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# Diversity, Equity, and Inclusivity–The Trident of Destiny

Nandini Chatterjee*

The Association of Physicians of India (API) was established in 1944 in the state of Madras, under the enthusiastic leadership of nine founding members, who came from diverse backgrounds representing the far-flung corners of our great nation: A nation with 28 states and 8 union territories; a nation which has boasted over the decades of *Unity in Diversity*, a colorful collage of faiths, traditions, culture, and 22 scheduled languages.

Our organization, with its nearly 24,000 members, is a piece of our nation. And it manifests gloriously in a diversity of race, gender, age, ethnicity, experiences, and perspectives. What we need is a sense of belonging that will lead to greater involvement and ultimately more productivity.

The key to this lies in inclusivity, where everyone feels respected and empowered to express their voices, leading to newer ideas and better outcomes.

The very concept of Diversity and Inclusivity (D&I) in every walk of life is the practice of incorporating individuals from different geographical and sociodemographic backgrounds, offering them equitable opportunities and tapping into a vast talent pool.[1]

The early foundations of D&I were laid in the 1940s–1960s, when the civil rights movements against racial segregation and discrimination led to groundbreaking legal landmarks. The Civil Rights Act of 1964 in the United States was a pathbreaking law prohibiting discrimination based on race, color, religion, sex, or national origin.

By the 1990s, there was a gradual change of direction from merely complying with legal dictates to acknowledging the value of a diverse workforce. The term "multiculturalism" was coined at this time, emphasizing incorporation and appreciation of different cultural backgrounds within organizations.

The decades following the 2000s saw globalization that uplifted the concept of inclusivity, pinpointing the immense significance of full participation and integration of diverse sociocultural groups into the basic weave of an organization.[2]

During the 2010s, the concept further expanded to include "equity" and "intersectionality," addressing unconscious biases and systemic inequities leading to unequal opportunities and outcomes for different groups.

Diverse viewpoints enhance creativity and innovation, without which there is stagnation and discontent. Broader representation and feedback *via* regular communication ensure that the communities are served with fairness and equity.[3]

This is a global enterprise, and our organization is not far behind. The dynamic leadership has ensured that inclusivity and equity be the cornerstone of API's future journey ahead.

A focused endeavor has been made for the following:

- Communication and interchange of ideas either physically, online, or through social media portals.
- Encouraging the celebration of cultural events and special days by state and city branches all over the nation simultaneously.
- Linking of all state websites with the mother website so that the whole nation is abreast of the activities of all the states.
- Creation of an updated member directory with corrected contact details, which will go a long way to smoothen journal/book delivery as well as creating a unified database of information.
- Making strategic changes in policies and standard operating procedures (SOPs) for better implementation of the principles of Diversity, Equity, and Inclusion (DEI).

Unity in Diversity should not be an ornament or a figure of speech but an actual accomplishment of a great organization. Greatness comes from the involvement and dedication of a body of passionate people who believe in the betterment and upheaval of the existing system to lead the organization to the path of glory.

Long live API

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ORIGINAL ARTICLE

# A Study to Assess the Prevalence, Risk Factors, and Role of Epicardial Fat Thickness in Prediction of Diabetic Retinopathy in Type 2 Diabetic Patients in a Tertiary Care Center in Western Uttar Pradesh

Shashank Shekhar[1*], Sandhya Gautam[2], Chhaya Mittal[3], Snehlata Verma[4], Priyank Garg[5], Chandra Bhushan Pandey[6]

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## Abstract

**Introduction:** Diabetic retinopathy (DR) is the most important risk factor causing blindness in diabetic individuals, and its risk progresses with increased disease duration. Epicardial fat thickness (EFT) is an emerging indicator of inflammation and metabolic derangement and has been proposed as a potential biomarker linked to the severity of DR. This study aims to assess the prevalence of DR, identify risk factors associated with DR, and evaluate the predictive role of EFT in detecting DR in subjects with type 2 diabetes mellitus (T2DM).

**Methods:** A cross-sectional observational study was conducted at LLRM Medical College, Meerut, from 2023 to 2024. The participants included 130 T2DM patients who were assessed clinically, radiologically, and biochemically. Demographic data, duration of diabetes, body mass index (BMI), HbA1c levels, and EFT were measured. The severity of DR was determined based on ophthalmic examination. Data were analyzed using Kruskal–Wallis and Chi-squared tests.

