08ng/mL and highest Kruskal-Wallis mean rank (104.08), indicating that the combined effects of prematurity and illness resulted in the higher 17α-OHP compared with other sub-groups. Similar findings have been reported by Vasundhara S. Chennuri, et al., who noted a median value of 34.0 ng/mL of 17α-OHP in sick full-term neonates compared with 8.4 ng/mL in healthy full-term neonate and 58.0 ng/mL in sick preterm infants compared with 20.0 ng/mL in healthy preterm infants, demonstrating that neonatal illnesses such as respiratory distress, sepsis, and perinatal asphyxia can significantly elevate 17α-OHP levels, emphasizing illness as an important confounding factor in neonatal screening.[10] The study conducted by Kum et al. demonstrated that synthesis of steroid hormone in adrenal glands was highly affected by the stress related neonatal conditions such respiratory distress leading to increase in 17α-OHP levels.[8] The large data of a 17α-OHP screening study by Yoon et al., 2023, also identified that preterm sick subgroup neonates have 17α-OHP cutoff values of approximately 35-45 ng/mL. This further strengthens the fact that neonatal health status along with gestational age can act as confounding factors while diagnosing CAH on the basis of serum 17 –OHP levels.[16]

In our study, birth weight and prematurity were also important determinants of serum 17α-OHP concentrations. Neonates weighing less than 2.5kg had median 17α-OHP levels of 22.50 ng/mL which was significantly higher than those weighing ≥2.5kg. This finding is in agreement with a study by de Castro et al. who documented 17α-OHP cutoff at 30.3 ng/mL and 18.1 ng/mL for neonates weighing <2.5kg and ≥2.5kg respectively.[17] Gestational age demonstrated an inverse relation with 17- OHP levels; the 17α-OHP levels were significantly higher in preterm than in term neonates. Our findings are supported by the study by Pode-Shakked et al. who used gestational age specific cutoff values for 17α-OHP: a cutoff of 23.1 ng/mL for preterm infants born at ≤37 weeks of gestation which was and 11.6 ng/mL for infants born > 37 weeks of gestation.[6] Multiple neonatal screening studies on 17α-OHP, have also reported elevated 17α-OHP levels in low-birth-weight infants, emphasizing that birth weight is a stronger predictor in determining 17α-OHP variability.[7,9,12,18] The review paper by Tsuji-Hosokawa et al. done in Japan also highlighted that results of 17α-OHP levels in neonates should be interpreted with caution, particularly in preterm and low-birth-weight infants.[19]

The correlation analyses further strengthened these findings. Serum17α-OHP showed a strong positive correlation with neonatal sickness, and a moderate negative correlation were seen with both gestational age and birth weight, indicating that 17α-OHP concentrations decrease as gestational age and birth weight increases. These findings of the present study are consistent with those reported by Yoon et al. who reported that), who demonstrated a significant negative correlation between 17-hydroxyprogesterone (17α-OHP) concentrations and both gestational age ($r = -0.560$, $p < 0.001$) and birth weight ($r = -0.488$, $p < 0.001$). These findings support the observations that elevated serum 17α-OHP levels in preterm and critically ill neonates reflects physiological immaturity and stress-related adrenal

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activation rather than congenital adrenal hyperplasia.[20]

Our study showed no statistically significant difference between the 17α-OHP levels in male and females. These findings are consistent with the study conducted by Claahsen-Van der Grinten HL et al. which emphasized that 17α-OHP levels in neonates are not affected by gender differences.[2] Speiser PW, et al, Endocrine Society clinical guidelines for congenital adrenal hyperplasia, based on systemic review and expert consensus indicated that gender does not significantly influence serum 17α-OHP levels in neonates.[21]

A retrospective cohort study, done in Canada conducted by Jomaa et al. highlighted that gestational age plays an important role in newborn screening for CAH, as it strongly influences the 17α-OHP concentrations in neonates, with preterm and low birth weight (BW) newborns exhibiting higher 17α-OHP levels that reduce the specificity of CAH screening cut-off value.[12] Similarly, Barra et al. suggested that screening could be made more cost effective by using the 17α-OHP cutoff values adjusted for birth weight, thereby reducing false-positive results.[7] These findings suggested that gestational age and birth weight adjusted interpretation of 17α-OHP, as recommended by the Endocrine Society guidelines can significantly improve diagnostic accuracy and reduce unnecessary recalls.[21] In tertiary care hospitals, the specificity of the test may be further improved by including the health status of neonates, as supported by recent screening strategy improvements and second-tier testing approaches.[18]

The limitations of this study included the absence of repeat 17α-OHP measurements, multivariable regression analysis and biochemical or genetic confirmation of CAH in neonates with elevated 17α-OHP levels. Additionally, the study was conducted at a single center with a small sample size. To validate these findings further studies involving multicentric populations and follow-up protocols are needed.

Conclusion

In conclusion, the present study supports existing evidence that neonatal illness, prematurity, and low birth weight significantly influence 17α-OHP levels. Therefore, these neonatal factors should be taken into account when interpreting 17α-OHP levels in the neonates.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORIGINAL ARTICLE

Maternal Knowledge, Attitudes, and Cultural-Religious Perceptions Regarding Human Milk Donation and Human Milk Banking: A Cross-Sectional Study among Postpartum Mothers at a Tertiary Care Hospital

Saira Zubair[1], Khurram Fayyaz[2], Anfal Hanan[3], Minahil Fatima[4], Sadia Rehman[5], Sadia Karim[6]

ABSTRACT

Objective: To assess maternal knowledge, attitudes, and cultural-religious perceptions regarding human milk donation and acceptance of donor human milk among postpartum mothers at a tertiary care hospital in Karachi, Pakistan.

Study Design: It was a descriptive cross-sectional study.

Place and Duration of Study: Over the course of six months, October 1, 2025, to March 30, 2026, this study was carried out at Pakistan Naval Ship (PNS) Shifa Hospital in Karachi.

Material and Methods: A total of 375 postpartum mothers with infants younger than six months were enrolled using non-probability consecutive sampling. Data were collected using a standardized interviewer-administered questionnaire covering sociodemographic characteristics, obstetric history, knowledge, attitudes, and cultural-religious perceptions regarding human milk donation and milk banking. Descriptive statistics and Chi-square test were used for data analysis, with $p \leq 0.05$ considered statistically significant.

Results: A total of 375 postpartum mothers participated in the study, with a mean age of 28.73 ± 4.43 years. Awareness of human milk banks was reported by 169 (45.1%) mothers, while 209 (55.7%) believed donor human milk was more nutritious than formula milk. Most participants (321; 85.6%) expressed concerns regarding infection transmission through donor milk, and 342 (91.3%) were aware of the concept of milk kinship (Rada'a). Only 82 (21.9%) mothers were willing to donate breast milk, and 49 (13.1%) were willing to receive donor human milk for their infants, indicating low acceptance despite moderate awareness.

Conclusion: Despite moderate awareness of human milk banking, willingness to donate or receive donor human milk remained low among postpartum mothers. Fears of infection and cultural-religious concerns were the principal barriers to acceptance.

Key Words: *Breast Feeding; Human Milk Banks; Knowledge; Attitude; Mothers; Pakistan.*

Introduction

Breastfeeding is widely recognized as the optimal method of infant feeding, providing complete nutrition and immunological protection essential for healthy growth and development. Human milk contains an ideal balance of macronutrients, micronutrients, bioactive compounds, enzymes, and immunoglobulins that reduce the risk of neonatal infections, improve neurodevelopment, and confer long-term health benefits for both infants and mothers. The World Health Organization (WHO) recommends exclusive breastfeeding for the first six months of life, followed by continued breastfeeding alongside complementary feeding up to two years of age or beyond.[1] However, breastfeeding may not always be possible because of maternal illness, inadequate milk production, maternal medication use, neonatal separation, or maternal death. In such circumstances, ensuring optimal nutrition for vulnerable infants becomes a significant public health challenge.[2,3] Pasteurized donor human milk is considered the preferred alternative when a mother's own milk is unavailable, particularly for preterm, low-birth-weight, and critically ill neonates. Human milk banks provide a safe mechanism for collecting, screening, pasteurizing, storing, and distributing donated breast milk according to standardized protocols. The use of donor human

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milk has been associated with a reduced incidence of necrotizing enterocolitis, late-onset sepsis, feeding intolerance, and other complications commonly encountered in premature infants when compared with formula feeding.[2,3] Consequently, several international organizations recommend donor human milk as the first alternative to maternal milk whenever breastfeeding is not feasible. Despite these established clinical benefits, the successful implementation of human milk banking depends largely on public awareness, parental acceptance, and confidence in the safety of donor milk.[3-5]

Studies conducted in different populations have demonstrated that acceptance of donor human milk is influenced by multiple factors, including educational status, knowledge of human milk banking, perceived risk of infection, cultural beliefs, and trust in healthcare systems.[4,5] Fear of disease transmission, concerns regarding donor screening, and misconceptions regarding the safety of donor human milk continue to limit its acceptance in many communities.[6] Healthcare professionals therefore play a critical role in providing evidence-based counselling to address misconceptions and improve community acceptance of donor milk.

In Muslim-majority countries such as Pakistan, religious and cultural considerations represent additional barriers to the establishment of human milk banks. This concern has generated considerable hesitation regarding donor human milk despite its proven medical benefits.[7] A previous attempt to establish a human milk bank in Pakistan encountered significant religious opposition, highlighting the importance of integrating Islamic principles into milk banking policies.[8] Nevertheless, experiences from countries such as Malaysia and Iran have demonstrated that culturally acceptable human milk banking systems can be successfully implemented by maintaining comprehensive donor-recipient records and involving religious authorities during policy development.[9,10]

Although donor human milk has become an integral component of neonatal care in many countries, evidence regarding maternal knowledge, attitudes, and cultural-religious perceptions toward human milk donation in Pakistan remains limited. Understanding these perceptions is essential for developing culturally appropriate educational interventions, strengthening community awareness, and guiding policies for the safe implementation of human milk banking. Therefore, the present study aimed to assess the knowledge, attitudes, and cultural-religious perceptions regarding human milk donation and human milk banking among postpartum mothers attending a tertiary care hospital in Karachi, Pakistan

Materials and Methods

This cross-sectional study was conducted at the Department of Pediatrics, Pakistan Naval Ship (PNS) Shifa Hospital, Karachi, Pakistan, over a period of six months from 1st October 2025 to 30th March 2026. The study was approved by the Institutional Ethical Review Committee of PNS Shifa Hospital (ERC/2026/PED/94) before the commencement of data collection. Written informed consent was obtained from all participants, and the confidentiality and anonymity of their information were strictly maintained throughout the study.

The study population comprised postpartum mothers attending the postnatal wards and pediatric outpatient department of PNS Shifa Hospital with infants younger than six months of age. Mothers who were willing to participate and provided written informed consent were included in the study. Mothers who declined participation, were unable to communicate effectively, or had incomplete questionnaire responses were excluded from the analysis.

The sample size was calculated using the single population proportion formula, $n = Z^2P(1-P) / d^2$, where $Z = 1.96$ for a 95% confidence level, $P = 73.4\%$ (0.734) based on the awareness of donor human milk banking reported by Tu et al., and $d = 5\%$ (0.05) margin of error.[11] The minimum required sample size was 341 participants. After adding a 10% allowance for non-response, the final sample size was 375 participants. Data were collected using a structured, interviewer-administered questionnaire developed after an extensive review of the published literature. To establish content validity, the questionnaire was reviewed independently by three faculty members with expertise in pediatrics, neonatology, and medical education. Their suggestions regarding wording, clarity, and relevance of the items were incorporated before data collection. A pilot test was conducted among 20 postpartum mothers to ensure

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comprehensibility and feasibility of administration. The pilot participants were excluded from the final study analysis.The questionnaire was administered in a private setting by trained investigators to ensure uniform data collection.

The primary outcome was the willingness of mothers to donate and receive donor human milk. Secondary outcomes included the level of knowledge regarding human milk banking, awareness of milk kinship (Rada'a), perceived safety of donor milk, and factors influencing attitudes toward human milk donation. Responses were initially recorded as Yes, No, or Maybe. For statistical analysis, "No" and "Maybe" responses were combined into a single category because both represented the absence of a definite willingness to donate or receive donor human milk.

Data were entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were presented as frequencies and percentages. Associations between willingness to donate breast milk and selected sociodemographic, obstetric, and knowledge-related variables were assessed using the Chi-square test. A p-value ≤ 0.05 was considered statistically significant.

Results

A total of 375 postpartum mothers participated in the study, with a mean age of 28.73 ± 4.43 years. The sociodemographic characteristics of the participants, including age, educational status, occupation, monthly family income, and place of residence, are presented in Table I. Obstetric and neonatal characteristics, including parity, mode of delivery, gestational age, and neonatal intensive care unit (NICU) admission, are summarized in Table II.

Knowledge and awareness regarding donor human milk and human milk banking are presented in Table III. Each participant identified a single primary source of information regarding human milk banking, with media being the most frequently reported source among those who were aware of human milk banking. Overall, 45.1% of participants had heard about human milk banks, while 55.7% believed that donor human milk was more nutritious than formula milk. The majority (85.6%) expressed concerns regarding the transmission of infections through donor milk. Awareness regarding breast milk storage was reported by 80.5% of mothers, whereas 91.3% were familiar with the concept of milk kinship (Rada'a). Nearly half (47.5%) had no prior source of information regarding human milk banking.

Table I: Sociodemographic Characteristics of the Participants (n = 375)

Variable	Category	n (%)
Age (years)	20–29	208 (55.5)
	30–39	165 (44.0)
	≥40	2 (0.5)
Educational Status	Primary	71 (18.9)
	Secondary	113 (30.1)
	Higher Secondary	88 (23.5)
	Graduate and above	104 (27.7)
Occupation	Housewife	265 (70.7)
	Employed	110 (29.3)
Socioeconomic Status	Low	67 (17.9)
	Middle	212 (56.5)
	High	96 (25.6)
Place of Residence	Urban	297 (79.2)
	Rural	78 (20.8)

Table II: Obstetric and Neonatal Characteristics of the Participants (n = 375)

Variable	Category	n (%)
Parity	Primiparous	78 (20.8)
	Multiparous	297 (79.2)
Mode of Delivery	Vaginal delivery	156 (41.6)
	Cesarean section	188 (50.1)
	Instrumental delivery	31 (8.3)
Gestational Age	Term (≥37 weeks)	228 (60.8)
	Preterm (<37 weeks)	147 (39.2)
NICU Admission	Yes	165 (44.0)
	No	210 (56.0)

Discussion

The present study assessed the knowledge, awareness, and attitudes of postpartum mothers regarding human milk donation and human milk banking at a tertiary care hospital in Karachi. Although breastfeeding is widely accepted as the optimal source of infant nutrition, awareness and acceptance of donor human milk remained limited among the participants. These findings highlight the need for structured educational initiatives and culturally sensitive policies before the successful implementation of human milk banking in Pakistan.

Table III: Knowledge and Awareness Regarding Human Milk Banking (n = 375)

Variable	Response	n (%)
Aware of human milk bank	Yes	169 (45.1)
	No	206 (54.9)
Donor human milk is considered more nutritious than formula	Yes	209 (55.7)
	No	166 (44.3)
Fear of infection transmission through donor milk	Yes	321 (85.6)
	No	54 (14.4)
Aware of breast milk storage devices	Yes	302 (80.5)
	No	73 (19.5)
Source of information	Media	92 (24.5)
	Healthcare provider	65 (17.3)
	Peers	40 (10.7)
	Not aware	178 (47.5)

In the present study, less than half of the participants (45.1%) were aware of human milk banks. This finding suggests inadequate public awareness regarding donor human milk despite increasing global recognition of its benefits. Similar findings have been reported from Malaysia, where only 55.9% of respondents had prior knowledge of human milk banking, indicating that awareness remains suboptimal even in countries where the concept is more established.[11] Likewise, studies from India have shown that limited knowledge remains one of the principal barriers to the successful establishment and utilization of human milk banks.[12] These observations emphasize the importance of educating mothers during antenatal and postnatal care through healthcare professionals and public health campaigns.

More than half of the mothers (55.7%) believed that donor human milk is more nutritious than infant formula, reflecting a reasonable understanding of the nutritional superiority of human milk. Nevertheless, this knowledge did not translate into a greater willingness to donate or receive donor milk. Similar discrepancies between knowledge and acceptance have been observed in Malaysian and Iranian populations, where participants acknowledged the health benefits of donor milk but remained hesitant because of safety concerns and cultural beliefs.[11,13] This finding indicates that improving knowledge alone may not be sufficient to enhance acceptance unless misconceptions and social concerns are simultaneously addressed.

A notable finding of the present study was that the majority of mothers (85.6%) expressed concern regarding the transmission of infections through donor milk. Similar concerns have consistently been reported in previous studies and represent one of the most frequently cited barriers to donor milk acceptance.[12,13] Mothers often perceive donated milk as a potential source of infectious diseases despite rigorous donor screening, pasteurization, and microbiological quality assurance practiced by accredited human milk banks. These findings highlight the need for healthcare professionals to educate families regarding established safety protocols and quality control measures used in human milk banking.[14,15]

Only 21.9% of participants expressed willingness to donate breast milk, while an even smaller proportion (13.1%) were willing to receive donor human milk for their own infants. These findings are also consistent with the observations of Bhutta et al.[16] in Pakistan, who highlighted that despite the recognized clinical benefits of donor human milk, implementation of human milk banking remains challenging because of limited public awareness, religious concerns, and the absence of an established regulatory framework. These contextual barriers may partly explain the low acceptance observed among mothers in the present study.[16] The lower acceptance observed in the present study may reflect the influence of sociocultural norms, religious considerations, and limited familiarity with human milk banking in Pakistan. Furthermore, approximately half of the participants selected "maybe" when asked about donation and acceptance, suggesting uncertainty rather than outright refusal. This finding indicates that targeted educational interventions could potentially improve acceptance among undecided mothers.

Religious and cultural beliefs remain an important consideration in Muslim-majority countries. Although awareness of the concept of milk kinship (Rada'a) was high among our participants,

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acceptance of donor human milk remained low. Similar findings have been reported from Iran and Malaysia, where concerns regarding milk kinship and Islamic jurisprudence significantly influenced parental attitudes toward donor milk.[13,14,16] Recent qualitative evidence suggests that these concerns can be addressed through transparent donor-recipient documentation, collaboration with religious scholars, and culturally appropriate counselling, thereby improving public confidence without compromising religious principles.[15,16]

The strengths of this study include a relatively large sample of postpartum mothers and the evaluation of both knowledge and attitudes within the local cultural context. However, the study has certain limitations. Being a single-center cross-sectional study, the findings may not be generalizable to all regions of Pakistan. In addition, the use of convenience sampling and self-reported responses may have introduced selection and response bias.

This study has several limitations. As a single-center descriptive cross-sectional study using non-probability consecutive sampling, the findings may not be generalizable to the wider population of postpartum mothers in Pakistan. The interviewer-administered questionnaire may have introduced interviewer and response bias, and although the questionnaire was developed following an extensive review of the literature, it did not undergo formal psychometric validation.

Chi-square test; multivariable logistic regression was not performed to identify independent predictors of willingness to donate or receive donor human milk. Future multicenter studies using validated instruments and multivariable analytical approaches are warranted to provide more robust evidence.

Conclusion

Postpartum mothers demonstrated moderate awareness of human milk banking; however, willingness to donate or receive donor human milk remained low despite recognition of its nutritional benefits. Fear of infection and cultural-religious concerns were the major barriers influencing maternal attitudes. Targeted educational initiatives, engagement of healthcare providers and religious scholars, and culturally sensitive policies are essential to improve knowledge, acceptance, and the safe implementation of human milk banking in Pakistan.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORIGINAL ARTICLE

Head and Neck Cancers in a Tertiary Care Setting: A Retrospective Analysis

Palwasha Faiz Khattak[1], Noor Ul Huda[2], Mirza Khizar Hameed[3], Mahum Khizar[4]

ABSTRACT

Objective: To determine the site-distribution and relative frequency of head and neck cancers and to study their age and gender distribution and histological types across different anatomical subsites.

Study Design: A Retrospective cross-sectional record-based descriptive study.

Place and Duration of Study: This study was conducted from July 01, 2020, to December 31, 2024, in the ENT Department, Fauji Foundation Hospital Rawalpindi.

Materials and Methods: The record of 305 patients, with age range of 14-79 years, and histologically confirmed cases of head and neck cancers, was reviewed over a period of four and a half years. Data on tumour sites, type, age, and gender were extracted and analyzed using SPSS v26. Descriptive statistics (frequencies and percentages) were calculated for each tumour category, and age was recorded as a range across the study population.

Results: Out of total 305 patients, 211 (69.2%) were males, while 94 (30.8%) were females. Oral cavity tumours were the most common, found in 76 cases (24.9%), followed by hypopharyngeal carcinomas, 51 cases (16.7%), oropharyngeal cancers, 47 cases (15.4%), nasal/paranasal carcinomas, 29 cases (9.5%) nasopharyngeal cancers, 27 cases (8.9%), laryngeal cancers, 23 cases (7.5%), salivary carcinomas in 18 cases (5.9%), thyroid carcinomas and neck tumours with unknown primary each 15 cases (4.9%) and aural cancers in 4 cases (1.4%).

Conclusion: Head and neck cancers predominantly involved the oral cavity, hypopharynx, and oropharynx, together accounting for nearly two-thirds of all cases. An overall male predominance was observed, consistent with reported regional patterns. However, the near-equal gender distribution of hypopharyngeal cancers among males and females was a noteworthy finding and warrants further investigations through larger multicentre studies to explore potential regional demographic, environmental, and risk factor influences.

Key Words*: Head and Neck Neoplasms, Hypopharyngeal Neoplasms, Mouth Neoplasms, Retrospective Studies; Tertiary Healthcare.*

Introduction

Head and neck cancers (HNC) constitute a heterogeneous group of malignancies arising from the oral cavity, pharynx, larynx, salivary glands, nasal cavity and paranasal sinuses, and thyroid gland. Because of this anatomical and histological diversity, patients with HNC may present with widely varying symptoms, ranging from painless neck swelling to hoarseness, nasal obstruction, dysphagia, or a mass discovered incidentally on imaging. Globally, HNC ranks among the common cancer groups making 4.7% of all the cancers. [1] The burden and subsite distribution of HNCs vary considerably by region, reflecting local differences in tobacco and areca nut (Paan & Chalya) use, alcohol consumption, Naswar use and human papillomavirus exposure. [2] In South Asia, including Pakistan, HNC constitute a disproportionately large share of the overall cancer burden. [3]

A recent analysis showed that there are approximately 890,000 new cases of HNC worldwide every year. [4] Lip and oral cavity cancers involve the most, followed by pharyngeal cancers. [5] Overall incidence has traditionally been higher in men, but in recent past a relatively higher incidence has been observed among women, particularly regarding thyroid tumours followed by pharyngeal cancers. [6]

Among these, the hypopharyngeal carcinoma is generally regarded as relatively uncommon, but

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associated with more morbidity, carrying the poorest prognosis of all head and neck sites. Traditionally the gender distribution has been described as 4:1 to 8:1 with a significant male predominance.[7]

Oral cavity cancer remains a major public health problem in South Asia, where it is among the leading causes of cancer-related mortality. It has been attributed largely to the widespread use of tobacco chewing (Naswar), areca (betel) nut, and paan.[8] Similarly, the incidence of thyroid cancer is increasing with women being affected more frequently than men.[9]

Local epidemiological data on the relative frequency and demographic pattern of HNC subsites are essential for guiding clinical suspicion, resource allocation, and screening priorities, since patterns observed in high-income settings do not always translate to South Asian populations. The present study was undertaken to document the incidence, site distribution, and demographic pattern of HNC in our region, reporting to the ENT department of Fauji Foundation Hospital Rawalpindi. The drainage area of this hospital spans from Azad Kashmir to the Potohar region down to Gujrat.

