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EDITORIAL

The Predicament of Postgraduate Medical Education in Pakistan

Irfan Ali Mirza

In Pakistan, it is dream of most parents to see their children pursuing medical carrier and doing post-graduation. Primary reason for this desire stems from the assumption that specialisation ensures financial freedom and is largely a status symbol. The desire to do post-graduation and path leading to this objective is full of struggle and obstacles. After doing MBBS, students feel less like the start of a career and more like a tough Journey with degree, ambition and investment they still do not have clear path ahead.

Young medical graduates are most of the times not aware of the problems that may come their way in pursuit of their post-graduation. College of physicians and Surgeons Pakistan (CPSP) with more than 80 specialities and sub-specialities is most sought after postgraduate medical education path in Pakistan. Following hindrances are frequently encountered by young doctors.

1. Bottleneck After Doing FCPS Part 1

The most visible problem is sheer volume. Every year, thousands of doctors clear Fellow of the College of Physicians and Surgeons (FCPS) Part 1 examination conducted by the CPSP. Clearing FCPS Part 1 does not guarantee a training slot. Training positions are limited, supervisor-dependent, and concentrated in a handful of accredited hospitals. This problem was highlighted few years back in a report published in leading newspaper of Pakistan. [1]

Core reason for this is supervisor availability. CPSP policy requires that supervisors hold at least the rank of Assistant Professor and should have five years of post-fellowship experience. In some specialties like orthopaedics, one supervisor often ends up overseeing ten to twelve trainees simultaneously, which is not justifiable. [2, 3] Supervisors are also not financially compensated for mentorship either, which is one of the strongest incentives to invest properly in trainees. Lack of incentives for supervisors in likely to affect the quality of training being imparted.

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2. Quality, Design and Process of Training

There is huge variation in the quality, design and process of training in various specialties across different accredited hospitals and institutes. Some hospitals have built strong training program and monitoring mechanisms to oversee the training. While quite a large number of hospitals. Just see this as an opportunity to have cheap labour to manage indoor and outdoor patient workload.

The exit examination of FCPS has unfortunately high failure rates, higher than comparable exams from the Royal Colleges in the United Kingdom (UK).

A recent study carried out in Lahore found that residents attributed their failures to a combination of factors including inadequate supervision, lack of structured training, and the burden of clinical responsibilities that left little time for focused study. [4] Unfortunately exam is also heavily dependent on spot performance on a single day, which adds enormous psychological pressure on trainees who have already spent five years in the program.

3. Monetary Factors

The trainees are often given a modest stipend during their 4-5 years of post-graduation which is difficult for them to support the family specially if they are married. Moreover, the expense of resources material, workshops and the examination fee add further economic burden. A recent report published in daily newspaper Dawn in 2025 highlighted that medical specialities even with double FCPS degrees often cannot find a job in public sector hospitals. [5]

4. Brain Drain

It is not surprising that migration to other countries is the dominant theme in conversations among young Pakistani doctors. About 60 to 95 percent of medical graduates from top Pakistani institutions intend to pursue postgraduate training abroad, primarily in the United States of America (USA) and UK. [6] A study carried out in 2024 on neurosurgery trainees found that 64 percent of those surveyed intended to leave Pakistan for fellowship training abroad. [7] They attribute this to better quality training, financial security, job opportunities, and the simple fact that a residency completed abroad opens doors for more opportunities. This is brain drain in its most

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expensive form. The country trains its best doctors at public expense and watches them leave because the system at home unfortunately cannot fulfil their objectives.

5. What is the Predicament, Really?

The predicament is not just about pass percentage or of supervisor's ratio. It is about a structural mismatch between the number of doctors Pakistan produces, the quality of training it offers them, and the opportunities available afterwards. Every part of the pipeline has a leak. A global system is thought to be more rewarding for our graduates who opt to move out.

To address this predicament, it would require coordinated actions between Ministry of Health services and regulations, provincial health departments, Pakistan Medical and Dental Council (PMDC) and CPSP. This would require expanding training capacity in a sustainable way compensating supervisors properly, standardising training quality across institutions and aligning the production of specialists with the actual job market. It would also require honest conversations about what doctors are paid and how the public sector retains them.

None of this is easy and none of it is happening at the speed it needs to be. Meanwhile, the most talented young doctors continue to make rational individual decisions that in aggregate hollow out the country's medical infrastructure. The predicament of postgraduate medical education in Pakistan is, in the end, a predicament about whether the country can offer its doctors a future worth staying for.

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

Spectrum of Ocular Pathologies Leading to Evisceration and Enucleation: A Tertiary Care Set Up Study

Muhammad Aneeq Haroon[1], Nadeem Qureshi[2], Ambreen Yousaf[3], Aiza Haroon[4], Aena Farooq[5], Mahwish Shahid[6]

ABSTRACT

Objective: To determine the spectrum of ocular pathologies leading to evisceration and enucleation in a tertiary care hospital.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: Al-Shifa Trust Eye Hospital, Rawalpindi, from 6th January 2025 to 12th January 2026.

Materials and Methods: A total of 120 patients who underwent evisceration or enucleation were included in the study. Non-probability consecutive sampling technique was used. Data was collected on a self-structured proforma that included sections for demographics, clinical presentation, diagnosis/indication for surgery, B-scan ultrasonography findings, histopathological findings, surgical procedure, implant placement, and preventability status**.** Data were analyzed using SPSS version 25. Age was expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. Chi-square test and Fisher–Freeman–Halton exact test were applied for association between categorical variables, and independent samples t-test was applied for comparison of mean age between traumatic and non-traumatic groups. A p-value of ≤0.05 was considered statistically significant.

Results: The mean age was 40.4 ± 17.9 years. Male patients were more frequently affected, 78 (65.0%). Evisceration was performed in 74 (61.7%) patients, while enucleation was performed in 46 (38.3%) patients. Ocular trauma was the most common indication, 52 (43.3%), followed by endophthalmitis/panophthalmitis, 24 (20.0%), and corneal ulcer/perforation, 16 (13.3%). Most patients presented with no perception of light, 88 (73.3%), and 92 (76.7%) cases were classified as preventable. Histopathology mainly showed inflammatory/infective changes, 62 (51.7%). A statistically significant association was observed between indication and preventability ($p < 0.001$), while gender showed no significant association with etiology, procedure, or visual acuity.

Conclusion: Ocular trauma and infective ocular pathologies were the leading causes of evisceration and enucleation, and most cases were potentially preventable. Early referral, timely treatment of ocular infections, and preventive eye safety measures may reduce severe ocular morbidity and the need for globe removal procedures.

Key Words*: Enucleation, Evisceration, Endophthalmitis, Pakistan.*

Introduction

Evisceration and enucleation are definitive globe-removal procedures performed and visual recovery is not possible when ocular tissue is severely damaged.[1] These procedures are commonly indicated in non-salvageable eyes affected by severe ocular trauma, uncontrolled intraocular infection, painful blind eye, and intraocular malignancy.[2] Although these surgeries may relieve pain, remove infected or malignant tissue, and improve socket rehabilitation, they represent the final stage of severe ocular morbidity and may have functional, cosmetic, and psychological consequences for patients.[3]

Preventable visual impairment remains an important global eye health concern.[4] Severe ocular trauma and advanced ocular infections are among the important

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causes of irreversible ocular damage that may ultimately require evisceration or enucleation.[5] In low- and middle-income countries, delayed presentation, inadequate use of protective measures, occupational injuries, limited access to specialist eye care, and delayed referral may increase the risk of progression to non-salvageable ocular disease.[6,7]

Ocular trauma is an important cause of monocular visual loss, particularly among young and economically active individuals.[8] Similarly, endophthalmitis, panophthalmitis, and microbial keratitis can result in irreversible structural damage when diagnosis or treatment is delayed.[9] Studies from South Asia and Pakistan have also shown that ocular trauma and infective ocular conditions contribute substantially to severe visual morbidity, supporting the need for timely referral, early treatment, and community-level preventive strategies.[10,11]

Despite advances in ophthalmic diagnosis and treatment, many patients in developing countries present to eye clinics or tertiary hospitals at an advanced stage, often with no perception of light and marked structural damage of the globe. Radiological assessment and histopathological examination in such cases may demonstrate findings such as disorganized globe, retinal detachment, necrosis, inflammation, infection, or tumor. Studying the pattern of clinical presentation, underlying diagnosis, imaging findings, histopathology, and preventability is important for identifying avoidable causes of globe removal and planning preventive eye care strategies.

In Pakistan, recent data remain limited regarding the spectrum of ocular pathologies leading to evisceration and enucleation in tertiary care hospitals. There is also limited local evidence on the relative contribution of traumatic and non-traumatic indications and the proportion of cases that may be preventable. Therefore, this study was conducted to determine the spectrum of ocular pathologies leading to evisceration and enucleation and to assess their preventability in a tertiary care hospital.

Materials and Methods

This descriptive cross-sectional study was conducted at Al-Shifa Trust Eye Hospital, Rawalpindi, from 6th January 2025 to 12th January 2026. Ethical approval was obtained before the start of data collection from the Ethical Review Committee of Al-Shifa Trust Eye Hospital, Rawalpindi, under reference number ERC-24/AST-24, dated 10 July 2024**.** The research synopsis was also approved by the Research Evaluation Unit, College of Physicians and Surgeons Pakistan, under reference number CPSP/REU/OPL-2023-114-2632, dated 21 August 2024.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from adult patients before recruitment. In patients below 18 years of age or those unable to provide consent, written informed consent was obtained from parents or legal guardians, and consent was taken where applicable. Patient identity and clinical information were kept confidential throughout the study.

The sample size was calculated using the WHO sample size formula for estimating a single population prevalence :$n = Z^2 p(1 – p) / d^2$, where Z = 1.96 at 95% confidence level, p = 0.12 was the anticipated population prevalence of ocular emergencies reported by Qayyum et al.[12], and d = 0.06 was the required absolute precision. The calculated sample size was approximately 113 patients. To improve the power of analysis and compensate for record incompleteness, the final sample size was increased to 120 patients.

A non-probability consecutive sampling technique was used. Patients aged 5–80 years, including pediatric ≥ 5 years and adolescent patients, and either gender who underwent evisceration or enucleation. Evisceration was defined as removal of intraocular contents with preservation of the scleral shell, while enucleation was defined as removal of the entire globe. Patients with incomplete medical records, missing operative details, unavailable histopathology reports where required, or previous globe removal surgery performed outside the study hospital were excluded.

Data were collected by a self-structured proforma developed after reviewing relevant literature and institutional clinical records[13, 14] and reviewed by the Ophthalmology Department for content relevance before data collection. It included separate sections for demographic details, clinical presentation, diagnosis/indication for surgery, radiological

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findings, surgical procedure, histopathological findings, implant placement, and preventability status.

Demographic variables included age, gender, and affected eye laterality. Age was recorded in completed years and categorized into class intervals of ≤20 years, 21–40 years, 41–60 years, and >60 years for analysis, as adapted from previous studies on destructive eye procedures and ocular morbidity patterns.[15, 16] Clinical variables included presenting visual acuity, etiology, diagnosis/indication for surgery, and type of globe removal procedure. The diagnosis/indication for surgery was categorized as ocular trauma, endophthalmitis/panophthalmitis, corneal ulcer/perforation, painful blind eye, intraocular tumor, and others. Tumor cases were recorded as a single broad category of "intraocular tumors." Specific tumor subtypes, such as retinoblastoma or melanoma, were not separately documented in the data collection proforma.

Clinical assessment included visual acuity at presentation, which was categorized as no perception of light, perception of light, or other visual status. B-scan ultrasonography was performed, where clinically indicated to assess posterior segment and globe status and radiological findings were categorized as disorganized globe, retinal detachment, vitreous hemorrhage, and intraocular mass lesion**.** Excised ocular specimens were sent for histopathological examination where indicated, and findings were categorized as inflammatory/infective changes, necrosis, tumor, and others.

Cases were further classified as preventable or non-preventable according to etiology, clinical diagnosis, and the possibility of prevention through early medical intervention, timely referral, protective measures, or appropriate infection control where "preventable causes" were defined as those potentially avoidable through early diagnosis, timely treatment, protective measures, or appropriate referral. Traumatic and infective causes were considered potentially preventable, while intraocular tumors, painful blind eye due to irreversible chronic pathology, and other unavoidable causes were categorized as non-preventable after clinical assessment. "Traumatic etiology" was defined as mechanical ocular injury leading to irreversible ocular damage and globe removal. "Non-traumatic etiology" included infective, inflammatory, neoplastic, degenerative, and other non-injury-related ocular causes.

Data were analyzed using Statistical Package for Social Sciences version 25.0. Quantitative variables such as age were presented as mean ± standard deviation. Categorical variables such as gender, laterality, procedure, etiology, indication, visual acuity, B-scan findings, histopathology, implant use, and preventability were presented as frequencies and percentages. Chi-square test was applied to assess associations between categorical variables, including gender with etiology, gender with procedure, gender with visual acuity, and indication with preventability. Fisher–Freeman–Halton exact test was applied where expected cell counts were less than 5. Cramér's V was used to measure the strength of association for categorical variables. Independent samples t-test was applied to compare mean age between traumatic and non-traumatic etiological groups. Effect size for the age comparison was reported using Cohen's d, Hedges' correction, and Glass's delta with 95% confidence intervals. A p-value of ≤0.05 was considered statistically significant.

Results

A total of 120 patients who underwent evisceration or enucleation were included in the study. The mean age of patients was 40.4 ± 17.9 years, with age range was 5–80 years. Most of the patients were 21–40 years of age group i.e. 49 (40.8%), whereas 16 (13.3%) patients were aged ≤20 years. Children below 5 years were not represented in the final cohort. On age-group analysis, no intraocular tumor was recorded in the ≤20 years age group. Among the 10 (8.3%) tumor cases, 3 (30.0%) were in the 21–40 years age group, 5 (50.0%) were in the 41–60 years age group, and 2 (20.0%) were in the >60 years age group. No statistically significant association was observed between age group and indication for surgery (χ^2 = 16.682, df = 15, p = 0.338; Fisher–Freeman–Halton exact p = 0.336). Male patients were more frequently affected, 78 (65.0%), compared with females, 42 (35.0%). Right eye involvement was observed in 66 (55.0%) patients, while left eye involvement was seen in 54 (45.0%) patients, showing slight right eye predominance

(Table I).

Evisceration was the most frequent surgical procedure, 74 (61.7%), compared with enucleation, 46 (38.3%). Non-traumatic etiologies were more common, 68 (56.7%), while traumatic etiology was present in 52 (43.3%) cases. The most common indication for globe removal was ocular trauma, 52 (43.3%), followed by endophthalmitis/panophthalmitis, 24 (20.0%), and corneal ulcer/perforation, 16 (13.3%). Overall, 92 (76.7%) cases were classified as preventable, while 28 (23.3%) were non-preventable (Table II).

Most patients presented severe ocular morbidity and irreversible visual loss at the time of presentation. No perception of light was recorded in 88 (73.3%) patients, perception of light in 22 (18.3%) patients, and other visual status in 10 (8.3%) patients. B-scan ultrasonography mainly showed disorganized globe in 50 (41.7%) patients and retinal detachment in 32 (26.7%) patients, indicating severe structural ocular damage. Histopathological examination mainly revealed inflammatory/infective changes in 62 (51.7%) cases, followed by necrosis in 30 (25.0%) cases. Orbital implant placement was performed in 82 (68.3%) patients (Table III).

No statistically significant association was observed between gender and etiology when Chi-square test was applied to assess associations between categorical variables. For gender it was $\chi^2 = 1.528$, df = 1, p = 0.216 whereas for the procedure it was $\chi^2 = 0.002$, df = 1, p = 0.969) and for gender and visual acuity it showed $\chi^2 = 0.130$, df = 2, p = 0.937. A statistically significant association was found between indication and preventability i.e. $\chi^2 = 120.000$, df = 5, $p < 0.001$, with Cramér's V = 1.000, indicating a very strong association. Because 5 cells (41.7%) had expected counts less than 5, Fisher–Freeman–Halton exact test was also applied and confirmed the significant association (exact $p < 0.001$) (Table IV).

The mean age of traumatic cases was 38.88 ± 17.94 years, while the mean age of non-traumatic cases was 41.59 ± 17.85 years. The difference was not statistically significant by applying independent samples t-test between traumatic and non-traumatic etiological groups (t = -0.820, df = 118, p = 0.414), with a mean difference of -2.70 years and 95% CI from -9.23 to 3.82 years. Effect size analysis showed a very small effect size, with Cohen's d = -0.151, 95% CI: -0.512 to 0.211, indicating that the age difference between traumatic and non-traumatic cases was clinically negligible (Tables V and VI).

Table I: Demographic Characteristics and Laterality of Patients (n = 120)

Variable	Category	n (%)
Age (years)	Mean ± SD	40.4 ± 17.9
	Range	5–80
Age group	≤20 years	16 (13.3%)
	21–40 years	49 (40.8%)
	41–60 years	36 (30.0%)
	>60 years	19 (15.8%)
Gender	Male	78 (65.0%)
	Female	42 (35.0%)
Laterality	Right eye	66 (55.0%)
	Left eye	54 (45.0%)

Statistical test applied: Descriptive statistics and frequency analysis

Table IA: Distribution of Indications According to Age Group (n = 120)

Age group	Corneal ulcer n (%)	Endophthalmitis n (%)	Ocular trauma n (%)	Others n (%)	Painful blind eye n (%)	Tumor n (%)	Total
≤20 years	3 (18.8%)	1 (6.3%)	10 (62.5%)	1 (6.3%)	1 (6.3%)	0 (0.0%)	16 (100.0%)
21–40 years	5 (10.2%)	9 (18.4%)	22 (44.9%)	2 (4.1%)	8 (16.3%)	3 (6.1%)	49 (100.0%)
41–60 years	4 (11.1%)	12 (33.3%)	11 (30.6%)	2 (5.6%)	2 (5.6%)	5 (13.9%)	36 (100.0%)
>60 years	4 (21.1%)	2 (10.5%)	9 (47.4%)	1 (5.3%)	1 (5.3%)	2 (10.5%)	19 (100.0%)

Chi-square test and Fisher–Freeman–Halton exact test. $\chi^2 = 16.682$, df = 15, p = 0.338; Fisher–Freeman–Halton exact p = 0.336.

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Table II: Surgical Procedure, Etiology, Indications and Preventability (n = 120)

Variable	Category	n (%)
Procedure	Evisceration	74 (61.7%)
	Enucleation	46 (38.3%)
Etiology	Traumatic	52 (43.3%)
	Non-traumatic	68 (56.7%)
Indication	Ocular trauma	52 (43.3%)
	Endophthalmitis / Panophthalmitis	24 (20.0%)
	Corneal ulcer / Perforation	16 (13.3%)
	Painful blind eye	12 (10.0%)
	Intraocular tumors	10 (8.3%)
	Others	6 (5.0%)
Preventability	Preventable	92 (76.7%)
	Non-preventable	28 (23.3%)

Statistical test applied: Frequency analysis

Table III: Clinical, Radiological, Histopathological Findings and Implant Use (n = 120)

Variable	Category	n (%)
Visual acuity	No perception of light (NPL)	88 (73.3%)
	Perception of light (PL)	22 (18.3%)
	Others	10 (8.3%)
B-scan findings	Disorganized globe	50 (41.7%)
	Retinal detachment	32 (26.7%)
	Vitreous hemorrhage	26 (21.7%)
	Intraocular mass lesion	12 (10.0%)
Histopathology	Inflammatory/Infective changes	62 (51.7%)
	Necrosis	30 (25.0%)
	Tumors	12 (10.0%)
	Others	16 (13.3%)
Implant use	Yes	82 (68.3%)
	No	38 (31.7%)

Statistical test applied: Frequency analysis

Table IV: SPSS-Based Inferential Analysis of Categorical Variables

Variables compared	Test applied	χ^2 value	df	p-value	Cramér's V	Interpretation
Etiology × Gender	Chi-square test	1.528	1	0.216	0.113	Not significant
Procedure × Gender	Chi-square test	0.002	1	0.969	0.004	Not significant
Visual acuity × Gender	Chi-square test	0.130	2	0.937	0.033	Not significant
Indication × Preventability	Chi-square test and Fisher–Freeman–Halton exact test	120.000	5	<0.001	1.000	Highly significant / very strong association

Statistical test applied: Chi-square test; Fisher–Freeman–Halton exact test where required; Cramér's V for effect size

Table V: Independent Samples T-test Comparing Age Between Traumatic and Non-Traumatic Etiology

Variable	Etiology	n	Mean ± SD	Mean difference	t-value	df	p-value	95% CI of mean difference
Age	Traumatic	52	38.88 ± 17.94	-2.70	-0.820	118	0.414	-9.23 to 3.82
	Non-traumatic	68	41.59 ± 17.85					

Statistical test applied: Independent samples t-test

Table VI: Effect Size Analysis for Age Difference Between Traumatic and Non-traumatic Etiology

Effect size measure	Point estimate	95% CI lower	95% CI upper	Interpretation
Cohen's d	-0.151	-0.512	0.211	Very small effect
Hedges' correction	-0.150	-0.509	0.209	Very small effect
Glass's delta	-0.151	-0.513	0.211	Very small effect

Statistical test applied: Independent samples effect size analysis

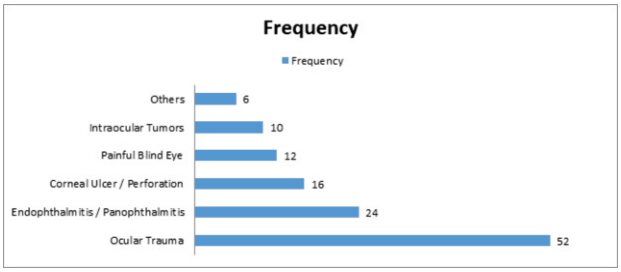

Figure 1: Distribution of Indications Leading to Evisceration and Enucleation

This bar chart illustrates the distribution of underlying indications among patients undergoing evisceration and enucleation. Ocular trauma was the most common indication (n = 52), followed by endophthalmitis/panophthalmitis (n = 24) and corneal ulcer/perforation (n = 16). Less frequent causes included painful blind eye (n = 12), intraocular tumors (n = 10), and other (n = 6).

Discussion

The present study showed that ocular trauma was

the leading indication for evisceration and enucleation, followed by infective causes including endophthalmitis/panophthalmitis and corneal ulcer/perforation. A clinically important finding was that more than three-fourths of the cases were preventable, and most patients presented with irreversible visual loss and severe structural ocular damage. These findings indicate that globe removal in this setting was not only the result of end-stage ocular pathology, but was also strongly linked with delayed presentation, preventable injury, and late management of ocular infection.

Ocular trauma accounted for 52 (43.3%) cases and was the most frequent indication for globe removal in the present study. Pediatric and adolescent patients were included in the present study, with 16 (13.3%) patients aged ≤20 years; however, children below 5 years were not represented in this cohort. This is relevant because retinoblastoma, an important pediatric indication for enucleation, commonly presents in younger children. In the present dataset, no tumor case was recorded in the ≤20 years age group, and tumor cases were observed mainly in adult age groups. This finding suggests that trauma and infection were more common contributors to globe removal among younger patients in this tertiary-care cohort. However, tumor subtype was not separately documented, so retinoblastoma-specific interpretation could not be performed. This finding is comparable with previous studies in which trauma was reported as a major cause of destructive eye procedures and severe ocular morbidity. Nuzzi et al, [17] reported that trauma remains an important indication for eye removal procedures, particularly in settings where occupational hazards and limited protective measures contribute to severe ocular injury. Similarly, Madan et al. [18] reported ocular trauma as a major cause of serious ocular damage in an Indian tertiary care setting, while Khan et al, [19] also highlighted ocular injuries as an important ophthalmic problem in Pakistan. The similarity between these studies and the present findings may be explained by shared regional factors such as occupational exposure, road traffic injuries, domestic injuries, lack of protective eyewear, and delayed referral after ocular injury. However, the high proportion of traumatic cases in our study is particularly important because most patients belonged to the productive age group and males were more frequently affected. This suggests that ocular trauma has both clinical and socioeconomic consequences, as globe loss in working-age individuals may affect earning capacity, family support, psychological well-being, and long-term rehabilitation needs.

Infective ocular pathologies also contributed substantially to globe removal in this study, with endophthalmitis/panophthalmitis and corneal ulcer/perforation together accounting for 40 (33.3%) cases. This finding is consistent with Ting et al, [20] who reported microbial keratitis as an important cause of severe visual impairment, especially where diagnosis or treatment is delayed. Obaid et al, [21] also reported resistance patterns in bacteria isolated from corneal ulcers in Pakistan, which is relevant because delayed or ineffective antimicrobial treatment can increase the risk of irreversible ocular damage. In the present study, the infective burden was further supported by histopathological findings, as inflammatory/infective changes were observed in 62 (51.7%) cases. This indicates that uncontrolled infection and inflammation played an important role in progression toward non-salvageable ocular damage. The similarity with previous studies may be due to delayed presentation, self-medication, poor follow-up, delayed referral from peripheral centers, and limited public awareness regarding warning symptoms such as ocular pain, corneal opacity, progressive visual loss, or ocular discharge.

The most important contribution of the present study is the assessment of preventability. Overall, 92 (76.7%) cases were classified as preventable, and a statistically significant association was observed between indication and preventability. This finding is clinically and publicly relevant because trauma, endophthalmitis, and corneal ulceration are conditions in which early intervention may prevent progression to irreversible visual loss and globe removal. Steinmetz et al, [22] reported that a substantial proportion of global blindness and visual impairment is avoidable. This supports the present finding that most globe-removal cases were linked to preventable causes such as trauma, endophthalmitis, and corneal ulceration. In Pakistan, Jadoon et al, [23] highlighted gaps in the reach of eye

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health programmes, showing that many patients may remain outside timely screening, referral, and specialist-care pathways. This finding is relevant to the present study because 76.7% of patients were classified as having preventable causes, and most presented with advanced disease and no perception of light. The high preventability rate in our study may therefore reflect not only disease severity but also delayed access to eye care, weak referral systems, limited community awareness, and insufficient coverage of preventive eye health services. Strengthening primary eye care, improving referral from peripheral centers, and increasing public awareness regarding ocular trauma and infection may reduce progression to irreversible ocular damage and the need for evisceration or enucleation.

Most patients presented with no perception of light, recorded in 88 (73.3%) cases, indicating irreversible visual loss at the time of presentation. This finding explains why evisceration or enucleation became necessary in many patients. Bourne et al,[24] described the continuing burden of blindness and visual impairment globally, while Wazir and Karim [25] reported delayed presentation as an important issue in severe ocular disease in a local setting. Compared with these studies, the present study shows that delayed presentation is not only associated with poor visual outcome but also with structural ocular destruction requiring globe removal. B-scan findings such as disorganized globe and retinal detachment further indicated severe structural ocular damage, while histopathology mainly showed inflammatory/infective changes and necrosis. These findings collectively suggest that many patients reached tertiary care after the eye had already become anatomically and functionally unsalvageable.