**Results:** In this study of 130 patients with T2DM, 64.61% ($n = 84$) had DR, including 33.84% ($n = 44$) with nonproliferative DR (NPDR) and 30.76% ($n = 40$) with proliferative DR (PDR), while 35.38% ($n = 46$) had no DR. Patients in the PDR group were older on average (60.5 ± 13.9 years), but age differences were not statistically significant ($p = 0.154$). The duration of diabetes was significantly longer in PDR patients (9.0 ± 3.01 years) compared with NPDR and non-DR groups ($p < 0.001$). BMI increased with DR severity, reaching 28.49 ± 2.07 kg/m$^2$ in the PDR group, in which 20% were obese and 72.5% were overweight. A higher waist–hip ratio (WHR) was significantly associated with more severe DR in males ($p < 0.001$) but not in females ($p = 0.099$). HbA1c levels increased with disease severity, from 6.1 ± 0.71% in non-DR to 8.6 ± 1.97% in PDR patients ($p < 0.001$). Similarly, EFT increased from 3.9 ± 0.47 mm in non-DR to 7.9 ± 1.09 mm in PDR ($p < 0.001$), suggesting EFT as a potential biomarker for DR severity. These findings highlight strong links between DR severity, poor glycemic control, obesity measures, and longer diabetes duration.

**Conclusion:** These findings suggest that in type 2 diabetes mellitus patients, EFT can serve as a significant marker for the severity of DR. It can be used as a noninvasive investigation to predict PDR. When considered alongside established risk factors such as BMI, HbA1c levels, and diabetes duration, EFT could enhance early identification of patients at risk, potentially helping to prevent advancement to the more severe proliferative stage (PDR). However, larger and more extensive studies are required to confirm these observations and strengthen their clinical relevance.

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## Introduction

Diabetes mellitus (DM) is a longstanding metabolic disorder associated with high blood sugar due to ineffective insulin secretion or action.[1] It ranks among the top 10 causes of global mortality and morbidity, alongside cardiovascular and respiratory diseases and cancer. In 2021, an estimated 74 million adults in India had diabetes, and this number is assumed to reach 125 million by 2045, according to the International Diabetes Federation (IDF).[2]

Diabetic retinopathy (DR) is one of the main causes of preventable blindness in adults. People with diabetes are about 2.4 times more likely to experience vision loss compared with those who do not have the condition.[3,4] DR prevalence increases with diabetes duration, rising from 28.8% in those with <5 years of diabetes to 77.8% in those with >15 years.[5] DR is subdivided into nonproliferative (NPDR) and proliferative (PDR). While NPDR is often asymptomatic, PDR includes neovascularization and can cause severe vision loss.

Despite screening and treatments such as laser therapy, corticosteroids, and anti-VEGF agents, many patients respond poorly, indicating the need for better predictors so that timely treatment can be started and complications can be prevented.

Epicardial adipose tissue (EAT), fat surrounding the heart, has gained attention for its role in inflammation and metabolic syndrome. Epicardial fat is metabolically active and releases inflammatory cytokines such as IL-6 and TNF-α. These cytokines promote vascular injury and worsening of insulin resistance, which is responsible for DR.[6]

Recent studies show that epicardial fat thickness (EFT) is more significantly associated with DR severity in type 2 diabetes.[7] PDR patients had higher EFT, with values above 5.90 mm predicting PDR with 74% sensitivity and 61% specificity. EFT can be measured using echocardiography, cardiac CT, or MRI.[8]

Considering the limited existing research on the correlation between DR and EFT, this study focuses on finding the prevalence of DR and its associated risk factors. This study aims to assess the prevalence of DR, identify its risk factors, and explore the predictive potential of EFT in type 2 diabetes subjects at a tertiary care center in Western Uttar Pradesh.

## Methods

This study was cross-sectional observational and was conducted in the departments of medicine, endocrinology, and ophthalmology at LLRM Medical College and SVBP Hospital, Meerut, during 2023–2024. It included young type 2 DM patients attending the

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OPD and IPD of medicine, endocrinology, and ophthalmology. Patients were evaluated clinically, radiologically, biochemically, pathologically, and via questionnaire. Ethical clearance was obtained from the institutional ethical committee with ref. no. SC-1/2025/2912, dated 23/04/2025.