Therefore, the aim of this study was to determine the distribution and relative frequency of HNC and to determine the age and gender distribution and histological types of HNC across different anatomical subsites.

Materials and Methods

This was a retrospective cross-sectional record-based descriptive study conducted in the ENT Department Fauji Foundation Hospital Rawalpindi from July 01, 2020, to December 31, 2024. Approval of the study was obtained from the Hospital Ethical Committee dated July 24, 2026. Hospital records, operation theatre registers, and histopathology reports of the ENT Department were reviewed.

All patients of either gender, in any age group, with a clinically and histologically confirmed diagnosis of HNC presenting to or admitted under the ENT department during this period were included in the study by census of eligible records. Patients with incomplete records, or with missing reports, and duplicate registrations and metastatic lesions from outside head and neck were excluded from the study.

A structured proforma was used to record patient demographic details (age, sex), site and sub site of the tumour, and histopathological diagnosis. HNCs were categorized into ten anatomical sub-sites: oral cavity, hypopharynx, oropharynx, nasal cavity/ paranasal sinuses, nasopharynx, larynx, neck tumours with unknown primary, salivary gland tumours, thyroid cancers and aural tumours. Tumours were classified histologically according to the WHO Classification of Tumours. Data was collected and analyzed using SPSS v26. Descriptive statistics (frequency and percentages) were calculated for each tumour category. Categorical variables (gender, tumour subsite, histological types) were summarized as frequencies and percentages. Age was recorded as a range across the study population. Age range was divided into seven groups; below 20 years, between 21- 30 years, between 31-40 years, between 41-50 years, between 51-60 years, between 61-70 years, and between 71-80 years. Chi-square test of independence was used to analyze association between gender and tumour site across the age range.

Results

Of the 305 patients recorded with HNC, 211 (69.2%) were males, while 94 (30.8%) were females as shown in Figure 1.

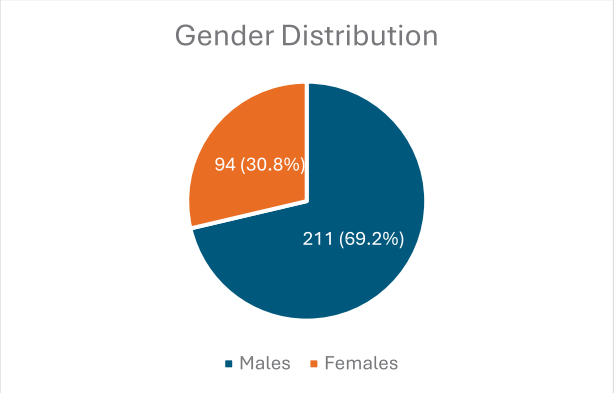

Figure 1: Gender Distribution (n= 305)

These cases fell in an age range of 14 to 79 years with a mean age of 61.76 years SD ± 9.392.

Oral cavity tumours were the leading contributors with 76 cases (24.9%). These were followed by hypopharyngeal carcinomas in 51 cases (16.7%), oropharyngeal cancers in 47 cases (15.4%), nasal/paranasal carcinomas in 29 cases (9.5%),

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nasopharyngeal cancers in 27 cases (8.9%), and laryngeal cancers in 23 cases (7.5%). Salivary carcinomas in 18 cases (5.9%), thyroid carcinomas and neck tumours with unknown primary 15 cases (4.9%) each, and aural cancers in 4 cases (1.4%) followed these. The site wise distribution of tumours is summarized in Table I.

Table I: Site wise Distribution of Head and Neck Tumours (n=305)

S. No.	Tumour Type	No. of Cases	Percentage (%)
1	Oral Cavity Tumours	76	24.9
2	Hypopharyngeal Tumours	51	16.7
3	Oropharyngeal Tumours	47	15.4
4	Nasal / Paranasal Sinus Tumours	29	9.5
5	Nasopharyngeal Tumours	27	8.9
6	Laryngeal Tumours	23	7.5
7	Salivary Gland Tumours	18	5.9
8	Neck Tumours with Unknown Primary	15	4.9
9	Thyroid Cancers	15	4.9
10	Aural Cancers	4	1.4
	Total	**305**	**100.00**

Gender Wise Tumour Site Distribution is shown in Figure: 2

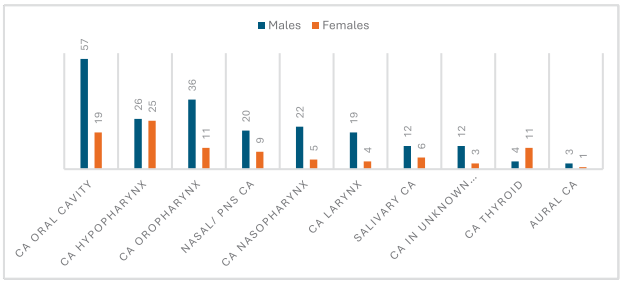

Figure 2: Gender-Wise Distribution of Head and Neck Cancers by Site (n= 305)

The male-to-female ratio was 2.2:1 (211 males and 94 females), most marked in laryngeal tumours (4.75:1) followed by nasopharyngeal tumours (4.4: 1) and neck tumours with unknown primary (4:1). Hypopharyngeal tumours presented with an almost equal gender distribution (1:1). But thyroid tumours showed a prominent female predominance (1: 2.75). The age of patients ranged from 14 to 79 years. Site incidence across the age range is shown in Table II.

Table II: Distribution Of HNC Across Age Groups (n=305)

TUMOUR SITE	AGE RANGE							
Site	Below 20 Yrs	21-30 Yrs	31-40 Yrs	41-50 Yrs	51-60 Yrs	61-70 Yrs	71-80 Yrs	Total
Ca Oral Cavity	0	0	0	2	13	59	2	76
Ca Hypopharynx	0	0	0	6	16	29	0	51
Ca Oropharynx	0	0	0	0	19	26	2	47
Nasal/ PNS Ca	0	3	0	0	3	23	0	29
Ca Nasopharynx	0	0	1	0	9	17	0	27
Ca Larynx	0	0	0	0	1	20	2	23
Ca Salivary Glands	0	0	0	2	1	12	3	18
Ca Unknown Primary	0	0	0	0	0	14	1	15
Ca Thyroid	4	2	0	0	2	3	4	15
Aural Ca	0	0	0	0	0	2	2	4
Total	4	5	1	10	64	205	16	305

There was a statistically significant association between gender and tumour site ($\chi^2(9) = 27.86$, $p = 0.001$, Cramer's V = 0.30). The thyroid and hypopharyngeal carcinomas significantly over-represented among females relative to males. Gender was also significantly associated with age at presentation ($\chi^2(6) = 43.53$, $p < 0.0001$, Cramer's V = 0.38), with males concentrated in the 61–70 year decade and females showing an earlier and broader age distribution.

Comparison of site incidence between both the genders across the age range is shown in Table III.

Well-differentiated squamous cell carcinomas were seen in 190 (62.3%) cases, poorly differentiated squamous cell carcinomas in 65 (21.3%) cases, undifferentiated carcinomas in 13 (4.3%) cases, adenocarcinomas in 15 (4.9%) cases, papillary/ follicular carcinomas in 14 (4.6%) cases, mucoepidermoid carcinomas in 5 (1.6%) cases, adenoid cystic carcinomas in 2 (0.65%) cases and medullary thyroid carcinoma in 1 (0.3%) case as shown in Figure 3.

Discussion

HNCs represent a diverse spectrum of pathology encountered routinely in otorhinolaryngology practice, and their pattern varies with geography, environmental exposures, tobacco and betel-nut use, viral aetiology. The present study describes the pattern of HNC in 305 cases, and highlights oral cavity, hypopharynx and oropharyngeal lesions as

Table III: Distribution of Tumour Sites by Gender and Age Group (n=305)

Gender	Tumour Site	Age Range							Total
		Below 20 Yrs	21-30 Yrs	31-40 Yrs	41-50 Yrs	51-60 Yrs	61-70 Yrs	71-80 Yrs	
Male	Ca Oral Cavity	0	0	0	2	5	49	1	57
	Ca Hypopharynx	0	0	0	0	5	21	0	26
	Ca Oropharynx	0	0	0	0	14	20	2	36
	Nasal/ PNS Ca	0	1	0	0	3	16	0	20
	Ca Nasopharynx	0	0	1	0	8	13	0	22
	Ca Larynx	0	0	0	0	1	16	2	19
	Ca Salivary glands	0	0	0	0	0	9	3	12
	Ca Unknown Primary	0	0	0	0	0	11	1	12
	Ca Thyroid	2	0	0	0	0	0	2	4
	Aural Ca	0	0	0	0	0	2	1	3
Total		**2**	**1**	**1**	**2**	**36**	**157**	**12**	**211**
Female	Ca Oral Cavity	0	0	0	0	8	10	1	19
	Ca Hypopharynx	0	0	0	6	11	8	0	25
	Ca Oropharynx	0	0	0	0	5	6	0	11
	Nasal/ PNS Ca	0	2	0	0	0	7	0	9
	Ca Nasopharynx	0	0	0	0	1	4	0	5
	Ca Larynx	0	0	0	0	0	4	0	4
	Ca Salivary glands	0	0	0	2	1	3	0	6
	Ca Unknown Primary	0	0	0	0	0	3	0	3
	Ca Thyroid	2	2	0	0	2	3	2	11
	Aural Ca	0	0	0	0	0	0	1	1
Total		**2**	**4**	**0**	**8**	**28**	**48**	**4**	**94**
GRAND TOTAL									**305**

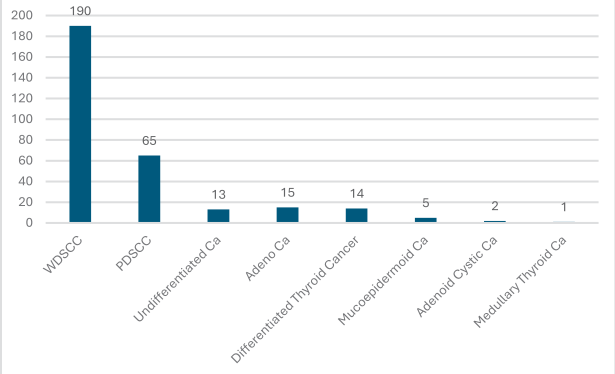

Figure 3: Histological Types of HNC

the leading categories. This pattern is not very different from results of other studies that report oral cavity and laryngeal squamous cell carcinoma as the dominant lesions. [10]

HNCs commonly occur in the fifth, sixth and seventh decades of life. [11] The age range in our study is 14 to 79 years. The occurrence of cases as young as 14 years is consistent with the recognized occurrence of salivary and sinonasal tumours in young adults and underscores the importance of maintaining head and neck tumours in the differential diagnosis across all age groups. [12] A local study also shows this high male predominance among HNC in Asia. [13] Other Pakistani studies also point towards this male predominance among the patients of HNC. [14]

The male predominance observed in this study 69.2% (male: female ratio of 2.2: 1) is in keeping with the well-documented worldwide trend of higher incidence of HNC in males, attributable in large part to the higher prevalence of tobacco smoking, alcohol use, and occupational exposures among men in most populations. [15] Globally, the male-to-female incidence ratio for HNCs typically ranges from about 3:1 to 5:1. [16] This lower ratio may partly be explained by the marked female predominance among patients with thyroid tumours in our study.

This marked male preponderance observed in laryngeal tumours (4.75:1) is consistent with the established association between laryngeal carcinoma and tobacco and alcohol use. [17]

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Nasopharyngeal carcinoma (4.4:1) and neck masses of unknown primary (4:1) also showed strong male predominance in our series, in keeping with regional and international trends of male-dominant upper aero-digestive tract malignancy.

In contrast, thyroid carcinoma showed a clear female predominance (1:2.75) in our study, which is similar to well-established global epidemiology. It shows papillary thyroid carcinoma incidence to be roughly 2.6 times higher in females than males, a disparity thought to relate to hormonal and reproductive factors rather than classical tobacco/alcohol-related carcinogenesis . [18] The predominance of thyroid tumours showing a marked female preponderance is consistent with the well-documented global rise in thyroid cancer incidence among women. Asia-specific data similarly show that thyroid cancer disproportionately affects women across all age groups, with incidence continuing to rise toward 2040. [9]

The near-equal gender distribution of hypopharyngeal tumours in males and females is a noteworthy finding, since this site is usually reported as one of the least common HNC, comprising only a small percentage of all squamous cell carcinomas of the region.[19] These finding merits particular attention, especially alongside our observation that a majority (17/25) of female hypopharyngeal cancer cases occurred in the fifth and sixth decades of life, a younger age bracket than typically expected. This may reflect true regional variation in risk factor exposure, as incidence of plummer vinson syndrome (PVS) due to iron deficiency anemia is quite common in this region.[20] Given the high prevalence of nutritional iron-deficiency anemia among women in the Indian subcontinent, this mechanism offers a biologically plausible explanation for this unusual gender pattern observed in the present study, although histopathological subtype and a formal assessment of iron status in these patients were beyond the scope of this study. Statistics confirm transformation of postcricoid mucosa towards malignancy in PVS. [21] Oral cavity carcinoma was the most frequently encountered subsite in our study (76 cases, 24.9%). It is consistent with the data that shows oral squamous cell carcinoma to be the most common HNC in South Asia despite an overall global decline in prevalence, largely attributed to the persistently high prevalence of areca nut, betel quid, and tobacco-chewing habits in the region. [22] The high proportion of oral cavity tumours in our study is consistent with the established pattern of dominance of oral cavity tumours due to a common habit of use of smokeless tobacco, betel quid, and areca nut. [4]

Hypopharyngeal (16.7%) and oropharyngeal (15.4%) carcinomas were the next most frequent sites in our series, followed by nasal/paranasal, nasopharyngeal, laryngeal, salivary gland, thyroid, neck node with unknown primary, and aural malignancies. This pattern differs somewhat from other Pakistani series in which nasopharyngeal carcinoma has been reported as the second most common site after the oral cavity. [11] Such variation between centers likely reflects differences in catchment population, referral patterns, and local dietary/occupational exposures, and highlights the value of institution-specific epidemiological data such as ours in guiding local screening and resource allocation strategies rather than relying solely on aggregated national figures.

Overall, these findings reinforce the importance of generating locally derived epidemiological data rather than depending solely on international series, since the demography and risk factors of HNC can vary substantially.

This study has several limitations that merit consideration. First, as a retrospective, single-center, record-based study, our findings are subject to the inherent limitations of incomplete or inconsistently documented clinical records and absence of individual level information on risk factor exposure. And lastly, the relatively small sample size limits the precision of subgroup comparisons and increases the influence of incidental findings.

Conclusion

Head and neck cancers predominantly involved the oral cavity, hypopharynx, and oropharynx, together accounting for nearly two-thirds of all cases. An overall male predominance was observed, consistent with reported regional patterns. However, the near-equal gender distribution of hypopharyngeal cancers among males and females was a noteworthy finding and warrants further investigations through larger multicentre studies to explore potential regional demographic,

environmental, and risk factor influences.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORIGINAL ARTICLE

Do Professional Attitudes Align with Academic Performance? A Cluster Analysis of Physical Therapy Students

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ABSTRACT

Objective: To identify professional attitudes among physical therapy graduates and to find patterns of professional attitudes in relation to academic performance using cluster analysis.

Study Design: An analytical cross-sectional study.

Place and Duration of Study: Department of Physical Therapy, Faculty of Medicine, Prince of Songkla University, Thailand between March 01, 2025 to September 30, 2025.

Materials and Methods: The study included 65 physical therapy graduates. Participants completed an adapted questionnaire assessing professional attitudes across four domains. Grade point averages (GPAs) were obtained from official academic records. K-means cluster analysis was used to identify clusters based on GPA and professional attitude scores. Cluster characteristics were summarized descriptively, followed by exploratory comparisons using the Kruskal–Wallis test with post-hoc Mann–Whitney U tests.

Results: Overall, participants reported favourable attitudes toward the physical therapy profession. Three distinct profiles emerged. The profiles were characterized by lower relative GPA with high attitude scores, higher GPA with high attitude scores, and intermediate GPA with comparatively lower attitude scores. These profiles illustrate that academic performance, and professional attitudes do not necessarily align.

Conclusion: The findings indicate variation in professional attitudes across levels of academic performance among physical therapy graduates. These findings highlight the importance of considering professional attitudes alongside academic performance in physical therapy education.

Key Words *Academic Performance, Cluster Analysis, Physical Therapy Education, Professional Attitude, Professional Identity.*

Introduction

Rehabilitation has become a global health priority because of population ageing, the increasing prevalence of chronic diseases, and the growing burden of disability worldwide. The World Health Organization (WHO) recognizes rehabilitation as an essential health service and a key component of Universal Health Coverage. Timely access to rehabilitation improves functioning, independence, participation, and quality of life for people of all ages. Despite this growing need, the WHO reports a substantial global shortage of rehabilitation professionals, particularly in low- and middle-income countries. [1]

Physical therapy plays an essential role in restoring function, reducing disability, promoting health, and improving quality of life across diverse populations. Physical therapists increasingly work within complex healthcare systems that require not only sound clinical knowledge and technical competence but also ethical judgment, effective communication, interprofessional collaboration, adaptability, and commitment to lifelong learning. [2] Consequently, physical therapy education is expected to prepare graduates who are academically competent and able to demonstrate professional values and attitudes that support responsible and patient-centered practice.

Professional attitude refers to individuals' positive or negative perceptions of their profession, including interest, satisfaction, and commitment. [3] Within health professions education (HPE), professional attitude is considered a key component of

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professionalism and professional identity formation. [4,5] Professional attitudes develop progressively through interactions between learners and their educational environment. Factors such as the formal curriculum, clinical education experiences, role modelling by faculty members and clinical mentors, reflective practice, and interactions with patients and healthcare teams all contribute to shaping students' professional values and attitudes. [4] A supportive learning environment that promotes professionalism, ethical practice, and reflective learning can further strengthen students' professional identity and commitment to the profession. [5] Positive attitudes have been associated with greater motivation, engagement in learning, commitment to professional standards, and resilience during academic and clinical training. [3,6] Conversely, negative attitudes may contribute to reduced motivation, disengagement from learning, and diminished persistence in professional development. [7]

Academic achievement, commonly measured by grade point average (GPA), remains a standard indicator of student performance. [8] However, GPA primarily reflects cognitive achievement and may not adequately capture students' professional values, engagement, or attitudes. Previous studies examining the relationship between academic performance and professional attitudes have reported inconsistent findings, with some demonstrating positive associations and others reporting weak or no relationships. [9,10] These inconsistencies may be attributable to differences in measurement instruments used to assess professional attitudes, variations in sample characteristics and sample sizes, differences in educational curricula and clinical training experiences, and cultural or institutional contexts across countries. Such methodological and contextual heterogeneity may partly explain why the reported relationships have varied across studies. Consequently, academic performance alone may not fully explain students' professional orientation.

Most studies in physical therapy education have relied on descriptive or correlational approaches, which focus on average relationships and may overlook meaningful differences among students.[11,12] Students with similar academic performance may differ substantially in motivation, professional commitment, and perceptions of the profession, while some lower-performing students may demonstrate strong professional values and engagement. [13-15] In contrast to conventional variable-centered methods such as correlation or regression, cluster analysis adopts a person-centered approach by identifying naturally occurring subgroups of individuals who share similar patterns of characteristics. This approach can reveal heterogeneity within a population that may be obscured when only average relationships are examined. The resulting profiles may provide educators with more meaningful information for designing targeted educational strategies, mentoring, and professional development initiatives tailored to the needs of different student groups. Person-centered approaches, such as cluster analysis, provide an alternative framework by identifying subgroups of students who share similar patterns of academic achievement and professional attitudes. [11,12] Although such approaches have gained attention in educational research, they remain underutilized in physical therapy education. [16,17]

In addition, evidence regarding physical therapy (PT) students' professional attitudes has been derived predominantly from Western and Middle Eastern settings, [18,19] whereas evidence from Southeast Asia, particularly Thailand, remains limited. Educational systems, cultural expectations, and societal perceptions of healthcare professions may influence both professional attitudes and their relationship with academic performance. Understanding these relationships within specific contexts is therefore important for developing targeted educational strategies and supporting professional identity formation.

Accordingly, this study examined PT students' attitudes toward their profession and explored their relationship with academic achievement using cluster analysis. By identifying distinct academic–attitudinal profiles, the study aimed to provide insights that may inform educational planning, student support, and professional development in physical therapy education. We hypothesized that distinct academic attitudinal profiles would emerge among PT students, reflecting heterogeneous patterns of academic achievement

and professional attitudes.

Materials and Methods

Analytical cross-sectional study examined PT students' professional attitudes and their relationship with academic performance. The study was conducted at the Department of Physical Therapy, Faculty of Medicine, Prince of Songkla University, Thailand. The university is a public university, and the study was conducted within a single institutional setting. Ethical approval was obtained from the institutional human research ethics committee (REC. 66-260-30-2; 28 July 2023). All procedures complied with the Declaration of Helsinki. Participation was voluntary, and informed consent was implied through completion of the online questionnaire.

No formal sample size calculation was performed because a total population sampling approach was used. All 100 eligible graduates were invited to participate; current final-year students were not included. Inclusion criteria were graduates who had successfully completed all academic requirements and mandatory clinical training of the physical therapy curriculum and graduated between 2023 and 2025. Of these, 65 completed the survey, resulting in a response rate of 65%. The remaining 35 graduates did not participate; no questionnaires were excluded because of incomplete responses, and there was no loss to follow-up. Specific reasons for non-participation were not collected.

Professional attitudes were assessed using a structured questionnaire adapted from a previously developed Thai-language instrument for PT students.[7] Because the original instrument was in Thai, language translation and forward–backward translation were not required. Content validity of the adapted questionnaire was evaluated by three experts using the Index of Item-Objective Congruence (IOC). IOC values for the retained items ranged from 0.67 to 1.00, indicating acceptable content congruence. Any item with an initial IOC value of 0.33 was revised based on the experts' recommendations before administration of the final questionnaire. The final questionnaire demonstrated excellent internal consistency, with a Cronbach's alpha of 0.910.

The instrument consisted of two sections: (i) demographic information, including age, sex, and year of graduation; and (ii) 27 attitude items covering four domains: social values (7 items), desirable characteristics of the profession (7 items), general professional practice (7 items), and relationships with colleagues, patients, and patients' relatives (6 items) (Table I).

Responses were rated on a 4-point Likert scale ranging from strongly disagree (1) to strongly agree (4). Negatively worded items were reverse scored so that higher scores indicated more positive attitudes. Although individual Likert items are ordinal, items within each domain were aggregated to form composite scores. Domain and overall attitude scores were calculated as the mean of their constituent items to preserve the original response metric and provide continuous composite measures for profile analysis.

Data were collected through an online questionnaire administered via Google Forms. The survey link and QR code were distributed through the official Facebook fan page of the Department of Physical Therapy and through LINE groups for each graduating cohort. Participants were informed of the study objectives, confidentiality procedures, and voluntary nature of participation before completing the survey. Cumulative GPA data were extracted from official academic records by authorized academic staff and provided to the research team in a de-identified form. Names, student identification numbers, and other directly identifying information were removed before the data were transferred to the researchers. Consequently, the researchers conducting the analysis were blinded to participants' identities and could not identify individual participants from the dataset.

Descriptive statistics were used to summarize participant characteristics, GPA, and attitude scores. Attitude scores were interpreted as very unfavourable (0.00–1.00), unfavourable (1.01–2.00), favourable (2.01–3.00), and very favourable (3.01–4.00). [7] K-means cluster analysis was performed using standardized (z-score) GPA and overall professional attitude scores. Candidate solutions with two to six clusters were evaluated using the elbow method, average silhouette coefficient, cluster size, and interpretability. The three-cluster solution was retained because it yielded the highest silhouette coefficient (0.439) and

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interpretable academic–attitudinal profiles. Cluster characteristics were summarized descriptively, with exploratory comparisons performed using the Kruskal–Wallis test, followed by pairwise comparisons were performed using Mann–Whitney U tests with Bonferroni correction for multiple comparisons. The $p \leq 0.05$ was considered statistical significant.