Histopathological examination in the present study most commonly showed inflammatory/infective changes, followed by necrosis. This finding supports the clinical observation that infection and inflammation were major contributors to globe loss. Lavaju et al,[26] reported histopathological patterns in severe ocular disease and emphasized the role of pathological confirmation in understanding destructive ocular conditions. In the present study, histopathology added important diagnostic value because it confirmed the underlying tissue changes responsible for irreversible ocular damage. Orbital implant placement was performed in 82 (68.3%) patients, reflecting the importance of post-surgical socket reconstruction and cosmetic rehabilitation. Talpur et al,[27] discussed visual outcomes and prognostic factors in open-globe injuries among Pakistani patients; in comparison, the present study extends the local evidence by focusing on patients who progressed to globe removal and required reconstructive planning after evisceration or enucleation.

The findings of this study have important clinical and public health implications. At the clinical level, ocular trauma and infection should be treated as urgent conditions requiring early recognition, prompt referral, and timely specialist management. At the public health level, the high preventability rate emphasizes the need for workplace eye safety education, use of protective eyewear, community awareness regarding ocular emergencies, improved management of corneal ulcers, and stronger referral links between primary, secondary, and tertiary eye care services. Based on these findings, preventive strategies should focus on both trauma-related and infection-related globe loss.[1,4,20,23]

For ocular trauma, mandatory protective eyewear should be promoted in high-risk settings such as industrial work, construction, welding, and agricultural activities. Workplace safety education, enforcement of occupational eye-safety regulations, and awareness regarding road traffic and domestic eye injuries may reduce preventable traumatic ocular morbidity.[1,2,18]

For infective causes, early referral protocols should be strengthened for corneal ulceration, suspected endophthalmitis, panophthalmitis, corneal perforation, progressive ocular pain, and rapidly worsening visual loss. Primary and secondary healthcare facilities should be encouraged to refer such cases urgently to tertiary ophthalmic centers before irreversible globe damage occurs.[3,4,20]

The strength of this study is that it included clinical, radiological, histopathological, surgical, and preventability-related variables in the same patient population, which provides a more complete assessment of globe removal procedures in a tertiary care setting.

Limitations

It was a single-center study with non-probability consecutive sampling, so the findings may not be generalizable to all regions. Preventability was assessed clinically, which may involve some subjective judgment. Although pediatric and adolescent patients were included, children below 5 years were not represented in this cohort, and tumor subtypes such as retinoblastoma were not separately recorded. Therefore, age-specific interpretation of pediatric tumor-related enucleation is limited. In addition, the type and setting of ocular trauma, such as blunt or penetrating injury and industrial, agricultural, domestic, or road traffic-related trauma, were not consistently available in the records; therefore, trauma subcategorization could not be performed. Future multicenter prospective studies with larger samples should record tumor subtype, mechanism and setting of trauma, and long-term psychological, cosmetic, rehabilitation and surgical outcomes to better define regional patterns and targeted prevention strategies.

Conclusion

Ocular trauma and infective ocular pathologies were the leading causes of evisceration and enucleation, and most cases were potentially preventable. Most patients presented with irreversible visual loss and advanced structural damage, reflecting delayed presentation. Early referral, timely treatment of ocular infections, use of protective eyewear, and stronger primary eye care services may reduce the need for globe-removal procedures.

Disclaimer: This study is part of compulsory research article for FCPS training and has been approved both by parent institute and REU department CPSP.

Conflict of Interest: Nil

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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

Determination of Upper Reference Limit for High-Sensitivity Troponin T in the Healthy Population of Balochistan

Sania Khan, Sayed Tanveer Abbas Gilani, Muhammad Zeeshan Rana, Muhammad Zubair, Usman Gulzar, Faiqa Mubeen

ABSTRACT

Objective: To determine the 99th percentile upper reference limit of high-sensitivity cardiac troponin T in the healthy population of North and South Balochistan, Pakistan, with age-and gender- stratification.

Study Design: Cross-sectional, Observational.

Place and Duration of the Study: Combined Military Hospital, Quetta, between July 1, 2024, to June 30, 2025.

Materials and Methods: Following the acquisition of informed consent, 217 healthy participants (110 men and 107 women) underwent cardiovascular evaluation included vital signs, chest auscultation, ECG, eGFR, and NT-proBNP to exclude subclinical disease. High-sensitivity cardiac troponin T (hs-cTnT) was measured using the Roche Diagnostics Cobas e-411 platform, with electrochemiluminescence technology. The 99th percentile upper reference limit (URL) for hs-cTnT was determined using non-parametric statistical methods, with age and gender-stratified comparisons.

Results: This study included 110 male (50.7%) and 107 female (49.3 %) participants. The median age, accompanied by interquartile ranges was 41.50 (32.00 – 53.00) years for males and 43.00 (32.00 – 57.00) years for females. The 99th percentile URL of hs-cTnT was 20.94 ng/L overall, with gender-specific values of 34.00 ng/L in men and 16.00 ng/L in women ($p < 0.001$). Median hs-cTnT concentrations were significantly higher in males [12.00 (8.00 - 29.25) ng/L] compared to females [4.00 (3.00 - 10.00) ng/L].

Conclusion: This study established the 99th percentile upper reference limit of hs-cTnT in a healthy population from Balochistan at 20.94 ng/L. Significant differences were observed by gender and age, with men and older individuals showing higher values, underscoring the need for gender- and age-specific diagnostic thresholds. No statistically significant differences were observed between Pathan and Baloch participants in this sample.

Key Words*: Acute Coronary Syndrome*, *High-Sensitivity Cardiac Troponin T, 99th Percentile, Myocardial Infarction, Upper Reference Limit.*

Introduction

Within this global health challenge, high-sensitivity cardiac troponin T (hs-cTnT) assays have become crucial for the early diagnosis and risk stratification of acute myocardial infarction, a major contributor to CVD morbidity and mortality.[1]

The gold standard biomarker for identifying myocardial damage is high-sensitivity cardiac troponin T assay. It is highly sensitive and specific, being released into the bloodstream following myocardial injury, thus reflecting even minimal damage to cardiac myocytes.[2] According to current international recommendations, the 99th percentile upper reference limit (URL) is crucial to the clinical interpretation of hs-cTnT findings and serves as a threshold for detecting acute myocardial infarction (AMI).[3] The introduction of hs-cTnT has significantly enhanced diagnostic capabilities, allowing for the detection of lower troponin concentrations in individuals with non-ST elevation myocardial infarction or those with mild symptoms.[4,5] Hs-cTnT levels rise within hours of myocardial injury; early detection enables timely intervention, often improving patient outcomes. Furthermore, high levels of hs-cTnT are associated with a higher risk of unfavorable outcomes like cardiac failure, arrhythmias, and death, making it a potent prognostic marker.[6] Another feature of high-sensitivity troponin tests is gender-specific upper reference limits. Because troponin levels are partly attributed to differences in cardiac mass and baseline troponin distribution, with higher values

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commonly observed in males.[7]

The 99th percentile upper reference limit (URL) for men is significantly higher than that for women, according to studies on reference intervals utilizing both high sensitivity troponin I and T (hs-Trop I, hs-Trop T) evaluation.[8,9] Recent studies emphasize the necessity of establishing gender and age-specific thresholds to improve the diagnostic accuracy of the hs-cTnT assay.[10] However, the generally applied universal cutoff value of 14 ng/L may not adequately reflect physiological variation across different populations.[11] Increasing evidence highlights significant biological variation in hs-cTnT concentrations based on demographic and geographic factors, including age, gender, and ethnicity.[11]

A study conducted in Multan, Pakistan, in 2022, reported that the 99th percentile hs-cTnT value was 18.20 ng/L in all cases, with men having higher levels (30.46 ng/L) than women (16.77 ng/L).[10] Similarly, research from Shenyang, China, employing improved selection criteria, established 99th percentile upper reference values of 18 ng/L for males and 13 ng/L for females.[6] The data underscore the significant influence of demographic factors, particularly gender, age and ethnicity on troponin concentrations. Data from the United States also demonstrate higher sex- and age-stratified URLs for fifth-generation hs-cTnT assays, generally ranging from approximately 18–20 ng/L.[12] Implementing tailored diagnostic thresholds could improve the specificity of myocardial infarction diagnoses, minimize overdiagnosis, and promote effective patient management. Continued research is crucial to validate these demographic-based cutoffs and refine clinical guidelines for diverse populations. Significant variation in the 99th percentile URL has been observed in population-based investigations, casting doubt on the applicability of a set diagnostic cutoff in a variety of groups. In resource-constrained and demographically unique regions such as Balochistan, Pakistan, the lack of population-specific reference data may compromise diagnostic accuracy and clinical decision-making.

To overcome this gap, our study aimed to establish age, gender and ethnicity specific 99th percentile upper reference limit for hs-cTnT using a cross-sectional design that reflects the demographic variety of North and South Balochistan. This study intends to improve the diagnostic accuracy of myocardial infarction by developing localized reference values, thereby supporting evidence-based cardiovascular decision-making in this underserved region.

Materials and Methods

This cross-sectional study was carried out at the CMH Quetta, Pakistan, from July 1, 2024, to June 30, 2025. The Institutional Ethical Review Board (IERB) approved the study protocol (Ethical Approval Number: CMH QTA-IERB/27/2024). The Clinical and Laboratory Standards Institute (CLSI) EP28-A3c guidelines recommend at least 120 individuals in each partition to perform non-parametric estimation of reference limits. In the current research, 217 presumably healthy subjects were recruited comprising 110 males and 107 females. Even though the sample size in the subgroups was slightly less than the suggested sample size to have valid estimation of extreme percentiles, strict inclusion and exclusion conditions were used to have the right sample of healthy reference population. The participants were recruited using non-probability convenience sampling method. Demographic details were recorded on a proforma. Participants were instructed to maintain their usual lifestyle and diet and avoid strenuous exercise. They fasted for 8-14 hours after dinner and consumed no more than 200 mL of water. Each participant provided two blood samples for analysis. Three milliliters of whole blood were collected in each ethylenediaminetetraacetic acid (EDTA) tube and used to estimate, HbA1c. Another 3 mL of blood was drawn into a clot activator tube for the analysis of lipid profile, serum creatinine, CK/CK-MB, Hs-cTnT and NT-Pro BNP levels. Each sample was processed on a Roche Diagnostics Cobas c-501 analyzer by photometry and e-411 by electro-chemiluminescence. Serum hs-cTnT concentrations were measured using the Roche Elecsys fifth generation high sensitivity cardiac troponin T assay on the Cobas e-411 analyzer employing electrochemiluminescence immunoassay (ECLIA) technology. Blood samples were centrifuged within 30 minutes of collection at 4000 rpm for 5 minutes, and serum was analyzed immediately. Limit of blank (LoB) of the assay is 2.5 ng/L, limit of detection (LoD)

is 3 ng/L and its measuring range is 3-10,000 ng/L. Internal quality control procedures were performed daily according to manufacturer recommendations.

All participants underwent a comprehensive cardiovascular assessment, including measurement of pulse rate, blood pressure, chest auscultation, and electrocardiography (ECG) performed to rule out diseases such as ventricular or atrial hypertrophy, myocardial infarction, and atrial fibrillation. After obtaining informed consent, this study included apparently healthy participants of both genders, aged 18 to 65 years, residing in North and South Balochistan, without any clinical cardiovascular conditions, such as atrial fibrillation, chronic heart failure, or coronary artery disease. Patients with history of hypertension, diabetes mellitus, heart failure (NT-pro BNP >125 pg/mL) was applied in accordance with European Society of Cardiology (ESC) guidelines, to exclude participants with possible underlying heart failure,[13] or chronic renal disease at stage III or greater (estimated glomerular filtration rate <60 ml/min/1.73m²) were excluded from the study to ensure the integrity of the healthy participants, because these conditions can result in hs-cTnT release independent of myocardial infarction.[11]

To statistically analyze the collected data, Statistical Package for Social Sciences (SPSS) software version 22 was used. Quantitative variable (Age, hs-cTnT) was presented as median interquartile range (IQR) after checking normality of data by Shapiro-Wilk test which showed that it was not distributed normally. Qualitative variables (Gender, Age groups, Ethnicity) were presented as frequency and percentages. The Mann Whitney U test used for the comparison of hs-cTnT with gender and Ethnicity and Kruskal Wallis test was used for the comparison of hs-cTnT with age groups.

The 99th percentile upper reference limit (URL) was calculated using a non-parametric method in accordance with CLSI EP28 A3c guidelines. After arranging hs-cTnT concentrations in ascending order, the percentile rank position was determined using the formula k = (n + 1) × 0.99. When the calculated rank position was non-integer, interpolation between adjacent observations was applied. Confidence intervals for the 99th percentile were estimated using bootstrap resampling with 5,000 iterations. Outlier detection was performed using Tukey's method based on the interquartile range (IQR). Observations exceeding 1.5 × IQR were examined carefully. No values were excluded after statistical and clinical review.

Results

Out of 217 participants, 110 (50.7%) were men and 107 (49.3%) women. Median age was 42.00 (32.00 – 52.00) years. Median was 41.50 (32.00 – 53.00) years for males and 43.00 (32.00 – 57.00) years for females. The 99th percentile upper reference limit (URL) for hs-cTnT in the overall population was 20.94 ng/L (90% CI: 18.5–23.8 ng/L). Gender specific analysis showed higher values in males (34.0 ng/L; 90% CI: 33.91–34.0 ng/L) compared with females (16.0 ng/L; 90% CI: 12.95–16.0 ng/L), ($p < 0.001$). Median of hs-cTnT was high in males [12.00 (8.00 - 29.25) ng/L] as compared to females [4.00 (3.00 - 10.00) ng/L] $p < 0.001$ as shown in Table I. Cardiac troponin concentrations demonstrated a significant age-related increase, with median values rising from 6.00 (3.00 – 11.00) ng/L in individuals aged 18–35 years to 11.50 (3.25 - 31.00) ng/L in those aged older than 50 years. Correspondingly, the 99th percentile increased from 15.3 to 34.0 ng/L, accompanied by broader 90% confidence intervals in older age groups, indicating greater variability. These findings were statistically significant ($p < 0.001$), highlighting age as a critical determinant of troponin levels shown in Table II. The median (IQR) hs-cTnT level in the Pathan group was 10.00 (3.00–13.75), while in the Baloch group it was 9.00 (3.00–12.00). The difference between the two groups was not statistically significant ($p = 0.439$), indicating that hs-cTnT levels were comparable across ethnicities. Furthermore, the 99th percentile upper reference limit was 33.93 in the Pathan group and 34.00 in the Baloch group, demonstrating nearly identical upper reference limits between the two ethnic groups as Shown in Table III. Confidence intervals for the 99th percentile were calculated for the overall population, gender groups, age groups, and ethnicity groups using bootstrap resampling.

Discussion

The 99th percentile URL of hs-cTnT was 20.94 ng/L in the overall population, with gender-specific values of 34.0 ng/L and 16.00 ng/L for men and women, respectively. These findings from our study revealed

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Table I: Distribution of High-Sensitivity Cardiac Troponin T Concentrations According to Gender (n=217)

Hs-cTnT (ng/L)	Male (n=110)	Female (n=107)	Total (n=217)	p-value
Median (IQR)	12.00 (8.00 - 29.25)	4.00 (3.00 - 10.00)	9.00 (3.00 – 13.00)	< 0.001$
99th percentile	34.00	16.00	20.94	-
90% CI	33.9–34.0	12.9–16.0	18.5–23.8	-

$= Mann Whitney U test, CI= Confidence Interval

Table II: Comparison of High-Sensitivity Cardiac Troponin T according to Age Distribution (n=217)

Hs-cTnT (ng/L)	Age in Years			*P* value
	18 - 35 (n=71)	36 – 50 (n=70)	> 50 (n=76)	
Median (IQR)	6.00 (3.00 - 11.00)	10.00 (5.50 - 13.00)	11.50 (3.25 -31.00)	< 0.001^
99th percentile	15.3	22.1	34.0	-
90% CI	13.0 -16.5	20.0 – 24.0	31.0 – 34.0	-

^ = Kruskal Wallis test, CI= Confidence Interval

Table III: Comparison of High-Sensitivity Cardiac Troponin T by Ethnicity (n=217)

Hs-cTnT (ng/L)	Pathan (n=108)	Baloch (n=109)	p
Median (IQR)	10.0 (3.00 - 13.75)	9.00 (3.00 - 12.00)	0.439$
99th percentile	33.93	34.00	-
90% CI	32.0 - 34.0	32.5 – 34.0	-

$= Mann Whitney U test

that males have considerably greater hs-cTnT levels than females, consistent with results from a U.S. study where the 99th percentile URL varied from 18.4 to 20.2 ng/L in all cases.[12]These results corroborate research by Anwar et al. and Apple et al. that indicates males have greater troponin levels, indicating the necessity of gender-specific reference ranges.[14,15] These results are also in line with a Singaporean study that found a 99th percentile cut-off value of 25.6 ng/L overall and 32.7 ng/L and 17.9 ng/L for males and females, respectively.[16] Another study by Romiti et al., in 2019, in Italy, compared various studies that looked at gender-specific 99th percentile cut-offs for hs-cTnT and discovered that men had higher values.[17] Similar to our results, another study by Khan et al. in Multan, Pakistan in 2022 found that the values of high sensitivity troponin T (hs-cTnT) were 18.20 ng/L in all patients, 30.46 ng/L for men, and 16.77 ng/L for women. However, they concluded that there were substantial differences in hs-cTnT levels across genders but not between age groups.[10]

We observed a considerable difference between the age groups in our study. Troponin levels increased with age, as evidenced by the rise in median values from 6 ng/L in younger individuals to 11.5 ng/L in older age groups. This trend was statistically significant ($p < .0001$) and corroborated findings from other studies that highlight age as a critical determinant of troponin levels. Our study found that the range of hs-cTnT values in the general population gradually increased with age.[18] These findings are also consistent with a study that showed hs-cTnT in three large cohorts and found this cut-off to be higher in males and increased with age. These findings are also consistent with a study that analyzed hs-cTnT in three large cohorts and discovered that the cut-off was greater in males and increased with age.[11] Another study in 2013, found a strong relationship between age, gender and 99th percentile URL values of hs-cTnI among healthy reference individuals.[19] Even in the absence of acute coronary syndrome or other diseases like diabetes and hypertension, older people have elevated troponin levels.[20]

Another study by Isiksacan et al. in 2018 reported that the values of hs-cTnT were significantly higher in men over 40 years of age (24 ng/L) compared to those under 40 years of age (10 ng/L).[21] These findings are consistent with our results that the broader confidence intervals in older age groups suggest greater variability, which may necessitate age-specific reference limits for accurate clinical assessment. With advancing age, subclinical myocardial changes such as fibrosis, increased left ventricular wall stress, and cardiomyocyte apoptosis contribute to higher baseline troponin release even in the absence of overt disease.[11,19] Declining renal clearance in older adults also prolongs troponin circulation.[20] Furthermore, cumulative exposure to cardiovascular risk factors across the lifespan leads to subtle myocardial injury, reflected in elevated hs-cTnT.[18] These findings are consistent with large cohort studies showing progressive increases in troponin concentrations with age, underscoring the need for age-adjusted diagnostic thresholds to avoid misclassification of myocardial infarction in older

individuals.

Our study found no significant difference for hs-cTnT levels between Pathan and Baloch participants, indicating that ethnicity does not require adjustment of diagnostic thresholds in this population (p = 0.439). This aligns with the research conducted by Burgio et al., in 2024, which suggested limited evidence for ethnicity-specific reference limits.[22]

Limitations

This study was conducted at a single tertiary care center using non-probability convenience sampling. Convenience sampling may limit representativeness of the general Balochistan population and may introduce selection bias including a potential healthy volunteer effect. The sample size was also smaller than the sample size recommended size in CLSI guidelines to provide stable estimation of extreme percentiles. According to CLSI EP28-A3c guidelines, at least 120 individuals per partition are recommended for non-parametric estimation of reference limits. In the present study, the gender-specific groups (110 males and 107 females) were slightly below this recommended number; therefore, the estimated reference limits should be interpreted cautiously.

Conclusion

Our study found significant gender-specific differences in high-sensitivity cardiac troponin T levels, with males exhibiting higher levels than females. Additionally, age significantly influences troponin levels, with median values increasing in older age groups, indicating the necessity for age-adjusted diagnostic thresholds. However, no statistically significant differences were observed between Pathan and Baloch participants in this sample.

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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

Assessment of Upper and Lower Limb Neural Tissue Extensibility in Asymptomatic Health Care Professionals

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ABSTRACT

Objective: To assess neural tissue extensibility of the upper and lower limb in asymptomatic healthcare professionals and to determine its association with work experience.

Study Design: Cross-sectional study.

Place and Duration of Study: The study was carried out at Fauji Foundation Hospital, Margalla Institute of Health Sciences, Pakistan Institute of Medical Sciences and Holy Family Hospital, Pakistan from 20th December 2024 to 4th August 2025.

Materials and Methods: A total of 384 asymptomatic healthcare professionals, aged 25–45 years were included, comprising physical therapists, dentists, and surgeons (128 participants from each category). The method of non-probability purposive sampling was applied. Upper and lower limb neural tissue extensibility was assessed using ULNT1, ULNT2, ULNT3 and slump test, prone knee bend test, and passive straight leg raise, respectively. Data were analyzed using descriptive statistics and spearman correlation to assess the relationship between work experience and neural extensibility via SPSS version 23.

Results: Among upper limb neurodynamics, median nerve showed highest positive response especially in dentists (Right: 35.9%, Left: 32%). While radial nerve depicted least positive response (10.4%) in all professionals. For lower limb neurodynamics, surgeons predominated in slump test results (Right: 22.7%, Left: 22.1%), while physical therapists showed the highest frequency for the passive straight leg raise (Right: 24.2%, Left: 21.1%). Moreover, work experience showed a statistically significant correlation ($p<0.05$) with neural tissue extensibility.

Conclusion: Occupational demands are associated with reduced neural tissue extensibility in healthcare professionals, particularly, dentists exhibited the greatest median nerve involvement, surgeons showed higher positive response in slump and prone knee bend tests and physical therapists demonstrated reduced extensibility in the passive straight leg raise test. These findings highlight the importance of early screening, ergonomic awareness, and preventive strategies in clinical practice.

Key Words*: Healthcare Professionals, Lower Extremity, Median Nerve, Neurodynamic Tests, Radial Nerve, Sciatic Nerve.*

Introduction

Healthcare professionals exposed to repetitive tasks and prolonged static postures may develop subclinical reductions in neural tissue mobility that remain undetected until functional impairment occurs. Although the human nervous system is designed to facilitate coordinated movement, prolonged or repetitive mechanical loading during professional activities may predispose peripheral nerves to dysfunction. These subclinical changes often manifest as a decrease in neural tissue extensibility, which can eventually hinder professional performance and lead to work-related musculoskeletal disorders (WMSDs).[1] WMSDs often progress through early stages of fatigue before resulting in a permanent reduction in work capacity.[2] Therefore, early detection of these neural changes is essential to prevent the transition from subclinical tension to chronic professional disability.[3]

Peripheral nerves are structurally designed to tolerate mechanical stress through protective

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connective tissue layers and organized fascicles. However, during routine activities, nerves are exposed to mechanical forces such as tension, compression, shear, and friction. When these forces exceed physiological limits or become prolonged, they may contribute to the development of peripheral neuropathies. Peripheral neuropathies are a major source of pain and functional limitation and are broadly classified into compressive and non-compressive types. Compressive neuropathies typically occur at anatomical sites where nerves traverse fibro-osseous tunnels or muscular and fibrous structures, whereas non-compressive neuropathies may result from trauma, infection, or inflammatory processes.[4] In healthcare settings, high-precision tasks and repetitive movements can lead to these stresses becoming chronic.

Neurodynamic tests are frequently applied to assess neural tissue mobility and mechanosensitivity. Upper Limb Neural Tension Tests (ULNTs), also referred to as neural tissue provocation tests, are established clinical tools for evaluating neural involvement by applying controlled mechanical stress. There are three main types of ULNTs, each assessing a specific nerve, with ULNT1 for the median nerve, ULNT2 for the radial nerve, and ULNT3 for the ulnar nerve. These tests apply controlled mechanical stress through specific limb positions to evaluate neural response and mobility.[5] While these tests are traditionally used in symptomatic populations to reproduce clinical pain, in asymptomatic individuals, they serve to provoke and identify subclinical neural responses, such as a stretching sensation or tingling, that indicate mechanical sensitivity. According to Wainner's criteria, a test is considered positive if it reproduces symptoms or provokes sensations, shows a discrepancy of more than 10 degrees in elbow extension or wrist flexion between both limbs, or if symptoms are modulated by structural differentiation, such as with contralateral cervical side-bending.[4,5]

Similarly, Lower Limb Tension Tests (LLTTs), including the Slump Test, Straight Leg Raise (SLR), and Prone Knee Bend test, are used to evaluate neural tissue mobility in the lower extremities, particularly involving nerve roots from L2 to S1. These tests assist in identifying early neural mechanosensitivity and nerve-related dysfunctions associated with pain, tingling, or numbness, and they play a vital role in clinical diagnosis and preventive screening.[6,7]

Beyond symptom identification, neurodynamic assessment focuses on evaluating nerve movement and length in relation to surrounding joints and soft tissues. When neural mobility restrictions are identified, interventions such as nerve gliding or mobilization are used to restore optimal neural function. Thus, the primary objectives of neurodynamic treatment include reducing neurological symptoms, improving range of motion, and normalizing neural responses to mechanical stress. Previous studies have demonstrated that neurodynamic techniques enhance neural mobility, leading to improved joint function and efficient movement patterns in both the upper and lower extremities.[8]

Although neurodynamic tests are well established in symptomatic populations, data on neural mobility and neurodynamic responses across occupational groups in asymptomatic healthcare professionals remains limited. Therefore, this study was carried out to evaluate neural tissue extensibility in both upper and lower limbs of asymptomatic healthcare professionals using standardized neurodynamic tests and to determine its association with years of work experience, aiming to identify early neural mobility limitations and to provide insight for preventive strategies.

Materials and Methods

The study was carried out at Fauji Foundation Hospital, Margalla Institute of Health Sciences, Pakistan Institute of Medical Sciences, and Holy Family Hospital, Pakistan, from 20th December 2024 to 4th August 2025. Ethical approval for the study (Project No. FF/FUCP/932-13/DPTF2008) was obtained from the Ethical Review Committee (FF/FUMC/215-512/Phy/24) and Institutional Research Committee (FF/FUCP/932-13/DPTF2008) of the Foundation University School of Health Sciences (FUSH).

A total of 384 participants was determined using the OpenEpi sample size calculator, considering a 95% confidence interval and 5% error margin, based on data obtained from registered professionals listed on the official websites of the Pakistan Medical and Dental Council and the World Physiotherapy

Association. A non-probability purposive sampling method was used to make three quotas including 128 physical therapists, 128 dentists, and 128 surgeons. The healthcare professionals having no pain in upper and lower limb in the last 3 months, of both genders, aged 25–45 years, with at least 1 year of clinical practice were included. While, the Individuals with current musculoskeletal injuries, neurological disorders, or prior surgeries affecting limb function were excluded.