The sample size for the study was determined using the standard formula: $n = 4\,pq/d^2$.

Where $p$ is the prevalence of type 2 diabetes mellitus, taken as 7.2%,[9] and absolute error was taken as 5%. On calculation, the sample size came out to be 113, and a 10% nonresponse rate was added, making the sample size nearly 130.

Inclusion criteria included all confirmed T2DM patients (according to WHO criteria) aged over 18. Exclusion criteria included age below 18, severely ill patients, liver or thyroid disorders, electrolyte imbalances, inflammatory or infectious diseases, pregnancy, and those who did not give consent.

Anthropometric measurements included weight, height, waist circumference, and hip circumference. From these data, BMI and WHR were calculated. Demographic and lifestyle data were recorded, including age, sex, address, education, occupation, addictions, family history, diet, physical activity, and diabetes duration. General and systemic examinations were performed.

Investigations included hemogram (Hb, TLC, DLC, platelet count, RBC count, and indices), reticulocyte count, FBS, PPBS, HbA1c, lipid profile, LFT, KFT, echocardiography, chest X-ray, ECG, and fundus examination.

In this study, subjects with DR were classified based on ETDRS (Early Treatment Diabetic Retinopathy Study) guidelines as follows: (1) nondiabetic retinopathy (NDR), (2) nonproliferative diabetic retinopathy (NPDR), and (3) proliferative diabetic retinopathy (PDR). Further, NPDR was categorized into mild, moderate, severe, and very severe stages, while PDR was classified as early or high risk.

Epicardial fat thickness was assessed through echocardiography. EFT was determined in the parasternal long-axis view. It is the echo-free space observed between the visceral pericardium and the outer wall of the myocardium. EFT was measured during end systole, in which the ultrasound beam was aligned to the free wall of the right ventricle and kept perpendicular to the aortic annulus. The aortic annulus served as the reference point.

All collected data were organized into tables, and appropriate statistical tests, including the Kruskal–Wallis test, Chi-squared test, Fisher's exact test, and one-way ANOVA, were used for analysis. The results were compared across 3 patient groups: those without retinopathy (non-DR), those with NPDR, and those with PDR.

## Results

In this study, all diabetic patients were divided on the basis of fundus examination into 3 groups. Group A contained patients who did not have any retinopathy. Group B included those having NPDR, and group C included those having PDR.

Figure 1 shows the prevalence of DR in type 2 diabetic patients in this study. Out of the 130 patients, 35.38% ($n$ = 46) had no retinopathy, and 64.61% ($n$ = 84) had DR. Of the 84 DR patients, 33.84% ($n$ = 44) were in the NPDR group, and 30.76% ($n$ = 40) were in the PDR group.

Table 1 shows the distribution of age. In the present study, out of 130 participants, 17.69% were under 40 years, 15.38% were in the 40–49 years age range, 20.77% were aged 50–59 years, and 46.15% were over 60 years. On analysis of age distribution in the 3 groups, the mean age was highest in the PDR group at 60.52 ± 13.9 years, followed by 58.52 ± 16.23 years in the NPDR group and 54.52 ± 14.45 years in the group without DR (non-DR). However, the differences in age among the groups were not statistically significant ($p$ = 0.154).

Table 2 shows the association of DR with duration of type 2 diabetes mellitus (T2DM). In the study population, 23.08% of subjects had diabetes for <5 years, 61.54% for 5–9 years, and 15.38% for ≥10 years. The mean duration of diabetes was longest in the PDR group (9.0 ± 3.01 years), which was significantly higher than in the NPDR (6.0 ± 2.15 years) and non-DR (6.0 ± 1.93 years) groups ($p$ < 0.001).

Subjects were divided based on BMI according to WHO criteria as normal (18.5–24.9), overweight (25–29.9), and obese (≥30).

Table 3 shows the association between DR, waist–hip ratio (WHR), and obesity (BMI). The mean BMI was highest in patients with PDR at 28.49 ± 2.07 kg/m$^2$, followed by those with NPDR at 27.0 ± 1.71 kg/m$^2$, and lowest in patients without DR at 26.0 ± 2.62 kg/m$^2$. When categorized by weight status, among patients without DR, 32.61% had normal weight, 67.39% were overweight, and none were obese.