Results

Of the 100 eligible graduates, 65 completed the questionnaire (response rate: 65%), and all completed questionnaires were included in the final analysis. Participant characteristics are presented in Table II. Most respondents were female (72.3%), with a mean age of 22.26 ± 1.44 years. Nearly half graduated in 2025 (49%), and the mean cumulative GPA was 3.58 ± 0.18 (range 3.15–3.95).

The mean overall professional attitude score was 3.48 ± 0.24, corresponding to the very favourable category based on the predefined scoring criteria. The highest ratings were observed for items related to clinical reasoning (3.91 ± 0.29), helping others (3.89 ± 0.31), patient care, including inquiry about patients' suffering (3.88 ± 0.33), responsibility (3.86 ± 0.34), and empathy (3.85 ± 0.36). Attitudes toward interpersonal relationships were also highly positive, including friendliness with patients and families (3.80 ± 0.40), collaboration with healthcare team members (3.75 ± 0.43), and friendliness with team members (3.74 ± 0.44) (Figure 1).

Within the social values domain, the highest-rated items were helping fellow humans (3.89 ± 0.31), responsibility (3.86 ± 0.34), being beneficial to self, family, and society (3.82 ± 0.39), and viewing physical therapy as an honourable profession (3.80 ± 0.40). In the desirable characteristic's domain, high scores were observed for empathy (3.85 ± 0.36), attention to detail (3.82 ± 0.39), creativity (3.78 ± 0.45), selflessness (3.74 ± 0.47), discipline and punctuality (3.73 ± 0.44), and enthusiasm (3.72 ± 0.48) (Figure 1).

In the general professional practice domain, the highest scores were observed for clinical reasoning (3.91 ± 0.29), evaluation before and after each treatment session (3.88 ± 0.33), equitable treatment of patients (3.78 ± 0.41), and pride in helping others (3.78 ± 0.41). Lower scores were observed for items concerning work-related injury risk (1.25 ± 0.50), infection risk (1.40 ± 0.55), and potential monotony of practice (2.37 ± 1.02). In the relationship's domain, scores were high for inquiring about patients' suffering (3.88 ± 0.33), friendliness with patients and families (3.80 ± 0.40), collaboration with healthcare team members (3.75 ± 0.43), friendliness with team members (3.74 ± 0.44), and cooperation from patients and families (3.69 ± 0.49) (Figure 1).

Candidate K-means solutions ranging from two to six clusters were evaluated. The average silhouette coefficients were 0.378, 0.439, 0.385, 0.390, and 0.391 for the two-, three-, four-, five-, and six-cluster solutions, respectively. The three-cluster solution yielded the highest silhouette coefficient (0.439) and produced distinct and interpretable academic–attitudinal profiles; therefore, it was retained for subsequent analysis (Table III).

Cluster 0 (n = 25) had a lower GPA (3.42 ± 0.11) than Cluster 1 (3.75 ± 0.10) but had the highest professional attitude score (3.60 ± 0.15). Cluster 1 (n = 28) had the highest GPA (3.75 ± 0.10) and a similarly high attitude score (3.58 ± 0.12). Cluster 2 (n = 12) had an intermediate GPA (3.56 ± 0.15) but the lowest professional attitude score (3.04 ± 0.19). As expected from the clustering procedure, the identified clusters differed in GPA and professional attitude scores. Exploratory statistical comparisons confirmed that these profiles were distinct (Kruskal–Wallis test, $p < 0.001$).

Pairwise Mann–Whitney U tests with Bonferroni correction showed significant differences in GPA between all cluster pairs (Cluster 0 vs 1, adjusted $p < 0.001$; Cluster 0 vs 2, adjusted $p = 0.007$; Cluster 1 vs 2, adjusted $p < 0.001$). For overall professional attitude scores, Cluster 2 differed significantly from both Cluster 0 and Cluster 1 (both adjusted $p < 0.001$), whereas no significant difference was observed between Clusters 0 and 1 (adjusted $p = 1.000$).

Figure 2 shows the differences in GPA and professional attitude scores across clusters, while Figure 3 demonstrates the distribution of individual participants according to cluster membership.

Discussion

This study examined professional attitudes among PT graduates and explored their relationship with academic achievement using a person-centered approach. Overall, participants reported favourable

Table I: Questionnaire Items Assessing Attitudes and Perceptions Toward the Physical Therapy Profession

Item
Social Values
1. PT is a profession beneficial to oneself, family, and society.
2. PT is an honourable profession.
3. PT offers welfare benefits comparable to those of other professions.
4. PT provides career advancement opportunities equal to other professions.
5. PT is a profession dedicated to helping others.
6. PT is a profession respected by society.
7. PT a profession in high demand.
Desirable Characteristics of the PT Profession
8. PT involves responsibility to oneself, the profession, and patients.
9. PT requires discipline and punctuality.
10. PT demands attention to detail.
11. PT encourages creativity.
12. PT fosters empathy toward others.
13. PT promotes selflessness.
14. PT inspires enthusiasm.
General Professional Practice
15. PT practice involves a risk of infection.*
16. PT practice may lead to work -related injury, e.g., back or leg pain from prolonged standing or walking.*
17. PT practice brings pride through helping others.
18. PT practice can lead to boredom.*
19. PT practice requires clinical reasoning and decision -making to provide appropriate care to each patient.
20. PT practice involves treating all patients equally.
21. PT practice requires evaluating patients before and after every treatment session.
Relationships with Colleagues, Patients, and Patients' Relatives
22. PT professionals maintain positive attitudes with interprofessional teams.
23. PT professionals collaborate with interprofessional teams.
24. PT is recognised as a valuable profession within the interprofessional team.
25. PT professionals maintain friendly relationships with patients and their families.
26. PT professionals inquire about patients' and families' concerns and well-being.
27. Most patients and families cooperate well during PT treatment.
Note: *For negatively worded items, the attitude score was assigned as follows: strongly agree = 1 point, agree = 2 points, disagree = 3 points, and strongly disagree = 4 points. PT: physical therapy.

Table II: Respondent characteristics (n = 65)

Variable	*n* (%) / Mean ± SD
Sex	
Male	18 (27.7)
Female	47 (72.3)
Age, years	22.26 ± 1.44 (range 21 – 24)
Year of graduation	
2023	16 (25)
2024	17 (26)
2025	32 (49)
Cumulative GPA	3.58 ± 0.18 (range 3.15–3.95)

Note: GPA: grade point average, SD: standard deviation.

Table III: Cluster-Specific Cumulative GPA And Professional Attitude Scores Derived Using K-Means Clustering (n = 65)

Cluster (n)	Cumulative GPA, Mean ± SD	95% CI	Overall professional attitude score, Mean ± SD	95% CI	Cluster characteristics
0 (25)	3.42 ± 0.11	3.37–3.46	3.60 ± 0.15	3.54–3.66	Lower GPA with a very favourable attitude score
1 (28)	3.75 ± 0.10	3.71–3.79	3.58 ± 0.12	3.53–3.63	Higher GPA with a very favourable attitude score
2 (12)	3.56 ± 0.15	3.47–3.66	3.04 ± 0.19	2.92–3.16	Intermediate GPA with a comparatively lower, but still very favourable, attitude score

GPA: grade point average, SD: standard deviation, CI: confident interval

Pairwise comparisons were performed using Mann–Whitney U tests with Bonferroni correction for three comparisons (adjusted significance threshold, $p < 0.0167$). For cumulative GPA, all pairwise comparisons were significant (Cluster 0 vs 1, adjusted $p < 0.001$; Cluster 0 vs 2, adjusted $p = 0.007$; Cluster 1 vs 2, adjusted $p < 0.001$). For overall professional attitude scores, Cluster 2 differed significantly from Clusters 0 and 1 (both adjusted $p < 0.001$), whereas Clusters 0 and 1 did not differ significantly (adjusted $p = 1.000$).

attitudes toward the profession. Cluster analysis identified three academic–attitudinal profiles based on different combinations of GPA and professional attitude scores. These profiles provide an exploratory description of heterogeneity within the sample rather than independent evidence of an

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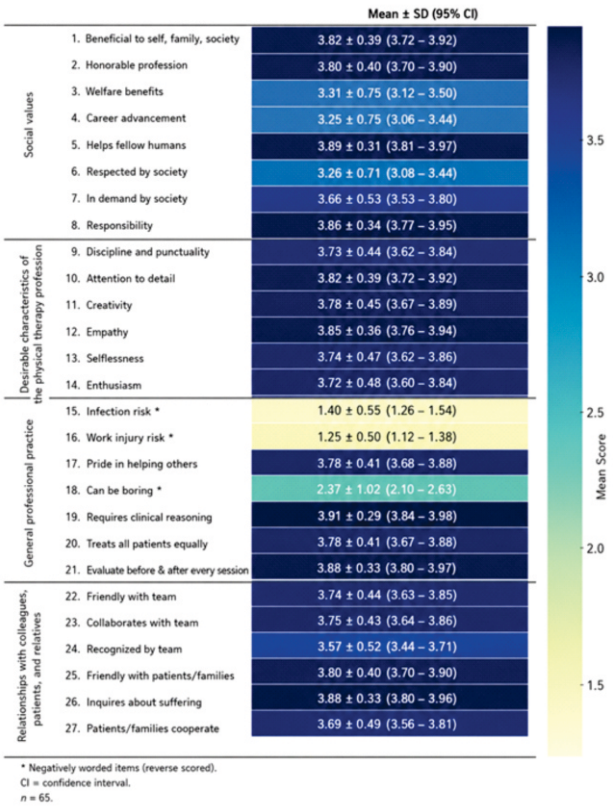

Figure 1: Heatmap Showing the Mean ± SD Of Scores for Attitude Toward the PT Profession by Questionnaire Item.

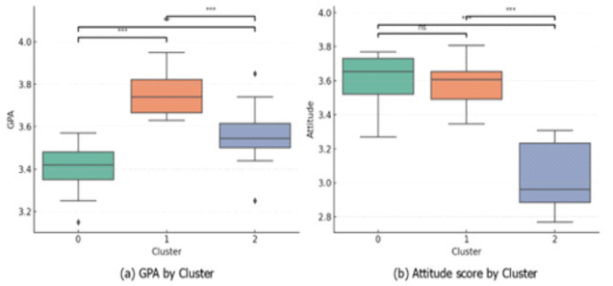

Figure 2: Comparison Of (A) GPA And (B) Professional Attitude Scores Across Clusters Based on K-Means Analysis

Note: Pairwise comparisons were performed using Mann–Whitney U tests with Bonferroni correction. For three pairwise comparisons, the adjusted significance threshold was set at $p < 0.0167$. ***$p < 0.001$, **$p < 0.01$, ns = not significant.

association between academic performance and professional attitudes.

Graduates demonstrated particularly positive attitudes toward the social and ethical dimensions of physical therapy, including helping others, responsibility, empathy, and clinical reasoning. These findings are consistent with previous studies reporting favourable professional attitudes among PT students in Thailand and other countries. [7,18,19] Participants also highly valued clinical reasoning, individualized patient management, and equitable treatment, reflecting positive attitudes toward key aspects of professional practice.

Despite these positive perceptions, graduates reported concerns regarding work-related injury and infection risks, reflected by low original scores on these negatively worded items (1.25 ± 0.50 and 1.40 ± 0.55, respectively). These items were reverse scored for the calculation of composite attitude scores. Similar occupational concerns have been reported among practicing physical therapists. [20,21] Attitudes toward communication and collaboration with patients, families, and healthcare teams remained favourable. [22]

The principal finding of this study was the identification of three distinct academic–attitudinal profiles among PT graduates. Cluster 1 was characterized by the highest GPA and a high professional attitude score. This profile represents the co-occurrence of relatively high academic performance and positive professional attitudes within this subgroup; however, the cluster analysis does not provide evidence regarding educational engagement, professional commitment, or other characteristics that were not directly measured.

Comparison of Clusters 0 and 2 illustrates heterogeneity in academic–attitudinal profiles. Cluster 0 had a lower GPA but higher professional attitude scores, whereas Cluster 2 had an intermediate GPA but lower attitude scores. Consistent with person-centered approaches, these profiles demonstrate that individuals can exhibit different combinations of academic and attitudinal characteristics that may not be apparent from average group-level relationships. [11,12] Professional identity formation may provide a theoretical context for understanding such variation because professional identity develops through interactions among personal values, educational experiences, and professional contexts. [4,5] However, professional identity, motivation, and educational experiences were not directly assessed in this study. Therefore, the identified clusters should be interpreted as exploratory academic–attitudinal profiles rather

than evidence of underlying professional commitment or identity development.

The identification of graduates with different combinations of academic performance and professional attitudes may have implications for educational planning. Professional attitudes have been associated with career perceptions, learning engagement, and responses to academic and clinical experiences, [3,6] whereas less favourable attitudes have been linked to adverse educational experiences and reduced professional engagement. [22] Evidence from medical and nursing education likewise suggests that professional attitudes, career motivation, and professional commitment may not correspond directly with academic performance. [3,9,10] Although these factors were not directly assessed in the present study, the identified profiles highlight the value of considering professional attitudes alongside academic indicators. Recognizing such heterogeneity may help educators develop more individualized approaches to student support and professional development.

These findings have several implications for PT education. Academic and professional attitude indicators may provide complementary perspectives on student development, as GPA alone may not capture differences in professional attitudes. However, the exploratory cluster profiles should not be used to classify or label individual students and further validation in larger and more diverse samples is needed before individual-level educational applications can be recommended. Educational approaches such as reflective learning, mentorship, role modelling, career guidance, and meaningful clinical experiences may support professional attitude and identity development, although these strategies were not directly evaluated in the present study. The present study illustrates the potential of person-centered analytical approaches for exploring heterogeneity in HPE. Cluster analysis identified subgroups with different combinations of academic performance and professional attitude scores that may not be apparent from group-level analyses. However, these profiles are exploratory and require external validation in larger and more diverse samples before their practical usefulness or application to educational interventions can be established.

Several limitations should be considered. First, the cross-sectional design precludes causal inferences regarding academic performance and professional attitudes. Second, professional attitudes were assessed using a self-report questionnaire with limited evidence of construct validity in the present population, and possible ceiling effects in attitude scores may have reduced discrimination between participants. Third, the 65% response rate raises the possibility of non-response bias, and the absence of information on non-participants prevented assessment of its potential influence. Fourth, the single-institution setting and relatively small sample limit generalizability. The cluster analysis should be considered exploratory given the modest sample size and the small number of participants in one cluster. Although the number of clusters was evaluated using the elbow method and silhouette coefficient, cluster stability and external validation were not assessed. Furthermore, because GPA and professional attitude scores were used both to construct and subsequently compare the clusters, between-cluster differences in these variables are expected by construction and should not be interpreted as independent validation of the cluster solution. The relatively narrow GPA range and possible ceiling effects in attitude scores may also have limited differentiation among participants. Finally, potentially relevant factors, including personality traits, clinical experiences, career expectations, and social support, were not assessed and may contribute to variations in professional attitudes. The identified profiles therefore require validation in larger and more diverse samples.

Despite these limitations, this study provides exploratory evidence on academic–attitudinal profiles among PT graduates in Thailand. The cluster analysis identified different combinations of GPA and professional attitude scores within the sample, although these profiles require validation before their educational relevance can be established. Future longitudinal and multi-institutional studies with larger samples are needed to examine changes in professional attitudes over time and their relationships with academic and professional outcomes. Further research should also investigate contextual and psychosocial factors that may contribute to variations in professional attitudes.

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Conclusion

PT graduates generally demonstrated favourable attitudes toward their profession. Cluster analysis identified distinct exploratory academic–attitudinal profiles, demonstrating heterogeneity in the combinations of academic achievement and professional attitude scores within the study population.

These results support the assessment of professional attitudes alongside academic performance and highlight the value of person-centered approaches for understanding student diversity. Educational strategies that foster professional identity formation and positive professional attitudes may support holistic student development and preparation for professional practice. These findings may also inform curriculum development.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORIGINAL ARTICLE

Prevalence of Selected Cardiovascular Risk Factors among Patients with Chronic Kidney Disease Receiving Maintenance Hemodialysis at a Tertiary Care Hospital in Islamabad

Muhammad Ali Riaz[1], Saima Bashir[2], Mobina Zafar[3], Bibi Taseer Fatima[4], Riaz[5]

ABSTRACT

Objective: To determine the prevalence of selected traditional cardiovascular risk factors and CKD-related cardiovascular risk-associated abnormalities among patients with chronic kidney disease receiving maintenance hemodialysis.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: Department of Nephrology, Shifa International Hospital, Islamabad, from 15 September 2020 to 14 March 2021.

Materials and Methods: A total of 150 patients with CKD on maintenance hemodialysis, aged 18–70 years, were recruited using consecutive sampling. Patients with congenital or rheumatic heart disease, chronic liver disease or malignancy were excluded. Demographic and clinical data were collected, and laboratory investigations included complete blood count, serum calcium, phosphate, and HbA1C. Data were analyzed using SPSS version 22. Continuous variables were presented as mean ± standard deviation, and categorical variables as frequencies and percentages. Associations were assessed using chi-square tests, with p ≤0.05 considered statistically significant.

Results: The mean age of participants was 51.2 ± 8.4 years; 103 (68.7%) were male and 47 (31.3%) female participants. Hypertension was present in 64.0% of patients, diabetes mellitus in 38.0%, hypocalcemia in 64.7%, hyperphosphatemia in 50.0%, and anemia in 72.0%. Anemia was slightly more prevalent in females (78.7%) than males (68.9%). Diabetes mellitus was significantly more common in males (44.7%) than females (23.4%) (p=0.013). Other risk factors did not show statistically significant sex or age differences.

Conclusion: Traditional cardiovascular risk factors and CKD-related abnormalities associated with cardiovascular risk were highly prevalent among patients receiving maintenance hemodialysis. Anemia was the most prevalent CKD-related cardiovascular risk-associated abnormality, followed by hypocalcemia, hypertension, hyperphosphatemia, and type 2 diabetes mellitus.

Key Words: *Anemia, Cardiovascular Diseases, Chronic Kidney Disease, Hemodialysis, Hypertension.*

Introduction

Chronic kidney disease (CKD) is a major global public health problem, with its prevalence and associated healthcare burden continuing to rise.[1] Available Pakistani studies have reported a substantial burden of CKD, with pooled or study-specific estimates varying according to the population and diagnostic criteria used. Diabetic nephropathy and hypertension are among the leading causes of CKD in the Pakistani population.[2] These figures highlight CKD as a significant public health challenge in the country.

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality among patients with CKD, particularly those receiving maintenance hemodialysis (HD).[3] The strong association between CKD and CVD is explained by shared traditional cardiovascular risk factors, including hypertension, diabetes mellitus, advanced age, and smoking, as well as CKD-specific factors such as uremic toxins, anemia, chronic inflammation, oxidative stress, and disturbances in calcium and phosphorus metabolism. Together, these factors contribute to structural and functional cardiac abnormalities and substantially increase cardiovascular risk among

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patients with end-stage renal disease (ESRD).[4–7]

Although several international studies have described the high burden of cardiovascular disease among patients undergoing hemodialysis, there is limited published evidence regarding the prevalence of cardiovascular disease risk factors among Pakistani patients with ESRD receiving maintenance hemodialysis. Given the high prevalence of CKD in Pakistan, the increasing burden of non-communicable diseases, and the resource constraints within the healthcare system, locally generated evidence is essential to guide risk stratification, preventive strategies, and clinical management.

Therefore, this study aimed to determine the prevalence of selected traditional cardiovascular risk factors and CKD-related cardiovascular risk-associated abnormalities among patients receiving maintenance hemodialysis.

Materials and Methods

This descriptive cross-sectional study was conducted from 15 September 2020 to 14 March 2021 at the Department of Nephrology, Shifa International Hospital, Islamabad. The sample size of 150 participants was calculated using the WHO sample size calculator, assuming a population proportion of 21 and a 91% confidence interval an a margin of error of 6.5%[2]. The population proportion used for the calculation was based on a previously reported prevalence of CKD in Pakistan. As this estimate was not specific to cardiovascular risk factors or CKD-related abnormalities among patients receiving maintenance hemodialysis, the sample-size calculation was not outcome-specific and is acknowledged as a limitation of the study. Participants fulfilling the inclusion criteria were recruited through consecutive sampling from both the Nephrology outpatient department and the hemodialysis unit of Shifa International Hospital, Islamabad.

Eligible participants included male and female patients aged 18–70 years with chronic kidney disease (CKD) receiving maintenance hemodialysis. Patients with congenital heart disease, rheumatic heart disease, chronic liver disease, or any malignancy were excluded from the study. In addition, the use of medications that influence mineral metabolism, such as phosphate binders, calcium supplements, vitamin D analogues, and calcimimetics, was not specifically recorded or analysed. As these medications may affect serum calcium and phosphate concentrations, they could have influenced the observed prevalence of hypocalcemia and hyperphosphatemia.

Ethical approval was obtained from the Institutional Review Board/Ethics Committee of Shifa International Hospital, Islamabad (IRB No. 457-1277-2020; approval date: 20 June 2020). Eligible patients were enrolled after providing written informed consent.

A detailed clinical history was obtained, followed by a comprehensive physical examination. Blood pressure was measured before the hemodialysis session using an automated blood pressure monitor, with the patient seated and rested for 5 minutes. Two to three blood pressure readings were obtained, and the average of the readings was used for analysis. Five milliliters of venous blood were collected under aseptic conditions before the hemodialysis session for laboratory investigations. Laboratory investigations included complete blood count (CBC), serum calcium, serum phosphate, and glycated hemoglobin (HbA1c). Demographic, clinical, and laboratory data were recorded using a predesigned proforma.

Hypertension was defined as a previous physician diagnosis of hypertension, current use of antihypertensive medication, or a blood pressure ≥140/90 mmHg. Type 2 diabetes mellitus (T2DM) was defined as a previous physician diagnosis of diabetes mellitus, current use of antidiabetic medication, or an HbA1c level ≥6.5%. Anemia was defined according to the World Health Organization criteria as a hemoglobin concentration of <13 g/dL in men and <12 g/dL in women. Hypocalcemia was defined as a serum calcium level of <8.4 mg/dL, while hyperphosphatemia was defined as a serum phosphate level of >4.5 mg/dL, based on the laboratory reference ranges used at Shifa International Hospital.

Data were analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), whereas categorical variables were presented as frequencies and percentages. Cardiovascular risk factors were stratified according to age group (18–45

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years and 46–70 years) and sex (male and female). Associations between categorical variables were assessed using the chi-square test. The assumptions for the chi-square test were verified before analysis. No confidence intervals were calculated. A p-value ≤0.05 was considered statistically significant.

Results

A total of 150 patients with chronic kidney disease (CKD) receiving maintenance hemodialysis were included in the study. The mean age was 51.2 ± 8.4 years. Most participants (111, 74.0%) were aged 46–70 years, while 39 (26.0%) were aged 18–45 years. There were 103 (68.7%) male and 47 (31.3%) female participants, giving a male-to-female ratio of 2.2:1.

Anemia was the most prevalent CKD-related cardiovascular risk-associated abnormality, affecting 108 (72.0%) patients, followed by hypocalcemia in 97 (64.7%), hypertension in 96 (64.0%), hyperphosphatemia in 75 (50.0%), and type 2 diabetes mellitus in 57 (38.0%) patients (Table I).