Upper limb neural tissue extensibility, as an outcome measure, was evaluated using neurodynamic testing. Participants were positioned supine on an examination couch to ensure comfort and an unrestricted range of motion, with the cervical region maintained in proper alignment. To isolate upper limb movements, joint positioning was progressed sequentially, beginning with shoulder movements followed by the forearm, wrist, fingers, and elbow, in accordance with Butler's guidelines. All tests were conducted bilaterally, and participants were instructed to report any discomfort or request test termination at any time.

Upper Limb Neurodynamics

The median nerve bias test (ULNT1) was performed with the shoulder girdle depressed and the glenohumeral joint abducted to approximately 90–110°, while extending the fingers and wrist and supinating the forearm as shown in Figure 1a. The sequence was completed by externally rotating the shoulder and extending the elbow to increase neural loading. Cervical lateral flexion, particularly contralateral side-bending, was added to further increase the sensitivity of test.[8]

Subsequently, the radial nerve bias test (ULNT2) was then performed in the same supine position, starting with the shoulder girdle depressed and the arm abducted about 10°, followed by flexing the fingers and wrist and pronating the forearm as shown in Figure 1b. Medial rotation of the shoulder was then applied, and the test concluded with elbow straightening, while the opposite side of the neck was laterally flexed to increase neural sensitivity.[8]

Finally, the ulnar nerve bias test (ULNT3) was conducted starting with shoulder depression and abduction ranging from 10° to 90°, followed by extending the fingers and wrist and pronating the forearm as shown in Figure 1c. The elbow was then flexed, and the shoulder rotated laterally in sequence, with contralateral cervical lateral flexion incorporated at the end.[8]

A test was considered positive according to Wainner's criteria if at least two criteria from below were met: the participant experienced symptoms such as tingling, paresthesia, or a stretching sensation; a difference of more than 10° while extending the elbow or flexing the wrist was observed between the limbs; or symptoms were altered by cervical side-bending, where ipsilateral side-bending reduced symptoms and contralateral side-bending increased symptoms.[4]

The reliability of the upper limb neurodynamic tests has been reported using the intraclass correlation coefficient (ICC), with ULNT1 and ULNT3 demonstrating moderate to good reliability (ICC 0.6–0.8) and ULNT2 showing high reliability (ICC 0.86–0.87).[9]

Lower Limb Neurodynamics

Following the assessment of upper limb neural extensibility, lower limb neural tissue extensibility was evaluated using standardized neurodynamic tests, including the Slump Test, Passive Straight Leg Raise (SLR) test, and Prone Knee Bend test as shown in Figure 2. These tests were performed using established protocols to assess neural tension and mobility of the lumbosacral and femoral nerve roots (L2–S1).

The Slump Test was performed with participants seated, with hands placed behind the lower back to maintain a neutral spinal position as shown in Figure 2a. The test was initiated by flexion of the thoracic and lumbar spine. In the absence of symptoms, cervical flexion was added, followed by extension of one knee. If pain was reported, the neck was returned to neutral; persistence of pain or inability to fully extend the knee with added ankle dorsiflexion was considered a positive test result.[6] The Slump Test has demonstrated acceptable inter-rater reliability, with an intraclass correlation coefficient (ICC) of 0.80.[10]

The Passive SLR test was performed with participants in a supine position as shown in Figure 2b. The examiner maintained the knee in full extension while passively flexing the hip to elevate the lower limb. The test was considered positive if pain radiating along the lower extremity occurred at an angle less

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than 60°, corresponding to the distribution of the lower lumbar nerve roots, most commonly L5 or S1.[11] The Straight Leg Raise test demonstrates excellent reliability, with reported intra- and inter-rater ICC values ranging from 0.97 to 0.99.[12]

The Prone Knee Bend test, used to assess femoral nerve tension, was performed with participants lying prone as shown in Figure 2c. The examiner stabilized the pelvis to prevent anterior rotation while passively flexing the knee to end range, ensuring contact between the heel and buttock and maintaining neutral hip rotation. The presence of unilateral neurogenic symptoms, such as pain or paresthesia in the lumbar region, buttock, or anterior thigh between 80° and 100° of knee flexion, was indicative of reduced neural extensibility.[13] This test demonstrated good intra-examiner reliability (ICC = 0.85) and excellent inter-examiner reliability (ICC = 0.92).[14]

Data collection was conducted in dedicated, quiet examination rooms across the participating hospitals to minimize external distractions. Healthcare professionals meeting the inclusion criteria were carefully enrolled, and prior to their involvement, all individuals gave their informed consent. To ensure standardization across all participants, all neurodynamic tests were performed on a high-low adjustable physiotherapy plinth by a single trained examiner (or specifically trained team) to maintain consistency in force and speed of movement. Participants were positioned according to standardized protocols, and a goniometer was used to precisely measure the 10° discrepancy in range of motion required for a positive result. Initially, demographic information was recorded for each participant, including name, age, gender, work experience, and body mass index (BMI). Following this, standardized upper and lower limb neural tension tests were performed on each limb to assess neural tissue extensibility. Descriptive statistics, including frequencies, percentages, means, and standard deviations, were used to summarize demographic data and neurodynamic test responses. To determine the correlation between work experience and neural tissue extensibility, the Spearman correlation (r_s) was applied. All analyses were performed using the IBM Statistical Package for Social Sciences (SPSS) version 23, with a p-value of <0.05 considered statistically significant.

Results

The sample consisted of 384 individuals mainly females 271 (70.6%), with the mean age of 29.33 ± 4.60 years. Most of the participants (34.1%) were within the normal weight range (18.5-22.9kg/m^2), while majority 216(56.3%) of them had 1-3 years of work experience, as shown in Table I. The analysis of neural tissue extensibility was primarily categorized by professional groups (surgeons, dentists, and physical therapists) and further evaluated based on years of work experience.

ULNT1 showed predominant median nerve involvement, with greater right-sided prevalence across all professions, especially dentists 46(35.9%). While ULNT2 demonstrated low positive response bilaterally, suggesting minimal right (10.4%) radial nerve involvement in all participants. ULNT3 also depicted positive response, reflecting ulnar nerve involvement, higher in surgeons 31(24.2%), particularly on the right side. In brief, dentists generally exhibited higher positive response for most of the neural tests of upper limb as shown in Table II. The prolonged static loading during repeated fine motor skills pose increased mechanical stress on neuronal structures leading to reduced neural flexibility over time.

Following the assessment of upper limb neural tension, lower limb neural extensibility was evaluated using the slump test to examine the lumbosacral and sciatic nerve pathways. Right 29(22.7%) and left 27(22.1%) sided slump test demonstrated positive response predominantly in surgeons followed by physical therapists and dentists. The prone knee bend test showed a relatively low response of femoral nerve tension across all participants as shown in Table III.

The surgeons exhibited relatively high prevalence for the right 20(15.6%) side and left 18(14.1%) side for prone knee bending. The occupational demands of surgeons, including prolonged standing with sustained static postures and frequent forward trunk inclination during lengthy surgical procedures may adversely affect lower limb neural extensibility. As physical therapists are routinely exposed to maintain a prolonged posture with repeated bending and frequent patient handling activities, thus in passive straight leg raise test, physical therapists

demonstrated a higher frequency of positive responses, with 31(24.2%) and 27(21.1%) for right and left side respectively. (Table III)

A statistically significant correlation between work experience and neural tissue extensibility of upper and lower limb is demonstrated in Table IV where the p-value was <0.05 for all the tests.

Table I: Demographic Profile of Research Participants (N= 384)

Age(years), mean(SD)			
Surgeons	29.94(4.96)		
Dentists	29.58(5.22)		
Physical therapists	28.46(3.28)		
Total	29.33(4.60)		
Gender n (%)			
	Male	**Female**	
Surgeons	64(50)	64(50)	
Dentists	21(16.4)	107(83.6)	
Physical therapists	28(21.9)	100(78.1)	
Total	113(29.4)	271(70.6)	
Body Mass Index n(%)			
Underweight (18.5kg/m2)	37 (9.6)		
Normal (18.5-22.9kg/m2)	131 (34.1)		
Overweight (23-24.9kg/m2)	94 (24.5)		
Obese (>25kg/m2)	122 (31.8)		
Work Experience n(%)			
	1-3 years	**4-6 years**	**7-9 years**	**>10 years**
Surgeons	69(53.9)	38(29.7)	9(7)	12(9.4)
Dentists	68(53.1)	28(21.9)	15(11.7)	17(13.3)
Physical therapists	79(61.7)	35(27.3)	7(5.5)	7(5.5)
Total	216(56.3)	101(26.3)	31(8.1)	36(9.4)

Table II: Descriptives Of Positive and Negative Responses in Upper Limb Tension Tests of Research Participants (N= 384)

Profession	Response	ULNT1 Right	ULNT1 Left	ULNT2 Right	ULNT2 Left	ULNT3 Right	ULNT3 Left
Surgeons	Positive	42 (32.8%)	37 (28%)	15 (11.7%)	17 (13.3%)	31 (24.2%)	24 (18.8%)
	Negative	86 (67.2%)	91 (71.1%)	113 (88.3%)	111 (86.7%)	97 (75.8%)	104 (81.3%)
Dentists	Positive	46 (35.9%)	41 (32%)	18 (14.1%)	17 (13.3%)	24 (18.8%)	23 (18%)
	Negative	82 (64.1%)	87 (68%)	110 (85.9%)	111 (86.7%)	104 (81.3%)	105 (82%)
Physical therapists	Positive	44 (34.4%)	36 (28.1%)	07 (5.5%)	07 (5.5%)	22 (17.2%)	17 (13.3%)
	Negative	84 (65.6%)	92 (71.9%)	121 (94.5%)	121 (94.5%)	106 (82.8%)	111 (86.7%)

Table III: Descriptives Of Positive and Negative Responses in Lower Limb Tension Tests of Research Participants (N = 384)

Profession	Response	Slump Test		Prone Knee Bend Test		Passive Straight Leg Raise Test	
		Right	Left	Right	Left	Right	Left
Surgeons	Positive	29 (22.7%)	27 (22.1%)	20 (15.6%)	18 (14.1%)	28 (21.9%)	21 (16.4%)
	Negative	99 (77.3%)	101 (78.9%)	108 (84.4%)	110 (85.9%)	100 (78.1%)	107 (83.6%)
Dentists	Positive	22 (17.2%)	20 (15.6%)	17 (13.3%)	17 (13.3%)	29 (22.7%)	26 (20.3%)
	Negative	106 (82.8%)	108 (84.4%)	111 (86.7%)	111 (86.7%)	99 (77.3%)	102 (79.7%)
Physical therapists	Positive	25 (19.5%)	26 (20.3%)	15 (11.7%)	11 (8.6%)	31 (24.2%)	27 (21.1%)
	Negative	103 (80.5%)	102 (79.7%)	113 (88.3%)	117 (91.4%)	97 (75.8%)	101 (78.9%)

Table IV: Association Between Work Experience with Upper and Lower Limb Neural Tissue Extensibility of Research Participants (N=384)

		Upper limb neural tissue extensibility					
		Dominant ULNT1	Non-dominant ULNT1	Dominant ULNT2	Non-dominant ULNT2	Dominant ULNT3	Non-dominant ULNT3
Work Experience	r_s	0.15	0.14	0.39	0.41	0.30	0.32
	*Sig. (2-tailed)	0.002	0.006	0.000	0.000	0.000	0.000
	N	384	384	384	384	384	384
		Lower limb neural tissue extensibility					
	r_s	Right Slump Test	Left Slump Test	Right Prone Knee Bend Test	Left Prone Knee Bend Test	Right Passive Straight Leg Raise Test	Left Passive Straight Leg Raise Test
		0.31	0.30	0.42	0.43	0.25	0.34
	*Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000
	N	384	384	384	384	384	384

*Correlation is significant at <0.05

a) ULNT1 b) ULNT2 c) ULNT3

Figure 1. Upper Limb Neurodynamic Testing (ULNT) Procedure

Discussion

Reduced nerve extensibility is commonly associated with occupations involving prolonged and repetitive work tasks. Among these, healthcare professionals exhibit a higher rate of musculoskeletal issues

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a) Slump Test b) Passive SLR c) Prone Knee Bend Test

Figure 2. Lower Limb Neurodynamic Testing Procedure

compared to other professions due to substantial job demands.

In current study, median nerve exhibited highest involvement 46(35.9%) particularly in dentists, followed by physical therapists 44(34.3%) and surgeons 42(32.8%). Previous literature elucidates multiple ergonomic risk factors in dental practice, including repetitive clinical procedures, sustained wrist postures and hand exertions. Prolonged contact stress from dental instruments further increases mechanical load over carpal tunnel contributing to the compression of median nerve.[15] Pejčić N et al. found that the dentists who maintained a static working posture (sitting or standing) were two times more likely to have musculoskeletal pain. The study also explored that 32.3% of dentists reported wrist pain and numbness after performing endodontic interventions.[16]

Sabike et al. reported that 24.2% of dentists experience median nerve compression at the wrist.[17] Julien et al. stated that surgeons, dentists and physical therapists report for wrist pain as 39.1%, 38.8% and 31.3% respectively. This may be attributed to high precision and procedural constraints of interventions, including limited access and tool-related risks while physical therapists perform manual therapies including patient handling and transfer, which place greater strain on the wrists and hands. Moreover, this study also explored that lower limbs were least affected for all health professionals, ranging from 15 to 25%.[18]

Another study reported that over 15% of surgeons were affected by carpal tunnel syndrome.[19] The findings are consistent with the previously published studies across various occupations, with wrist pain and numbness identified as the most prevalent conditions among the surveyed surgeons.

The results of the present study align with the previous study which indicated that median nerve involvement was the most prevalent (16.2%) in dentists, with greater involvement on the dominant side than the non-dominant side.[8] The present study showed involvement of median nerve as 42(32.8%) compared to the radial 18(14.1%) and ulnar nerves 24(18.8%) respectively.

For lower limb, 56(36%) and 38(24%) surgeons depicted high positive response for slump and prone knee bending test. The surgeons have long work hours, involving standing in awkward postures, challenges with various instrument design.[20] While, in current study, 58(45.3%) of physical therapists, exhibited high response in straight leg raise. Identifiable risk factors for the reduced lower limb extensibility in physiotherapists include bending or twisted postures during patient transfers or repositioning, prolonged standing postures as well as joint and soft-tissue mobilization requiring high force manual therapy.[21,22]

Furthermore, there was a statistically significant relationship ($p<0.05$) between years of experience and neurodynamics in current study. Likewise, Saad M Alqahtani et al. reported that the years of practice were associated ($p<0.001$) with work-related neuro-musculoskeletal symptoms in surgeons.[19] Goyal et al. reported no correlation ($p>0.05$) between the years of experience and the neural tissue extensibility in healthcare professionals.[8]

The findings indicate that median nerve tension is the most prevalent neural restriction among healthcare professionals, particularly in dentists, while lower limb nerves are commonly affected in long-standing position. All healthcare professionals consistently show higher neural involvement, supporting the protective role of physical activity and ergonomic practices. Early detection of subclinical neural tension through neurodynamic testing can guide preventive strategies to mitigate work-related neural disorders.

Key limitations of this study include its cross-sectional design, which restricts causal inference, and the absence of detailed ergonomic assessments. It is recommended to use longitudinal designs for future research to investigate the prolonged effects of occupational workload, including larger and more diverse samples. Incorporation of comprehensive ergonomic evaluations is essential. Additionally, the

impact of preventive exercises and workplace interventions on neural tension and musculoskeletal pain should be explored.

Conclusion

Occupational demands may contribute to reduce neural extensibility in healthcare professionals, particularly right and left median nerve as 34.4% and 29.7% in upper limb, while in lower limb, 22.7% and 18.2% for right and left passive straight leg raise respectively. Moreover, dentists exhibited highest response for median nerve, surgeons for slump and prone knee bending whereas physical therapists for passive straight leg raise test. Early screening through neurodynamic testing, combined with targeted preventive strategies such as ergonomic interventions and regular exercise, may help mitigate the development of work-related neural disorders and preserve long-term neuromuscular health.

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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

Caffeine Citrate Versus Aminophylline for Apnea of Prematurity: A Prospective Randomized Comparative Study

Hina Zaffar[1], Sidra Tul Muntaha[2], Aneesa Iqbal[3]

ABSTRACT

Objective: To compare the efficacy and safety of caffeine citrate versus aminophylline in the treatment of apnea of prematurity among preterm neonates.

Study Design: Prospective randomized comparative study

Place and Duration of Study: Department of Pediatrics, Benazir Bhutto Hospital, Rawalpindi from 4th July 2022 to 4th January 2023.

Materials and Methods: A total of 122 preterm neonates with gestational age of 34 weeks or less and at least four episodes of apnea were enrolled and randomly assigned to two groups. One group received a loading dose of 20 mg/kg of caffeine citrate followed by 10 mg/kg once daily, while the other group received a loading dose of 5 mg/kg of aminophylline followed by 2.5 mg/kg 12 hourly. The Treatment was continued for 34 weeks postmenstrual age. The primary outcome was complete resolution of apnea within 48 hours. Secondary outcomes included recurrence of apnea, changes in cardiorespiratory parameters, electrolyte stability and complications.

Results: Resolution of apnea within 48 hours occurred in 50(82%) of neonates receiving caffeine citrate compared with 40(65.6%) receiving aminophylline (p =0.040). Recurrent apnea was lower with caffeine citrate (3.3% versus 13.1%) with p value=0.048. Complications were also fewer in the caffeine group (13.1% versus 41%) with p value=0.026. Moreover, caffeine shortened NICU stay (14.2 ± 2.5 vs. 17.1 ± 3.1 days) with p value< 0.001, reduced the need for mechanical ventilation (18% vs. 31%) with p value=0.035, and improved early neurodevelopmental outcomes (87% vs. 72%) with p value =0.041.

Conclusion: Caffeine citrate is superior to aminophylline, demonstrating greater effectiveness in resolving apnea, reducing complications, shortening NICU stay, decreasing the need for mechanical ventilation and promoting improved early neurodevelopmental outcomes.

Key Words: *Apnea of Prematurity, Caffeine Citrate, Aminophylline, Preterm Neonates, Prospective Randomized Comparative Study.*

Introduction

Apnea of prematurity (AOP) is one of the most frequent and clinically significant complications among preterm neonates. It is defined as a cessation of breathing for more than 20 seconds or a shorter pause accompanied by bradycardia and desaturation, occurring primarily due to immaturity of the central respiratory control mechanisms in premature infants.[1] The condition is strongly inversely related to gestational age and birth weight, with reported occurrence in up to 85% of neonates born at ≤34 weeks of gestational age.[2] AOP contributes to prolonged neonatal intensive care unit stays, increased risk of hypoxemia related morbidities such as retinopathy of prematurity, bronchopulmonary dysplasia and adverse neurodevelopmental outcomes, thereby making it a major public health concern in resource limited settings.[3,4]

Historically, methylxanthines including theophylline, aminophylline and caffeine citrate have been the mainstay pharmacologic agents for the treatment of AOP.[5] Among these, caffeine citrate has gained increasing preference because of its longer half life, wider therapeutic window and fewer adverse effects compared to aminophylline and theophylline.[6]

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Several clinical studies and systemic reviews have shown that caffeine is effective in reducing apneic episodes, supporting extubation and improving neurodevelopmental outcomes in preterm infants.[7,8] Although previous studies have compared caffeine citrate and aminophylline for apnea of prematurity, much of the existing evidence has mainly addressed short term efficacy and safety outcomes, particularly apnea resolution and adverse effects. However, important gaps remain regarding their comparative performance in routine public sector NICU settings in Pakistan, where drug availability, affordability, monitoring capacity and hospitalization burden may influence treatment decisions. In many low and middle income countries, aminophylline continues to be used because of its lower cost and easier availability, despite concerns regarding its narrower therapeutic index and higher complication rates. In contrast, caffeine citrate may offer clinical advantages, but its wider adoption may be limited by cost and accessibility in local practice environments.[9,10]

Therefore, the present study was conducted to address this evidence gap by comparing caffeine citrate and aminophylline among preterm neonates with apnea of prematurity in a tertiary care public hospital setting. The study aimed to assess not only apnea resolution, but also recurrence of apnea, treatment related complications, NICU stay, requirement for mechanical ventilation and early neurodevelopmental status. The objective of this study was to compare the efficacy and safety of caffeine citrate and aminophylline in the treatment of apnea of prematurity among preterm neonates.

Materials and Methods

This Prospective Randomized Comparative Study was conducted in the Department of Pediatrics, Benazir Bhutto Hospital, Rawalpindi, from 4th July 2022 to 4th January 2023, after approval from the Institutional Review Board of Rawalpindi Medical University (Ref No 228/IREF/RMU/2022/ Dated:04/04/2022) and study was locally registered in Clinical Trial Unit Rawalpindi Medical University under registration number CTU 09/2023/0 13 RMU. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Sample size was calculated based on prior studies showing resolution rates of 80–85% with caffeine and 65–70% with aminophylline. To detect a 15% absolute difference with 80% power and 5% significance, 54 neonates were required per group; to allow for attrition, 61 were enrolled in each arm (n=122). Preterm neonates of either gender with gestational age ≤34 weeks and at least four episodes of apnea were eligible. Apnea was defined as cessation of breathing for ≥20 seconds or a shorter pause associated with bradycardia (heart rate <100 beats/min) and oxygen desaturation (<85%). Neonates with birth asphyxia, intraventricular hemorrhage (grade II or higher), sepsis, congenital malformations, metabolic abnormalities or necrotizing enterocolitis were excluded. Written informed consent was obtained from parents or guardians. Eligible neonates were allocated into two treatment groups using a computer-generated random sequence with allocation concealment through opaque sealed envelopes. Neonates were divided in two groups with 61 each group. Group 1 received caffeine citrate (loading dose 20 mg/kg followed by 10 mg/kg/day) and Group 2 received aminophylline (loading dose 5 mg/kg followed by 2.5 mg/kg every 12 hours). Therapy continued until 34 weeks of postmenstrual age. The primary outcome was complete resolution of apnea within 48 hours of treatment initiation. Additional outcomes included sustained resolution up to day 7, duration of NICU stay, requirements for mechanical ventilation and neurodevelopmental status at discharge and at 3 months corrected age. Secondary outcomes included recurrence of apnea, mean number of apneic spells at 24 hours, 25–72 hours and >72 hours, vital parameters (heart and respiratory rates), serum electrolytes and complications. Complications were prespecified as tachycardia (>180 bpm sustained), tachypnea (>70 breaths/min), feeding intolerance (≥2 gastric residuals >50% of feed or ≥2 vomiting episodes within 24 hours), restlessness requiring intervention and electrolyte imbalance confirmed on two consecutive samples. Blinding of caregivers was not feasible due to differences in dosing schedules; outcome data were collected by two pediatric residents using predefined operational definitions. Any discrepancy in outcome assessment was reviewed and resolved by consensus under consultant supervision before final data entry. Data were collected prospectively and analyzed

using SPSS v24. Continuous variables were expressed as mean ± SD and compared with independent t-tests; categorical outcomes were expressed as frequencies/percentages and compared with chi-square tests. Absolute risk differences with 95% confidence intervals (CIs) were reported. Logistic regression adjusting for gestational age and birth weight was applied to key outcomes, with p≤0.05 considered significant.

Results

A total of 122 neonates were randomized equally between caffeine citrate (61) and aminophylline (61). The baseline demographic and clinical profiles of neonates were similar in both groups. The mean gestational age was 29±1.5 weeks in the caffeine citrate group and 30±1.8 weeks in the aminophylline group with p value=0.12. Among those receiving caffeine citrate, 25(41%) were male and 36(59%) were female, whereas in the aminophylline group, 19(31%) were male and 42(69%) were female.

Apnea resolved within 48 hours in 82% of neonates treated with caffeine versus 65.6% with aminophylline (absolute risk difference 16.4%, 95% CI: 1.1–31.8) with p value=0.040. Recurrent apnea occurred in 3.3% of caffeine treated neonates compared with 13.1% of those receiving aminophylline (risk difference –9.8%, 95% CI: –18.8 to –0.8) with p value=0.048. The mean number of apneic spells at 24 hours did not differ significantly, but aminophylline showed fewer episodes at 25–72 hours, a difference that was temporary and disappeared beyond 72 hours (Table I).

By day 7, mean heart rates were similar between groups, while respiratory rates improved more with caffeine (56 vs. 58 breaths/min) with p value=0.003 . Serum electrolytes remained stable and comparable (Table II).

Complications occurred in 13.1% of caffeine treated neonates compared with 41% in the aminophylline group (risk difference –27.9%, 95% CI: –42.8 to –13.0) with p value=0.026. Tachycardia and feeding intolerance were significantly less common in the caffeine group, while other adverse events showed no statistical difference (Table II).

NICU and neurodevelopmental outcomes showed that caffeine shortened NICU stay (14.2 vs. 17.1 days) with p value=<0.001, reduced need for mechanical ventilation (18% vs. 31%) with p value=0.035 and improved early neurodevelopment (87% vs. 72% normal at discharge) with p value=0.041. Logistic regression confirmed caffeine as an independent predictor of higher apnea resolution (adjusted OR 2.45, 95% CI: 1.05–5.72) with p value=0.038 and lower complications (adjusted OR 0.29, 95% CI: 0.11–0.74) with p value=0.010. Table III.

Table I : Comparison Of Treatment Outcomes Between Study Groups (Aminophylline and Caffeine) n=122

Outcome	Caffeine Citrate (n=61)	Aminophylline (n=61)	p-value
Apneic episodes at 24h (mean ± SD)	2 ± 0.48	2 ± 0.49	0.193
Apneic episodes at 25–72h (mean ± SD)	2 ± 0.49	1 ± 0.50	0.018
Apneic episodes >72h (mean ± SD)	1 ± 0.39	1 ± 0.48	0.174
Complete resolution n (%)	50 (82%)	40 (65.6%)	0.040
Recurrent apnea n (%)	2 (3.3%)	8 (13.1%)	0.048
Mean heart rate (bpm, Day 7)	141 ± 6.1	143 ± 6.8	0.113
Mean respiratory rate (breaths/min, Day 7)	56 ± 2.9	58 ± 2.9	0.003*
Serum sodium (mmol/L, Day 7)	134 ± 1.65	134 ± 1.84	0.536
Serum potassium (mmol/L, Day 7)	4.3 ± 0.41	4.3 ± 0.45	0.642
Serum chloride (mmol/L, Day 7)	122 ± 1.56	121 ± 1.56	0.955
Mean heart rate (bpm, Day 7)	141 ± 6.1	143 ± 6.8	0.113
Mean respiratory rate (breaths/min, Day 7)	56 ± 2.9	58 ± 2.9	0.003*
Serum sodium (mmol/L, Day 7)	134 ± 1.65	134 ± 1.84	0.536
Serum potassium (mmol/L, Day 7)	4.3 ± 0.41	4.3 ± 0.45	0.642
Serum chloride (mmol/L, Day 7)	122 ± 1.56	121 ± 1.56	0.955

Table II:Comparison of Treatment related Complications between Study Groups (Aminophylline and Caffeine) n=122

Complication	Caffeine Citrate (n=61)	Aminophylline (n=61)	p-value
No complication n (%)	53 (86.9%)	36 (59%)	-
Tachycardia n (%)	1 (1.6%)	6 (9.8%)	0.05*
Feeding intolerance n (%)	1 (1.6%)	2 (3.3%)	0.559
Restlessness n (%)	2 (3.3%)	5 (8.2%)	0.243
Tachypnea n (%)	2 (3.3%)	8 (13.1%)	0.048
Electrolyte imbalance n (%)	2 (3.3%)	4 (6.6%)	0.402
Total Complications n(%)	8(13.1%)	25(41%)	0.026

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Table III: NICU and Neurodevelopmental Outcomes Between Study Groups (n=122)

Outcome	Caffeine (n=61)	Aminophylline (n=61)	p-value
NICU stay (days, mean ± SD)	14.2 ± 2.5	17.1 ± 3.1	<0.001*
Mechanical ventilation n (%)	11(18%)	19(31%)	0.035
Normal neurodevelopment at discharge n (%)	53(87%)	44(72%)	0.041
Developmental delay at 3 months n (%)	6(10%)	14(23%)	0.048

Discussion

In this prospective randomized comparative study, caffeine citrate was associated with better overall clinical outcomes than aminophylline in preterm neonates with apnea of prematurity. Apnea resolved within 48 hours in 82% of neonates receiving caffeine compared with 65.6% receiving aminophylline. Recurrence of apnea was also lower in the caffeine group, while overall complications were less frequent. In addition, caffeine was associated with shorter NICU stay, reduced need for mechanical ventilation and better early neurodevelopmental status.

The main contribution of our research is not simply to confirm that caffeine citrate is an effective methylxanthine, as this has already been reported in previous literature. Rather, this research adds practical evidence from a routine public sector NICU setting where aminophylline remains in use because of cost, availability and established prescribing practice. By evaluating apnea resolution together with recurrence, complications, NICU stay, ventilation requirement and early neurodevelopmental status, the present research provides clinically relevant information for treatment selection in resource limited neonatal care.

Apnea resolution remains the most important immediate treatment outcome in neonates with apnea of prematurity. In our study, caffeine citrate achieved a higher resolution rate than aminophylline. This finding is consistent with Xu et. al.,[11] who reported better apnea control with caffeine and with Shukla et. al.,[12] who also observed a higher treatment success rate in neonates receiving caffeine. Previous pooled research evidence has suggested that both caffeine citrate and aminophylline are effective methylxanthines for apnea of prematurity. However, caffeine appears to have a more favorable adverse effect profile. Therefore, the value of our research lies in showing that this expected benefit was also reflected in routine NICU outcomes in our setting.

Recurrence of apnea was significantly lower among neonates treated with caffeine citrate. This may be explained by the longer half life and more stable serum concentration of caffeine, which provides a more sustained therapeutic effect compared with aminophylline. Similar findings were reported by Zhang et. al.,[13] and Shivakumar et. al.,[14] who observed fewer recurrent apneic episodes in neonates treated with caffeine. Although aminophylline showed a temporary reduction in apneic spells during the early 25–72 hour period in our results, this effect did not persist beyond 72 hours. Clinically, this suggests that aminophylline may provide short term improvement, whereas caffeine offers more sustained control.

The safety profile also favored caffeine citrate. Overall complications were markedly lower in the caffeine group compared with the aminophylline group. Tachycardia, tachypnea, feeding intolerance, restlessness and electrolyte imbalance were all numerically less frequent among neonates receiving caffeine. These findings are in agreement with Schellack et. al.,[15] and Shivakumar et. al.,[14] who reported greater cardiovascular and systemic adverse effects with aminophylline. The narrower therapeutic index of aminophylline may explain its higher complication rate, whereas caffeine has more predictable pharmacokinetics and a wider therapeutic margin.

The present study also assessed outcomes beyond immediate apnea control. Neonates receiving caffeine had a shorter duration of nursery admission and a lower requirement for mechanical ventilation. These findings are clinically important in resource limited settings, where prolonged NICU stay increases treatment cost, bed occupancy and burden on families and healthcare systems. The CAP trial by Schmidt et al.[16] demonstrated important benefits of caffeine therapy in preterm infants, including improved longer-term outcomes. Similarly, Bruschettini et. al., [17] supported the role of caffeine in

improving clinically relevant neonatal outcomes. Our findings are consistent with this broader evidence, while adding local data from a public sector neonatal unit.

Early neurodevelopmental status was also more favorable in the caffeine group. A higher proportion of neonates having normal neurodevelopment at discharge and fewer showing developmental delay at three months. Although this follow up period was short and cannot establish long term neurodevelopmental benefit, the finding is clinically relevant and supports the need for extended follow up in future studies. Previous evidence, including the CAP trial, has suggested potential neurodevelopmental advantages of caffeine therapy in preterm neonates.[16]

Serum electrolyte levels remained comparable between the two groups, suggesting that neither drug produced clinically meaningful electrolyte disturbance in this cohort. This finding is consistent with Du et al.[18], who reported stable electrolyte parameters during caffeine therapy. Although routine monitoring remains important in critically ill preterm neonates, our result findings do not suggest a major difference between the two drugs in relation to sodium, potassium or chloride levels.

Recent researches from Pakistan and other low and middle income regions supports the practical relevance of our results. Umbreen et. al., and Afzal et. al., also found better outcomes with caffeine than aminophylline in apnea of prematurity, while Amponsah et. al., emphasized that the major challenge in low resource settings is improving access, affordability and routine use of caffeine. Therefore, the present research adds context by showing that caffeine's benefit is reflected not only in apnea resolution, but also in recurrence, complications, NICU stay, ventilation requirement and early neurodevelopmental outcomes in a public sector NICU setting.[19,20,10]

Overall, the findings support caffeine citrate as the preferred treatment option for apnea of prematurity because it was associated with better apnea resolution, fewer recurrences, lower complication rates, shorter NICU stay, reduced mechanical ventilation requirement and better early neurodevelopmental status. Aminophylline may still be used where caffeine is unavailable or unaffordable, but its higher adverse effect burden should be considered, especially in neonatal units with limited monitoring capacity.

This study has some limitations. It was conducted at a single tertiary care public hospital, which may limit generalizability to other settings. Blinding was not feasible because caffeine citrate and aminophylline have different dosing schedules; however, predefined operational definitions and consultant supervised verification were used to reduce observer bias. Long term neurodevelopmental outcomes beyond three months corrected age were also not assessed. Despite these limitations, the study provides useful real-world evidence from a setting where treatment choice is influenced by cost, availability, monitoring capacity and NICU resource burden.

Future multicenter studies with larger sample sizes and longer follow up are needed to confirm these findings and assess sustained neurodevelopmental outcomes. Cost effectiveness and implementation studies are also required in low resource settings, as caffeine citrate may offer clinical advantages, but wider adoption depends on affordability, availability and health system capacity.

Conclusion

Caffeine citrate is superior to aminophylline, demonstrating greater effectiveness in resolving apnea, reducing complications, shortening NICU stay, decreasing the need for mechanical ventilation and promoting improved early neurodevelopmental outcomes.

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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

Pre-analytical Laboratory Errors in the Department of Chemical Pathology at a Tertiary Care Hospital

Sadia Kirn[1], Lubna Ehtizaz[2], Sami Saeed[3], Tariq Masood Malik[4], Aamna Mahmood Gilani[5], Safina Saqib[6]

ABSTRACT

Objective: To determine the frequency and types of pre-analytical laboratory errors in the Department of Chemical Pathology at a tertiary care hospital in Pakistan.

Study Design: Prospective cross-sectional study.

Place and Duration of Study: This study was conducted at the Department of Chemical Pathology & Endocrinology, Fauji Foundation Hospital, Rawalpindi, from December 1, 2023, to March 1, 2024.

Materials and Methods: Following approval of the study protocol by the Institutional Review Board (IRB), data on laboratory requests (n=68,899) was assessed through the Laboratory Information System (LIS) in the Department of Chemical Pathology and Endocrinology. The study assessed all laboratory requests received during the study period to determine the prevalence of errors. Samples received from the Inpatient Department (IPD) and Outpatient Department (OPD) were examined physically and cross-checked for any specimen-related discrepancy against the records in the LIS.

Results: Of the total requests (n=68,899), 34,422 (49.96%) were received in the morning, 23,729 (34.44%) in the evening, and 10,748 (15.60%) at night. The overall incidence of pre-analytical errors was 5.53% (n=3,808). The most frequent errors were hemolyzed samples (52.65%) and lipemic samples (36.97%). Other errors included insufficient specimen quantity (6.80%), labeling errors (2.02%), and inadequate patient information (1.58%). From the total 68,899 requests, 41,340 were received from wards (IPD) and 27,559 from the OPD. The error rate was significantly higher in the IPD (6.75%, n=2,790) compared to the OPD (3.69%, n=1,018) ($p < 0.001$). A statistically significant difference was also observed in error frequencies between morning and evening sample requests ($p \leq 0.05$).

Conclusion: This study highlights the burden of pre-analytical errors in clinical chemistry samples and underscores the need for improved specimen collection and handling practices. Continuous training and strict adherence to protocols are essential to minimize these errors.

Key Words: *Clinical Chemistry, Laboratory Errors, Lipemic Samples, Pre-Analytical Error, Sample Requests.*

Introduction

Patient safety and medical diagnoses are significantly influenced by laboratory results, with studies showing that 60% to 70% of diagnostic decisions are affected by laboratory tests. [1] Clinical chemistry laboratory testing consists of three phases: pre-analytical, analytical, and post-analytical, together referred to as the Total Testing Process (TTP). Within this TTP, many errors occur outside the analytical phase: pre-analytical errors account for approximately 48–60%, while analytical errors comprise less than 10%, and post-analytical errors account for 19–47% of total laboratory errors.[2] The term pre-analytical factors was first introduced by Statland and Winkel in 1977. [3] It covers critical steps such as patient preparation and identification, specimen collection, transportation, centrifugation, and pre-analysis handling (such as dilution) in certain cases. Specimen collection errors include using inappropriate containers, inadequate sample volume, or improper labeling. Delayed or inappropriate transportation and inadequate centrifugation can lead to sample rejection, compromised analyte stability, and erroneous

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results. [4,5] Test requisition errors or incomplete patient information can also lead to misinterpretation of laboratory results.

The analytical phase involves the analysis of specimens followed by the verification and validation of test results. In clinical chemistry, this phase is frequently highly automated, reducing but not eliminating errors. The post-analytical phase includes the interpretation of results by laboratory consultants and the communication of these results to clinicians electronically or in printed format. [6]

Laboratory investigations are vital for clinical diagnosis and treatment decision-making. Since a significant portion of critical clinical decisions are based on laboratory results, errors in the testing process can have serious consequences, including the cost of repeat analysis, delayed diagnosis, and potentially worse patient outcomes. [7]

The importance of accurate and reliable laboratory outcomes for medical diagnosis and patient care is frequently underscored. [8] The laboratory staff must adhere to written policies for sample acceptance and rejection; non-adherence increases preventable pre-analytical errors. [1] The absence of adequate clinical information for routine laboratory tests leads to increased workload (e.g., through follow-up queries and repeated sampling) and healthcare costs. [9,10] The sample collection stage is a critical step in the testing process. This step is primarily manual and thus highly susceptible to human error. Effective quality management interventions are required to lessen errors and guarantee high-quality results. [10] The pre-analytical errors include test requisition mistakes, improper patient identification and preparation, untimely specimen collection, transportation delays, and processing faults that endanger sample integrity and accuracy. The importance of strict adherence to protocols in the pre-analytical phase is repeatedly emphasized. [6,11]

In Pakistan, variability in phlebotomy training and awareness may contribute to inaccuracies in specimen collection. Although general studies on laboratory errors have been conducted, there is limited focused research on pre-analytical errors within the specific environment of Pakistan's tertiary care hospitals, especially regarding error specificity and the impact on diagnostic accuracy and patient outcomes.

The aim of this study was to determine the frequency of pre-analytical errors in the Department of Chemical Pathology at a tertiary care hospital in Pakistan and to find improvement in diagnostic accuracy, patient care, and healthcare outcomes by identifying areas for improvement in laboratory processes.

Materials and Methods

This was a prospective cross-sectional study conducted at the Department of Chemical Pathology & Endocrinology, Fauji Foundation Hospital, Rawalpindi. A total of 68,899 laboratory requests were included. The study duration was three months, from December 1, 2023, to March 1, 2024.

Sample collection for routine and special chemistry tests was performed by phlebotomists in the Pathology lab for outpatients, while nursing staff and house officers collected samples from inpatient departments (IPD). Tests were allocated an ID number from the wards or OPD as per hospital protocol upon receipt in the Chemical Pathology Section. This study was conducted after approval from the Institutional Review Board (IRB) (Ref No. 618/RC/FFH/RWP).

The study assessed all laboratory requests (n=68,899) received during the study period to determine the prevalence of errors. Improper requests with incorrect or missing IDs, insufficient specimen volume, improper collection tubes, and hemolyzed, clotted, or lipemic specimens were categorized as errors. Laboratory requests with samples collected following standard operating procedures (SOPs) without pre-analytical errors were included in the total count to calculate error prevalence. A non-probability consecutive sampling technique was used. Statistical analysis was performed using SPSS Version 25. Chi-square test was applied to compare the proportions of pre-analytical errors between the outpatient and inpatient departments, as the outcome variables were categorical. A p-value of ≤ 0.05 was considered statistically significant.

Results

The overall incidence of pre-analytical errors was 5.53% (n=3,808). Of the total requests (n=68,899), 34,422 (49.96%) were received in the morning, 23,729 (34.44%) in the evening, and 10,748 (15.60%) at night. A statistically significant difference was

observed between morning and evening sample errors ($p < 0.05$), while no statistical difference was found between morning and night requests.

The majority of pre-analytical errors were due to hemolysis, accounting for 2,004 (52.65%) of total errors, followed by lipemic samples at 1,408 (36.97%). Inadequate sample volume comprised 259 (6.80%), labeling errors 77 (2.02%), and inadequate patient information 60 (1.58%) (Figure 1).

Comparison of Departments: Out of 68,899 lab requests, 41,340 were received from wards (IPD) and 27,559 were received from the OPD. Although the absolute number of errors was higher in the IPD (2,790 errors) compared to the OPD (1,018 errors), the error rate was also significantly higher in the IPD.

- **IPD Error Rate:** 6.75% (2,790 / 41,340 total sample requests)
- **OPD Error Rate:** 3.69% (1,018 / 27,559 total sample requests)

Chi-square test for independence was conducted to compare the incidence of pre-analytical errors which showed a significant association between the outpatient and inpatient departments ($p < 0.001$), confirming that the frequency of errors in the inpatient department was significantly higher than in the outpatient department (Figure 2).

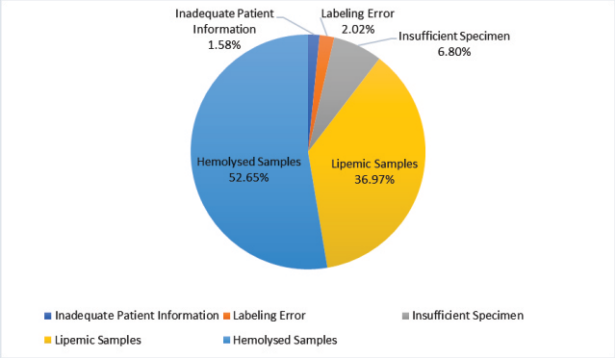

Figure 1: Distribution of Pre-analytical Error Types

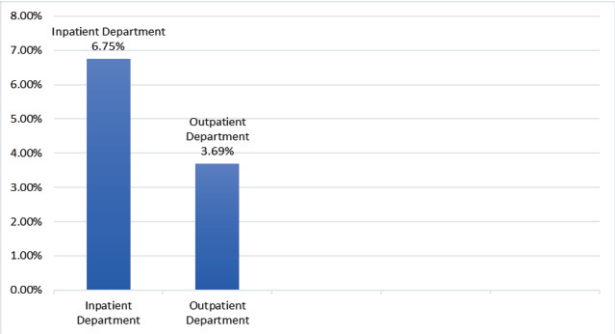

Figure 2: Comparison of Pre-analytical Error Rates

Discussion

Errors in laboratory sampling can significantly compromise result accuracy and adversely affect patient diagnosis and treatment. Systematic recognition and documentation of such errors are essential to improve the quality of laboratory medicine. [12] In the present study, the most frequent pre-analytical error was hemolysis. Comparable results were reported in a study at the Multan Institute of Kidney Diseases, where pre-analytical errors accounted for 0.67%, with hemolysis (41.6%) being the most frequent discrepancy. [13] The lower incidence observed in that study may reflect differences in case mix, workload, error definitions, or specimen handling protocols.

Another study conducted at CMH Kohat by Haroon Z et al. [14] found that pre-analytical errors accounted for 77% of all laboratory errors, primarily due to inadequate sampling methods. Our findings suggest that pre-analytical errors can be minimized by addressing factors such as mislabeling, insufficient volume, and transportation delays. Having skilled laboratory technicians and dedicated phlebotomy staff is essential for effectively minimizing these errors.

Hemolysis was also a major contributor in studies conducted in Doha, Qatar (24%) [15] and Jaipur, Rajasthan [16]. Hemolysis may occur due to traumatic venipuncture, use of small-gauge needles, vigorous tube mixing, or premature centrifugation. [13] Hemolysis interferes with laboratory results by releasing intracellular analytes (e.g., potassium, LDH, AST) and interfering with spectrophotometric measurements due to free hemoglobin. [17]

In a retrospective study conducted at the same hospital approximately eleven years ago, 41% of samples exhibited discrepancies, compared to the current study's rate of 5.53%.[18] This reduction suggests improvements in laboratory protocols over the decade. However, both studies found that the majority of errors originated from inpatient wards. This underscores the continuing need for targeted education for their staff regarding appropriate sampling techniques, proper container selection, and correct timing. Another cross-sectional questionnaire-based study conducted in a tertiary care setting stressed the importance of proper specimen collection, storage, and dispatch to assess

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the knowledge of junior doctors and nurses. The study showed that neither doctors nor nurses had enough knowledge about specimen collection and there was no statistically significant difference between their level of knowledge (p value = 0.435). [19]

The current study revealed a higher incidence rate of pre-analytical errors in samples received from the Inpatient Department (6.75%) compared with the Outpatient Department (3.69%). This higher error rate in the IPD may reflect differences in staffing, workload, patient acuity, and transport complexity in inpatient settings. Alcantara JC et al. [20] observed similar findings, noting that inpatient settings remain particularly vulnerable to pre-analytical errors.

Our study revealed a statistically significant difference in errors between morning and evening shifts. The higher error frequency in evening samples may be related to reduced supervision, increased workload, or staffing patterns; however, this was not formally evaluated in this study. Night shifts showed fewer errors, likely due to a lower volume of samples.

Conclusion

The pre-analytical errors identified in this study are primarily associated with sample collection and processing. This underscores the necessity for continuous and expanded educational initiatives that emphasize specimen quality issues and provide hospital staff with training in sample collection techniques. The data obtained from this research can be utilized to develop new strategies aimed at reducing pre-analytical errors. Reduction of the overall error rate can be achieved through regular training sessions, integrating suitable technologies, and routinely tracking quality indicators (QIs).

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

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ORIGINAL ARTICLE

Modified Above-Knee Versus Conventional Great Saphenous Vein Surgery Stripping for Lower Limb Varicose Veins: A Prospective Comparative Study

Zahid Rasheed, Muhammad Waqas Raza, Arsalan Manzoor Mughal, Aneeqa Jaleel, Umar Farooq

ABSTRACT

Objective: To compare the clinical outcomes, perioperative parameters, and postoperative complications of modified above-knee versus conventional full-length great saphenous vein stripping in patients with varicose veins.

Study Design: Prospective Comparative study.

Place and Duration of Study: The study was conducted at Holy Family, District Headquarters, and Benazir Bhutto Hospital, affiliated with Rawalpindi Medical University, from March 01, 2024 to March 31, 2025.

Materials and Methods: A total of 212 patients with Clinical-Etiology-Anatomy-Pathophysiology grades C2–C5 and Doppler-confirmed GSV reflux were enrolled and equally assigned to the modified above-knee group (n=106) and the conventional surgery group (n=106). Both procedures were completed with local or neuraxial anesthesia, performed by the same surgical group. Data on operative time, length of stay, incision type, intraoperative blood loss, and postoperative complications were collected.

Results: Baseline characteristics were comparable between the two groups. The modified above-knee stripping group showed significantly shorter operative time (48.7 ± 6.8 minutes vs. 59.1 ± 7.0 minutes, p<0.001) and shorter hospital stay compared with the conventional group. The incidence of postoperative saphenous nerve injury was significantly lower in the modified group (2.3% vs. 27.7%, p<0.001). Rates of surgical site infection, venous thromboembolism, and superficial thrombophlebitis were comparable between the groups. Technical and clinical success rates exceeded 97% in both groups.

Conclusion: Modified above-knee great saphenous vein stripping demonstrated favourable perioperative outcomes, including shorter operative time, reduced hospital stays, and lower incidence of saphenous nerve injury compared with conventional full-length stripping, while achieving comparable clinical and technical success rates.

Key Words: *Chronic Venous Insufficiency; Great Saphenous Vein; Postoperative Complications; Saphenous Nerve Injury; Varicose Veins; Vein Stripping.*

Introduction

Varicose veins are among the most common manifestations of chronic venous disease and result from venous valvular incompetence leading to venous hypertension and dilatation of superficial veins, particularly the great saphenous vein.[1,2] The disease commonly affects the lower extremities and presents with symptoms ranging from limb heaviness, pain, edema, and cosmetic concerns to skin pigmentation, lipodermatosclerosis, and venous ulceration in advanced stages.[1,3] These manifestations can significantly impair physical activity, work productivity, and overall quality of life.[4] Globally, the prevalence of varicose veins ranges from 20% to 60%, with higher occurrence reported among females, elderly individuals, prolonged standing workers, and patients with obesity or a family history of venous disease.[1] Incompetence at the saphenofemoral junction and reflux within the great saphenous vein are considered major contributors to disease progression and recurrence.[4,5] Because of the chronic nature of venous insufficiency and its associated healthcare burden, effective and durable treatment strategies remain an important surgical concern.

For decades, high ligation and stripping of the great saphenous vein have been regarded as a standard surgical procedure for symptomatic varicose veins.[4]

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Conventional full-length stripping effectively removes refluxing venous segments; however, it is associated with several postoperative complications, including hematoma formation, bruising, postoperative pain, delayed mobilization, recurrence, and particularly saphenous nerve injury due to stripping below the knee.[6,7] Previous studies have reported recurrence rates after conventional procedures ranging from 30% to 60%, which has encouraged continuous modification of surgical techniques and the development of minimally invasive alternatives.[8]

In recent years, endovenous techniques such as endovenous laser ablation, radiofrequency ablation, cyanoacrylate closure, foam sclerotherapy, and mechanochemical ablation have gained popularity because of shorter hospital stay, reduced postoperative pain, earlier ambulation, and favorable cosmetic outcomes.[4,9–12] Several comparative studies have demonstrated that minimally invasive approaches may provide outcomes comparable to or better than conventional surgery in selected patients.[13,14] However, the high cost of equipment, requirement for technical expertise, and limited availability in developing countries continue to restrict their widespread use, particularly in resource-limited healthcare systems.[15]

Consequently, modifications of conventional stripping techniques have been explored to reduce operative morbidity while preserving surgical efficacy. Among these approaches, modified above-knee great saphenous vein stripping has received attention because it avoids extensive below-knee dissection and may reduce the incidence of saphenous nerve injury without compromising venous reflux control.[16,17] *Hong et al*.[16] demonstrated improved postoperative recovery and patient satisfaction with modified stripping techniques compared with conventional procedures. Similarly, *Kusagawa et al*.[6] reported acceptable long-term clinical outcomes following modified great saphenous vein stripping with reduced postoperative complications.[17] Studies evaluating postoperative neural complications have further shown that limiting stripping below the knee may substantially decrease sensory nerve injury and associated morbidity.

Despite these advancements, controversy still exists regarding the optimal surgical approach for management of great saphenous vein reflux, particularly in settings where endovenous procedures are not routinely accessible. Much of the currently available literature focuses on comparisons between endovenous therapies and traditional surgery rather than comparisons between modified and conventional stripping techniques themselves.[4,8,10] Moreover, most studies originate from high-income healthcare systems, limiting the generalizability of findings to developing countries with different patient demographics, healthcare access, and economic limitations.

In Pakistan, chronic venous disease remains underreported despite its considerable impact on functional capacity and healthcare expenditure. Limited public awareness, delayed healthcare-seeking behavior, financial constraints, and restricted access to advanced endovenous interventions continue to influence treatment selection.[15] Under these circumstances, evaluating modified surgical techniques that are both clinically effective and economically feasible becomes particularly important. However, prospective local evidence comparing the modified above-knee stripping with conventional full-length stripping remains scarce. Therefore, this study was conducted to compare the clinical outcomes, perioperative parameters, and postoperative complications between modified above-knee and conventional full-length great saphenous vein stripping in patients with varicose veins.

Materials and Methods

This prospective comparative study was conducted in three tertiary care hospitals, including Holy Family, District Headquarters, and Benazir Bhutto Hospital, affiliated with Rawalpindi Medical University, in Pakistan, from March 01, 2024 to March 31, 2025. Patients presenting through outpatient vascular and general surgical clinics, as well as referrals from affiliated healthcare facilities, were screened for eligibility. During the study period, 212 patients with Doppler-confirmed great saphenous vein reflux who fulfilled the inclusion criteria and consented to surgery were enrolled consecutively and allocated equally into the two study groups. The diagnosis of varicose veins was verified in accordance with the

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International Committee of the American Venous Forum's (AVF) rationalized venous nomenclature and the clinical, etiologic, anatomic, and Pathophysiologic (CEAP) classification. [18] Patients of either gender between the ages of 18 and 75 who had CEAP grades C2–C5, a proximal GSV diameter larger than 8 mm, and a GSV reflux duration longer than 0.5 seconds when standing were eligible. Every participant felt discomfort and heaviness, which are signs of GSV reflux. The research excluded patients who had a history of venous surgery, venous thrombosis, post-thrombotic syndrome, significant deep vein reflux, Fontaine stage II–IV arteriosclerosis obliterans, organ dysfunction that precluded surgery, or a known intolerance to sodium tetradecyl sulfate (SST). Based on the surgical approach used, all eligible patients were split into two equal groups: the conventional surgery group (n=106) and the modified above-knee group (n=106). The proximal GSV diameter was measured 0.5 cm distal to the saphenofemoral junction, and the severity of chronic venous disease (CVD) was assessed using the CEAP method. Under local or neuraxial anesthesia, the same group of skilled surgeons carried out every surgical procedure. Doppler ultrasonography was used to map the varicose veins before surgery, and the skin was tagged with the locations that needed attention. At the level of the inguinal ligament, where its branches were ligated and split 0.5 cm distal to the femoral vein, the GSV was visible in both groups.