Table I: Frequency of Selected Traditional Cardiovascular Risk Factors and CKD-Related Cardiovascular Risk-Associated Abnormalities Among Patients Receiving Maintenance Hemodialysis (N = 150)

Selected Cardiovascular Risk Factors and CKD-Related Abnormalities	Frequency (%)	
	Yes	No
Hypertension	96 (64.0%)	54 (36.0%)
Type 2 Diabetes Mellitus	57 (38.0%)	93 (62.0%)
Hypocalcemia	97 (64.7%)	53 (35.3%)
Hyperphosphatemia	75 (50.0%)	75 (50.0%)
Anemia	108 (72.0%)	42 (28.0%)

Among patients aged 18–45 years, hypertension was present in 21 (53.8%) patients compared with 75 (67.6%) patients aged 46–70 years; however, the difference was not statistically significant (p=0.125). Similarly, type 2 diabetes mellitus was observed in 10 (25.6%) younger patients and 47 (42.3%) older patients, with no statistically significant difference (p=0.065). Hypocalcemia was present in 23 (59.0%) younger patients and 74 (66.7%) older patients (p=0.387), while hyperphosphatemia was identified in 22 (56.4%) and 53 (47.7%) patients, respectively (p=0.352). Anemia was observed in 30 (76.9%) patients aged 18–45 years and 78 (70.3%) patients aged 46–70 years (p=0.426). None of the cardiovascular risk factors showed a statistically significant association with age (Table II).

Table II: Association of Selected Cardiovascular Risk Factors and CKD-Related Abnormalities with Age Groups (N = 150)

		18-45 years (n=39)	46-70 years (n=111)	p-value
Hypertension	Yes	21	75	0.125
	No	18	36	
Diabetes Mellitus type 2	Yes	10	47	0.065
	No	29	64	
Hypocalcemia	Yes	23	74	0.387
	No	16	37	
Hyperphosphatemia	Yes	22	53	0.352
	No	17	58	
Anemia	Yes	30	78	0.426
	No	9	33	

Among male participants, hypertension was present in 67 (65.0%) patients compared with 29 (61.7%) female patients (p=0.692). Type 2 diabetes mellitus was significantly more common among males than females (46 [44.7%] vs. 11 [23.4%]; p=0.013). Hypocalcemia was present in 65 (63.1%) male and 32 (68.1%) female patients (p=0.554), whereas hyperphosphatemia was observed in 50 (48.5%) males and 25 (53.2%) females (p=0.597). Anemia was present in 71 (68.9%) male and 37 (78.7%) female patients; however, this difference was not statistically significant (p=0.215). No significant sex-based differences were observed for hypertension, hypocalcemia, hyperphosphatemia, or anemia (Table III).

Table III: Association of Selected Cardiovascular Risk Factors and CKD-Related Abnormalities with Sex (N = 150)

		Male (n=103)	Female (n=47)	p-value
Hypertension	Yes	67	29	0.692
	No	36	18	
Type 2 Diabetes Melitus	Yes	46	11	0.013
	No	57	36	
Hypocalcemia	Yes	65	32	0.554
	No	38	15	
Hyperphosphatemia	Yes	50	25	0.597
	No	53	22	
Anemia	Yes	71	37	0.215
	No	32	10	

Discussion

This study evaluated the prevalence of traditional cardiovascular risk factors (hypertension and type 2 diabetes mellitus) and CKD-related cardiovascular risk-associated abnormalities (anemia, hypocalcemia, and hyperphosphatemia) among patients with chronic kidney disease (CKD) receiving maintenance hemodialysis at a tertiary care hospital in Pakistan. The principal findings were that anemia was the most prevalent CKD-related cardiovascular risk-associated abnormality in our study (72.0%), followed by hypocalcemia (64.7%), hypertension (64.0%), hyperphosphatemia (50.0%), and type 2 diabetes mellitus (38.0%). With the exception of diabetes mellitus, which was significantly more common among male patients, no significant differences in cardiovascular risk factors were observed across age or sex groups.

The mean age of the study population was 51.2 ± 8.4 years, and most participants were between 46 and 70 years of age. A male predominance was observed, with a male-to-female ratio of 2.2:1, which is consistent with previous studies reporting a higher proportion of men among patients receiving maintenance hemodialysis.[14,15] This pattern may reflect differences in disease prevalence, healthcare access, and health-seeking behavior.

In the present study, hypertension was observed in 64.0% of patients receiving maintenance hemodialysis, while type 2 diabetes mellitus was present in 38.0%. These findings are broadly comparable with data from Pakistan, although some variation exists between study populations. A recent study among hemodialysis patients in Lahore reported hypertension in 58% and diabetes mellitus in 56% of participants, compared with 64.0% and 38.0%, respectively, in our cohort.[14] The higher prevalence of hypertension in our study may reflect differences in patient characteristics, referral patterns, dialysis-related factors, and diagnostic criteria, whereas the lower prevalence of diabetes may be related to differences in the underlying causes of CKD and demographic characteristics. In our study, diabetes mellitus was significantly more common among male patients than female participants (44.7% vs. 23.4%; p=0.013). However, the cross-sectional design of the study does not allow causal inferences regarding this association. The observed sex difference may reflect differences in underlying diabetes prevalence, lifestyle factors, healthcare utilization, or other unmeasured characteristics of the study population. Further multicenter studies with larger sample sizes are needed to determine whether this association is consistent across patients receiving maintenance hemodialysis.

Disorders of mineral metabolism were highly prevalent in this cohort. Hypocalcemia was observed in 64.7% of patients, while hyperphosphatemia affected 50.0%. These findings are consistent with the well-recognized disturbances in calcium–phosphate homeostasis that occur in advanced CKD due to impaired phosphate excretion, reduced vitamin D activation, and secondary hyperparathyroidism.[17] Although these abnormalities were slightly more frequent among female patients, the differences were not statistically significant.

Anemia was the most prevalent CKD-related cardiovascular risk-associated abnormality affecting nearly three-quarters of the study population. Reduced erythropoietin production, iron deficiency, chronic inflammation, and shortened red blood cell survival contribute to the high prevalence of anemia in patients with advanced CKD.[19] Although anemia was more common among female participants, the difference did not reach statistical significance. Early identification and appropriate management of anemia are important components of comprehensive care for patients receiving maintenance hemodialysis.[19]

The findings of this study have important clinical implications for the management of patients undergoing maintenance hemodialysis in Pakistan. Regular assessment of blood pressure, glycemic status, hemoglobin, and mineral metabolism should form an integral part of routine nephrology care to facilitate early identification and management of cardiovascular risk factors.

This study has several limitations. As a single-center, cross-sectional study, the findings may not be generalizable to the wider CKD population and causal relationships cannot be established. The relatively small sample size may have limited statistical power to detect significant subgroup differences. In addition, the sample size was calculated using the best available national CKD

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prevalence estimate during protocol development rather than an outcome-specific prevalence for cardiovascular risk factors in patients receiving maintenance hemodialysis, which should be considered when interpreting the findings. Laboratory parameters were measured at a single time point without longitudinal follow-up. In addition, dialysis adequacy, duration and frequency of hemodialysis, underlying causes of CKD, cardiovascular imaging findings, cardiac biomarkers, nutritional status, medication adherence, and socioeconomic factors were not evaluated. Future multicenter prospective studies incorporating these variables are warranted to provide a more comprehensive assessment of cardiovascular risk in patients with CKD receiving maintenance hemodialysis.

Conclusion

Traditional cardiovascular risk factors and CKD-related abnormalities associated with cardiovascular risk were common among patients receiving maintenance hemodialysis. Anemia was the most prevalent abnormality, followed by hypocalcemia, hypertension, hyperphosphatemia, and type 2 diabetes mellitus. Type 2 diabetes mellitus was more prevalent among male participants, whereas the remaining variables were not significantly associated with age or sex. Routine assessment and appropriate management of these abnormalities should be incorporated into the care of patients receiving maintenance hemodialysis to reduce cardiovascular risk.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORIGINAL ARTICLE

Evaluation Of Phenotypic and Genotypic Methods for Detection of Multidrug Resistant Tuberculosis in Pakistan

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ABSTRACT

Objective: To evaluate the phenotypic and genotypic approaches for the diagnosis of drug-resistant *Mycobacterium Tuberculosis*.

Study Design: A cross-sectional study.

Place and Duration of Study: This multicenter study was conducted over a period of six months, from April 01, 2025, to September 30, 2025, in collaboration with several healthcare and academic institutions across Pakistan. The study activities were carried out in the Biosafety Level 3 (BSL-3) Laboratory at the Department of Microbiology, Health and Population Department, Lahore; Tehsil Headquarters (THQ) Hospital Jand, district Attock; Health Services Academy, Islamabad; Islamabad Medical and Dental College, Barakaho; Metropole Laboratories, Islamabad; and Sindh Medical College, Karachi.

Materials and Methods: Eighty-Seven culture-positive samples of *Mycobacterium Tuberculosis* (MTB) isolates were evaluated for susceptibility to first-line anti-tuberculosis medications such streptomycin, isoniazid, ethambutol, and rifampicin, by using the Mycobacteria Growth Indicator Tube (MGIT) 960 system. The GeneXpert for MTB was used for its rapid detection and rifampicin resistance. Multiplex polymerase chain reaction (PCR) assays were optimized to amplify mutations in the *rpoB* gene associated with rifampicin resistance, the *katG* gene associated with isoniazid resistance, and the *embB* gene associated with ethambutol resistance. The amplified PCR products were analyzed by agarose gel electrophoresis to determine the banding patterns associated with multidrug-resistant (MDR) and extensively drug-resistant (XDR) MTB isolates.

Results: Among the 84 MTB isolates, 30 samples were single-drug resistance (SDR), while 4 samples were identified as MDR. Among the SDR isolates, resistance to isoniazid was the most common (n = 24), followed by ethambutol (n = 3) and rifampicin (n = 2). Three non-MTB samples (negative, positive, and normal controls) were included as controls. Additionally, 50 samples were identified as Mycobacteria Other Than Tuberculosis (MOTT).

Multiplex PCR analysis included the following diagnostic performance parameters for MDR: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy (DA) and it demonstrated 66.67%, 100%, 33.33%, 71.43%, and 80% for multidrug resistance; 66.67%, 100%, 50%, 75%, and 85.71% for isoniazid resistance; 80%, 96%, 50%, 84.50%, and 100% for rifampicin resistance; and 50%, 75%, 100%, 80%, and 80% for ethambutol resistance, respectively.

Conclusion: Genotypic methods for diagnosing drug-resistant MTB were faster than phenotypic methods to address important public health challenges in countries like Pakistan.

Key Words*: Genotypic, Mycobacterium tuberculosis, Phenotypic*.

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Introduction

Mycobacterium tuberculosis (MTB) is one of the major health problems with significant morbidity and mortality worldwide.[1] In 2020, the World Health Organization (WHO) published recommendations for tuberculosis (TB) diagnosis and treatment. For developing and low-resource nations alike, TB presents a serious problem. Approximately 25% of the world's population, or over 1.7 billion individuals, have MTB infections.[1,2] Pakistan is ranked fifth for TB and fourth for multi-drug-resistant

tuberculosis (MDR-TB) in the WHO's global report.[1,3-5] Laboratories and clinical radiological studies are used to diagnose TB. However, latent TB was identified by the tuberculin skin test (TST) and the interferon gamma release assay (IGRA). Culture-based testing, which yields phenotypic information, and molecular testing, which yields genotypic information, are two laboratory methods for determining drug susceptibility. The most accurate method now available for diagnosing drug-resistant tuberculosis is culture-based testing, which can take up to a month to complete. Drug resistance can be identified by comparing growth on the drug-containing media with growth on the control medium.[6]

Molecular testing yields results faster; they may now be acquired in a few hours. In the absence of a definitive culture-based Direct Susceptibility test (DST) these tests are useful in assisting with early therapeutic decisions. [7] The two broad categories into which molecular tests may be separated are sequencing-based assays and probe-based (non-sequencing) testing. Probe-based testing is also referred to as amplification assays for nucleic acids. Drug resistance genes can be found utilizing specialized nucleic acid amplification tests (NAAT) that amp up a certain nucleic acid sequence that can be identified with a nucleic acid probe. Assays that rely on sequencing are still being developed and have not been approved globally.[4,8]

The FDA has approved the use of two NAAT test platforms in the United States: The amplified Mycobacterium tuberculosis direct (MTD) test and the Xpert MTB/RIF test. The amplified MTD test is approved for respiratory specimens from people who have suspected TB, regardless of whether the specimens are smear-positive or smear-negative.[9] There are a few studies available which combine genotypic and phenotypic approaches for MTB detection; nevertheless, genotypic approaches aid in the prompt and precise identification of MTB and medication resistance for the patient's benefit.[9]

Therefore, the aim of this study was to evaluate the phenotypic approaches of Mycobacterium Growth Indicator Tube (MGIT) Culture and genotypic approaches (Gene Xpert and Multiplex PCR) for the diagnosis of drug-resistant MTB.

Materials and Methods

A cross-sectional study was carried out in BSL-3 at the Department of Microbiology, Health and population department Lahore, in collaboration with Shaheed ed Uzair Mehmood Tehsil Headquarters (THQ) Hospital Jand district Attock; Health services Academy, Islamabad; Islamabad Medical and Dental College, Barakahu; Metropol Hospital, Islamabad; Sindh Medical Collage Karachi. This multicenter study was conducted over a period of six months, from April 01, 2025 to September 30, 2025.

Using the WHO sample size calculator, the sample size was determined with a 95% confidence level, a 5% margin of error, and a reported sensitivity of 17.33% (GenoType) performance compared to phenotypic direct susceptibility test as reference technique). The sample size was 87 and the method of sampling was sequential, non-probability sampling.

Multidrug resistant (MDR) and single-drug resistant (SDR) samples were chosen for multiplex PCR and pulse field gel electrophoresis in Pakistan after MTB sample collection and Kinyoun Staining.

All smear-positive pulmonary and extrapulmonary drug-resistant MTB isolates, including those that were SDR and MDR were included in the study. We have also included Mycobacterium Other Than Tuberculosis (MOTT) and MTB isolates that are resistant to antituberculosis medications as a control. After the bar code was scanned, every MGIT was put into the system to be incubated. After an infected material produced a culture that was positive in the MGIT system, Digestion, decontamination, homogenization, and concentration were all aided by pretreating smear-positive only specimens using the conventional sodium hydroxide Nacetyl cysteine procedure.[10]

One step in the procedure was applying Kinyoun stain to a smear.

Following WHO guidelines, the smear's grade was established based on the quantity of acid-fast bacilli (AFB) visible under a microscope. A BACTEC MGIT 960 system was injected with a processed smear-positive sample to culture the MTB isolate. Culture media (7H9 broth, 7mL) was put into the barcoded MGIT.[10]

A growth supplement was provided to encourage the growth of MTB, and an antibiotic combination

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known as polymyxin, amphotericin, nalidixic acid, trimethoprim, azlocillin (PANTA) was added to stop other bacteria from infecting the MGIT tubes. Kinyoun staining and the BD MGIT TB identification test, a fast chromatographic immunoassay for MTB antigen detection, are used to confirm it. The MOTT were now identified and eliminated. Before the direct DST setup, 15 milliliters of SIRE supplements (Becton Dickinson Diagnostic Systems, Sparks, MD) were added to the lyophilized PANTA and thoroughly mixed. Two lyophilized drugs, isoniazid and rifampicin (the same ones used in MGIT), were fully blended and reconstituted using four milliliters of sterile deionized (DI) water. Regarding the direct Drug Susceptibility for every specimen, a set of three MGIT tubes was constructed. Three tubes had labels: one for growth control (GC), one for rifampicin and one for isoniazid. The PANTA-SIRE supplement combination (800µL) was added to the designated MGIT tubes. The appropriate drugs were then put into tubes with labels. In the tube designated rifampicin, 100µL of reconstituted rifampicin (1.0µg/mL) and 100µL of isoniazid drugs were added. The final concentration (0.1µg/mL) was added to the tube labeled isoniazid. Then, 500µL of the thoroughly mixed smear-positive processed material was added to each of the two medicine tubes. 500µL of water or sterile saline was diluted 1:10 with the material that has been treated prior to its introduction into the GC tube. The growth control, rifampicin, and isoniazid tubes were placed in the set carrier after being placed within the instrument. In the set carrier, the GC tube was always the first tube. When growth unit value was ≥ 400, the GC test was deemed finished. After scanning, the MGIT tubes were taken out of the device, and an inventory report was made. Any test result that had a growth unit (GU) value below 100 was deemed "susceptible."

The outcome was considered "resistant" if the GU value was more than or equal to 100. Recorded were the GU values. The growth was deemed insufficient if, after 21 days, the GU value in the control tube did not reach 400. On the other hand, GU above 400 prior to four days was considered a sign of contamination or over-inoculation. After thoroughly mixing with a pipette, 250µL of cell lysis solution was transferred to a 1.5µL Eppendorf tube containing 50µL of MTB culture isolates for DNA extraction. The tube was then heated to 65°C for 15 minutes. Following cell lysis, samples were allowed to cool to room temperature before being vortexed and mixed with 100µL of protein precipitation solution. The samples were centrifuged at 15,000rmp for 5 minutes after being allowed to reach room temperature. The DNA-containing supernatant and 250µl of 100% isopropanol were carefully transferred into a clean Eppendorf tube following the protein precipitation stage, and the mixture was gently agitated by inversion.

For three minutes, the samples were centrifuged at 15,000rmp. After discarding the supernatant, the particle was cleaned with 70%, and the data was examined using Medcalc. A 2x2 contingency table was used to calculate the sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy of genotypic and phenotypic testing.

Results

A total of 54 samples that satisfied the requirements were used to evaluate the multiplex PCR procedure for drug-resistant MTB strains. Details of the primer used for DNA extraction were mentioned in Table I and II.

Table I: The MTB Sample Genotyping Primers and PCR (n=87)

Anti-TB Drugs	Targets site	Primers	Tm (°C)	PS
RIF	*rpoB* (530)	F: GATCAGTTGATCAACATC R: TACGGCGTTTCGATGAAC	55	305
INH	*katG* (315)	F: GGCATGAGCGTTACAC R: CCCTCTGGCGGTGTATT	55	458
EMB	*embB* (344)	**F:** CGCGAACCCTGGTGGCTTC **R:** GGCGTTCACAACGACGACG	55	306

RIF: Rifampicin, INH: Isoniazid, EMB: Ethambutol, Tm: Temperature, PS: Product Size, Ref: Reference.

Table II: Multiplex PCR Reaction Mixture

Reagents	Concentration	Volume
Distilled water		16.1 µl
Forward primers for the genes *Kat G, emb B* , and *Rpo B*	1µM	3µ1
Reverse primers for *rpo B, Kat G*, and *emb B* genes	1µM	3µl
Magnesium chloride ($MgCl_2$)	2.5µM	2.5µl
Buffer without $MgCl_2$	2µM	2µl
dNTPS	0.5µM	0.5µl
Taq polymerase	0.5µM	0.5µl
DNA	1-5 ng	1.4µl
Total		25-27 µl

Table III: **Genotypic Values and Resistance Pattern of MDR, INH, RIF & EMB (n = 87)**

Resistance Pattern	Genotypic testing (Multiplex PCR)	Phenotypic testing (MGIT CULTURE) R	S	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	DA (%)
MDR	**R**	4	0	66.67%	100%	100%	33.33%	71.43%
	S	2	1					
RIF	**R**	2	0	66.67%	100%	100%	50%	75%
	S	1	1					
INH	**R**	24	1	85.71%	80%	96%	50%	84.50%
	S	4	4					
EMB	**R**	3	1	100%	50%	75%	100%	80%
	S	0	1					

MDR: Multi-drug resistant, INH: Isoniazid, RIF: Rifampicin, EMB: Ethambutol, PPV: Positive predictive value, NPV: Negative predictive value, DA: Diagnostic accuracy

Three samples used control positive and negative and normal control as non-MTB samples, whereas four of the 54 MTB samples were multidrug resistant and thirty were single drug resistant (SDR). Two of the thirty SDR were resistant to rifampicin, twenty-seven to isoniazid, and three to ethambutol. According to multiplex PCR, the genotypic values of MDR were 66.67 %, 100 %, 33.33 %, 71.43 %, PPV, NPV, and DA; rifampicin were 66.67 %, 100 %, 100 %, 50%, 75.00 %; isoniazid were 85.71 %, 80 %, 96 %, 50 %, 84.50 %; and ethambutol were 100 %, 50 %, 75 %, 100 %, and 80 %, respectively. (Table III)

Using primer pairs for the rifampicin (*rpoB*), isoniazid (*katG*), and ethambutol (*embB*) genes, multiplex PCR created a distinctive banding pattern on a 1% agarose gel stained with ethidium bromide. Resistance to rifampicin is specified by a single 305 bps band. Resistance to isoniazid is specified by a single 458 bps band. Resistance to ethambutol is specified by a single band of 306 bps. The resistance to rifampicin and isoniazid is defined by two bands, 305 bps and 458 bps. Two bands, measuring 458 bps and 306 bps, respectively demonstrate the resistance to isoniazid and ethambutol. Lane M is a 100 and DNA as seen in figures 2; Lanes 1 through 8 are SDR (isoniazid), SDR (rifampicin) and MDR (rifampicin and isoniazid) in Lanes 3-5 and 7, as shown in Figure 1 & 2

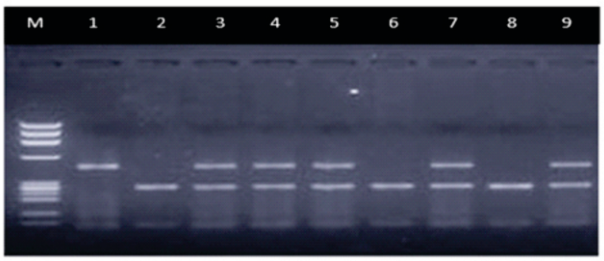

Figure 1: Gel Electrophoresis to Identify the Resistance Pattern of MDR *RpoB* Rifampicin and Isoniazid for *KatG* Genes

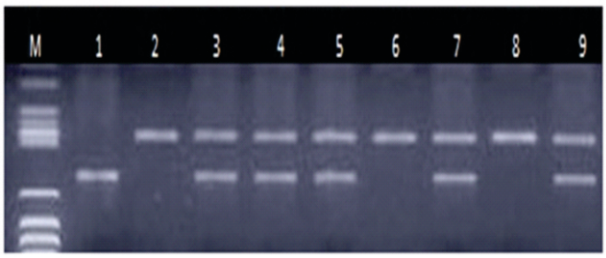

Figure 2: Resistance Pattern of the Extensive Drug Resistant *RpoB, KatG, InhA,* And *EmbA* Genes in Gel Electrophoresis

Discussion

Humans have had TB for thousands of years. For TB, drug susceptibility testing and culture-based diagnostic techniques remain the gold standard. The results of these techniques take at least six to eight weeks. On the other hand, molecular techniques show promise for a quick and precise diagnosis of drug-resistant TB. Most of the newly created molecular techniques are costly, need a high level of quality control, and are unavailable in distant regions with high illness incidence. Furthermore, only rifampicin resistance may be diagnosed using the WHO-approved GeneXpert. Due in large part to delayed diagnosis, drug-resistant tuberculosis is becoming more common in Pakistan. To quickly diagnose drug-resistant tuberculosis in distant places, a simple, affordable molecular technique is needed.

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Rifampicin resistance may be detected via multiplex PCR with varying sensitivity depending on the location. Sensitivity and specificity in our analysis were found to be 100% equivalent to Chinese data. [11] The bulk of rifampicin resistance in MTB is caused by mutations in the *rpoB* gene. [12] According to earlier findings from Pakistan, most isolates had a mutation at codon S531L of the *rpoB* gene. [12]

Finding a mutation within the rifampin drug resistant of *rpoB* is crucial for the quick identification of MTB isolates. This is supported by the following lines of evidence: first, the molecular mechanism of rifampin resistance is the most fully understood of the anti-tuberculosis drugs currently in the market. It has been found that 95% or more of rifampicin-resistant isolates had a second mutation inside the rifampicin drug resistant of *rpoB*. In our investigation, 17 (26%) isolates with rifampicin resistance were found to have a mutant band in multiplex PCR 100% diagnostic accuracy.