In the conventional surgery group, high ligation with full-length great saphenous vein stripping was performed, while the modified group underwent above-knee stripping only using standard described techniques. [16,17] The branch varicosities ≥4 mm were treated with stab avulsion/phlebectomy where required. Postoperative compression therapy with elastic bandaging and antithrombotic stockings was advised for all patients for three months. The assessed variables included operative time, intraoperative blood loss, duration of hospital stay, technical and clinical success, recurrence, and postoperative complications, including saphenous nerve injury, surgical site infection, venous thromboembolism, and superficial thrombophlebitis. Operative time was recorded from skin incision to completion of compression dressing, while intraoperative blood loss was estimated using the stained gauze area method. Clinical success was defined as improvement or absence of symptoms and visible varicosities at one-month follow-up. Saphenous nerve injury was assessed clinically based on postoperative pain or numbness along the medial aspect of the leg.

Patients were followed up postoperatively at 1, 6, 12, and 24 months after surgery for incision healing disturbances, sensory alterations, recurrence of varicose veins, and CEAP-C grade progression. Follow-up visits were conducted through outpatient clinic appointments, supplemented by telephone contact when in-person assessment was not feasible. Contact information was verified at discharge, and reminder calls were made before scheduled visits to enhance compliance and minimize loss to follow-up. Patients who missed a scheduled visit were contacted and offered rescheduled appointments whenever possible. The number of patients lost to follow-up at each time point was recorded and accounted for in the final analysis.

Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Quantitative variables were described as mean ± standard deviation and analyzed using independent or paired t-tests; categorical variables were compared with the Pearson, Chi-square test or continuity correction where appropriate. Recurrence-free survival was estimated using the Kaplan–Meier method. Statistical significance was defined as a p-value ≤ 0.05.

Results

A total of 212 patients diagnosed with great saphenous vein (GSV) reflux were included in this study, where 106 patients were enrolled in the modified above-knee and conventional surgery groups, respectively. Patients in the above-knee group had a mean age of 49.6 ± 13.9 years (range, 25 to 83) versus that of the conventional group at 52.1 ± 13.5 years (p = 0.06). The groups were comparable regarding gender, with males making up 46.2% of the above-knee group and 54.7% of the conventional group (p = 0.08). There were no significant differences between the two groups with respect to laterality, body mass index (BMI), duration of disease, smoking history, or proximal GSV diameter

(all p > 0.05) (Table I). This demonstrated that baseline characteristics had been well balanced. As for clinical grades according to CEAP-C classification, the distribution was not significantly different between groups, with most of the patients having C2 disease (51.2% in the above-knee group and 50.9% in the conventional group). The same was true when comparing the higher grades (C3–C5) between groups (Table II).

Of the entire study population, 96.5% (n = 205) had surgery under local anesthesia, and just 3.5% (n = 7) received neuraxial anesthesia. The modified group's technical success rate was 99.1%, whereas the traditional group was 98.1%. Additionally, there was no change in the clinical success rate (97.2% vs. 97.7% above the knee versus traditional). The modified technique offered distinct advantages over conventional stripping in terms of perioperative outcomes. Mean operation time, hospitalization duration, number of incisions, total incision length, and intraoperative blood loss were all significantly less in the modified group (All p < 0.001), revealing a minimally invasive and efficient surgical technique (Table III).

Although both groups experienced a few short-term postoperative problems, the modified group saw a considerably decreased rate of subcutaneous hematoma and saphenous nerve injury (p < 0.05). Saphenous nerve injury was seen in only 2.3% of the patients in the above-knee group compared with 27.7% of those in the conventional group. Mild injuries were more common, whereas severe injuries were infrequent and considered not statistically significant. Surgical site infection rate, venous thromboembolism, and superficial thrombophlebitis were not statistically significant between the two groups (Table IV).

Discussion

Baseline demographic and disease severity characteristics were comparable across the two study groups, indicating appropriate group allocation and minimizing selection bias. The observed age distribution and predominance of middle-aged patients are consistent with established epidemiological patterns of varicose veins reported in previous literature. [2] Similar CEAP classification profiles between groups further confirm comparable baseline disease severity, thereby allowing valid

Table I: Comparison of General Data of Patients (n = 212)

Variable	Modified (Above-knee)	Conventional Surgery	t/χ²	p-value
Age (years)	49.6 ± 13.9	52.1 ± 13.5	–1.89	0.06
Male (%)	49 (46.2%)	58 (54.7%)	3.04	0.08
Left side (%)	54 (50.9%)	55 (51.9%)	0.09	0.77
BMI	25.9 ± 3.5	26.3 ± 3.6	–1.22	0.22
Course of disease (months)	12.4 ± 7.4	13.0 ± 7.1	–0.84	0.40
Smoking (%)	42 (39.6%)	53 (50.0%)	2.73	0.10
Proximal GSV diameter (mm)	11.7 ± 2.0	12.0 ± 1.9	–1.72	0.09

BMI: Body Mass Index, GSV: Great Saphenous Vein

Table II: CEAP-C Grade Distribution (n = 212)

CEAP-C Grade	Modified (Above-knee)	Conventional Surgery
C2	54 (51.2%)	54 (50.9%)
C3	17 (16.0%)	21 (19.8%)
C4	18 (17.0%)	19 (17.9%)
C5	17 (16.0%)	12 (11.3%)

CEAP-C: Clinical, Etiologic, Anatomic, and Pathophysiologic Classification

Table III: Comparison of Perioperative Outcomes (n = 212)

Variable	Modified (Above-knee)	Conventional Surgery	t/χ²	p-value
Operation time (min)	48.7 ± 6.8	59.1 ± 7.0	–15.67	≤0.001
Length of hospitalization (days)	2.5 ± 0.5	4.9 ± 1.4	–23.68	≤0.001
Number of cuts	3.1 ± 1.5	4.2 ± 1.5	–7.30	≤0.001
Total incision length (cm)	3.8 ± 1.3	4.8 ± 1.2	–7.96	≤0.001
Intraoperative blood loss (mL)	14.6 ± 3.2	36.2 ± 9.1	–32.77	≤0.001

Table IV: Comparison of Postoperative Complications (n = 212)

Variable	Modified (Above-knee)	Conventional Surgery	t/χ²	p-value
Saphenous nerve injury (overall)	2.3	27.7	53.620	≤0.001
Mild	2.3	25.8	48.450	≤0.001
Severe	0.0	1.9	2.270	0.137
Surgical site infection	0.9	0.9	0.000	1.000
Venous thromboembolism	0.0	0.9	0.500	0.483
Subcutaneous hematoma	1.4	7.5	7.933	0.005
Superficial thrombophlebitis	8.9	6.6	0.821	0.365

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intergroup comparison of perioperative and postoperative outcomes.

Operative time was significantly shorter in the modified above-knee stripping group than in the conventional full-length stripping group. This finding is consistent with previous reports demonstrating improved procedural efficiency with modified stripping techniques due to reduced extent of dissection and avoidance of below-knee venous exposure. [16] The reduction in operative duration is likely attributable to decreased surgical field extension and simplified vein retrieval. From a clinical standpoint, shorter operative time reduces anesthesia exposure and may improve operating room efficiency without compromising procedural success.

Intraoperative blood loss was significantly lower in the modified above-knee group. Similar reductions have been reported in studies evaluating less extensive venous stripping procedures, where limited tissue dissection reduces collateral vessel injury and soft tissue trauma. [13,17] The reduced stripping length and minimized subcutaneous dissection likely account for decreased bleeding in the modified technique. This reduction in blood loss reflects a lower surgical burden and contributes to improved perioperative safety.

Hospital stay was significantly shorter in patients undergoing modified above-knee stripping. This finding aligns with existing literature on minimally invasive and modified venous procedures, which consistently demonstrate earlier mobilization and reduced inpatient recovery time.[4,12] The likely explanation includes reduced postoperative pain, lower tissue trauma, and fewer wound-related concerns in the modified group. Clinically, shorter hospitalization translates into reduced healthcare utilization and improved patient turnover.

A markedly lower incidence of saphenous nerve injury was observed in the modified above-knee group compared with conventional stripping. This finding is strongly supported by anatomical and clinical evidence indicating that the saphenous nerve is closely associated with the great saphenous vein below the knee, making full-length stripping a major risk factor for nerve injury. [6] Avoidance of below-knee stripping in the modified technique provides a clear anatomical rationale for this reduction. These results support modified stripping as a nerve-sparing surgical approach.

Postoperative subcutaneous hematoma occurred less frequently in the modified group. Similar findings have been reported in studies evaluating refined surgical techniques, where reduced dissection and limited tissue trauma result in fewer postoperative bleeding complications. [13] The decreased incidence in the modified group likely reflects reduced disruption of subcutaneous venous tributaries. This contributes to improved early postoperative comfort and wound recovery.

Both surgical techniques demonstrated high and comparable clinical success rates exceeding 97%. This indicates that limiting stripping to the above-knee segment does not compromise short-term symptomatic relief or eradication of visible varicosities. These findings are consistent with previous studies showing comparable efficacy between modified and conventional surgical approaches for great saphenous vein reflux. [11,17] The results suggest that modified above-knee stripping maintains therapeutic effectiveness while reducing perioperative morbidity.

Overall, modified above-knee great saphenous vein stripping demonstrated clear advantages in perioperative outcomes, including reduced operative time, blood loss, hospitalization duration, and saphenous nerve injury, while maintaining equivalent clinical success compared with conventional full-length stripping. These findings indicate that procedural modification can optimize the balance between surgical efficacy and safety. In routine vascular surgical practice, particularly in resource-constrained healthcare systems, such modifications may offer a pragmatic and cost-effective alternative without compromising treatment outcomes.

Limitations of the Study

Although the study was conducted across three tertiary care hospitals, all participating centers were located within the same geographical region, which may limit the generalizability of the findings to other populations and healthcare settings. Additionally, the non-randomized study design may also introduce selection bias despite comparable baseline characteristics between groups. Further large-scale multicenter randomized controlled

studies with extended follow-up periods are warranted to validate these findings and assess long-term effectiveness and recurrence rates.

Conclusion

Modified above-knee great saphenous vein stripping demonstrated favourable perioperative outcomes, including shorter operative time, reduced hospital stays, and lower incidence of saphenous nerve injury compared with conventional full-length stripping, while achieving comparable clinical and technical success rates.

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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

Detection of Reduced eGFR among Adults with Normal Serum Creatinine: A Cross-Sectional Study

Saman Sarwar, Muhammad Dilawar Khan, Hijab Batool[3], Tayyaba Rashid[4], Akhtar Sohail Chughtai[5], Asma Rasheed [6]

ABSTRACT

Objective: This study aimed to determine the prevalence of reduced glomerular filtration rate (eGFR) among adults with normal serum creatinine and to compare eGFR values calculated using the Cockcroft–Gault (CG) and Modification of Diet in Renal Disease (MDRD) equations.

Study Design: Cross-sectional study

Place and Duration of Study: Department of Pathology, Services Hospital, Lahore, from 1st August 2023 to 31st October 2023.

Materials and Methods: A total of 180 adults aged between 45 and 70 years were enrolled in the study. To reduce potential confounding effects on renal function assessment, participants with normal serum creatinine levels were included, whereas those who were fat or malnourished were omitted. After obtaining each participant's informed written consent, data were gathered using a systematic, pre-designed Performa. Relevant demographic characteristics and clinical information were systematically recorded and subsequently analyzed. Serum creatinine was measured, and estimated glomerular filtration rate (eGFR) was calculated using the Cockcroft–Gault (CG) and MDRD equations.

Results: The mean serum creatinine level was 0.65 ± 0.15 mg/dL. The mean eGFR calculated using the Cockcroft–Gault equation was 117.03 ± 27.55 mL/min, whereas the mean eGFR using the MDRD equation was 112.23 ± 24.20 mL/min/1.73 m². The difference between the two equations was statistically significant ($p < 0.001$).

Conclusion: The findings demonstrate that reduced eGFR may occur in adults with normal serum creatinine levels. Reliance solely on serum creatinine may lead to underdiagnosis of renal impairment. Routine reporting of eGFR, particularly using the MDRD equation, may facilitate earlier detection of renal dysfunction.

Key Words*: Cockcroft–Gault Equation, Estimated Glomerular Filtration Rate, MDRD Equation, Renal Insufficiency, Serum Creatinine.*

Introduction

Chronic kidney disease (CKD) is defined as the presence of kidney damage or reduced kidney function for at least three months, regardless of the underlying cause. Kidney damage may be identified through imaging findings, histopathological abnormalities, or clinical markers such as increased albuminuria or abnormal urinary sediment. Reduced kidney function is generally assessed by measuring the glomerular filtration rate (GFR), which is commonly estimated (eGFR) from serum creatinine levels. CKD is a progressive condition affecting more than 10% of the global population and is particularly prevalent among older individuals, females, and patients with diabetes mellitus or hypertension. It is also an important contributor to increased morbidity and mortality worldwide.[1,2]

Renal impairment significantly increases the risk of complications such as hypertension, cardiovascular disease, and bone mineral disorders, which may ultimately require renal replacement therapy. Early identification of renal dysfunction, therefore, allows timely interventions that can slow disease progression and reduce associated complications.[3–8]

Serum creatinine (SCr) is widely used in clinical practice as an endogenous marker to assess renal function due to its simplicity and availability. However, SCr is relatively insensitive for detecting early kidney dysfunction. Serum creatinine levels may remain within the normal range despite

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substantial nephron loss, as they are influenced by factors such as age, muscle mass, and delayed accumulation in the bloodstream. Consequently, reliance solely on serum creatinine may lead to underdiagnosis of early renal impairment.[9–11]

Glomerular filtration rate (GFR) is considered the most reliable indicator of kidney function. Direct measurement of GFR using exogenous filtration markers such as inulin clearance is regarded as the gold standard; however, this method is complex, costly, and impractical for routine clinical use.[13] Therefore, various equations have been developed to estimate GFR using serum creatinine along with demographic variables such as age and sex.[12,14]

Among these equations, the Modification of Diet in Renal Disease (MDRD) equation is widely used for estimating eGFR in clinical practice, whereas the Cockcroft–Gault (CG) equation estimates creatinine clearance and has historically been used for drug dosing adjustments in renal impairment. Although these equations are useful for estimating renal function, differences between them may influence the detection of reduced kidney function.[12,15]

According to Kidney Disease Improving Global Outcomes (KDIGO) guidelines, CKD is classified into stages based on eGFR values, ranging from normal kidney function (≥90 mL/min/1.73 m²) to end-stage renal disease (<15 mL/min/1.73 m²).[18] Reduced eGFR becomes increasingly common with advancing age due to structural and functional changes in the kidney.

Therefore, this study aimed to determine the prevalence of reduced eGFR among adults with normal serum creatinine and to compare eGFR values calculated using Cockcroft–Gault and MDRD equations.

Materials and Methods

After obtaining approval from the institutional review board (IRB Num # IRB/2023/1144/SIMS), this cross-sectional study was conducted in the Department of Pathology, Services Hospital Lahore, over a three month period from 1st August 2023 to 31st October 2023.

The sample size of 180 participants was calculated using a 95% confidence level , 7% margin of error, and an expected prevalence of moderate renal impairment of 21% based on previously published literature. A consecutive non-probability sampling (Convenient Sampling) technique was used.

Adult Pakistani patients aged 45-70 years attending the outpatient department with normal serum creatinine levels (male ≤ 1.3 mg/dL and female ≤ 1.1 mg/dL) were included in the study. Patients on dialysis and those with obesity or malnutrition were excluded to minimize confounding due to altered muscle mass affecting serum creatinine and eGFR estimation.

Information regarding risk factors such as diabetes mellitus and hypertension was obtained through patient history. During clinical assessment, participants' weight and height were measured, and body mass index (BMI) was calculated using the formula weight (kg)/height (m²).

Serum samples were collected from all participants for the estimation of serum creatinine. A fully automated chemistry analyzer (Beckman Coulter AU480) was used to measure serum creatinine using the Jaff kinetic technique (photometric measurement) following standard laboratory quality control procedures.

In our study, renal function was determined using the MDRD equation and CG formula, and eGFR of all patients was calculated using both equations.

According to KDIGO guidelines, renal function was classified based on eGFR values as follows:
G1 ≥90 mL/min/1.73 m² (normal),
G2 60–89 mL/min/1.73 m² (mild decreased),
G3a 45–59 mL/min/1.73 m², (mild to moderately decreased)
G3b 30–44 mL/min/1.73 m²,(moderately to severely decreased
G4 15–29 mL/min/1.73 m² (severely decreased).[19, 20]

Data analysis was performed using SPSS version 28. Quantitative variables like age, height, weight, BMI, and Serum Creatinine were presented as mean ± standard deviation, while categorical variables like ethnicity, gender, hypertension, diabetes, obesity, and family history, were presented as frequency or percentages. Stratification was performed for potential effect modifiers, and the Chi-square test was applied for comparison of categorical variables. P-value <0.05 considered statistically significant.

Results

In our study, 38.33% (n=69) were female and 61.67% (n=111) male. The mean age was 52.40 ±5.35 years, with a range of ages between 45 and 70. In this study,

106 (59%) hypertensive and 74 (41%) non-hypertensive patients were enrolled, with 53 (29.44%) diabetic and 127 (70.56%) non-diabetic patients, as shown in Fig. 1. The mean body mass index (BMI) was 26.47 ± 3.48. The serum creatinine estimated was 0.65 ± 0.15mg/dl.

The mean eGFR by the C-G equation was 117.03 ± 27.55 ml/min and 112.23 ± 24.20 ml/min/1.73m^2 by the MDRD equation. Patients falling in G1, G2, and G3a stages of renal insufficiency were 146 (81.1%), 31 (17.2%), and 3 (1.7%), respectively, by C-G formula (Table I). Patients with renal insufficiency in stages G1, G2, and G3a were 154 (85.6%), 24 (13.3%), and 2 (1.1%), respectively, by the MDRD equation (Table II). A comparison of both equations according to stages by chi square is given in Table III.

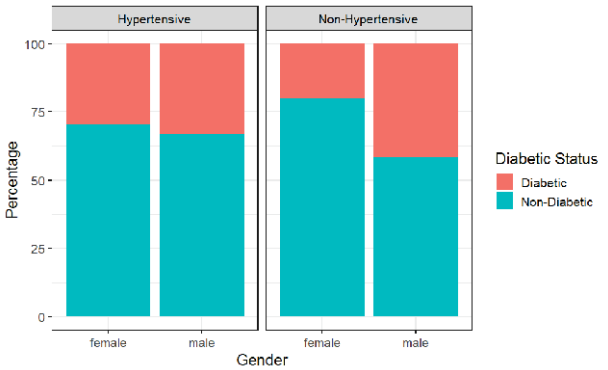

Figure 1. Prevalence of Hypertension and Diabetes Across Gender Groups

Table I: Frequency of Renal failure by CG Formula (N= 180)

	G1 n(%)	G2 n(%)	G3a n(%)	G3b n(%)	G4 n(%)	P-Value
Male	60 (87%)	9 (13%)	0 (0%)	0 (0%)	0 (0%)	0.528
Female	94 (84.7%)	15 (13.5%)	2 (1.8%)	0 (0%)	0 (0%)	
Total	154 (85.6%)	24 (13.3%)	2 (1.1%)	0 (0%)	0 (0%)	

Table II: Frequency of Renal failure by MDRD Equation (N= 180)

	G1 n(%)	G2 n(%)	G3a n(%)	G3b n(%)	G4 n(%)	P-Value
Male	55 (79.7%)	13 (18.8%)	1 (1.4%)	0 (0%)	0 (0%)	0.892
Female	91 (82%)	18 (16.2%)	2 (1.8%)	0 (0%)	0 (0%)	
Total	146 (81.1%)	31 (17.2%)	3 (1.7%)	0 (0%)	0 (0%)	

Table III: Comparison of Renal Failure by CG and MDRD Equation (N= 180

CKD Stages	CG n (%)	MDRD n (%)	P-Value
G1	146 (81.11%)	154 (85.56%)	<0.001
G2	31(12.22%)	24(13.33%)	
G3a	3 (1.67%)	2(1.11%)	
G3b	0 (0%)	0 (0%)	
G4	0 (0%)	0 (0%)	

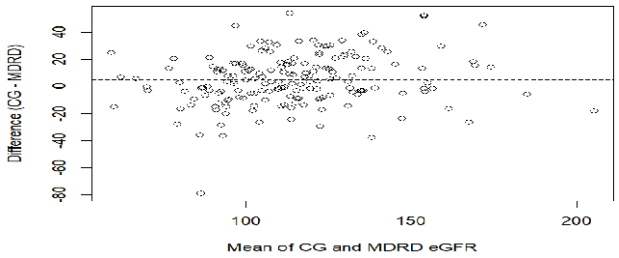

Figure 2. Bland–Altman Plot: CG vs MDRD eGFR

Discussion

The early detection of any renal damage can be ensured through clinical guidelines that suggest using estimation equations like MDRD and CG formulas to estimate eGFR on the basis of SCr levels.[6] The reason why estimation equations need to be used in the detection of early-stage renal damage is because SCr levels, which by themselves are considered insensitive markers in the determination of kidney damage, can be normal even if renal impairment occurs. In view of this problem, the current study seeks to establish the prevalence of kidney insufficiency and CKD staging among adults with normal SCr levels.[20]

Our results show that the mean SCr of the patients is 0.65 ± 0.15, which aligns with the literature findings, with a mean Serum Creatinine of 0.88 ± 0.26 in patients without renal insufficiency, which displays that the serum creatinine is normal in both studies. In our study, the mean eGFR by the CG formula was 117.03 ± 27.55, and by the MDRD formula was 112.23 ± 24.20. Similar findings for $eGFR_{MDRD}$ (ml/min/1.73 m^2) were found in a previous study, i.e., 116.8 ± 43.5, which supported our results. However, the mean $eGFR_{CG}$ (ml/min/1.73 m^2) was 90.5 ± 33.1, which is different from our findings of $eGFR_{CG}$ equal to117.03 ± 27.55.[21,22]

Bland-Altman plot displayed in Fig. 2 revealed the presence of a mean bias of 4.80 mL/min/1.73m2 which shows that the CG formula is somewhat biased towards an overestimation of eGFR with respect to

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MDRD formula. On the other hand, the limits of agreement were observed between -32.14 and 41.74 mL/min/1.73m2, suggesting a considerable extent of spread between the two formulas. However, the magnitude of the overall bias seems relatively low. Thus, it can be said that the CG and MDRD formulae present a fair degree of agreement and interchangeability.

Our findings shows that, according to $eGFR_{CG}$, there were 146 (81.11%) patients, and according to $eGFR_{MDRD}$, 154 (85.56%) patients were in G1 (Normal) group, while 31(12.22%) and 24(13.33%) according to CG and MDRD respectively were in G2 (Mild) stage. 3 (1.67%) patients by CG and 2 (1.11%) by MDRD in the G3a (Mild-Moderate) group. However, no patient was found in the G3b and G4 (Severe) groups. P-value was significant <0.001, making both equations significantly different as based on different formulas. Another study demonstrates that by MDRD equation with normal SCr, 21.5% of and by C-G formula, 38.2% of patients were in the mild renal impairment group. According to the MDRD and CG equations, 7.7% and 9.2% of study subjects had mild to moderate kidney dysfunction, respectively. The MDRD and CG formula found moderate renal insufficiency in 7.7% and 16.9% of patients, respectively. Additionally, 7.7% of patients had moderate to severe renal impairment despite having SCr in the reference range when eGFR was predicted by the CG formula.[23]

CG equation overestimates eGFR as body weight is used as a marker of muscle mass; in obese people, it will overestimate the eGFR value. Moreover, standardization of the Creatinine assay used in the equations is not available to reference method. Cockcroft -Gault formula has yet to be modified for body surface area. The eGFR by the MDRD equation can be calculated without body weight, and this equation is adjusted for body surface area. UK chronic kidney disease guidelines endorse it. The MDRD equation is more reliable for the calculation of eGFR as compared to the CG formula. However, the MDRD equation shows negative bias when eGFR calculated is over 60 ml/min/1.73 m^2.[25] A study that evaluated the MDRD equation in a sizeable, diverse population demonstrated that at eGFR less than 60 ml/min / 1.73 m^2, the MDRD equation had lower bias than at eGFR more than 60 ml/min / 1.73 m^2. The MDRD equation, therefore, provides precise and unbiased estimates when eGFR is <60 ml/min/1.73 m^2. Clinicians should carefully infer GFR estimates around 60 ml/min /1.73 m^2 to avoid erroneous CKD staging during the patient's evaluation.[26] Furthermore, in 2005, MDRD's four-factor equation (age, gender, ethnicity) was proposed for precise estimation of GFR, and this re-expressed equation has serum creatinine traceability to isotope dilution mass spectrometry (IDMS). The benefit of MDRD over CG is that it was verified against measured GFR using 125 I-iothalamate and does not require body weight because it is already corrected in the calculation.[27]

Conclusion

Reduced eGFR can be present in adults with normal serum creatinine levels, indicating that reliance on serum creatinine alone may underestimate early renal dysfunction. Although both the Cockcroft–Gault and MDRD equations were effective in identifying reduced kidney function, significant differences were observed between the eGFR values generated by the two formulas. Routine calculation and reporting of eGFR, particularly in middle-aged and older adults, may improve early recognition of chronic kidney disease and support timely clinical intervention.

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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

Clinical Features and Outcome of Mucormycosis Cases in A Tertiary Care Hospital: A 10-year Experience

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ABSTRACT

Objective: The study aimed to describe the clinical features and outcomes of mucormycosis and identify variables associated with in-hospital mortality.

Study Design: Retrospective observational study.

Place and Duration of Study: Rehman Medical Institute, Peshawar between 1st January 2015 and 31st December 2024.

Materials and Methods: The study included all histopathological confirmed cases of mucormycosis diagnosed during the study period. Demographic data, clinical and radiological features, treatment, and outcomes at discharge were retrieved from patient hospital records. Data were summarized as frequencies and percentages for categorical variables and as median (interquartile range) for continuous variables. Mann–Whitney U test and Fisher's exact test were applied to do mortality analysis between two groups.

Results: Of 35 study participants, the median (IQR) age was 55(21) years, 68.6% (n=24) were male. Rhino-orbito-cerebral mucormycosis (ROCM) was the most common presentation in 94.2% (n=33). About 77.1% (n=27) of patients had diabetes mellitus (DM), 85.7% (n=23) had poor glycemic control. Surgical intervention was performed in 97.1% (n=34). About 20% (n=7) of patients required ICU care. The in-hospital mortality was 17.1% excluding patients who left against medical advice. ICU admission, intracranial extension, and septic shock were strongly associated with mortality ($p < 0.001$ each). Higher C -reactive protein (CRP) (49.26 vs 7.75 mg/L, $p= 0.007$), neutrophilia (87.8 vs 73.3%, $p = 0.005$) and HbA1c (13.9% Vs 11.4%. $P= 0.03$) were associated with high mortality.

Conclusion: Mucormycosis mostly affected middle aged adults with poorly controlled DM. ROCM was common with worse outcomes. Extensive organ involvement at presentation, high CRP, HbA1c levels, and neutrophil counts were associated with mortality.

Key Words: *Mucormycosis, Diabetes Mellitus, Mortality.*

Introduction

Mucormycosis is a rare, opportunistic, life-threatening fungal infection. The causative organisms belong to the Rhizopus genus. Rapid tissue invasion and necrosis are the hallmark of this disorder from its ability to invade blood vessels. Rhino-orbito-cerebral mucormycosis (ROCM) is the most common form worldwide, followed by pulmonary, cutaneous, gastrointestinal, and disseminated forms.[1]

Immunocompromised individuals are most prone to developing the disease.[1,2] Uncontrolled diabetes mellitus, absolute neutropenia, organ transplantation and/or immunosuppressant drug use, hematological malignancies, and steroid use are major risk factors in this patient cohort. A significant surge in mucormycosis was reported during the COVID-19 pandemic as well. The use of steroids, presence of hypoxemia and poor glycemic control in patients with diabetes, was deemed to be responsible for this surge during COVID-19.[2] Indian researchers reported, around 40,000 cases during the COVID-19 pandemic, further reiterating the statistics reported worldwide.[3]

Prevalence of mucormycosis is significantly higher in underdeveloped countries (140 vs 1.7 cases per million population) compared to the developed world.[4] Globally, the prevalence of mucormycosis has been estimated as 0.005 to 1.7 per million people for the year 2019-2020, with the incidence in India

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about 80 times greater than in industrialized nations.[5,6] A significant burden on healthcare systems has been reported in the underdeveloped countries in mucormycosis infections. Tens of thousands of US dollars are often needed for effectively treating a single episode of the disease due to the need for prolonged hospitalization, unavailability and costly antifungal medications (e.g., liposomal amphotericin B), multiple surgeries, and post-recovery rehabilitation.[7]

Mucormycosis carries a high mortality rate of 25-87%, despite the advancements in diagnosis and treatment.[8] Prognosis, including mortality rates, is influenced by several variables. Disseminated disease, brain involvement, radiological bone erosions, diagnostic delays, underlying medical conditions like uncontrolled diabetes and presence of hematological malignancies etc., and inadequate surgical resection were associated with increased mortality rates. Reports of improvement in the disease outcome have been associated with the early introduction of antifungal therapy and aggressive surgical debridement if warranted.[8]

To date, studies highlighting the clinical and radiological presentations, and outcomes of mucormycosis in Pakistan are limited. Data from our region is important as patients frequently present in advanced stages of disease due to delayed access to healthcare centers. Moreover, limited availability of anti-fungal drugs and advance surgical procedures also effect disease outcome. The scarcity of data in Pakistan, underscores the need for research to understand the epidemiology, risk factors, outcomes and management challenges within this region. This study aims to help identify trends in the clinical features and outcomes of this rare but fatal disease. The aim of the study is to evaluate the demographic, clinical, and radiological characteristics, clinical course, and discharge outcomes of mucormycosis and to identify factors associated with poor outcomes.

Materials and Methods

This retrospective observational study was conducted at Rehman Medical Institute, Peshawar, and included all histopathological confirmed cases of mucormycosis diagnosed and managed between 1st January 2015 and 31st December 2024. Ethical approval was obtained from the institutional review board and ethics committee of Rehman Medical Institute before data collection under reference number RMI/IRB-EC/approval/259 dated June 18th,2025. Patient consent for the use of their data was routinely taken at the time of admission. Only cases with complete medical records were included, while cases with incomplete data or those diagnosed outside the institution were excluded.

Data were collected from the hospital medical records, laboratory databases, radiology reports, and surgical records. The variables retrieved included demographic information, clinical presentation (symptoms and site of involvement), underlying risk factors such as diabetes, Hypertension (HTN), history of COVID-19 infection. Diagnostics such as CT or MRI scans and histopathological characteristics were also recorded. Data regarding treatment modalities, including medical and surgical management, and clinical outcomes such as recovery, complications, or death at the time of discharge were also retrieved. All data was anonymized and handled with strict confidentiality.

Statistical analysis was performed using SPSS 27. Normality of continuous variables was assessed using the Shapiro–Wilk test. As data was non-normally distributed hence whole cohort or subgroup continuous data were presented as median (IQR) or Median (range) where IQR was unstable. Categorical variables were expressed as frequencies and percentages. Comparative analyses between groups (survivors vs. non-survivors) at discharge (after excluding patients who left against medical advice) were conducted using Fisher's exact test and Mann-Whitney U test due to unequal group size. Mortality association analysis was performed after excluding patients who left against medical advice. A p-value of less than 0.05 was considered statistically significant.

Results

During the study period, a total of 35 patients with confirmed mucormycosis were identified from the hospital records. Of these, 68.6% (n=24) were males and 31.4% (n=11) were females. The median (IQR) age of patients was 55 (21) years. In terms of nationality, 80% (n=28) were Pakistani, while 20% (n=7) were Afghan citizens.

The predominant clinical presentation was Rhino-

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orbito-Cerebral mucormycosis in 94.2% (n=33), whereas 2.8% (n=1) each had pulmonary and isolated orbital involvement. Patients were mainly admitted under the care of ENT division 65.7% (n=23), followed by Maxillofacial Surgery 22.9% (n=8), Neurology 5.7% (n=2), Cardiothoracic surgeon or physicians 2.9% (n=1), and Pediatrics 2.9% (n=1).

Radiological evaluation included CT sinuses and brain in 48.6% (n=17), MRI brain in 25.7% (n=9), while the remaining patients underwent alternative or combined imaging modalities.

The most common underlying risk factor was diabetes mellitus in 77.1% (n=27). Among patients with diabetes, 85.7% (n=23) had poorly controlled diabetes (HbA1c > 9%), 9.5% (n=3) had inadequately controlled diabetes (HbA1c 7–9%), and 4.7% (n=1) had well-controlled diabetes (HbA1c < 7%). Regarding hypoglycemic therapy, 28.6% (n=10) were on oral hypoglycemic agents, 34.3% (n=12) were on insulin, and 11.4% (n=4) were on dual therapy with insulin and oral agents. Other comorbidities included hypertension in 37.1% (n=13), ischemic heart disease in 14.3% (n=5), and a history of COVID-19 in 34.3% (n=12) patients.

Surgical intervention was performed in 97.1% (n=34) of patients. FESS being the most performed procedure i.e. 71.4%(n=25). All the patients were treated with amphotericin B. ICU care was required in 20% (n=7) patients.

Mortality association was performed on 31 patients with known discharge outcomes (25 survivors, 6 non-survivors); 4 patients discharged against medical advice hence excluded from mortality associated analysis.

Overall, complications were observed in 37.1% (n=13) patients, including drug-induced acute kidney injury in 11.4% (n=4). All patients who developed AKI received conventional amphotericin B. (p value 0.11 yielded by Fisher's exact test)

The in-hospital mortality rate was 17.1% (6/35), while the outcome of 11.4% (n=4) patients could not be ascertained as they took early discharge against medical advice (DAMA). These DAMA cases were excluded from further analysis between survivor and non-survivor groups. Further details of demographic, clinical and radiological features, management, and clinical outcomes are provided in Table 1 and 2.

Multiple factors were assessed for their association with mortality. ICU admission was significantly associated with mortality in our cohort. This association likely reflects underlying disease severity among critically ill patients rather than a causal relationship between ICU admission and adverse outcome. Out of 7 patients admitted to ICU 5 (71.4%) died, compared to 1 of 24 non-ICU patients (4.2%) ($p < 0.001$). Similarly, presence of complications such as shock or brain extension was significantly linked to

Table I: Demographic, Clinical, laboratory and radiological features of the patients with mucormycosis (n=35)

Demographic features	**n (%) or median (IQR)**
Age (years), mean ± SD	55(21)
Gender	
Male	24 (68.6)
Female	11 (31.4)
Comorbidities	**n (%)**
Diabetes mellitus	27 (77.1)
Hypertension	13 (37.1)
Ischemic heart disease	5 (14.3)
Clinical features	**n (%)**
Visual loss	12 (34.3)
Epistaxis (nosebleed)	2 (5.7)
Sinusitis	22 (62.9)
Oral involvement	9 (25.7)
Eye swelling	14 (40.0)
Facial pain	16 (45.7)
Focal neurological deficit	5 (14.3)
Third nerve palsy	4 (11.4)
Central retinal artery occlusion	2 (5.7)
Drooping of eyelid (ptosis)	6 (17.1)
Laboratory features	**Median (IQR)**
Total leukocyte count ($\times 10^9$/L)	14.06(8.82)
Neutrophils (%)	79.2(16.1)
C-reactive protein (mg/L)	25(39.48)
HbA1c (%)	11.50(2.40)
Radiological Features	**n (%)**
Sinus involvement	34(97.1)
Maxillary sinus	32(91.4)
Ethmoid sinus	22(62.9)
Frontal sinus	9(25.7)
Sphenoid sinus	13(37.1)
Cavernous sinus	5(14.3)
Bone erosion	17(48.6)
Orbit involvement	17(48.6)
Brain involvement	9(25.7)

mortality (p < 0.001). Among laboratory markers, CRP was substantially elevated in non-survivors (median ≈ 49.26 mg/L) versus survivors (median ≈ 7.75 mg/L), with a highly significant difference (p= 0.007). Neutrophilia was also significantly higher in those who died (median ≈ 87.8%) than in survivors (median ≈ 73.3%; p = 0.005). HbA1c was significantly higher in non-survivor group (median ≈ 13.9% vs 11.4%, P = 0.03). No significant differences were noted in gender, age, comorbidities, or total leukocyte count by outcome. Details are described in Table III.

Table II: Management strategies and in-hospital outcomes of patients with mucormycosis (n = 35).

Factors		n (%)
Management		
Medical Treatment		
Conventional amphotericin B		20(57.1)
Liposomal amphotericin B		15(42.9)
Surgical treatment*		
FESS		25(71.4)
Orbital exenteration		3(8.6)
Maxillectomy		6(17.1)
Debridement		3(8.6)
Pneumonectomy		1(2.9)
Clinical outcome		
Mean Duration of stay in hospital (median (IQR))		3(2.50)
Complications	Acute kidney injury	4(11.4)
	Extension to brain	8(22.9)
	Septic shock	2(5.7)
Death		6(17.1)

* Some patients underwent more than one surgical intervention (e.g., FESS with maxillectomy and debridement); hence, the total percentage exceeds 100%.

Discussion

We identified 35 confirmed cases over a 10 year period, which is comparable to reports from the various regions i.e., 33 cases in 11 years reported by Abanamy et al, from 3 tertiary care hospitals of the Kingdom of Saudi Arabia (KSA)[9] and 43 cases over a 14 year period by Allaw et al, from a tertiary care hospital, Lebanon.[10] These findings may reflect a similar disease burden across the Middle Eastern and

Table III: Comparison Of Clinical and Laboratory Variables Between Survivors and Non-Survivors with Known Outcomes (n=31) (4 DAMA cases excluded)

Categoric variables		Survivors (25/31) n%	Non-survivors (6/31) n%	P value (Fisher's exact test
Gender	Male	20 (80%)	5 (83.3%)	0.20
	Female	5 (20%)	1 (16.7%)	
Diabetes Mellitus		21 (84%)	5 (83.3%)	0.85
Hypertension		10 (40%)	2 (33.3%)	0.76
COVID positive		10 (40%)	1 (16.7%)	0.28
ICU admission		2(8%)	5 (83.3%)	<0.001
Complications	AKI	3(12%)	1 (16.7%)	0.75
	Brain extension	4(16%)	4(66.7%)	0.002
	Septic Shock	0(0%)	2(33.3%)	<0.001
continuous variables		Median(min-max) *	Median(min-max) *	P value (Mann Whiteny U test)
Age (years)		60 (33-67)	40 (31-50)	0.12
Duration of admission (days)		2.5(2-6)	3.5(3-4)	0.27
TLC($(\times 10^9/L)$)		14.02(7.16-16.3)	14(4.63-23.5)	0.13
Neutrophilia (%)		73.3(56-81.3)	87.8(83.2-92.4)	0.005
CRP (mg/L)		7.75 (0.8-29.7)	49.26(42-58)	0.007
HbA1c (%)		11.4(9.5-12.8)	13.9(13-14.8)	0.03

*Due to small sample in non-survival group, IQR was unstable so median (min-max) is used

South Asian cohorts. However, Priya et al[11] from India, reported a disproportionately higher incidence of mucormycosis compared to the rest of the regional statistics i.e. 38 cases in 4years. In a review on epidemiology of mucormycosis in India, has reported the rising incidence of the disease in India, exceptionally higher than regional and international statistics.[12] The relatively high prevalence of poorly controlled diabetes in India, may be one of the reasons for this statistical deviation compared to reports from the rest of the region. Additionally, the widespread use of glucocorticoids, especially during COVID-19, and environmental factors favoring disease transmission may have played a role as well.

The middle-aged adults and male predominance of our patient cohort align with the international data[9,11-15] suggesting a comparable, predominant disease trend among middle-aged adults worldwide. The gender predominance may be attributed to both a higher diabetes burden and increased

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environmental exposure to fungal spores among men.

In our study, Rhino-orbito-cerebral mucormycosis constituted 94.2% of cases, which is substantially higher than reported globally. Rhino-orbito-cerebral mucormycosis is widely recognized as the most common type of mucormycosis due to the site of entry of the organism. However, the number of cases seen in our cohort is still higher than reported elsewhere in literature. International data show lower frequencies. Allaw et[10] al. reported 74.4% of Rhino-orbito-cerebral mucormycosis in their patient cohort, while a meta-analysis of 66 studies over a period of 62 years has reported Rhino-orbito-cerebral mucormycosis in 75.2% of cases.[16] Some other studies have reported even lower rates of Rhino-orbito-cerebral mucormycosis. For example, 67.1% reported by A. Petal and colleagues in a multicenter study from India 2019[17] and 58.2% by Petal et al in 2021.[15] Pulmonary mucormycosis has been reported as a predominant form (~22%), followed by Rhino-orbito-cerebral mucormycosis (~20%) in a review by Alqahiri et al. [18] Data from Middle East reflects a different distribution, with cutaneous mucormycosis being most prevalent (27.2%), followed by localized sinusitis (21.21%), while pulmonary and Rhino-orbito-cerebral mucormycosis each accounted for 18.1% of cases[9]. These findings accentuate a distinctly skewed clinical distribution of Rhino-orbito-cerebral mucormycosis in this study, in comparison to the studies from neighboring regions and global cohorts. Several factors may explain this divergence, including a very high burden of poorly controlled diabetes in our region, differences in the patterns of healthcare access and a patient referral bias. Our tertiary level center frequently receives cases of mucormycosis with disseminated ENT, orbital, and cranial involvement. Environmental exposure and post-COVID susceptibility may have additionally predisposed patients in our study population to Sino-nasal inoculation and subsequent rhino-orbito-cerebral mucormycosis.

Diabetes mellitus emerged as the predominant comorbid condition in this study's cohort, aligning with the trends reported worldwide. However, the magnitude of contribution of diabetes to the disease burden varied for different regions. In our population, nearly all diabetic patients had poor glycemic control, affirming it as the leading predisposing factor for mucormycosis as documented in literature.[19] Comparable studies from Ghavami et al. reported diabetes in 76.5% of cases[13], Mishra et al, from India reported 87% of the study participants with mucormycosis, had poorly controlled diabetes.[20] A meta-analysis by Osaigboro et al, reported diabetes in 49.8%[16] and Abanamy et al, reported diabetes in 48% of the cases of mucormycosis in a multicenter study from KSA.[9] These regional differences, are likely influenced by disparities in metabolic health and healthcare availability . In contrast, studies from the western regions revealed results that are significantly different to our study cohort. In Europe, hematological malignancies are the most common risk factor for mucormycosis.[21] Hariprasath et al, described hematological malignancy as the most common risk factor for mucormycosis in Europe and the United States, ranging from 38% to 62%.[6] In another review, hematological malignancy has been recorded as a leading predisposing factor for mucormycosis in Europe i.e. 50% in France and 62% in Italy.[5] A French cross sectional prospective surveillance program on 550 patients reported hematological malignancies as the primary risk factor (65.1%), and diabetes only contributed to 7.5% of their patient population, [14] emphasizing that mucormycosis epidemiology mirrors local health burdens. Reports from Australia show 49% of the cases are in patients with hematological malignancies.[5]

Mortality in mucormycosis remains high globally and is strongly influenced by comorbidity profiles, diagnostic timelines, as well as sites of organ involvement. Mortality rates ranging from 25% to 87% have been reported with a clear predilection for higher mortality rates in disseminated disease involving the respiratory system.[8] Burak et al, documented a 48.4% one-year mortality and 46% overall mortality,[22] whereas a meta-analysis by Osaigboro et al, recorded 49.9% mortality associated with mucormycosis.[16] In a systematic review by L. Shamithra M. Sigera et al, mortality in untreated patients was 100%, whilst those treated with antifungal medications and having undergone surgical intervention, the mortality rates decreased

by 25.7% and 21.8% respectively.[23] These findings indicate that despite advances in antifungal therapy and surgical debridement techniques, outcomes still remain poor, particularly in settings with delayed presentation or advanced underlying illness. The high burden of poorly controlled diabetes in our study population likely contributes significantly to both disease susceptibility and adverse outcomes. A broad spectrum of clinical, laboratory, and host-related predictors of mortality has been consistently recognized in the literature, thus reflecting the diverse nature of disease presentation in mucormycosis. Phenotypes with disseminated infection at presentation, renal impairment, central nervous system involvement, and poor response to antifungal therapy are major determinants of mortality.[8] Abdulkadir et al. demonstrated that involvement of the orbit (HR 2.0), intracranial extension (HR 2.6), ICU admission (HR 6.4), poor glycemic control (HR 2.3), and multiple comorbidities (HR 1.6) independently predicted fatal outcome,[24] underscoring the combined influence of disease burden and host physiology. Burak et al. further highlights the prognostic value of immune-inflammatory markers, with non-survivors exhibiting significantly lower lymphocyte counts, markedly elevated neutrophil–lymphocyte ratios, and substantially higher rates of ICU admission and the need for mechanical ventilation. COVID-19–associated mucormycosis also showed disproportionate mortality rates, attributed to profound immune dysregulation.[22]A study by Amina Al-Jardani et al, reported a statistically significant association between COVID-19 status and patient survival (p = 0.024), and no correlation with age or diabetes control.[25]

Ghavami et al described rising age (P-value= 0.037), platelet count (P-value=0.006), C-reactive protein (CRP) levels (P-value= 0.001), and treatment duration (P-value= < 0.001) were significantly associated with higher mortality rates while the association of diabetes control with mortality in their study population remained insignificant. [13] Our findings align closely with the evidence reported in the studies mentioned above. Laboratory markers, such as CRP and neutrophil levels, were markedly higher in our study cohort supporting prior studies by Ghavami et al[13] and Mishra et al, [20] highlighting the prognostic relevance of inflammatory markers.

To conclude, both the published literature and findings from our cohort demonstrate that mortality in mucormycosis is driven predominantly by markers of severe disease, systemic complications, immune-inflammatory dysregulation, and the severity of the disease, which necessitates intensive care. These findings reinforce the importance of early recognition, aggressive and timely management of high-risk patients with clinical features suggesting aggressive disease, to improve survival rates. It is also vital to recognize the fact that prognostic predictors such as demographic and metabolic variables have a diverse influence worldwide, especially diabetes control which has been found to be a main player as a risk factor in Southeast Asia and showed strong impact on prognosis.

There are certain limitations to our study**.** Being a single center study, findings may not be observable or generalizable to other regions of Pakistan, where referral patterns, microbiological profiles, or healthcare infrastructure may be dissimilar to this center. Although consistent with the rarity of the disease, the small sample size limits statistical power, particularly in subgroup analyses. Reliance on documentation may have resulted in incomplete symptom reporting and limited assessment of certain clinical variables. Outcomes were recorded only at discharge; hence, recurrence, delayed complications, and long-term mortality could not be assessed.

Conclusion

This 10-year review highlights that mucormycosis in our region predominantly affects middle-aged individuals with poorly controlled diabetes and predominantly presented as rhino-cerebral disease. Despite surgical intervention, mortality remained substantially high and was strongly associated with markers of severe disease, particularly ICU admission, intracranial extension, septic shock, and elevated inflammatory markers. These findings emphasize the urgent need for early and accurate diagnosis. Furthermore, aggressive glycemic control, timely surgical debridement, and escalation of care for high-risk patients should be included in the management guidelines for mucormycosis, to help reduce preventable mortality.

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Declarations

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Conflict of Interest / Competing Interests
The authors declare no competing interests.

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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

Vasorelaxant Effect of Metformin in Human Umbilical Artery In Vitro

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ABSTRACT

Objective: To evaluate the effect of metformin on the vasomotion of human umbilical artery (HUA) in vitro.

Study Design: Experimental study (Ex-vivo Pharmacodynamic study).

Place and Duration of the Study: Department of Pharmacology of Azra Naheed Medical College - Superior University, and Combined Military Hospital, Lahore from Nov 13, 2024 to Apr 30, 2025.

Materials and Methods: A total of 24 HUA samples were obtained from 24 different donors to ensure biological independence, and were divided into 4 groups with a sample size of 6 in each. Fresh HUA tissues with intact-endothelium, and without endothelium were suspended in Kreb's solution, pH 7.4. A force transducer (MLT0420) connected with an amplifier (AD Instruments Bridge Amps) was used to record isometric tension. The vasorelaxant responses of HUA to metformin (1-20 µM/l) were noted. In another setup the responses of metformin in KCl (60µM) precontracted HUA rings were recorded. The response of KCl + PGE_2 were taken as standard.

Results: The effect of metformin (1-20 µM/l) on HUA with intact endothelium showed significant relaxation ($p<0.0001$) IC_{50} was 7.031. The HUA ring with intact endothelium presented with better relaxation than rings without endothelium. The IC_{50} were 7.031 and 0.6821 respectively. In case of KCl (60µM) pretreated HUA rings, the findings of metformin and PGE_2 were comparable showing IC_{50} 5.739 and 6.248 respectively.

Conclusion: Metformin with KCl exhibited the strongest vasorelaxation followed by PGE_2. Metformin alone without endothelium had comparatively weaker relaxation. These findings highlight the significant efficacy of metformin under vasoconstrictive stress. It has potential to protect fetoplacental circulation during GDM pregnancies.

Key Words*: Endothelium, Human Umbilical Artery, Metformin, PGE2, Vasorelaxation, Verapamil.*

Introduction

Diabetes mellitus is primarily classified into two distinct etiologies: Type 1 (T1DM), characterized by an autoimmune-mediated destruction of pancreatic beta-cells leading to absolute insulin deficiency, and Type 2 (T2DM), a complex metabolic disorder involving peripheral insulin resistance and progressive secretory dysfunction.[1] Gestational diabetes mellitus (GDM) is the form of diabetes in which there is abnormal or uncontrolled rise in blood sugar levels during pregnancy, compromising the health of both fetus and mother. 2 In the past, diabetes was diagnosed in adulthood but it is now increasingly observed in children as well.[3]

During pregnancy, placental hormones induce insulin resistance that is most pronounced in the last trimester. [4] Glucose Tolerance Test (OGTT) with glycated hemoglobin (HbA1c) are used as diagnostic tests. The normal level of HbA1c is < 5.7%, whereas the value ≥ 6.5% is indicative of GDM. [5]

The global prevalence of GDM is estimated at approximately 14% according to a World Health Organization (WHO) report. The prevalence of GDM in Pakistan is 16.7% and, the Middle East countries have about 27.6%.[6,7] Family history, advanced maternal age, low socioeconomic status, illiteracy, multiparity and obesity are the risk factors of GDM. [8]

The growing fetus has a high energy demands and utilizes glucose at an average rate of 6 mg/kg/min, which is nearly three times the adult requirement of 2 mg/kg/min. The increased fetal requirement is regulated by transplacental transfer from the mother to the fetus during normal pregnancy.

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Consequently, the fetal blood glucose levels are directly proportional to maternal glycemic levels. [9]

GDM can lead to serious complications such as obesity, increased rate of pre-eclampsia, pregnancy induced hypertension (PIH), antepartum hemorrhage, cesarean births in mothers as well as results in newborns, such as large for gestational age, macrosomic babies and hypoglycemia in neonates. Infants of mothers with GDM are much prone to have severe chemical imbalances, such as low serum calcium and magnesium levels.[10]

In cases where lifestyle modifications, including medical nutrition therapy and physical activity, fail to achieve glycemic targets, insulin therapy is indicated to optimize maternal-fetal outcomes and mitigate gestational complications.[11,12]

Metformin, a biguanide is used for T2DM. Its main mechanism is to activate AMP activated protein kinases, which in turn reduces hepatic glucose production. Metformin is preferred over insulin in GDM because it passes through placental barrier via organic cation transporters.[13] In addition to antidiabetic effects, metformin has well established antioxidant role in preclinical animal models. [14,15] However, a significant knowledge gap persists regarding the translatability of these pleiotropic effects in human clinical cohorts. This study was designed to bridge this gap by evaluating the vasoactive effect of metformin on human umbilical artery (HUA). The aim of this study was to compare the vasodilator effect of metformin against potassium chloride (KCl) induced vasoconstriction whereas the vasorelaxant effect was compared against standard vasodilator on HUA of pregnant patients.

Materials and Methods

Ex-vivo experimental study was carried out in the Department of Pharmacology of Azra Naheed Medical College - Superior University, CMH Medical College and Institute of Dentistry, Lahore from Nov 13, 2024 to Apr 30, 2025. Ethical approval was taken from the Board of Advanced Studies & Research (BASR) Superior University vide Ltr. no. Acad/BASR/42/2024 dated 13th November, 2024; and Ethical Review Committee, CMH Lahore Medical College vide Ltr. No. 73/ERC/CMH/LMC dated 23rd September 2024.

A sample size of 6 blood vessels for each group was calculated using data of similar studies where sample size varied from 6-8 blood vessels.[16] Fresh samples were taken from the Obstetric Units of CMH Lahore, and CMA Teaching Hospital and Research Institute, Azra Naheed Medical College. A total of 30 healthy pregnant women of 18-35 years having full term pregnancy were enrolled, out of which 24 best samples were included in the study. Pregnant females, having PIH, cardiovascular, respiratory, renal & liver diseases were excluded. Consent was taken during admission according to Declaration of Helsinki. A 10 cm piece of umbilical cord from the portions for biological disposal were taken. The samples were placed in modified Kreb's solution at a temperature of 37°C and instantly transported to the Laboratory of Pharmacology Department. A piece of 3cm was taken, Wharton's jelly, connective and adipose tissues were cleaned from the HUA rings. The HUA ring was used with intact endothelium. A piece of HUA ring was denuded by passing a thread through the lumen.[17]

For the solutions for tissue bath system, the research grade chemicals were procured from the market. Kreb's solution was used in the organ bath where HUA ring was placed. All the salts were dissolved in distilled water and the following concentration (mM) were achieved: KCl 4.7, NaCl 118.3, KH2PO4 1.2, NaHCO3 25, glucose 11, MgSO4 1.2 and CaCl2, 2.5mM. The solutions were held at < 40 ºC, pH was adjusted at 7.4 with NaOH and HCl.[18]

The tension exerted on the HUA rings was determined by the HUA ring, that was dipped in the organ bath and suspended between two parallel stainless-steel wires. The tension exerted by the rings was measured through isometric transducers (MLT0420) to record isometric tension. An analog digital converter platform installed on a computer was connected with an amplifier (AD Instruments Bridge Amps) linked with a transducer. The organ bath solution was replaced after every 15 min during the rest periods. A 10 ml of Kreb's solution with a continuous oxygen in a carbon-oxygen mixture (95% O2;5% CO2) at a temperature of 37°C was used. After every 15 minutes of suspended artery rings, the new solution was added. Contractions were recorded after stabilization of human umbilical artery rings. Isometric contraction of the tissue was recorded using the force displacement transducer connected

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to a Power Lab data acquisition system. [18]

Total number of patients were divided into four groups. For Group I, tissue was stabilized in normal Kreb's solution and metformin (1-20 µM) was used to develop graded dose-response relationship with HUA (intact endothelium) rings. For Group II, tissue was stabilized in normal Kreb's solution and vasorelaxant effect of metformin (1-20 µM) was observed on HUA (denuded endothelium) rings. For Group III: Tissue of HUA (intact endothelium) ring was pretreated with KCl (60mM) and vasorelaxant effect of metformin was observed. For Group IV the HUA rings were pretreated with KCl (60 µ M) and graded dose response relationship of prostaglandin E_2 was observed.