Genotypic testing for isoniazid resistance is much more challenging than rifampin. First, isoniazid resistance is linked to changes in at least three genes: *katG, inhA*, and *ahpC.* Second, a mutation in the regulatory regions of either *ahpC* or *katG*. Third, whereas certain *katG* mutations may not provide resistance to clinically relevant concentrations of isoniazid others are linked to low level resistance (deletion or S315T). Fourth, focusing on codons that are most modified globally may miss local strain populations and/or epidemic strains, which may be reflected in the relative frequency of a certain mutation. Fifth, it is unclear how the overexpression of the *ahpC* gene and *inhA* are related. [13] The 458 bp section of *katG* that includes codon 315 was employed in our investigation. 28 (42%) of the 39 isoniazid-resistant isolates detected in MGIT 960 had *katG* mutations. On the other hand, the target region of the *katG* gene included no mutations in the remaining 11 (17%). Threonine replacing serine amino acid 3 was, as expected, the most common *katG* modification.

We used a 458 bp segment of the *kat G* gene for INH and a 305 bp segment of *rpoB* for rifampicin in our investigation of MDR-TB. The 18 MDR isolates were identified using the MGIT 960 technique; 11 (17%) of these isolates showed characteristic banding patterns of concurrent *rpoB* and *katG* mutations in multiplex PCR followed by gel electrophoresis. In China, a similar resistance pattern was discovered by a similar experiment on MDR MTB. [14] This study's primary goal was to determine how much time MTB genotypic testing saves in comparison to phenotypic testing. [15] Genomic testing saved a maximum of 8 to 13 days (depending on the genotype) in comparison to MGIT culture or phenotypic test. The most plausible reason could be that the study was limited to one location and that the sample size was appropriately sized. Doctors can begin early therapy and prevent the spread of drug-resistant TB isolates thanks to this time-saving technique.

Overall, our findings suggest that genotypic testing is a reliable method, demonstrating a high level of agreement with MGIT culture and drug susceptibility testing. Given its potential for rapid detection, genotypic testing may serve as a valuable complementary diagnostic approach for TB and drug resistance in the Pakistani population.

Our research had two limitations. Initially, specimens that were tested positive for smears were used. Afterwards, just 21 percent of the isolates had multidrug resistance.

Conclusion:

Genotypic methods for diagnosing drug-resistant MTB were faster than phenotypic methods to address important public health challenges in countries like Pakistan.

Disclaimer: The research is conducted by the author using available resources with no funding. The opinions and content are solely the responsibility of the author and not the journal's editors or publisher.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORIGINAL ARTICLE

Comparative Analysis of Dyslipidemia and Atherogenic Indices among Controlled and Uncontrolled Diabetes Mellitus Patients at a Tertiary Care Center

Hafiz Osama Amjad Iqbal[1], Shehzina Hafeez[2], Kainaat Mahzaib John[3], Sadaf Farzand[4], Ambereen Anwar[5]

ABSTRACT

Objective: To compare the prevalence, patterns, and atherogenic indices associated with dyslipidemia among patients with controlled and uncontrolled diabetes mellitus.

Study Design: Cross-sectional observational study.

Place and Duration of Study: The study was conducted at the Punjab Institute of Cardiology, Lahore, from October 2 to December 29, 2025.

Materials and Methods: Fasting blood samples of 503 eligible patients were analyzed for their lipid profiles and HbA1c levels. Participants were grouped into controlled and uncontrolled diabetes groups based on ADA criteria, while dyslipidemia classification was made using NCEP ATP III cutoffs. Clinical characteristics, lipid profile parameters, and atherogenic indices were compared between the two groups using independent t-test for parametric variables while Mann-Whitney U test was employed for non-parametric variables. Logistic regression analysis was used to assess the association of specific dyslipidemia phenotypes with glycemic control status.

Results: The overall prevalence of dyslipidemia in the study population was 87.3%, which was significantly higher in the uncontrolled group (89.5% vs. 82.8%, χ^2=4.510, *p=0.034*). Patients with uncontrolled diabetes showed significantly higher median values of HbA1c, triglycerides, duration of diabetes, Castelli Risk Index I and II, atherogenic coefficient, and cardiometabolic index, while HDL-C levels were lower. Uncontrolled diabetes was a strong, independent predictor of the combined dyslipidemia phenotype (OR: 3.43, 95% CI: 1.61–7.31, *p=0.001*), while LDL-C did not differ significantly between groups.

Conclusion: Uncontrolled diabetes mellitus is strongly associated with a more atherogenic lipid profile. Atherogenic indices should be incorporated into routine lipid profile along with a detailed assessment of dyslipidemia patterns, especially in the diabetic population.

Key Words: *Diabetes Mellitus, Dyslipidemias, Atherosclerosis, Lipoproteins, Cardiometabolic Risk Factors.*

Introduction

Dyslipidemia is defined as an abnormal concentration of lipids and lipoproteins in the bloodstream.[1] These lipids primarily include triglycerides and cholesterol, which are absorbed from the intestine and transported in circulation through lipoproteins such as chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) for tissue utilization, storage, and excretion.[1] Any imbalance or defect altering the lipid metabolism pathway may result in a disturbed lipid profile or dyslipidemia.[2]

Dyslipidemia is a major risk factor for cardiovascular disease, which is responsible for an estimated 19.8 million deaths globally.[3] Elevated levels of these lipids, particularly high LDL and low HDL can result in the deposition of fats in the vessels, resulting in narrowing of the vessel-lumen and compromised tissue perfusion.[4] The formation of atherosclerotic plaques due to fat deposition can result in ischemia and stroke, both of which may be fatal.[4]

Dyslipidemia can develop due to hereditary/genetic defects (primary dyslipidemia) or due to poor lifestyle choices and underlying medical conditions (secondary dyslipidemia).[5] Of the many disorders that can disturb lipid metabolism, diabetes mellitus is of prime importance due to its high prevalence and strong association with dyslipidemia and cardiovascular disease.[6] Insulin resistance increases adipose tissue lipolysis, leading to enhanced free

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fatty acid (FFA) delivery to the liver and increased hepatic VLDL synthesis, which contributes to dyslipidemia.[7] Furthermore, VLDL produced transfers triglycerides to HDL and LDL forming small dense LDL, which is readily oxidized and can deposit in arteries causing atherosclerosis.[7,8] All of these changes can result in a characteristic lipid profile pattern known as diabetic dyslipidemia.

Pakistan bears one of the highest burdens of diabetes mellitus globally, with an estimated 34.5 million adults suffering from the condition.[9] Pakistan also has above global average mortality rates from CVD and other heart-related illnesses.[10] Studies conducted in different health-care centers in the country have consistently reported a high frequency of dyslipidemia among individuals with diabetes mellitus.[11,12] The high prevalence of dyslipidemia among the Pakistani diabetic population warrants further investigation into patterns of lipid abnormalities and their association with glycemic control.

Despite the high frequency of dyslipidemia among Pakistani adults in general and among diabetic individuals, much of the existing literature is either dated or does not compare lipid abnormalities between controlled and uncontrolled diabetes, particularly in the population of Lahore. This study aims to address these gaps by comparing the prevalence and patterns of dyslipidemia along with associated cardiovascular risk between individuals with controlled and uncontrolled diabetes mellitus.

Materials and Methods

This cross-sectional observational study was conducted at the Department of Pathology, Punjab Institute of Cardiology, Lahore, Pakistan, from October 2 to December 29, 2025. Ethical approval for the study was obtained from the institutional ethical review committee (Ref. No: RTPGME-Research-358; August 18, 2025).

The sample size was calculated using the Kirkwood's formula for comparing two independent continuous means $n = 2\sigma^2 (Z\alpha + Z\beta)^2 / d^2$. Sample size estimation was based on previously reported HDL-C values among controlled and uncontrolled type 2 diabetes mellitus patients reported by Rashid and Haider (1.05 ± 0.27 mmol/L vs. 0.91 ± 0.23 mmol/L).[13] Assuming a two-sided significance level of 5% ($Z\alpha$ = 1.96) and 80% power ($Z\beta$ = 0.84), the minimum required sample size was calculated to be 51 participants per group, yielding a total minimum sample size of 102 participants.

Consecutive sampling was employed. Patients with diagnosed cases of diabetes mellitus who visited the diagnostic laboratory for routine testing and fulfilled the study eligibility criteria were recruited. The inclusion criteria consisted of individuals with an established diagnosis of type 2 diabetes mellitus (T2DM) who were on an overnight fast and were not taking any lipid-lowering drugs. Patients with chronic liver disease, chronic kidney disease, thyroid disorders, known familial dyslipidemias, pregnancy, nephrotic syndrome, corticosteroid use, alcohol abuse, or other identifiable causes of secondary dyslipidemia were excluded from the study.

After obtaining written informed consent, participants were interviewed using a structured questionnaire to collect demographic and clinical information, including age, gender, duration of diabetes, comorbidities, antidiabetic medication or insulin use, physical activity, smoking status, and family history of diabetes mellitus or cardiovascular disease. Height and weight were measured using standard procedures, and body mass index (BMI) was calculated as weight (kg)/height (m^2).

Blood samples for HbA1c estimation were collected in EDTA vacutainers, while samples for lipid profile analysis were collected in lithium heparin tubes. Samples were transported to the laboratory within one hour of collection at room temperature (25°C). Blood samples in heparin vacutainers were centrifuged at 3000 rpm for 10 minutes prior to analysis. Lipid parameters were analyzed using the Atellica CH Analyzer, while HbA1c levels were measured using the Bio-Rad D-100 Hemoglobin Testing System. Patient lab results were later retrieved using the Hospital Information and Management System (HIMS).

Based on HbA1c levels, patients were grouped into controlled and uncontrolled diabetes according to the guidelines set by the American Diabetes Association (i.e., HbA1c < 7 = controlled diabetes, HbA1c ≥ 7 = uncontrolled diabetes .[14] Dyslipidemia was defined according to NCEP ATP III cut-off values (Triglycerides: ≥150 mg/dL, HDL-C: <40 mg/dL in men, <50 mg/dL in women).[15] Atherogenic dyslipidemia was defined as the presence of

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hypertriglyceridemia and low HDL-C. Combined dyslipidemia was defined as the combined presence of increased triglycerides, total cholesterol, LDL-C, and low HDL-C levels.

Laboratory results along with patient demographics were entered onto excel spreadsheets, which were later exported to SPSS for analysis. Clinical characteristics, lipid profile parameters and atherogenic indices including non-HDL cholesterol, remnant cholesterol, the atherogenic index of plasma (AIP), Castelli risk index I, Castelli risk index II, the atherogenic coefficient (AC), and the cardiometabolic index (CMI) were compared between the two groups using independent t-test for parametric variables and Mann-Whitney U test for non-parametric variables. Chi-square was used to assess the association of dyslipidemia in relation to glycemic control. Dyslipidemia was stratified into specific phenotypes (e.g., isolated High TG, Combined Dyslipidemia, etc.), and multivariable binary logistic regression analysis was employed to assess the association of uncontrolled diabetes with specific dyslipidemia phenotypes after adjusting for confounding variables.

Results

A total of 503 eligible patients with type 2 diabetes mellitus were included in the study, comprising 47.3% males and 52.7% females. Approximately two-thirds of the study population (66.4%) had poor glycemic control (HbA1c ≥7%), while 33.6% had controlled diabetes (HbA1c <7%). Overall, 87.3% of participants demonstrated some form of dyslipidemia.

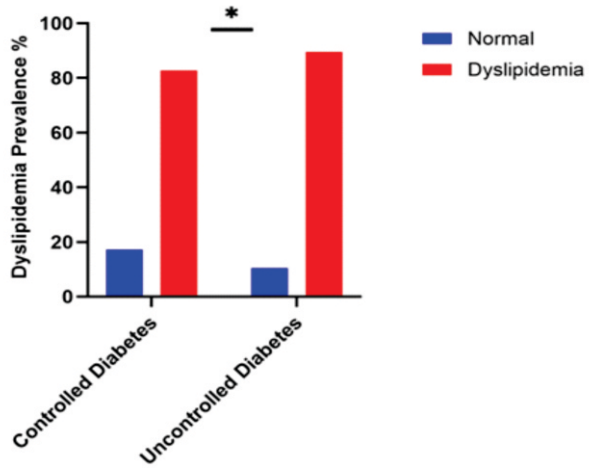

Figure 1: Comparison of Dyslipidemia Prevalence Between Controlled and Uncontrolled Diabetes.

Comparison of dyslipidemia frequency in controlled and uncontrolled diabetes is shown in Figure 1. Individuals with poor glycemic control had a higher prevalence of dyslipidemia (89.5 %) compared to those with adequate glycemic control (82.8%). Uncontrolled diabetes was found to be significantly associated with dyslipidemia $\chi^2 = 4.510$, $p = 0.034$.

Table I: Comparison of lipid parameters, atherogenic indices, and clinical characteristics between controlled and uncontrolled type 2 diabetes mellitus (n = 503)

Parameter	*Controlled Diabetes (n=169)*	*Uncontrolled Diabetes (n=334)*	*p-value*
Parametric Variables (Mean ±SD)			
Total Cholesterol (mg/dl)	178.81 ± 43.20	185.09 ± 39.14	0.101
LDL-Chol (mg/dl)	108.61 ± 35.63	112.58 ± 32.64	0.212
Non-HDL Chol (mg/dL)	135.44 ± 41.45	144.63 ± 37.86	**0.013**
AIP (Atherogenic index of Plasma)	0.177 ± 0.26	0.297 ± 0.26	**<0.001**
Age (Years)	53.89 ± 11.46	50.96 ± 10.87	0.005
Non-Parametric Variables (Median [IQR])			
HbA1c	6.50 (0.60)	8.75 (2.70)	**<0.001**
Triglyceride (mg/dl)	145.00 (92.00)	170.50 (111.00)	**<0.001**
HDL-Chol (mg/dl)	43.00 (12.00)	39.00 (11.00)	**<0.001**
BMI	29.39 (6.64)	29.70 (6.45)	0.784
Duration	3.00 (7.68)	6.00 (8.00)	**<0.001**
Non-Parametric Variables (Median [IQR])			
Castelli Risk Index I	4.12 (1.51)	4.74 (1.65)	**<0.001**
Castelli Risk Index II	2.57 (1.08)	2.81 (1.15)	**<0.001**
Remnant Cholesterol	26.00 (20.50)	29.00 (22.25)	0.180
Atherogenic Coefficient	3.12 (1.51)	3.74 (1.65)	**<0.001**
Cardiometabolic Index	100.78 (85.86)	131.54 (109.46)	**<0.001**

Data are presented as mean ± standard deviation (SD) for parametric variables and median (interquartile range [IQR]) for non-parametric variables. Parametric variables were analyzed using the independent t-test, while non-parametric variables were analyzed using the Mann-Whitney U test. Statistical significance was set at p <0.05.

Abbreviations: T2DM = Type 2 Diabetes Mellitus; LDL-Chol = Low-Density Lipoprotein Cholesterol; HDL-Chol = High-Density Lipoprotein Cholesterol; HbA1c = Glycated Hemoglobin; BMI = Body Mass Index; AIP = Atherogenic Index of Plasma; Castelli Risk Index I = Total Cholesterol/HDL-Cholesterol ratio; Castelli Risk Index II = LDL-Cholesterol/HDL-

Cholesterol ratio.

Phenotypic stratification demonstrated atherogenic dyslipidemia (High TG + Low HDL-C) to be the most frequent pattern in both controlled (23.1%) and uncontrolled diabetes mellitus (26.3%). Isolated low HDL levels were more prevalent in patients with adequate glycemic control (21.9%) compared to poor glycemic control (17.4%). High frequency of combined dyslipidemia was observed in individuals with uncontrolled diabetes (14.7%) vs. controlled diabetes (5.3%). The distribution of different patterns of dyslipidemia in relation to glycemic control is shown in Figure 2.

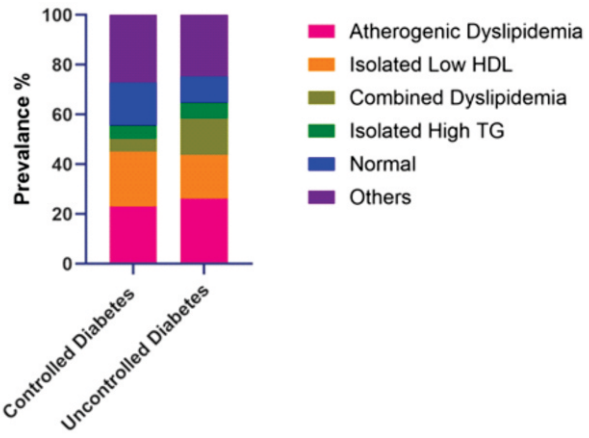

Figure 2: Distribution Of Dyslipidemia Patterns in Controlled and Uncontrolled Diabetes

The results of the logistic regression analysis are presented in Table II. Female gender was found to be associated with higher odds of having isolated low HDL (OR=1.92, p = 0.009), whereas uncontrolled diabetes was strongly associated with increased odds of combined dyslipidemia (OR=3.43, p = 0.001). Longer duration of diabetes slightly reduced the odds of isolated low HDL (OR=0.96, p = 0.036).

Table II: Multivariable Binary Logistic Regression Analysis Showing Independent Predictors of Dyslipidemia Phenotypes Among Patients with Diabetes Mellitus (n = 503)

Predictor	*Atherogenic Dyslipidemia OR (95% CI)*	*Isolated Low HDL OR (95% CI)*	*Isolated High TG OR (95% CI)*	*Combined Dyslipidmia OR (95% CI)*
Age (years)	0.98 (0.96–1.00)	1.04 (1.01–1.06) *	0.98 (0.95–1.01)	0.98 (0.96–1.01)
Female gender	1.06 (0.70–1.61)	1.92 (1.18–3.13) *	0.31 (0.14–0.71)	1.48 (0.83–2.62)
Duration of DM (years)	1.01 (0.98–1.04)	0.96 (0.92–0.99) *	—	0.94 (0.89–0.99)
Uncontrolled diabetes	1.10 (0.70–1.71)	0.91 (0.56–1.47)	1.03 (0.45–2.33)	3.43 (1.61–7.31) *
Exercise	1.20 (0.79–1.82)	1.26 (0.79–2.02)	—	—
BMI (kg/m²)	1.02 (0.99–1.04)	0.98 (0.94–1.02)	—	0.996 (0.98–1.02)

- Bolded ORs are statistically significant (p < 0.05).
- "—" indicates the predictor was not included in that model due to low events.
- *Statistically significant at p <0.05 in multivariable binary logistic regression analysis.

Discussion

This study demonstrates a high prevalence of dyslipidemia in patients with T2DM, specifically highlighting the profound impact of glycemic control. The principal finding is that uncontrolled diabetes is strongly associated with a more atherogenic dyslipidemic profile, as reflected by elevated composite risk indices and high-risk dyslipidemia phenotypes like combined dyslipidemia. These may provide additional insight into atherogenic risk profiles beyond isolated LDL-C levels.

The major abnormalities in the lipid profile of both controlled and uncontrolled diabetes were elevated triglycerides and low HDL-C. The observed pattern of lipid abnormalities, including hypertriglyceridemia and low HDL-C, is consistent with diabetic dyslipidemia. Although LDL-C differences were not significant, diabetic dyslipidemia is often associated with qualitative LDL abnormalities, particularly small dense LDL particles, which may contribute to residual atherogenic risk.[16] The high prevalence of dyslipidemia (87.3%) in our study population aligns with previous studies that have consistently shown > 90% prevalence of dyslipidemia among Pakistani diabetic population.[11,17] This is in agreement with the

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pathophysiological models where insulin resistance in diabetes drives hepatic VLDL overproduction and impaired lipolysis.[18]

In our study, all of the evaluated atherogenic indices were elevated in individuals with poor glycemic control. This aligns with recent clinical evidence that AIP and TyG index are significantly higher in the uncontrolled diabetic group, suggesting an association with an adverse atherogenic risk profile.[19] Elevated atherogenic ratios (TG/HDL-C, LDL-C/HDL-C, AIP) have also been observed in uncontrolled diabetes mellitus, which highlights their role as surrogate markers of lipid metabolism abnormalities that accompany worsening glycemic control.[20] High levels of Non-HDL-C in uncontrolled diabetes represent the total burden of atherogenic lipoproteins, while remnant cholesterol, carried by triglyceride-rich lipoproteins, is increasingly recognized as an independent risk factor for atherosclerosis.[21] These findings highlight the importance of non-LDL-C atherogenic fractions, which may represent additional markers of dyslipidemia burden that are often overlooked when deciding the treatment regimen and management of dyslipidemias in diabetic individuals, as these lipids may carry residual cardiovascular risk.[22] Nonetheless, in the absence of direct cardiovascular outcome assessment, these findings should be interpreted as indicators of an unfavourable lipid risk profile rather than predictors of cardiovascular events.

A key finding of our study is the association of uncontrolled diabetes with severe dyslipidemia phenotypes like combined dyslipidmia (OR=3.43). A study conducted in Riyadh also found significantly higher triglycerides, cholesterol, and lower HDL levels in admitted patients with poor glycemic control, while the differences in LDL-C were not statistically significant.[23] These findings indicate that poor glycemic control increases the likelihood of developing these highly atherogenic phenotypes characterized by elevated triglycerides, low HDL, and increased cholesterol levels.

Our regression analysis also revealed that female gender was associated with higher odds of developing isolated low HDL and decreased odds of having a normal lipid profile. This is supported by global epidemiological data that shows a higher prevalence of low HDL levels in women compared to men.[24] This increased prevalence of specific dyslipidemia phenotypes might be due to sex related differences in lipid metabolism, fat distribution, hormonal differences, and socioeconomic determinants.

Our study found no association of BMI with dyslipidemia. Prior research has also shown a limited ability of BMI to correlate with the severity of CVD and other metabolic disorders, including T2DM.[25] This indicates that insulin resistance may have a more direct effect on deranged lipid metabolism than adiposity alone. However, these associations may be influenced by potential confounders such as menopause, obesity-related heterogeneity, socioeconomic factors, and treatment adherence, which were not fully controlled in the present analysis.

The findings of this study highlight that achieving glycemic control (e.g., HbA1c < 7%) is associated with a more favourable lipid profile and reduced burden of atherogenic dyslipidemia. The results of this study also support the perspective that standard lipid panels and isolated LDL-C targets may not fully capture the complex lipid derangements present in uncontrolled diabetes. Incorporating calculated atherogenic indices into routine lab reporting could provide clinicians with a more comprehensive overview of a patient's lipid status.

Future studies should focus on prospective longitudinal designs to determine whether these atherogenic indices and dyslipidemia phenotypes translate into actual cardiovascular outcomes. Further research is also needed to evaluate optimal lipid-lowering strategies in different dyslipidemia phenotypes among diabetic patients, particularly in relation to glycemic control status.

Limitations

The single center nature of this study limits the generalizability of its findings to the general population. Also, the information regarding specific diabetic drugs (e.g., SGLT2 inhibitors, GLP-1 RAs, which can affect lipid levels), dietary habits, and level of physical activity was limited, which are potential confounding factors.

Conclusion

In conclusion, poor glycemic control is associated with more severe forms of dyslipidemia. Patients

with uncontrolled diabetes demonstrate a more atherogenic lipid profile and worse cardiometabolic indices. These findings suggest that dyslipidemia in diabetes is more pronounced in uncontrolled glycemic states and may warrant more comprehensive lipid assessment, including evaluation of specific dyslipidemia patterns and calculated atherogenic indices, in addition to standard lipid parameters. Improved glycemic control may help reduce the severity of dyslipidemia and associated cardiometabolic risk.

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Disclaimer The research was conducted independently and the views expressed in this study, regarding the interpretation and conclusions of results do not represent the official position or policy of Punjab Institute of Cardiology.

Conflict of Interest All the authors declare that there is no conflict of interest that could have influenced the work.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

GRANT SUPPORT AND FINANCIAL DISCLOSURE

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORIGINAL ARTICLE

Frequency and Antimicrobial Susceptibility Patterns of ESBL-Producing Uropathogens in Culture-Confirmed Pediatric Urinary Tract Infections

Sidra Tul Muntaha[1], Muhammad Ibrahim[2]

ABSTRACT

Objective: To determine the frequency of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae isolated from culture-confirmed pediatric urinary tract infections and to evaluate their antimicrobial susceptibility patterns according to age and sex.

Study Design: Analytical cross-sectional study employing consecutive sampling.

Place and Duration of Study: Holy Family Hospital, Department of Pediatrics from 1st April 2023 to 1st April 2024.

Materials and Methods: Children aged 1 month to 12 years with clinical features of urinary tract infection and a positive urine culture were recruited. Urine specimens were processed for bacterial identification, antimicrobial susceptibility testing and confirmation of ESBL production. Age, sex, isolated organism, ESBL status and antimicrobial susceptibility findings were recorded and analyzed.

Results: Among 384 children, 236 (61.5%) were female and 148 (38.5%) were male. *Escherichia Coli* was the most frequently isolated organism, accounting for 284 (74.0%) cases, followed by *Klebsiella Pneumoniae* in 62 (16.1%) cases. Of 346 *Enterobacteriaceae* isolates, 208 (60.1%) produced ESBL. ESBL production was detected in 162 (57.0%) *Escherichia coli* isolates and 46 (74.2%) *Klebsiella pneumoniae* isolates. Infections were more frequent among children aged 1 month to <5 years and among females. Resistance was highest to ampicillin, co-amoxiclav and ciprofloxacin, whereas meropenem and fosfomycin retained complete antimicrobial activity.

Conclusion: Extended-spectrum beta-lactamase producing *Enterobacteriaceae* were common among children with urinary tract infections, particularly in younger children and females. The high resistance to commonly prescribed antibiotics supports regular antimicrobial surveillance and culture guided treatment. Routine urine culture and antimicrobial susceptibility testing should be encouraged before initiation of definitive therapy to optimize antibiotic stewardship.

Key Words: *Antibiotic Resistance, Child, Enterobacteriaceae, Escherichia Coli, Klebsiella Pneumoniae, Urinary Tract Infection*.

Introduction

Recurrent urinary tract infection remains an important concern in children because repeated episodes may lead to additional investigations, prolonged treatment and increased antimicrobial exposure. Non antibiotic preventive measures have been evaluated, although the available evidence does not support the routine use of a single intervention for all children.[1] Antibiotic prophylaxis is still used in selected patients, however its benefit must be balanced against the increased likelihood of infection with resistant uropathogens.[2] Management is particularly complex in children with primary vesicoureteric reflux, for whom conservative and surgical approaches may be considered according to clinical severity.[3] Long term antibiotic prophylaxis may reduce recurrence in some children, but its overall benefit is modest and prolonged exposure may contribute to antimicrobial resistance.[4]

Escherichia Coli is the predominant organism isolated from pediatric urinary tract infections, while *Klebsiella pneumoniae* is also frequently identified, particularly among infections caused by extended-spectrum beta-lactamase (ESBL) producing organisms.[5] Studies conducted in different clinical settings have similarly shown *E. coli* to be the leading

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urinary pathogen and have documented substantial resistance to commonly prescribed antimicrobial agents.[6] ESBL production further complicates treatment because it reduces susceptibility to penicillins, extended spectrum cephalosporins and monobactams and may coexist with resistance to other antibiotic classes. A recent review highlighted the growing clinical and public health importance of ESBL producing uropathogens in children, including restricted treatment options, delayed effective therapy and the potential spread of multidrug resistant organisms. [7]

Antimicrobial resistance among urinary *E. coli* isolates in children has been associated with previous antibiotic exposure, emphasizing the need for careful empirical prescribing and timely culture based treatment.[8] Concerns regarding resistance have also encouraged investigation of non antibiotic approaches for preventing recurrent urinary tract infection, although their role in pediatric practice remains incompletely established.[1,9]

Limited data are available in Pakistan regarding Enterobacteriaceae causing urinary tract infections in the pediatric population and their antimicrobial susceptibility patterns. This study is particularly relevant in the context of UTIs, where inappropriate or excessive antibiotic use contributes to rising resistance rates. Given the limited local data on pediatric ESBL infections, the rationale of the study was to provide evidence based insights to help pediatricians select appropriate antibiotics, reduce resistance development and improve treatment outcomes in children under 12 years of age. Additionally, by identifying age and gender specific trends, the study can inform targeted infection control and preventive strategies, ultimately improving pediatric healthcare management in tertiary care settings. The objective of this study was to determine the frequency of ESBL-producing Enterobacteriaceae isolated from culture-confirmed pediatric urinary tract infections and to evaluate their antimicrobial susceptibility patterns according to age and sex.

Materials and Methods

An analytical cross-sectional study employing non probability consecutive sampling was conducted in the Holy Family Hospital Department of Pediatrics, Rawalpindi from 1st April 2023 to 1st April 2024. Children aged 1 month to 12 years presenting with clinically suspected urinary tract infection and culture confirmed urinary tract infection were enrolled. Ethical approval was obtained from the Institutional Research and Ethics Forum of Rawalpindi Medical University through Letter No. 307/IREF/RMU/2022, dated 12 February 2022. Written informed consent was obtained from the parent of each child before enrolment.

The sample size was calculated using OpenEpi version 3.01, assuming a 95% confidence level, 5% absolute precision, and an anticipated prevalence of 49.3% for ESBL-producing uropathogens among children hospitalized with urinary tract infection, as reported by Koçak et al., yielding a calculated sample size of 384 participants.[10]

Clinically suspected urinary tract infection was defined by the presence of fever without an identifiable alternative focus or at least one age-appropriate urinary symptom. In infants and younger children, relevant features included fever, poor feeding, vomiting, irritability or failure to thrive. In older children, clinical features included dysuria, increased urinary frequency, urgency, suprapubic or abdominal pain, flank pain or new onset urinary incontinence. Children were included when they had compatible clinical features and a culture confirmed urinary tract infection. Children who had received antibiotics during the preceding two weeks, had contaminated or insignificant urine cultures or had urine collected using a collection bag were excluded. Urine specimens were obtained according to the child's age, toilet training status and clinical condition through midstream clean catch collection or urethral catheterization.

Specimens were transported to the microbiology laboratory within one hour of collection. In the laboratory, each urine specimen was mixed thoroughly and a 2-µL aliquot was inoculated onto cystine lactose electrolyte deficient (CLED) agar using a sterile calibrated 2-µL inoculating loop. The inoculated plates were incubated aerobically at 35–37°C for 18–24 hours. Following incubation, colonies were counted and multiplied by 500 to express the bacterial concentration as colony forming units per millilitre (CFU/mL). Colony growth and colony morphology were also assessed. Significant bacterial growth was defined as the

isolation of a single uropathogen at a concentration of at least 10^5 CFU/mL in a midstream clean catch specimen or at least 10^4 CFU/mL in a catheter obtained specimen. Following confirmation of significant bacterial growth, representative pure colonies were subjected to preliminary identification based on colony morphology and Gram staining.

Enterobacterales isolates were identified using the API 20E identification system, whereas non fermenting Gram negative isolates were identified using the API 20NE identification system, according to the manufacturer's instructions. Antimicrobial susceptibility testing of the identified isolates was performed using the Kirby–Bauer disk-diffusion method on Mueller–Hinton agar in accordance with Clinical and Laboratory Standards Institute disk diffusion procedures. Inhibition-zone diameters were measured and interpreted according to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, 33rd edition (CLSI M100, 2023). ESBL production in *Escherichia coli* and *Klebsiella pneumoniae* isolates was assessed using the combination disk method. Ceftazidime and cefotaxime disks were tested alone and in combination with clavulanic acid. An increase of at least 5 mm in the inhibition zone diameter around either antimicrobial disk combined with clavulanic acid, compared with the corresponding disk without clavulanic acid was interpreted as confirmation of ESBL production.[11]

Clinical information was obtained from the patient's history and medical record. Following completion of urine culture, bacterial identification and antimicrobial susceptibility testing, the principal investigator manually entered the clinical and microbiological findings into a structured data collection proforma. Recorded variables included age, sex, presenting clinical features, method of urine collection, isolated organism, ESBL status and antimicrobial susceptibility results.

Data were analyzed using SPSS version 26. Continuous variables were presented as mean and standard deviation or median and interquartile range, as appropriate. Categorical variables were summarized as frequencies and percentages. The overall distribution of cultured organisms was compared between females and males using Pearson's Chi-square test. Age group distributions were compared between sexes separately for *Escherichia coli* and *Klebsiella pneumoniae* isolates. ESBL-production was compared between the two bacterial species. Pearson's Chi-square test was used when its assumptions were satisfied, while Fisher's exact test or the Fisher–Freeman–Halton exact test was used when expected cell frequencies were inadequate. All statistical tests were two sided. A p value of <0.05 was considered statistically significant. These analyses were unadjusted bivariate comparisons, and no multivariable regression model was fitted

Results

Our study enrolled a total of 384 children with culture confirmed urinary tract infections. Of these, 236 (61.5%) were female and 148 (38.5%) were male. The mean age was 6.4±5.3 years, with participants ranging from 1 month to 12 years. Because ESBL testing and organism-specific antimicrobial resistance analyses focused on *Escherichia coli* and *Klebsiella pneumoniae,* these analyses included 346 isolates.

Escherichia coli was the predominant cultured organism, accounting for 284 (74.0%) isolates, followed by *Klebsiella pneumoniae* with 62 (16.1%) isolates. The remaining 38 (9.9%) were other Gram-negative isolates. The overall distribution of organism categories differed between females and males (p=0.028). (Table I)

Resistance to commonly prescribed antimicrobial agents was high in both major bacterial species. Among *E. coli* isolates, resistance was highest against ampicillin (253/284, 89%), co-amoxiclav (239/284, 84%), and ciprofloxacin (230/284, 81%). The corresponding resistant counts among *K. pneumoniae* isolates were 59/62 (95%), 56/62 (91%), and 52/62 (84%), respectively. No resistance to meropenem or fosfomycin was recorded in either organism (Table II).

Among the 346 *Escherichia coli* and *Klebsiella pneumoniae* isolates, 181 (52.3%) were recovered from children aged 1 month to <5 years, 86 (24.9%) from children aged 5 to <9 years, and 79 (22.8%) from children aged 9 to 12 years.

The age distribution of *Escherichia coli* isolates differed between females and males (p = 0.006). No difference in the age distribution of *Klebsiella*

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pneumoniae isolates was observed between the sexes (p = 0.609). When both bacterial species were considered together, their distribution across age categories differed according to sex (p = 0.016). (Table III).

Overall, 208 of the 346 isolates (60.1%) were ESBL-positive. ESBL production was detected in 162 of 284 (57.0%) *Escherichia coli* isolates and 46 of 62 (74.2%) *Klebsiella pneumoniae* isolates. The proportion of ESBL-positive isolates differed significantly between the two bacterial species (p = 0.012; Table IV).

Table I: Distribution Of Cultured Uropathogens According To Sex (n=384)

Organism	Females: n(%) (n=236)	Males:n(%) (n=148)	Total :n(%)	*P* value
Escherichia coli	183 (77.5)	101 (68.2)	284(74)	
Klebsiella pneumoniae	37 (15.6)	25 (16.8)	62(16.1)	
Other Gram-negative organisms	16(6.8)	22(14.9)	38(9.9)	
Total	236(100)	148(100)	384(100)	0.028

Pearson's chi-square test was used to compare the overall distribution of cultured organism categories between females and males. The reported P value represents the overall three category comparison

Table II: Antibiotic Resistance Patterns Of *Escherichia Coli* And *Klebsiella Pneumoniae* Isolates

Antibiotic	*Escherichia coli Resistance*: n(%), (n=284)	*Klebsiella pneumoniae* Resistance: n(%), (n=62)
Amikacin	26 (9)	7 (11)
Ampicillin	253 (89)	59 (95)
Cefixime	111 (39)	17 (27)
Ceftazidime	162 (57)	46 (74)
Ceftriaxone	88 (31)	33 (53)
Ciprofloxacin	230 (81)	52 (84)
Co-Amoxiclav	239 (84)	56 (91)
Co-Trimoxazole	196 (69)	54 (87)
Fosfomycin	0 (0)	0 (0)
Gentamicin	102 (36)	25 (41)
Meropenem	0 (0)	0 (0)
Nitrofurantoin	82 (29)	43 (69)
Piperacillin–Tazobactam	31 (11)	19 (31)

Discussion

This study demonstrated a high burden of ESBL production and resistance to commonly prescribed antibiotics among pediatric uropathogens. Although

Table III :*Escherichia coli* and *Klebsiella pneumoniae* distribution by age group and sex (n = 346)

Organism	Female: 1 month to <5 years, n (%)	Female: 5 to <9 years, n (%)	Female: 9 to 12 years, n (%)	Male: 1 month to <5 years, n (%)	Male: 5 to <9 years, n (%)	Male: 9 to 12 years, n (%)	p-value
Escherichia coli	82 (44.8)	45 (24.6)	56 (30.6)	64 (63.4)	21 (20.8)	16 (15.8)	0.006
Klebsiella pneumoniae	21 (56.8)	13 (35.1)	3 (8.1)	14 (56.0)	7 (28.0)	4 (16.0)	0.609
Both organisms	**103 (46.8)**	**58 (26.4)**	**59 (26.8)**	**78 (61.9)**	**28 (22.2)**	**20 (15.9)**	**0.016**

Percentages were calculated across age categories within each organism-and-sex group. Pearson's chi-square test was used for the E. coli and combined comparisons. The Fisher-Freeman-Halton exact test was used for K. pneumoniae because of small expected cell frequencies. All p-values are two-sided

Table IV: ESBL production in *Escherichia coli and Klebsiella pneumoniae* isolates (n = 346)

Organism	Total Isolated Organisms	ESBL-positive n (%)	Non-ESBL n (%)	P value
Escherichia coli	284	162 (57%)	122 (43%)	
Klebsiella pneumoniae	62	46 (74%)	16 (26%)	
Total	346	208 (60%)	138 (40%)	**0.012**

Footnote:Other gram-negative isolates (n = 38) such as *Pseudomonas spp*. were excluded from ESBL analysis. *Pearson's chi-square test was used to compare the proportion of ESBL-positive isolates between Escherichia coli and Klebsiella pneumoniae. ESBL: Extended spectrum beta-lactamase.*

E. coli remained the predominant organism, ESBL production was proportionally more frequent in *K. pneumoniae*. The marked resistance to commonly used oral agents highlights the importance of local antimicrobial surveillance and culture-directed treatment.

The female predominance observed in the current study is consistent with the established pattern of pediatric urinary tract infection beyond early infancy. Tamas et al. described a similar shift towards female predominance after infancy, while also identifying *E. coli* as the principal pediatric uropathogens.[12] A recent research from Karachi reported that 80% of culture positive children were female and that *E. coli* accounted for 73.9% of isolates, which is almost identical to the 74.0% observed in the present study.

However, *Klebsiella species* constituted only 6.5% of isolates in that study compared with 16.1% in the present series.[13] A nationwide study by Shkalim Zemer et al. also identified *E. coli* as the dominant organism, although its proportion of 82.1% was higher than ours.[14] These differences may reflect variation in referral patterns, participant age, urine collection methods and the proportion of children with complicated or recurrent infections. Lin et al. reported a male predominance among hospitalized children with *E. coli* urinary tract infection, but their patients had a median age of seven months.[15] The contrasting sex distribution may therefore be explained partly by their concentration on infants, among whom urinary tract infection is more frequently recognized in boys, whereas the present study included children up to 12 years of age.

Children aged 1 month to <5 years contributed more than half of the *E. coli* and *K. pneumoniae* isolates in the present study. This finding is compatible with the young age profile reported by Lin et al., whose participants had a median age of seven months.[15] Heshmat et al. similarly studied a predominantly young hospitalized population with a median age of 12 months.[16] Nevertheless, direct comparison requires caution because those investigators included febrile hospitalized children, whereas the present study included culture confirmed urinary tract infections across a broader pediatric age range.

The overall ESBL frequency of 60.1% was considerably higher than most recently published pediatric estimates. Among *E. coli* isolates, our ESBL proportion of 57.0% was close to the 53.6% reported by Lin et al. in hospitalized children with community acquired urinary tract infection.[15] In contrast, Abdelgalil et al. identified ESBL production in 20.7% of pediatric *E. coli* urinary isolates in Saudi Arabia.[17] Heshmat et al. reported ESBL production in 5.8% of *E. coli* and *K. pneumoniae* isolates,[16] while Rizik et al. found ESBL producing Enterobacterales in 9.8% of children with community acquired urinary tract infection.[18] Further pediatric studies reported frequencies of 14.1% in Taiwan and 19.0% among *E. coli* isolates in Romania.[19,20]

The higher frequency in the present study may reflect differences in local antibiotic pressure, tertiary care referral, prior healthcare exposure, underlying urinary abnormalities and regional circulation of resistant strains. Study definitions and laboratory methods may also influence the reported frequency. Children exposed to antibiotics more than two weeks before enrolment were not excluded from the present study and such earlier exposure was not analyzed separately. In addition, the research did not record recurrent infection, previous hospitalization or structural urinary tract disease, all of which may influence the likelihood of an ESBL producing infection. The significantly greater ESBL proportion in *K. pneumoniae* than in *E. coli* may also reflect species related resistance patterns or local hospital ecology. However, the smaller number of *K. pneumoniae* isolates and absence of molecular typing prevent conclusions regarding clonal transmission or a particular resistance mechanism.

Resistance to ampicillin and co-amoxiclav was high in both principal organisms. This pattern was consistent with the Karachi study, in which amoxicillin-clavulanate was the least active tested agent, although that study reported substantially greater ciprofloxacin susceptibility than the present study.[13] Pakistani data reported by Iqbal et al. also demonstrated extensive resistance among pediatric uropathogens, including resistance of 61% to amoxicillin-clavulanate, 94% to co-trimoxazole and 37% to gentamicin.[21] The gentamicin result was close to the 36% resistance in *E. coli* and 41% in *K. pneumoniae* observed in the present study, whereas the differences for the other agents indicate considerable variation even between centres within the same country.

Among ESBL producing *E. coli* isolates, Abdelgalil et al. reported resistance of 56% to quinolones and 64% to Trimethoprim-Sulfamethoxazole.[17] These values were lower than our ciprofloxacin resistance of 81% but close to the 69% co-trimoxazole resistance among *E. coli*. Their nitrofurantoin resistance of 8% was also lower than the 29% found in the present study. Rizik et al. reported resistance of 62.5% to amoxicillin-clavulanate, 58.9% to trimethoprim-sulfamethoxazole and 33.9% to ciprofloxacin among ESBL producing Enterobacterales, all lower than the corresponding findings in our population. Their piperacillin-tazobactam resistance of 26.8% was, however between the rates observed for *E. coli* and *K. pneumoniae* in the present study.[18]

The difference in nitrofurantoin resistance between

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E. coli and *K. pneumoniae* was substantial in the present study. Heshmat et al. reported a comparable organism specific pattern, with nitrofurantoin resistance of 15.7% in *E. coli* and 73.3% in *K. pneumoniae*.[16] Their *K. pneumoniae* finding was close to our rate of 69%, supporting the need to interpret nitrofurantoin susceptibility according to the isolated organism rather than treating all urinary Enterobacterales as a single group.

No resistance to meropenem or fosfomycin was observed in the present study. Abdelgalil et al. similarly reported no meropenem resistance among their pediatric ESBL producing *E. coli* isolates.[17] Păcurar et al. found complete susceptibility to fosfomycin and high susceptibility to carbapenems and amikacin among ESBL producing *E. coli*.[20] These findings are reassuring, but the preserved activity of these agents should not justify unrestricted empirical use. The absence of resistant isolates in a single centre sample does not exclude their presence in the wider population.

Previous antibiotic exposure and contact with urological services have been identified as independent risk factors for community acquired pediatric ESBL producing *E. coli* urinary tract infection.[22] These unmeasured factors may partly explain the high ESBL frequency in the present study. Choi et al. also documented a progressive increase in resistance among pediatric urinary isolates over a ten-year period, including a rise in ciprofloxacin resistance.[23] Their findings reinforce the importance of updating hospital antibiograms rather than depending on resistance estimates from other centres or earlier periods.

The high resistance to commonly prescribed oral agents in the present study makes empirical selection difficult. Culture and susceptibility testing should therefore guide definitive therapy whenever possible. Piperacillin-tazobactam retained greater activity than ampicillin, co-amoxiclav and ciprofloxacin, particularly against *E. coli*. Recent pediatric evidence suggests that it may have a role as a carbapenem sparing option in selected hospitalized children, although treatment must be individualised according to age, illness severity, infection site and susceptibility results.[24] Carbapenems should remain reserved for patients with severe infection or isolates for which narrower effective alternatives are unavailable.

The study has several strengths, including a relatively large sample of culture confirmed pediatric infections, organism specific resistance reporting and standard phenotypic assessment of ESBL production. However, its single centre design limits generalisability. Molecular characterisation of ESBL genes was not performed, and multivariable analysis was not undertaken. Information regarding antibiotic exposure occurring more than two weeks before enrolment was not collected; therefore, the possible association between earlier antimicrobial exposure and ESBL production could not be evaluated. Despite these limitations, the study demonstrates a substantial local burden of ESBL producing pediatric uropathogens and provides clinically relevant data for antimicrobial surveillance and culture directed treatment.

Conclusion

Extended-spectrum beta-lactamase producing Enterobacteriaceae were common among children with urinary tract infections, particularly in younger children and females. The high resistance to commonly prescribed antibiotics supports regular antimicrobial surveillance and culture guided treatment. Routine urine culture and antimicrobial susceptibility testing should be encouraged before initiation of definitive therapy to optimize antibiotic stewardship.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORIGINAL ARTICLE

Optimizing Platelet Transfusion Practices: A Comprehensive Analysis of Adherence to Guidelines in a Tertiary Care Hospital

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ABSTRACT

Objective: To evaluate adherence of platelet transfusion practices to British Society for Haematology (BSH) guidelines – 2024 and identify patterns of non-compliance across clinical settings.

Study Design: A descriptive cross-sectional study.

Place and Duration of Study: Department of Haematology and Blood Bank, Fauji Foundation Hospital, Rawalpindi, from July 01, 2025 to December 31, 2025.

Materials and Methods: A total of 260 patients who received platelet transfusions during the study period were included. Demographic details, clinical diagnoses, indications for transfusion, and requesting departments were recorded. The appropriateness of platelet transfusion practices was assessed in accordance with the 2024 BSH platelet transfusion guidelines. Data were analyzed using SPSS v29. Guideline adherence was classified as adherent or non-adherent, and associations between diagnosis and adherence were assessed using the Chi-square test.

Results: Adults constituted 56.2% of study population, whereas paediatric patients accounted for 43.8%. Females constituted 66.5% of the study population. Most platelet transfusions were administered in the NICU (18.1%), followed by oncology units (15.4%), ICU (14.2%), Medical wards (14.2%), paediatric wards (14.2%) and others (23.9%). Overall, 161 platelet transfusions (61.9%) were found to be compliant with the recommended transfusion guidelines, whereas 99 transfusions (38.1%) were identified as non-compliant.

Conclusion: Despite reasonable overall compliance, a substantial proportion of platelet transfusions were inappropriate, particularly in non-haematology settings. These findings highlight the need to strengthen transfusion management, to improve guideline adherence, enhance patient safety and optimize platelet utilization.