GraphPad Prism version 9.0 was used for statistical analysis. After analyzing the data, through non-linear regression, fit curves were achieved. The effects of metformin, and PGE_2 on HUA ring were evaluated by applying row statistics to get IC_{50} values. Degree of freedom explored R-squared (R^2) for Good Fit values showing the reliability of concentration-response relationship.

Results

Metformin had graded dose response relationship in HUA ring and reduced basal tone by up to 76.34% ($p<0.0001$). The R-squared value was 0.5906 (Table I & II). Nonlinear regression analysis showed value of 7.031 with 95% confidence interval (CI). When metformin (1-20 µM) was used in HUA ring with denuded endothelium; the vasorelaxation response was 28%, and IC_{50} of 0.6521. The R-squared value was 1.787, showing good curve fit on GraphPad Prism (Table II)

To get the response of metformin with intact endothelium for pharmaco-mechanical excitation-contraction coupling (ECC), the smooth muscle contraction of HUA was induced with KCl (60µM). Metformin showed relaxation response on HUA when its increasing concentrations were added in the presence of high KCl (60µM) showing CI 95% value of 7.135%, ($p<0.0001$) and R squared value of 0.8291 (Table.II).

Graded dose response relationship was carried out on isolated HUA ring. PGE_2 produced vasorelaxation under precontracted KCl (60µM). One-way ANOVA followed by post hoc Tukey's multiple comparison test was used. There was significant difference between baseline and higher doses of PGE_2 ($p<0.05$). The maximum vasorelaxation achieved with metformin and PGE_2 in precontracted vessels with KCl were almost the same (Figure.1).

Table I: Dose-response relationship of drugs on isolated human umbilical artery (n=24)

µM/l	Metformin (intact endothelium) (n=6)	Metformin (without endothelium) (n=6)	KCl + metformin with (intact endothelium) (n=6)	KCl + PGE_2 (intact endothelium) (n=6)
1	11.07914	2.56712	7.36435	8.93425
4	24.18774	13.08751	22.67973	22.7087
8	36.98836	20.14684	37.9108	45.32477
12	46.85018	20.79666	53.09538	50.41856
16	61.74670	21.89781	71.6646	63.60850
20	76.34016	28.4789	78.98972	78.13234

Table II: Non-linear regression analysis for comparison of effects of metformin (intact and denuded endothelium) with KCl and PGE_2 on HUA in vitro (n=24)

Parameters	Metformin with Intact Endothelium (n=6)	Metformin without Endothelium (n=6)	KCl + Metformin with Intact Endothelium (n=6)	KCl + PGE_2 with Intact Endothelium (n=6)
95% CI* IC_{50}	7.031	0.6821	5.739	6.248
P-Value	<0.0001	<0.0001	<0.0001	<0.0595
R-Squared	0.5906	-1.787	0.8291	0.6185
* CI: Confidence Interval				

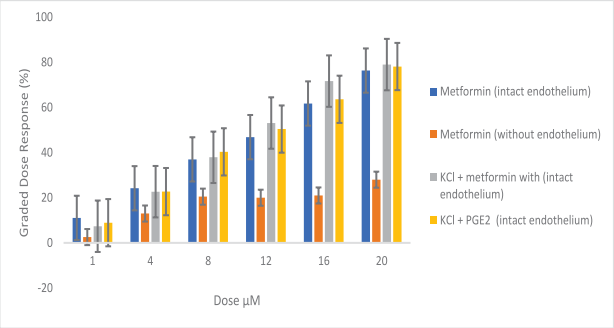

Figure 1: Vasorelaxant Effect of Drugs on Human Umbilical Artery Ring (n=24)

Discussion

It was found that metformin produced dose dependent direct vasorelaxation in isolated HUA (In-Vitro). Metformin has marked vasodilator effect on systemic blood vessels as well as coronary blood vessels. Current studies show that metformin reduces the burden of ischemic heart diseases (IHD) by its beneficial effect on vascular smooth muscle cells (VSMCs). [20,21]

Metformin administered alone produced dose-dependent vasorelaxation with an IC_{50} of 7.03 µM and a moderate goodness of fit ($R^2 = 0.59$). In another

study on human internal mammary arteries from cardiac patients, metformin at 10 µM reversed angiotensin 2-induced endothelial dysfunction. This effect of metformin is comparable to the effect of metformin on HUA in the present study.[22] Relaxation of HUA rings in the presence of intact endothelium is caused by multiple endothelial mediators such as NO, PGI_2, and endothelium-derived hyperpolarizing factors (EDHFs). Metformin improves vascular endothelial function by activating 5-adenosine monophosphate activated protein kinase (AMPK), increase nitric oxide synthesis, and inhibit cardiac remodeling and cardiac fibrosis. Nitric oxide causes vasodilation through cGMP-dependent pathway. [23] It also works through cGMP independent pathway by S-nitrosylation of proteins and activate calcium pumps. Prostacyclin I_2 released from vascular endothelial cells causes vasodilation by reducing Ca^{2+} concentration in the vascular smooth muscle cells.[24]

The metformin induced relaxation in the present study suggests the modulation of ion channels and reducing intracellular Ca^{+2} in smooth muscle through voltage-gated Ca^{+2} channels. [25] Another possibility is metformin has physical action on L-type Ca^{+2} channels and reduce calcium influx as we have seen with nifedipine, verapamil, and diltiazem. These findings suggest metformin has both direct action through the release of endothelial mediators as well as indirect action by modulation of calcium and potassium channels. These findings are consistent with those reported by Sahinturk S et al., [26] in a study demonstrating effect of metformin on thoracic artery in rats.

Metformin in the presence of KCl (60 µM) exhibited more potent and dose dependent vasorelaxation response, replicated with IC_{50} value of 5.74 µM and a markedly higher R^2 (0.83). These findings are suggestive of therapeutic effectiveness in relieving pathophysiological vasoconstrictive ailments such as GDM. This effect of metformin is similar to the vasorelaxant effect of nifedipine in HUA of pre-eclampsia patients.[27]

Higher extracellular KCl changes the membrane potential of cells, and causes persistent depolarization. Depolarized membrane inhibits the influx of Ca^{++} as well as disrupt the internal release and sequestration system that controls contractile proteins such as actin and myosin. In the present setup metformin activated membrane depolarization and voltage gated calcium channels activation; consequently, the stronger relaxation seen with metformin is suggestive of interference with calcium influx or calcium dependent contractile mechanism.[28] A similar hypothesis is proven by a recent study on human and mouse mesenteric arteriole, where metformin induced vasorelaxation through endothelium-dependent hyper-polarization.[29]

Conclusion

Metformin revealed more vasorelaxation with intact endothelium than denuded HUA rings suggesting endothelial-dependent mechanisms are involved. In the presence of KCl induced contractions with intact endothelial tissues of HUA, metformin exhibited the strongest vasorelaxation followed by PGE_2. Metformin has the potential to protect fetoplacental circulation during GDM pregnancies.

The study limits in elaborating direct measurements of biochemical markers like endothelial mediators (NO, PGI_2, K^+ channels, L-type Ca^{++} channels, etc.) to assess NO levels, gene expression, oxidative stress markers, prostaglandin levels etc.

Conflict of Interest: None

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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.

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ORIGINAL ARTICLE

Association of Serum Calcium and Vitamin D Levels with Genitourinary Microbiological Shifts in Post-Menopausal Women: A Tertiary Care Center Study

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ABSTRACT

Objective: To assess serum calcium and vitamin D levels and identify their relationship with genitourinary microbiological changes in post-menopausal women visiting a tertiary care center.

Study Design: Cross-sectional, observational.

Place and Duration of Study: This study was done at Sadaf Yahya Hospital, a tertiary care center in Daska, Pakistan, between 3rd March 2025 and 29th August 2025.

Material and Methods: There were 217 post-menopausal women (over the age of 45 years) enrolled through non-probability consecutive sampling. Serum vitamin D and Serum calcium levels were measured. The vaginal flora was assessed using high vaginal swabs, followed by Gram staining with Nugent scoring and aerobic and anaerobic culture techniques. Data were analyzed using SPSS version 29. The Spearman's rank test was used to assess the associations. The level of statistical significance is taken at p-value below 0.05.

Results: The mean age of the participants was 54.0 years. 52.1% of the women were found to be deficient in vitamin D, and 68.7% of the women had low calcium levels in their serum. Bacterial vaginosis (Nugent score 7-10) was identified in 34.1% of participants. A significant association was found between vitamin D status and Nugent score categories ($p < 0.001$). Both vitamin D deficiency ($p<0.001$) and low calcium levels ($p=0.001$) were significantly associated with alterations in vaginal flora (as indicated by Nugent score). Serum vitamin D ($r=-0.509$) and calcium levels ($r=-0.199$) showed significant negative correlations with Nugent score ($p<0.001$).

Conclusion: Low serum vitamin D and calcium levels are significantly associated with altered vaginal flora consistent with bacterial vaginosis in post-menopausal women. However, further longitudinal and interventional studies may be done before clinical screening recommendations are made.

Key Words: *Bacterial Vaginosis, Calcium, Menopause, Vaginal Microbiota, Vitamin D.*

Introduction

Post-menopausal phase is characterized by a series of different psychological, physical, and emotional changes in a woman. Females undergoing menopause experience physiological changes that not only affect the genitourinary anatomy but also influence mucosal immunity and microbial balance of the genitourinary area.[1] Altered hormonal balance, especially a lack of estrogen, leads to mucosal thinning and an altered epithelial barrier function. This eventually leads to dryness in the vaginal area, painful intercourse, and repeated urinary tract infections (UTI). The constellation of these symptoms is also referred to as the genitourinary syndrome of menopause (GSM).[2]

Along with hormonal changes, some minerals and vitamins may also influence the female genitourinary environment. Notably to mention here are calcium metabolism and vitamin D status, which are believed to influence epithelial integrity. The role of Vitamin D is believed to be due to receptors that are located across urogenital tissues. These receptors play a role in regulating antimicrobial peptides and tight-junction proteins.[3] Serum calcium is believed to have a role in epithelial cell signaling and muscular activity. Deficiency of these factors can lead to shifts in vaginal and urinary microbial flora. These shifts make an individual

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susceptible to genitourinary infections and inflammation.[4]

With a global rise in life expectancy globally, the burden of genitourinary morbidity in older women is substantial and growing as life expectancy rises. Recurrent UTIs and GSM impair quality of life, increase healthcare utilization, and drive antibiotic exposure, with important public-health consequences including antimicrobial resistance. Previous microbiological studies demonstrate that post-menopausal vaginal microbiological composition shows increased diversity and reduced Lactobacillus dominance compared with premenopausal states, patterns associated with symptomatic disease and recurrent cystitis.[5] In parallel, epidemiological and meta-analytic data suggest associations between low serum vitamin D and increased risk of UTIs and lower urinary tract symptoms, mediated via immune modulation and mucosal barrier effects; professional societies note high prevalence of hypovitaminosis D in older women and call for targeted evaluation.[6] These international results indicate that micronutrient status and the microbial flora patterns are modifiable factors that may help to decrease the risk of infection and enhance genitourinary prognosis.[7]

Regionally, particularly in South Asia, data on the knowledge and information about the interaction of vitamin D, calcium, and genitourinary microbial flora in post-menopausal women has not been limited.[8] Pakistan as well as other neighboring countries' population studies report high rates of vitamin D deficiency in all age groups, including middle-aged and elderly women, but regular tests of vitamin D or calcium in women with recurring urinary symptoms are not a common practice.[9] Local microbiological practice often focuses on culture-based pathogen identification and antibiotic susceptibility without integrating culture-independent investigations or host nutritional status. This gap in regional evidence and clinical practice creates missed opportunities to understand context-specific drivers of recurrent UTIs and GSM.[10]

Given these gaps, our study evaluated serum calcium and 25-hydroxyvitamin D concentrations, along with genitourinary microbiological assessment using Nugent scoring and culture in postmenopausal women at a tertiary care center. We aimed to clarify whether low vitamin D levels or altered calcium levels are associated with vaginal microbiological changes and/or a higher infection burden. We hoped that the findings could inform targeted screening, non-antibiotic preventative strategies (nutritional optimization, topical/systemic vitamin D approaches, probiotic or estrogenic therapies), and stewardship-friendly care pathways that would reduce morbidity and antibiotic exposure.

Materials And Methods

This cross-sectional observational study was conducted at the Sadaf Yahya Hospital, a tertiary care center in Daska, Pakistan from 3rd March 2025 till 29th August 2025, having received approval from the Institutional Review Board (IRB) under reference number SYH-IRB/2025/125. Based on extensive literature search, a sample size of 217 participants was calculated using the world health organization (WHO) sample size calculator. It comprises 5% margin of error, confidence level of 95% and prevalence rate of bacterial vaginosis in post-menopausal women as 16.93%.[11] The sampling was performed through a non-probability convenience method of sampling.

All women aged 45 years or older with natural menopause for at least 12 months were eligible to participate in this study.

Any female with history of antibiotics, antifungals, probiotics, or hormone replacement therapy intake within the past four weeks of sampling was excluded from the study. Women with history of chronic illnesses such as kidney disease, endocrine diseases, malabsorption syndromes, immunosuppressive therapy and those who were not willing to give informed consent were also excluded.

All patients were enrolled with prior written permission. Confidentiality of patients was ensured at all levels. Data were collected in the outpatient clinics on pre-approved forms via direct consultation. Demographic details (age, BMI, parity), lifestyle (dietary calcium consumption, sun exposure, supplementation), and medical history (duration of menopause, comorbid conditions such as diabetes, genital symptoms such as vaginal dryness, itching, dysuria, discharge), and a history of recurring UTI were recorded in the interview questionnaire.

After the interview, all participants were sampled by

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venipuncture following strict aseptic technique. 5 mL of blood was collected in EDTA sampling tube. After separating out the serum by centrifugation, both serum 25-hydroxyvitamin D (25(OH)D levels) and serum calcium levels were measured. Serum Vitamin D levels were analyzed on Roche Cobas® 6000 Series by Electrochemiluminescence immunoassay technique using the Elecsys® Vitamin D total III reagent kit. Roche-provided reagent inserts indicate interpretation of 25(OH)D levels for vitamin D status assessment as: Deficient: < 20 ng/mL (< 50 nmol/L), Insufficient: 20–30 ng/mL (50–75 nmol/L), Sufficient: > 30 ng/mL (> 75 nmol/L), Potential High/Excess: > 100 ng/mL (> 250 nmol/L).[12]

Serum Calcium levels were also measured on the same analyzer by Photometric colorimetric assay using Calcium Gen.2 (CA2) reagent Kit. The typical Machine-Associated Reference Interval for serum calcium levels followed in our lab is as follows: Low (hypocalcemia): < 8.6 mg/dL (< 2.15 mmol/L), Normal: 8.6–10.2 mg/dL (2.15–2.55 mmol/L), and High (hypercalcemia): > 10.2 mg/dL (> 2.55 mmol/L).[13]

Vaginal microbiota was assessed using the Nugent scoring system. High vaginal swabs were collected from the posterior fornix, and a thin smear was prepared on a clean glass slide, air-dried, and heat fixed. Gram staining was performed using crystal violet (1 minute), Gram's iodine (1 minute), decolorization with acetone–alcohol (10–15 seconds), followed by counterstaining with safranin (30–60 seconds). Slides were examined under oil immersion at 1000× magnification, and 10–20 representative high-power fields were evaluated. Bacterial morphotypes were quantified semi-quantitatively, including large Gram-positive rods (Lactobacillus morphotypes), small Gram-variable rods (Gardnerella/Bacteroides morphotypes), and curved Gram-negative or Gram-variable rods (Mobiluncus morphotypes). A composite score ranging from 0 to 10 was calculated based on the relative abundance of these morphotypes. Interpretation was as follows: scores 0–3 were considered normal flora, 4–6 intermediate flora, and 7–10 consistent with bacterial vaginosis, according to CDC-referenced standards.[14]

Data was analyzed using SPSS version 29. The Shapiro-Wilk test was applied to check the normality of continuous data. Categorical data were presented as frequencies and percentages, while continuous data were presented as mean and standard deviation if normally distributed and median and IQR for non-normally distributed data. Chi-square test was applied to check the association between categorical variables. Pearson correlation was applied to check the correlation between continuous variables. Statistical significance was considered as a p-value less than 0.05.

Demographic and clinical variables, including BMI, diabetes status, sun exposure, and calcium supplementation, were recorded; however, these variables were not included as covariates in multivariable modeling and were analyzed descriptively.

Results

A total of 217 postmenopausal women were included in this study. The median age of the participants was 54.00 (50.00–59.00) years. The baseline demographic, clinical and microbiological characteristics of study participants are shown in Table-I.

The association between Nugent score and Vitamin D is shown in Table-II. A significant association was found between vaginal microbiota composition and Vitamin D status ($p<0.001$). This indicates that the prevalence of bacterial vaginosis decreased with improving vitamin D status, indicating a strong inverse association between serum vitamin D levels and Nugent score categories.

Table-III presents the association of vitamin D and calcium status with genitourinary microbiological shift among postmenopausal women. A statistically significant association was observed between vitamin D status and microbiological shift ($p < 0.001$). Among vitamin D deficient women, 51.3% exhibited microbiological shift, compared to 12.9% in insufficient and 18.2% in sufficient groups. Similarly, serum calcium status showed a significant association with microbiological shift ($p = 0.001$), with 40.9% of women with low calcium levels demonstrating microbiological shift compared to 16.2% among those with normal calcium levels.

The correlation of vitamin D and calcium with Nugent score is shown in Table-IV. Spearman´s rank correlation analysis showed a statistically significant negative correlation between serum vitamin D levels

and Nugent score (r = −0.509, 95% CI: −0.60 to −0.40, p < 0.001), indicating that lower vitamin D levels were moderately associated with higher Nugent scores and greater vaginal microbiological imbalance. Similarly, serum calcium levels showed a significant inverse correlation with Nugent score (r = −0.199, 95% CI: −0.32 to −0.07, p < 0.001), suggesting that low calcium levels were associated with increased severity of vaginal microbiological imbalance.

Table I: Baseline Demographic, Clinical and Microbiological Characteristics of Postmenopausal Women (n = 217)

Variables		Median, IQR
Age (years)		54.00 (50.00–59.00)
Menopausal Duration (years)		5.00 (1.00–9.00)
Parity		3.00 (2.00–5.00)
Nugent Score		5.00 (4.00–7.00)
		Mean ± SD
BMI (kg/m²)		26.62 ± 3.80
Vitamin D (ng/mL)		19.36 ± 6.40
Calcium		8.27 ± 0.39
		n (%)
Diabetes	No	150 (69.1%)
	Yes	67 (30.9%)
Vitamin D status	Deficient (<20 ng/mL)	113 (52.1%)
	Insufficient (20–29 ng/mL)	93 (42.9%)
	Sufficient (≥30 ng/mL)	11 (5.1%)
Calcium Status	Low	149 (68.7%)
	Normal	68 (31.3%)
Nugent Category	Normal (0–3)	32 (14.7%)
	Intermediate (4–6)	111 (51.2%)
	BV (7–10)	74 (34.1%)
Microbiological shift	No	143 (65.9%)
	Yes	74 (34.1%)
Lactobacillus Dominance (Lactobacillus spp.)	No	185 (85.3%)
	Yes	32 (14.7%)
BV Associated Anaerobes *(Gardnerella, Prevotella, Mobiluncus)*	No	140 (64.5%)
	Yes	77 (35.5%)
Uropathogens *(E. coli, Klebsiella, Enterococcus, Proteus)*	No	152 (70.0%)
	Yes	65 (30.0%)

Table II: Association between Vitamin D Status and Nugent Score Categories (n=217)

Vitamin D Status	Nugent Score Category			Total	p Value
	Normal	Intermediate	BV		
Deficient	4 (3.5%)	51 (45.1%)	58 (51.3%)	113 (52.1%)	**<0.001**
Insufficient	29 (31.2%)	52 (55.9%)	12 (12.9%)	93 (42.9%)	
Sufficient	3 (27.3%)	6 (54.5%)	2 (18.2%)	11 (5.1%)	
Total	**36 (16.6%)**	**109 (50.2%)**	**72 (33.2%)**	**217 (100%)**	

Table III: Association of Vitamin D and Calcium Status with Genitourinary Microbiological Shift (n=217)

Variables		Microbiological Shift		*p*-value
		No n (%)	Yes n (%)	
Vitamin D Status	**Deficient**	55 (48.7%)	58 (51.3%)	**<0.001**
	Insufficient	81 (87.1%)	12 (12.9%)	
	Sufficient	9 (81.9%)	2 (18.2%)	
Calcium Status	**Low**	88 (59.1%)	61 (40.9%)	**0.001**
	Normal	55 (80.9%)	13 (19.1%)	

Table IV: Correlation of Serum Vitamin D and Calcium Levels with Nugent Score (n=217)

Variable	Nugent score		
	r	CI	p Value
Vitamin D	−0.509	−0.60 to −0.40	**<0.001**
Calcium	−0.199	−0.32 to −0.07	**<0.001**

Discussion

This cross-sectional study demonstrates a significant association between serum vitamin D and calcium levels with genitourinary microbiological imbalance among postmenopausal women. Our findings indicate that lower vitamin D and calcium levels are associated with higher Nugent scores, increased prevalence of bacterial vaginosis (BV), and loss of Lactobacillus dominance. The existence of such associations is becoming biologically plausible, as supported by international literature.[15] It has been demonstrated by several studies that vitamin D is very important in supporting mucosal immunity via the regulation of antimicrobial peptides like cathelicidin and defensins, which are needed to maintain Lactobacillus-dominant vaginal ecosystems.[16] Calcium may also contribute to epithelial barrier integrity and cellular signaling pathways that influence mucosal defense mechanisms within the genitourinary tract.[17]

Neugent et al. proved that a postmenopausal estrogen deficiency with a change in host immunity predisposes to BV-related anaerobes, which further predispose to genitourinary infection.[18] Similarly,

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Hugenholtz et al. established that the abundance of Lactobacillus is lower in postmenopausal women with or without symptomatic infection, highlighting the importance of systemic host factors including micronutrient status.[19] Our results of high negative correlation between the vitamin D level and the Nugent score (r = -0.509) are consistent with meta-analytical findings of vitamin D deficiency with a higher risk of BV and UTIs.[20,21] Although calcium has received comparatively less research attention in relation to genitourinary microbiota, emerging evidence suggests that it plays an important role in epithelial cell signaling, tight junction stability, and mucosal barrier integrity.[22] These mechanisms may influence the maintenance of a healthy vaginal microbiological environment. The relatively high proportion of low serum calcium observed in this study may reflect regional nutritional patterns and laboratory-specific reference ranges and therefore should be interpreted cautiously.

Regionally, there are limited data treating micronutrient status and genitourinary microbiota in postmenopausal women. Pakistani and South Asian studies continue to indicate that vitamin D deficiency is commonly found among women, in some cases reaching over 50% and this is similar to our results, where 52.1% of the participants were vitamin D deficient.[23] Nevertheless, the majority of regional research concentrates on bone health or metabolic health, and in most cases, it pays little attention to urogenital health.[24] Local microbiological research remains mostly based on culture-based detection of pathogens without the addition of Nugent scoring and host nutritional parameters. The gap that our research fills is the inclusion of biochemical, microbiological, and clinical evidence to provide region-specific data showing that micronutrient deficiencies can contribute to genitourinary dysbiosis in postmenopausal women.

Overall, this study demonstrates that lower serum vitamin D and calcium levels were associated with higher Nugent scores and increased prevalence of microbiological imbalance among postmenopausal women. Evaluation of micronutrient status, including both vitamin D and calcium, may be considered in future research investigating genitourinary health in postmenopausal women reporting with recurring genitourinary symptoms by determining modifiable systemic variables associated with the presence of microbiological imbalance. These findings highlight the importance of considering host nutritional status alongside microbiological findings when evaluating postmenopausal women presenting with recurrent genitourinary symptoms. On the level of public health, these interventions might minimize frequent infections, exposure to antibiotics, and the burden of antimicrobial resistance.

Limitations

There are some limitations to this study. As a cross-sectional, single-center study, causal associations cannot be drawn, and the results may not be applicable to all groups. Potential confounders such as dietary calcium intake, sunlight exposure, and metabolic comorbidities were not controlled through multivariable analysis, which may influence micronutrient levels and microbiological composition. Also, no molecular microbiome sequencing was carried out, which would have given more profound insights into microbial diversity and functional modification.

Conclusion

In conclusion, low serum vitamin D and calcium levels are potentially associated with genitourinary microbiological dysbiosis in post-menopausal women. These findings highlight a potential association between micronutrient status and vaginal microbiological dysbiosis and warrant further multicenter and interventional studies.

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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 and Neonatal Outcomes Following Elective Versus Emergency Repeat Cesarean Section in Women with One Previous Cesarean Delivery: A Comparative Cohort Study

Nadia Naureen, Farah Asghar, Fardous Alshangiti, Mona Al Airan, Rafad Nizar B Abdu, Shahad Essa Alrehaili

ABSTRACT

Objective: To compare maternal and neonatal outcomes between elective and emergency repeat cesarean section in women with previous cesarean delivery.

Study Design: Retrospective cohort study

Place and Duration of Study: The study was conducted in the obstetrics and gynecology unit of National Guards hospital Madinah Munawara, KSA from July 2025 to December 2025.

Materials and Methods: A total of 130 women with one previous cesarean section who underwent repeat cesarean delivery were included in the study. Among them, 65 underwent elective cesarean section and 65 chose trial of labour as their mode of delivery but later underwent emergency cesarean section due to complications arising during labour. Maternal outcomes assessed included uterine tears, postpartum hemorrhage, blood transfusion, bladder injury, puerperal wound infection, thrombosis, maternal death, and prolonged hospital stay. Neonatal outcomes included respiratory distress, aspiration of liquor, perinatal death, low Apgar score, and NICU admission. Demographic variables including maternal age, BMI, diabetes, parity, and fetal weight were also analyzed. To determine the odds of adverse outcomes between emergency versus elective caesarean groups, odds ratios with confidence interval were calculated for all variables. Chi square test was applied to assess the association of maternal and fetal outcomes with emergency cesarean.