Key Words: *Clinical Audit, Guideline Adherence, Platelet Transfusion, Tertiary Care Centers.*

Introduction

Platelet transfusion is critical supportive therapy for preventing and treating bleeding in patients with thrombocytopenia or platelet function disorders. Platelet concentrates are routinely administered in surgical, obstetric, haemato-oncological, and intensive care units based on clinical indications and platelet dose requirements. [1] However, platelet demand and supply remain challenging due to stringent storage requirements and a storage duration of up to 7 days. [2]

Furthermore, platelet concentrates are associated with the highest risk of adverse transfusion reactions among all blood components due to their pro-inflammatory effects. Patients receiving platelets face an elevated risk of severe complications, including transfusion-associated acute lung injury (TRALI), anaphylactic reactions, transfusion-associated circulatory overload (TACO), and febrile non-haemolytic transfusion reactions (FNHTR). [3,4] Additionally, because platelets must be stored at room temperature (approximately 20°C–22°C), they present a significant risk for bacterial proliferation. While the implementation of nucleic acid testing (NAT) has drastically minimized viral transmission risks, bacterial contamination remains a persistent complication.[5,6] To mitigate these clinical risks and

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manage scarce resources, international bodies like the Association for the Advancement of Blood and Biotherapies (AABB) and the International Collaboration for Transfusion Medicine Guidelines (ICTMG) strongly advocate for restrictive platelet transfusion practices.[7,8,9] In Pakistan, clinical settings generally align with the frameworks established by BSH.[10,11] The BSH recommends strict, evidence-based triggers based on clinical indications, such as thresholds of <100 $x10^9$/L for ophthalmic and neurosurgery, <50 $x10^9$/L for major surgeries or active bleeding, and <20 $x10^9$/L for central venous catheter insertion, while explicitly discouraging prophylactic transfusions in immune thrombocytopenic purpura (ITP). BSH guidelines similarly recommend restrictive practices, with specific thresholds for various procedures. Most platelet transfusions are administered prophylactically rather than therapeutically.[11]

While audits of platelet transfusion practices are vital for patient blood management in haematology patients, detailed audits involving thorough medical record review remain limited. There is insufficient evidence on adherence to BSH guidelines in Pakistani tertiary care settings, and no comprehensive analysis of non-compliance patterns across different clinical departments.[12,13] What remains unknown or poorly understood are the specific clinical drivers, systemic factors, and departmental habits that lead clinicians to deviate from established protocols. This study addresses the critical gap in understanding platelet transfusion practices in Pakistan. By systematically evaluating adherence to BSH guidelines and identifying non-compliance patterns, our findings will inform targeted strategies to improve guideline adherence, optimize patient blood management, and reduce transfusion-related complications in patients requiring haematological care. The aim of this study was to evaluate adherence of platelet transfusion practices to BSH guidelines (2024) and identify patterns of non-compliance across clinical settings.

Materials and Methods

This descriptive cross-sectional study was conducted at the Department of Haematology and Blood Bank, Fauji Foundation Hospital, Rawalpindi, after obtaining approval from the Institutional Ethical Review Board (Ref. No. 779/RC/FFH/RWP). The study was carried out over a six-month period, from July 01, 2025 to December 31, 2025. A sample size of 260 was calculated using the WHO sample size calculator at a 95% confidence level, an anticipated prevalence of 50%, and a margin of error of 5%).

Patients receiving their first platelet transfusion during the study period were included. Patients who received multiple platelet transfusions during the same admission/study period were excluded. Consecutive non-probability sampling was employed. Data on demographic characteristics, clinical indications for platelet transfusion, pre-transfusion platelet count, transfusion thresholds, requesting department, and the presence of active bleeding or planned invasive procedures were collected using a structured data collection proforma.

Appropriateness of platelet transfusion was assessed using BSH platelet transfusion guidelines (2024), based on pre-transfusion platelet count and clinical indication. Platelet transfusions were considered adherent when the indication and pre-transfusion platelet count fulfilled its recommendations. Adherence to these guidelines were the primary outcome and the secondary outcome includes diagnosis-wise adherence and department wise adherence. Compliance with guideline thresholds was assessed across participating hospital departments. A summary of therapeutic and prophylactic clinical scenarios in which platelet transfusion was deemed appropriate under the BSH guidelines is presented.

The data were analyzed on SPSS v29. Normality of data was assessed using the Shapiro-Wilk test. Continuous variables are presented as mean ± SD or median (IQR), while categorical variables are reported as frequencies and percentages. The proportions of platelet transfusions that were compliant and non-compliant with the BSH guidelines were calculated. Associations between categorical variables were analyzed using the Chi-square test or Fisher's Exact test where appropriate. A p value ≤ 0.05 was considered statistically significant.

Results

A total of 260 patients who received platelet transfusions during the study period were included

in the analysis. Of these, 146 (56.2%) were adults, while 114 (43.8%) were pediatric patients. Females constituted the majority of the study population, accounting for 173 (66.5%) cases, whereas 87 (33.5%) were males. Platelet transfusions were most frequently administered in the Neonatal Intensive Care Unit (NICU), representing 47 (18.1%) of all transfusions, followed by oncology units with 40 (15.4%) cases. Intensive Care Unit, Medical wards, and Paediatric ward each contributed 37 (14.2%) transfusions, while the remaining 62 (23.8%) were distributed across other departments. Overall, 161 (61.9%) transfusions were adherent to the BSH platelet transfusion guidelines, whereas 99 (38.1%) were found to be non-adherent. The most common indication for platelet transfusion was thrombocytopenia, reported in 177 (68.1%) patients, followed by surgical procedures in 36 (13.8%) cases and post-chemotherapy support in 19 (7.3%). Neonatal sepsis (16.9%) and acute leukemia (15.8%) were the leading diagnoses associated with transfusion. A statistically significant association was observed between diagnosis and guideline adherence (Pearson's $\chi^2 = 46.30$, $p = 0.003$).

Table I: Baseline Demographic Characteristics, Clinical Department Distribution, and Overall Guideline Adherence among Patients Receiving Platelet Transfusions (n = 260).

Characteristic	Category	n (%)
Gender	male	87 (33.5)
	female	173 (66.5)
Age Group	paediatric (<18 years)	114 (43.8)
	adult (≥18 years)	146 (56.2)
Department	NICU	47 (18.1)
	oncology	40 (15.4)
	ICU	37 (14.2)
	medical Ward	37 (14.2)
	paeds Ward	37 (14.2)
	Other*	62 (23.8)
Guideline Adherence	adherent	161 (61.9)
	non-adherent	99 (38.1)

Table II: Distribution of Underlying Diagnoses among Patients Receiving Platelet Transfusions (n = 260).

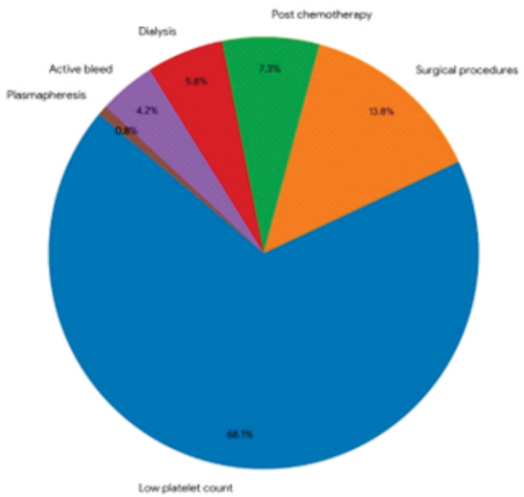
Diagnosis	Frequency, n (percentage, %)
Neonatal sepsis	44 (16.9)
Acute leukemia	41 (15.8)
Dengue Fever	29 (11.2)
Solid tumors	21 (8.1)
Infection	17 (6.5)
DIC	15 (5.8)
CKD	18 (6.9)
All others	75 (28.8)
Others (Abdominal Surgery, Multiple Myeloma, Myelofibrosis)	

TABLE III: Diagnosis-Wise Adherence to BSH Platelet Transfusion Guidelines (n = 260).

Diagnosis	Adherent, n (%)	Non-adherent, n (%)	Total, n
Neonatal sepsis	29 (65.9)	15 (34.1)	44
Acute leukemia	32 (78.1)	9 (21.9)	41
Dengue Fever	22 (75.9)	7 (24.1)	29
Solid Tumors	9 (42.8)	12 (57.2)	21
CKD	5 (27.8)	13 (72.2)	18
Infection	9 (52.9)	8 (47.1)	17
DIC	12 (80)	3 (20)	15
ITP	6 (54.5)	5 (45.5)	11
Caesarean Section	3 (33.3)	6 (66.6)	9
Others*	34 (61.8)	21 (38.2)	55
Total	161 (61.9)	99 (38.1)	260
Others (Abdominal Surgery, Multiple Myeloma, Myelofibrosis)			

Table IV: Association Between Clinical Diagnosis and Platelet Transfusion Guideline Adherence (Chi-Square Analysis, n = 260)

Test	χ^2 value	df	*p* value
Pearson Chi-Square	46.298	23	0.003*
Likelihood Ratio	52.516	23	<0.001*



Indications For Platelet Transfusion (n = 260)

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Discussion

Platelet transfusion plays a critical role in the prevention and management of bleeding. However, inappropriate utilization may expose patients to unnecessary risks and place an additional burden on limited blood bank resources. In the current study, 61.9% of platelet transfusions were found to be in accordance with BSH guidelines, whereas 38.1% did not meet the recommended criteria, reflecting considerable non-compliance in transfusion practices. These observations are comparable to findings reported in both regional and international studies, where inappropriate platelet transfusion practices continue to persist despite the implementation of standardized evidence-based guidelines. [14,15,16]

Audits from Pakistan have reported varying rates of appropriate platelet transfusion, ranging from 55% to 75%, depending on the clinical setting and patient population. [11] Similar variability has been observed in Indian tertiary care hospitals, where inappropriate platelet transfusion rates between 20% and 45% have been documented. [11,14] The level of non-adherence observed in our study therefore aligns with existing South Asian data and reinforces the need for sustained transfusion stewardship programs.

Our results revealed marked department-wise variation in guideline adherence, with significantly higher compliance observed in haematology-driven diagnoses such as acute leukemia (78.1%) and DIC (80%). This higher compliance in haematology-related diagnoses may reflect greater familiarity with transfusion thresholds in specialized services. Studies from both Pakistan and India have similarly shown that transfusions ordered by haematology or oncology services are more likely to be appropriate compared to those initiated in surgical or general medical wards. [13,15]

Conversely, solid tumors, chronic kidney disease (CKD), obstetric cases, and ICU settings demonstrated poorer adherence. In CKD patients, 72.2% of transfusions were non-adherent, reflecting a common misconception that uremia-associated platelet dysfunction necessitates prophylactic platelet transfusion. However, BSH and other international guidelines do not support routine platelet transfusion in CKD in the absence of active bleeding or invasive procedures. Similar patterns of over-transfusion in renal units have been reported in Indian studies, where clinicians concern regarding bleeding often overrides guideline-based decision-making. [7,17]

One of the most concerning findings was the low adherence in obstetric cases, particularly cesarean sections, where only 33.3% of transfusions were appropriate. This mirrors findings from audits conducted in Pakistan and India, which consistently demonstrate liberal platelet use in obstetrics due to fear of postpartum haemorrhage, medicolegal concerns, and lack of familiarity with evolving thresholds. [18,19] Evidence suggests that prophylactic platelet transfusion is unnecessary at platelet counts ≥ 50 × 10^9/L for vaginal delivery and ≥ 70–80 × 10^9/L for neuraxial anesthesia, yet these thresholds are frequently exceeded in routine practice.

Surgical indications accounted for 13.8% of transfusions in our study, with many administered above recommended platelet counts. Previous regional audits have highlighted similar trends, emphasizing that surgeons often transfuse platelets pre-emptively rather than based on procedure-specific bleeding risk. [14,16] This underscores the need for closer collaboration between surgical teams and transfusion medicine services.

Dengue fever represented a significant proportion of platelet transfusions in our cohort (11.2%), with adherence of 75.9%. Although relatively better than some other diagnoses, nearly one-quarter of dengue-related transfusions were still inappropriate. South Asian literature consistently demonstrates overuse of platelets in dengue fever without active bleeding, driven largely by public anxiety, pressure from attendants, and misconceptions regarding platelet counts as a marker of disease severity. [20] Several studies from Pakistan and India suggest limited clinical benefit of prophylactic platelet transfusion in dengue fever and, in some cases, increased transfusion-related complications. [21]

Neonatal sepsis was the most common diagnosis in this study, accounting for 16.9% of transfusions, with adherence of 65.9%. While this reflects moderate compliance, over one-third of neonatal transfusions were still non-adherent. Neonatal platelet transfusion thresholds have evolved significantly in

recent years, with increasing evidence supporting restrictive strategies to reduce morbidity and mortality. Studies from neonatal intensive care units in Pakistan have reported wide variability in platelet transfusion thresholds, often influenced by clinician experience rather than standardized protocols. [22] These findings highlight the urgent need for neonatal-specific transfusion education and protocol implementation.

The high rate of non-adherent platelet transfusions observed in this study has important clinical and economic implications. Platelets are associated with the highest rate of transfusion-related adverse events, including allergic reactions, febrile non-haemolytic reactions, transfusion-associated circulatory overload, transfusion-related acute lung injury, and bacterial sepsis. In resource-limited settings such as Pakistan, inappropriate platelet use also exacerbates supply shortages and increases healthcare costs.

Evidence from transfusion stewardship programs in India has demonstrated that education, audit-feedback mechanisms, and mandatory indication documentation can significantly reduce inappropriate platelet transfusions without increasing bleeding complications. [13] Implementing similar interventions locally could substantially improve adherence and patient safety.

This study has certain limitations. The sample size was relatively modest, and the study was conducted at a single tertiary care center, which may limit the wider generalizability of the findings. A multicenter design with a larger and more diverse patient population would provide more robust estimates of guideline adherence across different clinical settings. In addition, repeated transfusion episodes and long-term clinical outcomes such as bleeding complications or transfusion reactions were not assessed, which restricts evaluation of the direct impact of inappropriate transfusion practices. Furthermore, some diagnostic subgroups included small numbers of patients, which may reduce the statistical strength of comparisons. Future studies incorporating multicenter data, larger cohorts, and outcome-based assessment are recommended to validate and expand upon these observations.

Conclusion

This study demonstrates that while the majority of platelet transfusions in a tertiary care hospital were compliant with the BSH (2024) guidelines, a considerable proportion (38.1%) were inappropriate, with higher non-compliance observed in non-haematology settings such as CKD, obstetrics, solid tumors, and selected surgical cases. Guideline adherence varied across clinical specialties and was influenced by underlying clinical indication.

These findings underscore the importance of strengthening transfusion governance within institutions. Implementation of structured transfusion stewardship measures, including regular clinician education, dissemination of simplified guideline-based protocols, prospective audit with feedback, and active engagement of transfusion committees, is essential to improve adherence to evidence-based platelet transfusion practices, optimize utilization, and enhance patient safety.

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CONFLICT OF INTEREST

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORIGINAL ARTICLE

Perspectives of Undergraduate Medical Students Towards Electronic Portfolios: A Mixed-Methods Study from Pakistan

Rida Hassan, Ayesha Aleem

ABSTRACT

Objective: To explore undergraduate medical students' perspectives on the adoption of electronic portfolios (e-portfolios), focusing on perceived benefits, challenges, and influence on learning and professional growth.

Study Design: A convergent mixed-methods design was employed.

Place and Duration of Study: Bahria University College of Medicine, Bahria University Health Sciences Campus, Islamabad, Pakistan, from May 27, 2025 to January 30, 2026.

Materials and Methods: All 100 first-year MBBS students were invited through census sampling and all participated. A structured questionnaire assessed awareness, perceptions, perceived benefits, and barriers to e-portfolio use. Semi-structured interviews with 21 purposively selected students explored these experiences in greater depth. Quantitative data were analysed using descriptive and inferential statistics; qualitative data underwent thematic analysis.

Results: Only 8% of students used the institutional e-portfolio. Educational background was not associated with use (Fisher's Exact Test, p = 1.000). Overall perceptions were positive (mean composite score 23.84 of 30): 63% high, 36% moderate, and 1% low. Female students scored higher than male students on the Mann-Whitney U test (p = 0.042), although this should be interpreted cautiously because the parametric analysis was not significant (Welch's t-test, p = 0.069). Lack of awareness (42.4%) was the commonest barrier, followed by perceived irrelevance to undergraduate education (19.0%) and uncertainty regarding expected use (12.9%). Greater support and guidance (46.8%) and evidence of educational benefit (26.0%) were the measures most often endorsed to encourage future use. Thematic analysis generated four themes: Knowledge Acquisition and Self-Awareness, Skill Development and Reflective Practice, Learning from Mistakes and Growth Mindset, and Necessary Support Structures.

Conclusion: Medical undergraduates held predominantly positive perceptions of e-portfolios despite limited experience of their use. Improving awareness, structured orientation, faculty support, and curricular integration may enhance meaningful adoption in undergraduate medical education.

Key Words: *Education, Medical, Undergraduate; Portfolio; Self-Assessment; Students, Medical; Pakistan.*

Introduction

Electronic portfolios (e-portfolios) are defined as digital collections of documents, reflections, multimedia, and other artefacts that demonstrate an individual's learning progression, skills, achievements, and professional development. [1] In medical education, e-portfolios support competency-based medical education (CBME) by enabling students to document clinical performance, track competency attainment, link learning activities with intended outcomes, and compile evidence of achievement. [2] They also promote self-regulated learning by encouraging learners to set goals, monitor their progress, reflect on feedback, and identify areas for improvement. [3]

E-portfolios facilitate critical appraisal of learning experiences and contribute to lifelong learning through structured reflective practice, while continuous documentation of personal and professional growth supports professional identity formation. [4] They offer flexibility, portability, and real-time updating, overcoming many limitations of paper-based documentation. Internationally, undergraduate and postgraduate programmes have

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adopted e-portfolios to support assessment for learning, enhance reflection, and foster professional development. However, their integration at the undergraduate level remains inconsistent in many countries, including Pakistan, where no national framework currently guides their use.[5]

Consistent with these educational principles, existing literature has shown that although e-portfolios have the potential to improve engagement, self-regulated learning, reflective capacity, competency development, and professional readiness, student utilisation often depends on institutional support, technological reliability, training, and clarity of expectations. [6] Studies report that many medical undergraduates perceive e-portfolios as an added burden because of variability in technology, perceived usability issues, limited faculty involvement, and uncertain relevance to assessments. [7,8] Evidence also suggests that early integration within the curriculum, structured reflection opportunities, and mentor guidance promote deeper engagement and more meaningful learning experiences. [9-11] Additional findings highlight variability in the quality of entries and challenges related to originality and standardisation of portfolio content. [3,12]

Despite documented benefits, several barriers remain common globally. Students frequently cite time constraints, lack of structural clarity, technological difficulties, and insufficient training as key impediments to sustained use. [13-15] Concerns regarding privacy, consistency in portfolio design, and fear of misinterpretation of personal reflections also hinder engagement. [16] Faculty-related factors further influence adoption; inadequate faculty training, inconsistent feedback practices, and limited understanding of the pedagogical role of portfolios adversely affect student perceptions. [17-19] Successful implementation therefore requires institutional commitment, clear guidelines, robust technical infrastructure, and continuous faculty development.[20-22] Innovative approaches such as gamification, social-media-supported portfolios, and enhanced feedback mechanisms have been proposed to strengthen motivation, collaboration, and learning.[13,23]

Despite growing global interest, limited research exists on e-portfolio perceptions among Pakistani medical undergraduates. The absence of empirical data restricts informed planning and hinders the development of structured, context-appropriate e-portfolio systems aligned with national curricular needs. This gap underscores the need to explore student attitudes, barriers to adoption, and factors influencing engagement in the local setting. Therefore, there was research gap regarding context-specific insights that could guide curriculum developers and institutional leaders in designing effective, student-centred e-portfolio systems.

The aim of this study was to identify medical undergraduates' perceptions of e-portfolios, identify barriers to their adoption, and determine factors influencing engagement, in order to inform effective integration into undergraduate medical education in Pakistan.

Materials And Methods

A convergent mixed-methods research study was conducted at Bahria University College of Medicine, Bahria University Health Sciences Campus, Islamabad, Pakistan, over a period of eight months from May 27, 2025 to January 30, 2026. Ethical approval was obtained from the Ethics Review Committee of Bahria University College of Medicine (Approval No. BUCM/ERC/2502, dated 26 May 2025).

No formal sample size calculation was performed because the entire target population was included. First-year students were selected because institutional implementation of e-portfolios was planned to commence from first-year, making this cohort the most relevant population for evaluating the acceptability and feasibility of the proposed initiative. The study employed census sampling. whereby quantitative survey findings and qualitative interview findings were collected concurrently, analysed separately, and integrated during interpretation

All eligible first-year MBBS students (n = 100) were invited to participate, and all students responded, resulting in a response rate of 100%. Informed consent was obtained from all participants. Participation was voluntary, and confidentiality and anonymity were maintained throughout the study. Participants were informed of their right to withdraw from the study at any stage without any consequences.

Undergraduate medical students enrolled in the first-year MBBS programme at Bahria University College of Medicine who provided informed consent were included in the study. Students who declined to participate or did not provide informed consent were excluded.

Data were collected using a structured questionnaire adapted from previously published studies by Belcher et al. [24] and Bangalan et al. [25] The instrument comprised demographic characteristics, system utilisation, barriers to non-use, and two distinct Likert-scale subscales. To ensure psychometric soundness, internal consistency was evaluated separately for both subscales using Cronbach's alpha (α). The first subscale, consisting of six items measuring student perceptions of e-portfolio utility, demonstrated good internal consistency (α = 0.824). The second subscale, consisting of seven items evaluating prospective behavioural intentions and reflective learning applications, demonstrated excellent internal consistency (α = 0.891).

Because demographic items and categorical barrier checklists represent independent nominal variables rather than continuous scale, reliability metrics were not mathematically applicable to those sections. The analyses reported in this paper are based on the six-item perception subscale, which was combined into a single composite perception score. The questionnaire was administered electronically using Google Forms.

In addition, semi-structured interviews were conducted with 21 purposively selected students, using an interview guide derived from the open-ended questionnaire items, to explore their experiences and perceptions in greater depth.

Quantitative data were analysed using IBM SPSS Statistics v27. Descriptive statistics were reported as frequencies, percentages, means, and standard deviations. Categorical variables were summarised as frequencies and percentages. Because e-portfolio use was reported by only eight participants, and more than 20% of cells in the relevant cross-tabulations had an expected count below 5, the Pearson Chi-square test was not appropriate and all cross-tabulations involving e-portfolio use were analysed using Fisher's Exact Test.

The composite perception score (six items, reverse-coded so that a higher value indicated a more positive perception; possible range 6 to 30) was assessed for normality using the Shapiro-Wilk test and, being non-normally distributed, was compared between two groups, male versus female and e-portfolio users versus non-users, using the Mann-Whitney U test, with the independent-samples t-test reported for corroboration.

Homogeneity of variance was assessed using Levene's test, and Welch's t-test was used in place of the Student t-test where variances were unequal. Differences in perception scores across the three educational backgrounds (FSc, A Levels, and IB) were examined using the Kruskal-Wallis test, with one-way ANOVA reported alongside. Effect sizes were calculated as Cohen's d. Statistical significance was set at a two-sided p-value of <0.05, and exact p-values were reported.

Qualitative interview data were analysed using Braun and Clarke's six-phase reflexive thematic analysis approach. Interviews were transcribed with verbatim, and transcripts were read repeatedly to achieve familiarisation. Meaningful units of text were coded independently by two members of the research team, and coding was performed manually. The coders compared their coding frameworks, and discrepancies were resolved through discussion until consensus was reached. Related codes were grouped into categories, and broader themes were generated iteratively.

Data collection and analysis continued until data saturation was reached, defined as the point at which no new codes or themes emerged from successive interviews. Saturation was achieved at participant 18, but interviewing continued with the last participant. Reflexivity was maintained through regular team debriefing, with the researchers acknowledging their position as medical educators to limit the influence of preconceptions on interpretation. The trustworthiness of the qualitative findings was addressed in accordance with the criteria of Lincoln and Guba: credibility through investigator triangulation and the use of participant-derived verbatim quotations, dependability and confirmability through an audit trail of coding decisions, and transferability through a detailed description of the study setting and participants.

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Results

The study included 100 undergraduate medical students aged 18 to 24 years (mean = 19.41, SD = 1.14). Females constituted 68% (n = 68) of the participants, whereas males represented 32% (n = 32). Most students reported were with Faculty of Science (FSc) educational background at entry (93%, n = 93), followed by A Levels (6%, n = 6) and the International Baccalaureate (1%, n = 1).