Results: Odds ratio in Table II and III showed that patients in group I (emergency cesarean) had higher odds for adverse maternal and neonatal outcomes in the emergency caesarean group compared with the elective group except maternal death due to zero cases in the elective group. while Chi square test (Table IV) showed significant association of maternal outcomes like uterine tears p=.027, and puerperal wound infection p=.015 while border line association of PPH p=.049, blood transfusion p=.049 and low Apgar score p=.05 with emergency cesarean group I.

Conclusion: These findings indicate that although emergency cesarean section may have a greater odd of adverse maternal and neonatal outcomes, only uterine injury, postpartum hemorrhage, need for blood transfusion, puerperal wound infection and low Apgar score related morbidity showed statistically significant association with emergency cesarean compared to elective cesarean.

Key Words: *Emergency Cesarean, Maternal Morbidity, Neonatal Morbidity, Previous Cesarean Delivery.*

Introduction

Cesarean section (CS), once avoided because of its historically high mortality rate little more than a century ago, has evolved into one of the most frequently performed surgical procedures worldwide. It currently accounts for approximately one in three births in the United States[1] and as many as four out of five deliveries in certain regions globally.[2] Women with a history of a single prior cesarean section constitute a substantial proportion of this increasing rate.[3]

Determining the optimal mode of delivery for women with one previous cesarean birth presents a complex clinical dilemma for both patients and healthcare providers. Neither elective repeat cesarean delivery nor a trial of labor after cesarean (TOLAC) is entirely devoid of risk. Consequently, a pregnant woman with a prior cesarean section is

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invariably considered high risk—not only from an obstetric standpoint, but frequently from a psychological perspective as well.[4]

Although a trial of labor after cesarean (TOLAC) is advocated for appropriately selected women with a single prior cesarean delivery, repeat cesarean section remains a common practice in subsequent pregnancies. Multiple factors have been identified as increasing the probability of cesarean delivery, including advanced maternal age (particularly over 40 years)[5,6] obesity with its attendant obstetric complications such as fetal macrosomia[7,8] and both gestational and pregestational diabetes mellitus.[5]

Despite its general safety in modern obstetrics, cesarean delivery is associated with greater maternal morbidity and mortality when compared with vaginal birth.[9] Moreover, the timing and urgency of the procedure play a critical role in determining maternal and neonatal outcomes. Evidence from several studies indicates that emergency cesarean sections are linked to higher rates of intraoperative and postoperative complications, as well as prolonged recovery periods, relative to elective procedures[10,11,12.] Despite extensive research, limited evidence specifically compares maternal and neonatal outcomes among women with one previous cesarean delivery. The present study was designed to evaluate and compare maternal and neonatal outcomes between elective and emergency repeat cesarean sections in women with a history of one prior cesarean birth. Through systematic analysis of this specific population, the study aims to clarify the relative risks associated with each approach and to inform evidence-based clinical decision-making and patient counseling.

Materials and Methods

This cohort study was conducted on women with one previous caesarean section who underwent repeat caesarean delivery between July 2025 and December 2025. Ethical approval for the study was obtained from the hospital's Ethical Review Board. The Institutional Review Board (IRB) number was NRM26/003/2. All participants were informed about the study and provided written informed consent prior to enrollment.

Patients were divided into two groups: Emergency caesarean section (Group I) and elective caesarean section (Group II). Patients in both groups had a history of one previous cesarean. Group I consisted of women who opted for trial of labour after caesarean (TOLAC) but subsequently required emergency caesarean section due to fetal distress or failure to progress in labour. Patients who delivered normally were not included in the study. Group II comprised women who directly opted for elective repeat caesarean section following one previous caesarean delivery. They did not experience any labour.

The sample size was calculated using G*Power version 3.1 for comparison of two independent proportions.[13] Assuming a 95% confidence level, 80% study power, and an equal allocation ratio (1:1), with expected complication rates of 35% in the emergency caesarean section group and 15% in the elective caesarean section group, the minimum required sample size was calculated to be 63 participants per group. After accounting for incomplete data, 65 patients were included in each group, resulting in a total sample size of 130. Initially 500 patients were enrolled with history of one previous cesarean were examined out of which 260 did not meet the inclusion criteria while 40 patients showed incomplete data so 300 patients were excluded from the study. The remaining 200 patients were divided in 2 groups.125 patients opted for trial of labour so included in group I. Remaining 75 patients were added in group II. In group I all patients waited for the labour to start naturally. Out of 125 patients 60 were delivered normally so excluded from the study and remaining 65 underwent emergency cesarean due to poor progress of labour or fetal distress. The final sample in group I was 65. In group II 75 patients opted for elective cesarean, out of which follow up of 10 patients could not be maintained so we were left with 65 patients in group II. Strobe diagram is shown in Figure 1. Participants in both groups were recruited using consecutive sampling until the required sample size for each group was achieved. Equal allocation (1:1 ratio) was selected to maximize statistical efficiency and facilitate comparison between groups. The inclusion criteria were: history of one previous caesarean section with a low transverse uterine incision, gestational age ≥37 weeks, singleton pregnancy, and a clinically adequate pelvis. Exclusion criteria included lack of antenatal care, fetal malformations,

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cephalopelvic disproportion, multiple gestations, previous uterine surgery for non-obstetric indications, fetal malpresentation, placenta previa, estimated fetal weight >4,000 g, and interpregnancy interval of less than 12 months from the previous caesarean delivery. Patients with a history of previous vaginal delivery or those with more than one prior caesarean section were also excluded. All patients were followed regularly during antenatal care and received detailed counseling regarding the risks and complications associated with TOLAC, along with the likelihood of successful vaginal birth. All patients in Group I provided informed consent for TOLAC. A Pfannenstiel incision was used in all caesarean sections across both emergency and elective groups.

Statistical analysis was performed using IBM SPSS Statistics version 16. Various intraoperative and postoperative maternal and neonatal outcomes were assessed. Maternal outcome variables included uterine tears, postpartum hemorrhage (PPH), blood transfusion, bladder injury, wound infection, prolonged hospital stay, and thrombosis. Neonatal outcomes included respiratory distress, aspiration of liquor, perinatal death, low Apgar score, and neonatal intensive care unit (NICU) admission. Hospital stay was classified as prolonged if it exceeded two days. Apgar score was considered low if it was less than 7 at 5 minutes after birth. Both variables were converted into categorical variables and coded as yes/no.

The Shapiro–Wilk test was performed to assess the normality of data distribution. To determine the odds of adverse outcomes between emergency versus elective caesarean groups, odds ratios with confidence interval were calculated. A p-value <0.05 indicated that the data was not normally distributed. A p-value ≤0.05 was considered statistically significant. Chi Square test was performed to study the association of maternal and fetal outcomes with emergency cesarean. A p-value ≤0.05 was considered statistically significant.

Results

Out of 130 patients, 65 (50%) underwent emergency caesarean section (Group I) and 65 (50%) underwent elective caesarean section (Group II). Regarding anesthesia, 27 patients (20.7%) received general anesthesia, of whom 20 (74%) belonged to the emergency group I and 7 (25.9%) to the elective group II. Spinal anesthesia was used in 103 patients (79.2%), including 25 (24.3%) emergency cases and 78 (75.7%) elective cases. Demographic characteristics are presented in Table I. Advanced maternal age (≥35 years) was more common in the emergency group (93.8%) than in the elective group (61%). Similarly, BMI >30 was more frequent in the emergency group (40%) compared with the elective group (27.6%).

Emergency caesarean section was associated with higher percentage of maternal morbidity compared with elective cesarean. Uterine tears occurred in 13.8% of emergency cases versus 3.1% of elective cases. Postpartum hemorrhage was observed in 16.9% versus 6.1%, blood transfusion in 18.5% versus 4.6%, and wound infection in 15.4% versus 1.5% in group I and II, respectively. Bladder injury occurred in 3.1% of emergency cases and 1.5% of elective cases. Thrombotic events were reported 3 (4.6%) and (1.5%) in group I and II respectively. One maternal death occurred in the emergency group (1.5%), while none occurred in the elective group. Neonatal complications were also more frequent in the emergency group. Respiratory distress occurred in 6.1% versus 3.1%, liquor aspiration in 6.1% versus 1.5%, and perinatal mortality in 3.1% versus 1.5% in emergency and elective groups, respectively. Low Apgar score was (12.3% & 1.5%). and NICU admission rates were (9% & 3%) in group I and II respectively.

All odds ratios (Table II&III) were greater than 1, indicating higher odds of adverse maternal and neonatal outcomes in the emergency caesarean group compared with the elective group except maternal death due to zero cases in the elective group. Similarly, the chi-square test (Table V) demonstrated significant association of uterine tears ($p = 0.027$) as well as puerperal wound infection ($p = 0.015$). Borderline significance was observed for postpartum hemorrhage ($p = 0.049$), blood transfusion ($p = 0.049$) and low Apgar score ($p = 0.057$).

Discussion

Caesarean section is a well-established obstetric surgical procedure performed to reduce maternal and fetal morbidity and mortality. Despite being classified as a major abdominal surgery, its global prevalence has increased considerably over recent

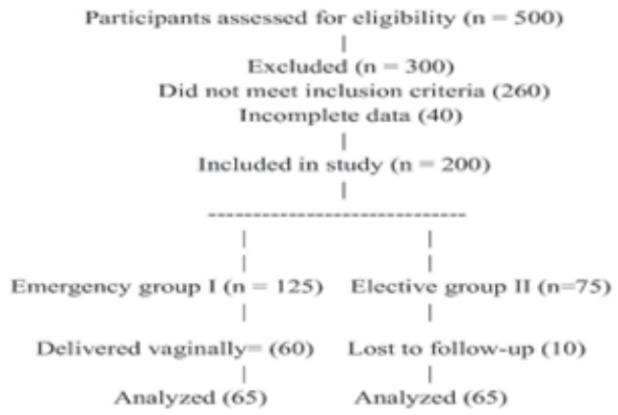

Figure 1: Strobe Diagram

Table I: Frequency Of Demographic Characteristics of Emergency and Elective Cesarean Groups

Demographic characteristics	Em CS N=65	Percentage	El CS N=65	Percentage
Maternal age < 25	4	6.15%	8	12.3%
Maternal age ≥ 35	61	93.8%	39	60%
Maternal BMI ≥ 30	26	40%	18	27.6%
Gestational diabetes	6	9.2%	7	10.7%
Fetal weight more than 3.5 kg	12	18.4%	6	9.2%

Table II: Odds Ratio of Maternal Complications

Complication	Odds ratio	Confidence interval
Uterine tears	1.83	1.354-2.483
PPH	1.613	1.143-2.27
Blood transfusion	2.071	1.615-2.656
Bladder injury	1.344	.592-3.308
Puerperal wound infection	1.788	1.300-2.458
Thrombotic event	1.524	.842-2.758
Prolonged hospital stay	1.72	.721-2.239
Maternal death	.000	.000

Table III: Odds Ratio of Fetal Complications

Complication	Odds ratio	CI
Respiratory distress	1.355	.749-2.453
Liquor aspiration	1.693	1.021-2.632
Perinatal death	1.344	.592-3.049
Low APGAR score	1.887	1.40-2.543
NICU admission	1.367	.832-2.247

Table IV: Chi Square Test in Emergency Cesarean Group

MATERNAL COMPLICATIONS	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Uterine tear	4.866[a]	1	.025	.054	.027
PPH	3.693[a]	1	.055	.097	.049
Blood transfusion	3.69[a]	1	.055	.097	.049
Bladder injury	.341[a]	1	.559	1.00	.500
Puerperal wound infection	5.876[a]	1	.015	.030	.015
Thrombotic event	1.032[a]	1	.310	.619	.310
Hospital long stay	.533[a]	1	.465	.718	.359
Maternal death	.000[a]	1	1.00	1.00	.752
FETAL COMPLICATIONS					
Respiratory distress	.699[a]	1	.403	.680	.340
Liquor aspiration	1.872[a]	1	.171	.365	.183
Perinatal death	.341[a]	1	.559	1.000	.500
Low APGAR score	3.775[a]	1	.052	.115	.057
NICU admission	1.07[a]	1	.300	.492	.246

decades.[14] A recent study by Gavial and Thomas reported a notable rise in caesarean section delivery rates in India over the past decade, increasing from 17% during National Family Health Survey-4 (NFHS-4, 2015–2016) to 21.5% during National Family Health Survey-5 (NFHS-5, 2019–2021.[15]

In our study, emergency deliveries accounted for 50% of cases, while elective procedures also comprised 50%. This was because we took elective cesarean group as control to calculate the odds ratio, so maintained the sample size equal in both groups. As odds ratio in our study was greater than 1, this shows that group 1 has higher odds for maternal and neonatal complications. These observations are in agreement with the results reported by Khawaja et al.[16] who found that cesarean delivery is associated with excessive bleeding, postoperative infection, thromboembolic events, and prolonged convalescence. However, from a neonatal perspective, cesarean birth is linked to an increased likelihood of respiratory complications, including respiratory distress syndrome which is contradictory to our results.[17]

In our sample, emergency cesarean sections were marked by a higher percentage of adverse maternal and fetal outcomes in group I. These findings align with RCOG guidelines which recognize emergency cesarean section as a higher-risk procedure due to urgent clinical circumstances, increased operative complexity, and greater maternal and fetal compromise at the time of delivery.[18] Similarly according to recommendations by the World Health

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Organization, caesarean section is a critical life-saving intervention when clinically indicated. However, unnecessary or emergency caesarean deliveries may increase the risk of both short-term and long-term maternal and neonatal complications, including hemorrhage, infection, surgical trauma, and neonatal respiratory morbidity.[19]

Emergency cesarean sections are associated with significantly higher number of postpartum hemorrhage in our study which is consistent with the findings of Sajjad et al.[14] who documented an elevation in the risk of hemorrhage in emergency cesarean deliveries compared with elective operations. Similarly, a meta-analysis conducted by Lee and Park[20]identified a significantly greater overall risk of maternal morbidity in emergency cesarean sections relative to planned procedures.

However, when chi square test was applied to our data adverse maternal outcomes such as uterine tears, and puerperal wound infection were observed statistically significantly associated with emergency cesarean group I, while post-partum hemorrhage and blood transfusion requirement showed a borderline association. No association was found between fetal outcomes and emergency cesarean. In contrast to these findings, earlier research indicates that the overall incidence of bladder injury during cesarean delivery ranges from 0.22% to 0.44%. Reported rates vary between 0.11% and 0.42% for primary cesarean sections and 0.27% to 0.81% for repeat procedures.[21] Similar to our study the pooled prevalence of surgical infection in post-cesarean section was 3 % (95 % CI: 2 %–4 %) in Saudi sample.[22] Hansen et al.[23] documented increased respiratory morbidity in infants delivered before 39 weeks of gestation by emergency cesarean. Edipoglu and Celik[24] also reported less favorable neonatal outcomes when emergency cesarean delivery was performed under general anesthesia compared to regional anesthesia techniques. This investigation is distinctive in that it exclusively evaluated maternal and neonatal outcomes among women with a history of a single prior cesarean delivery, comparing emergency and elective repeat procedures in a local Saudi hospital. The findings consistently demonstrated that patients undergoing emergency cesarean section were significantly associated only with uterine tears, post-partum hemorrhage, blood transfusion and puerperal wound infection indicating significant differences between the group I and II for these outcomes.

Despite its contributions, the study has several limitations. Confounding variables such as maternal age, BMI, parity, diabetes status, and fetal weight were adjusted but still all confounding variables were not adjusted which may have introduced bias in the estimated associations. As the study was conducted in a single healthcare center, the findings may reflect local clinical practices and patient characteristics, limiting external validity to other settings or populations. The relatively small sample size may have reduced statistical power, increased the likelihood of Type II error and limited the precision of odds ratio estimates, particularly for rare outcomes. Differences in patient selection between emergency and elective cesarean section groups may have influenced outcome comparisons, potentially affecting the validity of the results.

Conclusion

These findings indicate that although emergency cesarean section may have a greater frequency of adverse maternal and neonatal outcomes, only uterine tears, post-partum hemorrhage, need for blood transfusion, and puerperal wound infection related adverse maternal outcomes while in fetal outcomes low Apgar score is statistically significantly associated with emergency cesarean. These findings underscore the critical role of meticulous antenatal surveillance in the early detection of high-risk pregnancies and in facilitating timely, well-structured delivery planning. When cesarean section is clinically warranted, scheduling the procedure electively under controlled and optimal conditions may markedly enhance outcomes for both mother and neonate.

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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.

CASE REPORT

Fluoroscopy-Guided Repositioning of Malposition Central Venous Catheter During Cardiac Surgery: A Case Report

Saad Ahmed Naved[1], Abid Ur Rehman[2], Sana Asif[3], Madiha[4], Khalid Rasheed[5]

ABSTRACT

Central venous catheter (CVC) malposition remains a recognized complication, even with ultrasound-guided insertion, and may not be detected by routine functional assessment. We present a case involving a 61-year-old male patient undergoing elective coronary artery bypass grafting, in which a right internal jugular vein CVC exhibited selective distal lumen dysfunction after insertion. Although three lumens were aspirated and flushed normally, the inability to aspirate blood from the distal lumen suggested catheter malposition. Fluoroscopic imaging confirmed the deviation of the catheter tip into the ipsilateral subclavian vein. The catheter was successfully repositioned under fluoroscopic guidance by withdrawing it with continuous aspiration, followed by saline-assisted re-advancement, which restored full lumen function. In this patient, fluoroscopic repositioning eliminated the need for repeat cannulation and its associated procedural risks. This case underscores that the normal function of some catheter lumens does not preclude malposition and that selective distal lumen dysfunction should prompt early imaging confirmation and corrective intervention.

Key Words*: Catheter Malposition, Catheter Repositioning, Central Venous Catheter, Fluoroscopy, Internal Jugular Vein.*

Introduction

The placement of a central venous catheter (CVC) is a crucial aspect of cardiac anesthesia, enabling hemodynamic monitoring, administration of vasoactive medications, and perioperative fluid management.[1] The right internal jugular vein is typically preferred because of its direct anatomical path to the superior vena cava and its association with lower complication rates. Although the use of ultrasound guidance has enhanced the safety and success of venous cannulation, it does not consistently confirm the final position of the catheter tip, and catheter malposition remains a recognized complication even with image-guided insertion.[2,3] Reported malposition rates vary from approximately 3% to 15%, with catheter tips potentially deviating into adjacent venous structures despite seemingly uncomplicated placements.[4]

Detecting malposition can be challenging because routine functional assessments, such as aspiration and flushing of the catheter lumens, do not always accurately indicate the correct tip location. Selective dysfunction of a single lumen, particularly the inability to aspirate blood from the distal port while other lumens remain functional, may serve as an early but under-recognized indicator of catheter malposition.[5]

We present a case of a mispositioned right internal jugular vein CVC in a patient undergoing coronary artery bypass grafting. In this case, selective distal lumen dysfunction prompted early imaging diagnosis and successful fluoroscopy-guided catheter repositioning.

Case Report

A 61-year-old man with triple-vessel coronary artery disease was scheduled for elective coronary artery bypass grafting. Following the induction of general anesthesia, a quad-lumen central venous catheter (8.5 Fr, 16 cm) was inserted into the right internal jugular vein under ultrasound guidance using the Seldinger technique. During guidewire advancement, mild resistance was encountered, which was resolved with minor adjustments. Post-catheter placement, three lumens were aspirated and flushed normally; however, the distal (brown) lumen failed to aspirate blood despite allowing easy saline injection, suggesting possible catheter

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malposition. Given the necessity for accurate central venous pressure monitoring, fluoroscopic imaging was performed, revealing catheter malpositioning into the ipsilateral subclavian vein (Figure 1A). Under fluoroscopic guidance, the catheter was withdrawn while continuous negative aspiration was applied to the nonfunctional lumen. Blood aspiration was restored at an insertion depth of approximately 9 cm, after which the catheter was re-advanced with saline flushing. Repeat fluoroscopy confirmed correct positioning at the superior vena cava–right atrial junction, with restoration of function in all lumens (Figure 1B). The remainder of the procedure and postoperative course were uneventful, and the patient was discharged on postoperative day six without catheter-related complications.

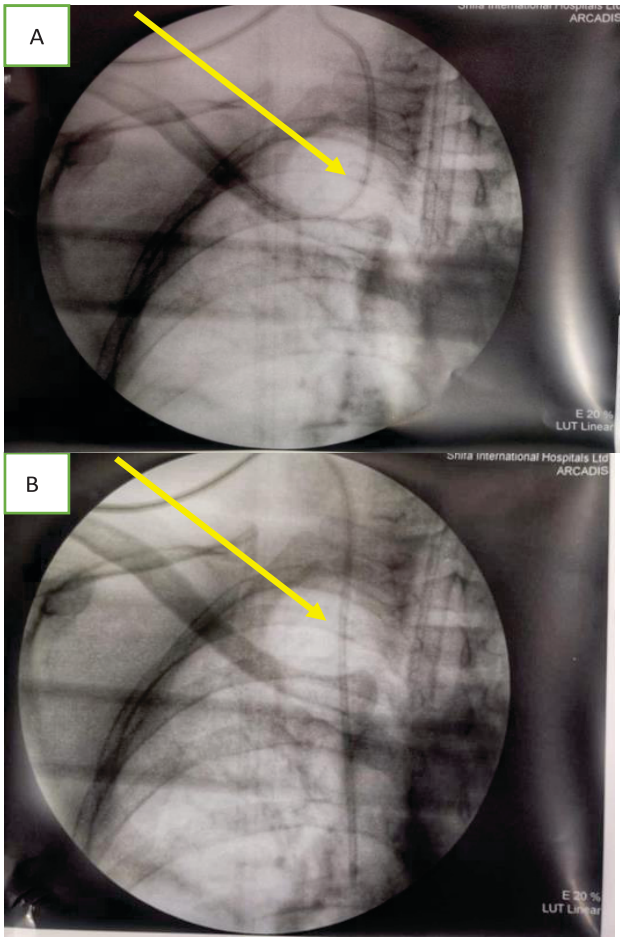

Figure 1: Fluoroscopic images of central venous catheter positioning. (A) Malposition of the catheter with deviation from the expected course toward the superior vena cava. (B) Post-repositioning image demonstrating appropriate catheter alignment within the superior vena cava.

Discussion

Central venous catheter (CVC) malposition remains a recognized complication despite the widespread use of ultrasound-guided venous cannulation.[4] The educational value of this case lies in highlighting the diagnostic clues that prompted further evaluation and ultimately led to the identification of catheter malposition. Although the three catheter lumens functioned normally in terms of aspiration and flushing, the inability to aspirate blood from the distal lumen prompted further investigation, ultimately revealing malposition of the catheter tip into the ipsilateral subclavian vein. This finding underscores a critical clinical principle; satisfactory function of one or more lumens does not reliably exclude catheter malposition, and selective lumen dysfunction should not be dismissed as a minor technical issue.

The reported rates of CVC malposition range from approximately 3% to 15%, even when ultrasound guidance is used during insertion.[4] Malposition may occur because of anatomical variations, vessel angulation, guidewire deviation, or resistance during advancement. Common malposition sites following internal jugular vein cannulation include the ipsilateral subclavian vein, contralateral brachiocephalic vein, azygos vein, and internal mammary veins.[4] In the present case, transient resistance was encountered during guidewire insertion, prior to successful catheter placement. Although this can be overcome with minor adjustments, it may represent an early indication of deviation into an unintended venous pathway. Recognition of such procedural clues should prompt heightened vigilance and consideration for early imaging confirmation.

Selective distal lumen dysfunction is an under-recognized but potentially important indicator of catheter malposition. In multilumen catheters, the distal port is located at the catheter tip and is therefore most susceptible to impaired aspiration when the tip abuts the vessel wall, enters a smaller tributary vein, or assumes an abnormal orientation. Consequently, the proximal lumen may remain functional, whereas the distal lumen fails to aspirate blood. Previous reports have similarly identified the inability to aspirate from the distal ports as a warning sign of catheter malposition.[5] However, clinicians

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should recognize that selective lumen dysfunction is not specific to malposition and may also result from catheter kinking, thrombus formation, or fibrin sheath development.[5] Nevertheless, in the setting of newly inserted catheters, unexplained distal port dysfunction should prompt immediate investigation rather than relying on routine flushing and aspiration tests alone.

This case highlights that although ultrasound guidance facilitates vessel identification and safe venous access, it fails to confirm the final catheter tip location.[6] Consequently, additional methods are required when the position is uncertain. Chest radiography remains the most commonly used confirmation technique; however, it only provides indirect assessment.[7] Intracavitary electrocardiography offers real-time localization without radiation exposure[4], whereas contrast-enhanced ultrasound[6] and bubble-test techniques demonstrate high accuracy.[6] Transesophageal echocardiography is the most accurate bedside method for directly visualizing the superior vena cava–right atrial junction.[8] In this case, fluoroscopy was employed as it was the sole modality immediately available to verify the catheter tip's position and facilitate its real-time adjustment. At that time, our institution did not routinely use advanced ultrasound-based techniques for tip confirmation. Despite the radiation exposure and the need for specialized equipment associated with fluoroscopy, it enabled prompt diagnosis and correction without delaying surgery. The real-time visualization provided by fluoroscopy was beneficial in this case, allowing for immediate diagnosis and correction without interrupting the surgical procedure. Several approaches are used to manage malpositioned CVCs, including guidewire-assisted repositioning, catheter exchange, and removal, followed by repeat cannulation[1,4]. However, in patients undergoing cardiac surgery, repeat cannulation may increase the complexity and risks, particularly when systemic anticoagulation is anticipated. In this case, catheter position was restored by fluoroscopy-guided withdrawal followed by saline-assisted re-advancement, without catheter replacement. This minimally invasive technique preserved vascular access, avoided repeated cannulation, and minimized risks associated with the procedure.

Conclusion: Selective dysfunction of the distal lumen may indicate an early sign of central venous catheter (CVC) malposition. Prompt imaging confirmation and fluoroscopic repositioning can avert the necessity for repeat cannulation in specific high-risk patients.

Consent Statement: Written informed consent was obtained from the patient for the publication of this case report and any accompanying images.

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Learning Points

- Normal aspiration from some catheter lumens does not exclude catheter malposition.
- Selective distal lumen dysfunction should prompt immediate imaging confirmation.
- Ultrasound guidance facilitates venous access but does not reliably confirm final tip location.
- Fluoroscopy-guided repositioning may avoid repeat cannulation in selected high-risk cardiac surgical patients.

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.

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At times the article may occasionally be retracted for correction of errors in submission or publication and will be replaced with the corrected one.

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  - o CONSORT 2010 flow diagram
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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.

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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:

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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
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- 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.
- Compare your work with other's work.
- Describe limitations of your study.
- Suggest future work if necessary.

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REFERENCES

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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