Only 8% (n = 8) of all participants reported any use of the institutional e-portfolio system, while 92% (n = 92) had never used it. Among users, the most frequently accessed section was educational background (87.5%, n = 7), followed by co-curricular activities (37.5%, n = 3) and distinctions (25%, n = 2). Reflective and feedback-oriented sections showed minimal engagement (≤12.5%, i.e. ≤1 user).

Cross-tabulation of e-portfolio use by educational background demonstrated similar levels of non-usage across FSc (91.4%, n = 85), A Levels (100%, n = 6), and IB (100%, n = 1). Because more than 20% of cells had an expected count below 5, all cross-tabulations were assessed using Fisher's Exact Test. Use of the e-portfolio was not significantly associated with educational background (p = 1.000) or with gender (p = 0.708), nor with agreement on either of the two single-item statements examined (p = 0.385 and p = 1.000). Results of all cross-tabulations are presented in Table I.

A composite perception score was calculated by summing responses to six five-point Likert-scale items, with each item reverse-coded so that a higher value reflected stronger agreement and, therefore, a more positive perception of e-portfolios (possible range 6 to 30). The six items showed good internal consistency (Cronbach's alpha = 0.824), supporting their combination into a single scale.

Scores were categorised a priori into equal intervals as low (6 to 14), moderate (15 to 22), and high (23 to 30). The mean perception score was 23.84 (SD = 3.75), with scores ranging from 14 to 30, indicating an overall positive orientation toward e-portfolios relative to the scale midpoint of 18. Based on these categories, 63% (n = 63) of students demonstrated high perception, 36% (n = 36) moderate perception, and 1% (n = 1) low perception. The distribution of responses to each of the six individual perception statements is shown in Figure 1.

Because perception scores were not normally distributed (Shapiro-Wilk p = 0.007), between-group differences were examined using the Mann-Whitney U test (Table II). Female students reported more positive perceptions than male students (median 24 versus 22; mean 24.37 versus 22.72; Mann-Whitney U, p = 0.042; Cohen's d = 0.45). Although the Mann-Whitney U test reached statistical significance, this finding should be interpreted cautiously, because the parametric analysis did not (Welch's t-test, p = 0.069) and the associated effect size was small.

E-portfolio users reported more positive perceptions than non-users (mean 26.12 versus 23.64; Cohen's d = 0.67), although the difference was not statistically significant (Mann-Whitney U, p = 0.083), a finding consistent with the small number of users (n = 8).

Perception scores did not differ significantly across educational backgrounds (FSc 23.86, A Levels 22.83, IB 28.00; Kruskal-Wallis p = 0.347; one-way ANOVA p = 0.439), as shown in Table III.

Among non-users, the most frequently reported reason for non-engagement was lack of awareness (42.4%, n = 39). Other reasons included perceptions of irrelevance to the undergraduate phase (19.0%, n = 17), uncertainty about expected use (12.9%, n = 12), time constraints (8.6%, n = 8), and technical challenges (7.2%, n = 7). Confidentiality concerns (3.4%, n = 3) and peer non-use (2.4%, n = 2) were rarely cited.

When asked which measures would encourage future use, students most frequently endorsed greater support and guidance (46.8%, n = 44). Additional suggestions included providing evidence of training benefit (26.0%, n = 24), making the portfolio compulsory (14.9%, n = 14), and hearing experiences from junior doctors (12.3%, n = 12).

The qualitative component included 21 semi-structured interviews, which produced four main themes.

Knowledge Acquisition and Self-Awareness: participants described the e-portfolio as a tool that enabled them to monitor their academic development. One participant stated, *"It helps me see how much I have improved over time and what I still need to work on."* (Participant 5).

Skill Development and Reflective Practice: students highlighted that documenting experiences encouraged reflection. One participant commented,

"Writing down my experiences made me think about what went well and what I could improve next time." (Participant 12).

Learning from Mistakes and Growth Mindset: participants viewed the e-portfolio as an opportunity to reflect on mistakes and use feedback for continuous improvement. One participant commented, *"It helps me identify where I went wrong so I can learn from my mistakes and avoid repeating them in the future."* (Participant 9).

Necessary Support Structures: participants consistently emphasised the need for institutional guidance. As one student explained, *"We need clear instructions and someone to guide us in using the portfolio effectively."* (Participant 18).

Within these themes, the most frequently mentioned motivator was progress tracking (42.9%, n = 9). Other frequently expressed motivators included learning awareness (33.3%, n = 7) and goal setting (23.8%, n = 5). Regarding support needs, students most requested clearer instructions (52.4%, n = 11), followed by regular tutor guidance (28.6%, n = 6) and access to exemplar portfolios (19.0%, n = 4). Representative participant quotations illustrating each theme are presented in Table IV, and the thematic structure is summarised in Figure 2.

Table I: Association Between E-Portfolio Use and Student Characteristics or Single-Item Responses (Fisher's Exact Test) (n = 100)

Cross-tabulation	Fisher's Exact p	% cells with expected count < 5
e-portfolio use × gender	0.708	25%
e-portfolio use × educational background	1.000	50%
e-portfolio use × agreement with the statement "e-portfolios are easier to use in the postgraduate phase"	0.385	50%
e-portfolio use × agreement with the statement "e-portfolios should be used in future assessment (mini-CEX)"	1.000	70%

p values from Fisher's Exact Test. Chi-square were not used because more than 20% of cells had an expected count below 5. The final two rows report agreement with single questionnaire statements and not composite constructs.

Table II: Composite Perception Score by Gender and by E-Portfolio Use (Mann-Whitney U Test and Parametric T-Test) (n = 100)

Comparison	Mean ± SD	Median [IQR]	Mann-Whitney p	Parametric p
Male (n = 32)	22.72 ± 4.49	22.0 [19.5-25.5]	0.042 *	Welch 0.069
Female (n = 68)	24.37 ± 3.25	24.0 [22.0-26.2]		Student 0.040
User (n = 8)	26.12 ± 3.00	25.5 [23.8-28.5]	0.083 (ns)	Welch 0.055
Non-user (n = 92)	23.64 ± 3.76	24.0 [21.8-26.0]		Student 0.072

*Composite perception score is reverse-coded, so a higher score indicates a more favourable perception (possible range 6 to 30). IQR, interquartile range. Gender: Levene's test showed unequal variances (p = 0.040), so Welch's test is the appropriate parametric comparison. Effect sizes: gender Cohen's d = 0.45 (small); user status Cohen's d = 0.67 (moderate to large). * Statistically significant on the non-parametric test only; see text for interpretation*

Table III: Composite perception score across educational background (Kruskal-Wallis test and one-way ANOVA) (n = 100)

Group	n	Mean ± SD	Median [IQR]
FSc	93	23.86 ± 3.73	24.0 [22.0-26.0]
A Levels	6	22.83 ± 4.17	21.5 [21.0-24.2]
IB	1	28.00	28.0

One-way ANOVA F = 0.830, p = 0.439. Kruskal-Wallis H = 2.116, p = 0.347. Kruskal-Wallis is the defensible primary test given the small, unequal groups; no post-hoc test is meaningful because the IB group has a single respondent. Higher score indicates a more favourable perception.

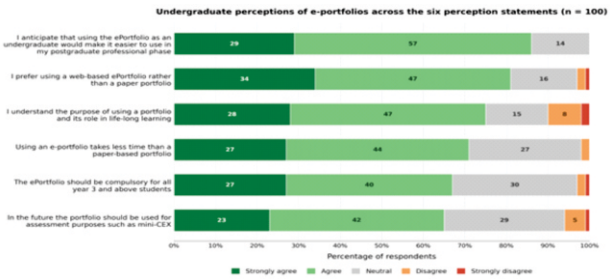

Figure 1: Distribution of Responses to the Six E-Portfolio Perception Statements (n = 100).

Discussion

The present study identified a marked discrepancy between attitude and behaviour: undergraduate medical students held positive attitudes toward e-portfolios despite very low utilisation (8%) of the institutional system. This gap indicates that deploying digital software alone is not sufficient to

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Table IV: Qualitative Themes, Subthemes, and Representative Participant Quotations (n = 100)

Theme	Subthemes	Representative Quotation
Knowledge Acquisition and Self-Awareness	Progress tracking; learning awareness; goal setting	"It helps me see how much I have improved over time and what I still need to work on." (Participant 5)
Skill Development and Reflective Practice	Reflection; self-assessment; professional development	"Writing down my experiences made me think about what went well and what I could improve next time." (Participant 12)
Learning from Mistakes and Growth Mindset	Learning from mistakes; continuous improvement; goal setting	"It helps me identify where I went wrong so I can learn from my mistakes and avoid repeating them in the future." (Participant 9)
Necessary Support Structures	Clear instructions; tutor guidance; exemplar portfolios	"We need clear instructions and someone to guide us in using the portfolio effectively." (Participant 18)

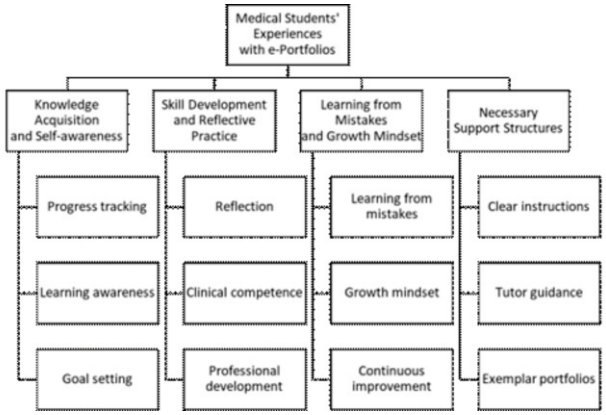

Figure 2: Thematic Representation of Medical Students' Experiences With E-Portfolios

generate active engagement. Students appear more likely to adopt these tools when they are introduced early, integrated into the curriculum, and actively supported by faculty. Our findings are consistent with those of Belcher et al. [24] and Bangalan et al., [25] who reported that e-portfolios gain traction only when they are treated as an active medium for teaching and assessment rather than a peripheral task. The low engagement observed in our cohort is therefore most plausibly explained by the absence of formal orientation and by limited curricular integration.

Our data show that 63% of students recorded high perception scores, 36% moderate scores, and 1% a low score, giving a mean composite score of 23.84 out of 30. Although actual users viewed e-portfolios more favourably than non-users, the difference in composite perception scores did not reach statistical significance. This most likely reflects the small number of users (n = 8) rather than a true absence of difference, a pattern commonly observed in early-stage implementation. A similar note of caution applies to the gender comparison; although the Mann-Whitney U test reached statistical significance ($p = 0.042$), this finding should be interpreted cautiously because the parametric analysis did not (Welch's t-test, $p = 0.069$) and the effect size was small (Cohen's $d = 0.45$). Existing literature suggests that students recognise the value of reflective portfolios mainly after they begin using them consistently. [8,9] This supports embedding portfolio work directly into the learning timeline rather than leaving it as an optional, self-guided activity.

The finding that 92% of Pakistani medical undergraduates had never used the institutional e-portfolio, with 42.4% reporting a lack of awareness of its existence, points to a substantial implementation gap. This lack of awareness is consistent with wider Pakistani and South Asian medical education literature, in which digital initiatives frequently fail to gain traction when introduced without structured onboarding. [7,18,19] Reviews of portfolio implementation also describe a time-versus-benefit trade-off, in which assessment-driven medical students give low priority to platforms that are not linked to assessment. [14] Within a competency-based medical education framework that relies on continuous programmatic assessment such as the mini-Clinical Evaluation Exercise (mini-CEX), an e-portfolio that is neither linked to assessment nor compulsory is readily overlooked by students managing a heavy workload. Our data do not support the assumption that students who are familiar with digital technology will spontaneously adopt reflective portfolios. To narrow this gap, and to ease the transition to postgraduate training, where

portfolio-based competency tracking is increasingly adopted, [12,16] institutions need to move from passive availability to active integration, embedding e-portfolios in clinical schedules, summative assessment, and structured faculty feedback cycles.

The quantitative and qualitative components of this study were mutually explanatory rather than independent. The interview accounts help explain why lack of awareness emerged as the dominant quantitative barrier (42.4%), since students repeatedly reported that they did not know the platform existed or how it was intended to help them. Similarly, the qualitative theme Necessary Support Structures corresponds directly to the survey finding that 46.8% of students wanted structured guidance and tutor support. The quantitative observation that users held more favourable perceptions was likewise reflected in the interviews, in which students who had logged in described concrete benefits such as tracking progress, reflecting on clinical skills, and learning from mistakes. This convergence of quantitative and qualitative findings supports the interpretation that low uptake indicates the need for structured guidance and clearer communication, rather than a rejection of the value of the tool.

Limitations

This study has several limitations that should be kept in mind when interpreting the findings. First, the study included a single first-year MBBS cohort at one medical college, recruited through census sampling. Consequently, the findings may not represent the experiences of students in later clinical years, or those at other institutions with different digital resources. Second, the data rely on self-reported questionnaires, which are inherently vulnerable to social desirability and recall bias. Third, because only 8% of the sample had actual experience of using the institutional e-portfolio, most responses represent prospective expectations and theoretical perceptions rather than hands-on, long-term experience with the platform. Fourth, the cross-sectional design captures a single snapshot of student views and cannot track how perceptions, digital literacy, or engagement levels evolve over time. Fifth, the study assessed subjective student perceptions rather than actual educational outcomes; objective markers of success, such as clinical performance, reflective writing quality, or academic grades, were not measured, so it is not possible to determine whether e-portfolio use translates into improved clinical competence.

Future Recommendations

To establish the e-portfolio as an active educational anchor rather than an underused resource, future initiatives should focus on the following strategies. Firstly, mandatory hands-on e-portfolio orientation modules should be implemented during the first week of medical school to address the lack of awareness identified in this study. Moreover, mentor-guided reflective cycles should be designated, in which faculty review portfolio entries and provide timely, formative feedback.

Furthermore, portfolio tasks should be formally linked to workplace-based clinical assessments such as the mini-CEX, so that the tool is directly tied to academic progress. Finally, multicentre comparative studies should be conducted across Pakistani medical colleges using different integration models. This, alongside longitudinal research, would help determine whether early e-portfolio integration yields long-term benefits for clinical reflective practice, competency tracking, and professional identity development.

Thus, introducing e-portfolios early in the curriculum, supported by structured orientation, faculty mentorship, and integration with competency-based learning and assessment, may improve student engagement and encourage reflective learning. Further multicentre longitudinal studies are needed to evaluate the long-term impact of e-portfolios in undergraduate medical education in Pakistan.

So, this systems would support undergraduate learning needs, strengthen professional development, and address contextual challenges related to technology and training.

Conclusion

Undergraduate medical students recognised the potential educational value of e-portfolios and held predominantly positive perceptions of their use, with 63% scoring in the high-perception category and a further 36% in the moderate category. However, most students had little or no experience with the institutional e-portfolio, resulting in limited utilisation. Lack of awareness, insufficient guidance,

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and minimal curricular integration emerged as the main barriers to adoption.

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The material submitted for publication may be in the form of an original research (Randomized controlled trial – RCT, Meta-analysis of RCT, Quasi experimental study, Case Control study, Cohort study, Observational Study with statistical support, etc.), a Review Article, a Case Report, Recent Advances, New Techniques, Debates, Book/CDs Review on Clinical/Medical Education, Adverse Drug Reports or a Letter to the Editor. Survey Articles and Studies more than five years old at the time of submission are not accepted for publication in JIIMC. Non-English articles are not accepted for publication in JIIMC.

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REVIEW ARTICLE should consist of structured overview of relatively narrow topic providing background and recent development with reference of original literature. An author can write a review article only if he/she has written a minimum of three

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LETTERS TO THE EDITOR should normally not exceed 400 words, have no more than 05 references and be signed by all the authors-maximum 3 are allowed. Preference is given to those that take up points made in contributions published recently in a journal. Letters may be published with a response from the author of the article being discussed. Discussions beyond the initial letter and response will not be entertained for publication.

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  - o CONSORT 2010 checklist
  - o CONSORT 2010 flow diagram
- Templates for these can be readily accessible here or on the CONSORT website, which also describes several CONSORT checklist extensions for different designs and types of data beyond two group parallel trials
- Authors should ensure that your article, at minimum, reports content addressed by each item of the checklist. Meeting these basic reporting requirements will greatly improve the value of your trial report and may enhance its chances for eventual publication.
- As per recommendation of ICMJE, Journal of Islamic International Medical College requires registration of clinical trials in a public trials registry as a prerequisite for publication of all clinical trials.
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  A clinical trial is any research study that prospectively assigns human participants or groups to one or more health-related interventions to assess their effects on health outcomes. These interventions can include drugs, surgical procedures, devices, behavioral treatments, dietary changes, and modifications in care processes. Health outcomes encompass any biomedical or health-related measures collected from patients or participants, including pharmacokinetic data and adverse events. Purely observational studies (those in which the assignment of the medical intervention is not at the discretion of the investigator) do not require registration.

GENERAL ARCHIVAL INSTRUCTIONS

The manuscript should be typed in MS Word. Each manuscript should include a title page (containing email address, cell numbers, institution, and postal address of the corresponding author), abstract, key words, text, acknowledgements (if any), references, tables (each table, complete with title and footnotes) and legends for illustrations and photographs. Each component should begin on a new page. Sub-headings should not be used in any section of the script except in the abstract.

TEXT ORGANIZATION

All manuscripts except Short Communication and Letter to the Editor should be divided into the following sections.

ABSTRACT

Abstracts of original article should be in structured with following sub-headings:

- Objective
- Study Design
- Place & Duration of Study
- Materials & Methods
- Results
- Conclusion

Four elements should be addressed: "why did you

start?", "what did you do?", "what did you find?" and "what does it mean? “.” Why did you start?" is addressed in the objective. "What did you do?" constitutes the methodology and could include design, setting, patients or other participants, interventions, and outcome measures. "What did you find?" is the 'results', and "what does it mean?" would constitute the conclusions. Please label each section clearly with the appropriate sub-headings. Structured abstract for an original article, should not be more than 250 words. At least 3 key words should be written at the end of the abstract. Review articles, case reports and others require a short, unstructured abstract. Commentaries do not require an abstract.

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Write this section with references as per following instructions:

1. Give background information about the subject matter and the issues your study intends to address. Only strictly pertinent references should be cited, and the subject should not be extensively reviewed.
2. Describe what is known (in the literature) and what is not clear about the subject with reference to relevant literature thus identifying the literature gap.
3. You write the rationale (justification) of your study.
4. Finally, you mention the objective of your study

MATERIALS AND METHODS

Methodology is written in past tense.

Follow this sequence **without headings:**

- Study design
- Place and Duration of Study
- Sample size
- Sampling technique
- Mention about permission of the ethical review board and other ethical issues addressed.
- Inclusion and Exclusion Criteria
- Data collection procedure-
- Type of data: parametric or nonparametric
- Data analysis: including Statistical Software used, and statistical test applied for the calculation of p value and to determine the statistical significance. Exact p-values and 95% confidence interval (CI) limits must be mentioned instead of only stating greater or less than level of significance. All percentages must be accompanied with actual numbers.

RESULTS

These should be presented in logical sequence in the text, tables, and illustrations. All the data in the tables or illustrations should not be repeated in the text; only important observations should be emphasized or summarized. No opinion should be given in this portion of the text.

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This section should include the author's comments on the results. Write in present tense, active voice except for results, which are written in past tense. It should be written in following sequence:

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- Discuss key studies relevant to your study.
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- Describe limitations of your study.
- Suggest future work if necessary.

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References must be written in Roman Number and in the Vancouver Style only. References should be numbered in the order in which they are superscripted in the text. At the end of the article, the full list of references should give the names and initials of all authors (unless there are more than six when only the first six should be given followed by et al). The author's names are followed by the title of the article; title of the journal abbreviated according to the style of the Index Medicus (see “List of Journals Indexed”, printed yearly in the January issue of Index Medicus); year, volume, and page number, e.g., Hall, RR. The healing of tissues by C02 laser. Br J. Surg: 1970; 58:222-225. References to books should give the names of editors, place of publication, publisher, and year. The author must verify the references against the original documents before the article. References to papers accepted but not yet published should be designated as “in press” or “forthcoming”; authors should obtain written permission to cite such papers as well as verification that they have been accepted for publication.

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Tables and illustrations should be merged within the text of the paper, maximum number of tables and illustrations should not exceed four, and legends to illustrations should be typed on the same sheet. Tables should be simple and should supplement rather than duplicate information in the text; tables repeating information will be omitted. Each table should have a title and be typed in double space without horizontal and vertical lines on an 8 ½" x 11' paper. Tables should be numbered consecutively with Roman numerals in the order they are mentioned in the text. Page number should be in the upper right corner. If abbreviations are used, they should be explained in footnotes and when they first appear in text. When graphs, scattergrams, or histograms are submitted, the numerical data on which they are based should be supplied. All graphs should be made with MS Excel and be sent as a separate Excel file even if merged in the manuscript. For scanned photographs the highest resolution should be used.

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System International (SI) Unit measurements should be used. All drugs must be mentioned in their generic form. The commercial name may however be mentioned within brackets, if necessary.

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- JIIMC Checklist
- JIIMC Conflict of Interest Performa
- JIIMC CopyRight and Undertaking Agreement
- IRC Certificate
- Bank draft as initial processing fee (Original bank draft send in JIIMC office)

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- If there is no Methodology section, the role of the funding source should be stated as an acknowledgment.
- The corresponding author should confirm that he/she had full access to all the data in the study and had final responsibility for the decision to submit for publication.
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1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; and
2. Have been involved in drafting the work or

revising it critically for important intellectual content; and
3. Have given final approval of the version to be published; and
4. Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. We strongly discourage gift or ghost authorship. Mere supervision, collection of data, statistical analysis and language correction do not grant authorship rights. To avoid any dispute regarding authorship authors are advised to consult COPE guidelines to avoid authorship problems.

CORRIGENDUM

Correction of Author Affiliation and Department

- **Article Title:** Modified Above-Knee Versus Conventional Great Saphenous Vein Stripping for Lower Limb Varicose Veins: A Prospective Comparative Study
- **Journal:** Journal of Islamic International Medical College (JIIMC)
- **Issue:** June 2026

The *Journal of Islamic International Medical College* (JIIMC) wishes to issue a correction regarding the institutional affiliation and department of Dr. Arsalan Manzoor Mughal in the above-mentioned article, published in the June 2026 issue.

In the published article, the affiliation was incorrectly stated as: **Department of Surgery, Holy Family Hospital**

The correct affiliation is: **Department of Anatomy, Rawalpindi Medical University, Rawalpindi, Pakistan**

Accordingly, the author's details should read as follows: **Dr. Arsalan Manzoor Mughal** Associate Professor of Anatomy, Department of Anatomy, Rawalpindi Medical University, Rawalpindi, Pakistan

This corrigendum is issued to accurately reflect the author's department and institutional affiliation. The correction does not affect the scientific content, methodology, results, conclusions, or interpretation of the published article.

The Editorial Office sincerely regrets this error and apologizes to the author and readers for any inconvenience caused.

Editorial Office *Journal of Islamic International Medical College (JIIMC)*

CORRIGENDUM

Correction of Author Name

- **Article Title:** Clinical Features and Outcome of Mucormycosis Cases in A Tertiary Care Hospital: A 10-year Experience
- **Journal:** Journal of Islamic International Medical College (JIIMC)
- **Issue:** June 2026

The *Journal of Islamic International Medical College* (JIIMC) wishes to issue a corrigendum regarding the spelling of an author's name in the above-mentioned article, published in the June 2026 issue.

In the published article, the author's name was incorrectly printed as: **Mawra Iftikhar**

The correct spelling of the author's name is: **Mawara Iftikhar**

This correction is strictly limited to the author's name and does not affect the scientific content, methodology, results, or conclusions of the article.

The Editorial Office regrets this error and apologizes to the author and readers for any inconvenience caused.

Editorial Office *Journal of Islamic International Medical College (JIIMC)*