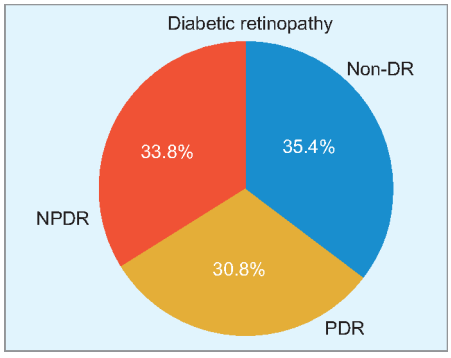


**Fig. 1:** Pie chart showing distribution of DR in our study

**Table 1:** Table showing the age distribution of subjects in our study

| *Parameter* | | *Group* | *Frequency* | *Percent* | *p-value* |
|---|---|---|---|---|---|
| Age-group | | <40 years | 23 | 17.69% | |
| | | 40–49 years | 20 | 15.38% | |
| | | 50–59 years | 27 | 20.77% | |
| | | ≥60 years | 60 | 46.15% | |
| | | Total | 130 | 100% | |
| Age-group | | Non-DR ($n$ = 46) | NPDR ($n$ = 44) | PDR ($n$ = 40) | |
| | Mean (SD) | 54.52 (±14.45) | 58.52 (±16.23) | 60.52 (±13.9) | H statistic: 3.746 $p$-value: 0.154[a] |
| | Median (IQR) | 55.5 (44.5–64.0) | 59.5 (42.5–76.0) | 62.0 (50.0–75.25) | |
| | Min–max | 29.0–76.0 | 34.0–76.0 | 34.0–76.0 | |

[a]Kruskal–Wallis test

**Table 2:** Table showing the association between DR and duration of T2DM

| *Parameter* | | *DR* | | | *Total* | *p-value* |
|---|---|---|---|---|---|---|
| | | *Non-DR (n = 46)* | *NPDR (n = 44)* | *PDR (n = 40)* | | |
| Duration of T2DM (years) | Mean (SD) | 6.0 (±1.93) | 6.0 (±2.15) | 9.0 (±3.01) | | H statistic: 25.991; *p*-value: <0.001[a] |
| | Median (IQR) | 7.0 (4.0–8.0) | 5.5 (4.0–8.0) | 8.5 (6.75–11.25) | | |
| | Min–max | 2.0–8.0 | 4.0–11.0 | 5.0–14.0 | | |
| Duration of T2DM (years) | <5 years | 13 (43.33%) | 17 (56.67%) | 0 (0%) | 30 (23.08%) | $\chi^2 = 43.908$; *p* value: <0.001[b] |
| | 5–9 years | 33 (41.25%) | 24 (30%) | 23 (28.75%) | 80 (61.54%) | |
| | ≥10 years | 0 (0%) | 3 (15%) | 17 (85%) | 20 (15.38%) | |

[a]Kruskal–Wallis test; [b]Chi-squared test

**Table 3:** Table showing the association of DR with obesity and waist-to-hip ratio

| *Parameter* | | *DR* | | | *Total* | *p-value* |
|---|---|---|---|---|---|---|
| | | *Non-DR (n = 46)* | *NPDR (n = 44)* | *PDR (n = 40)* | | |
| BMI (kg/m$^2$) | Mean (SD) | 26.0 (±2.62) | 27.0 (±1.71) | 28.49 (±2.07) | | H statistic: 21.571; *p*-value: <0.001[a] |
| | Median (IQR) | 26.39 (24.23–28.22) | 27.15 (25.6–28.51) | 28.48 (26.98–29.86) | | |
| | Min–max | 21.34–29.95 | 23.93–29.64 | 23.6–31.91 | | |
| Obesity | Normal (18.5–24.9) | 15 (32.61%) | 9 (20.45%) | 3 (7.5%) | 27 (20.77%) | <0.001[a] |
| | Overweight (25–29.9) | 31 (67.39%) | 35 (79.55%) | 29 (72.5%) | 95 (73.08%) | |
| | Obese ≥30) | 0 (0.0%) | 0 (0.0%) | 8 (20.0%) | 8(6.15%) | |
| Waist–hip ratio | | | | | | *p*-value |
| Male | Excellent (<0.85) | 6 (23.08%) | 0 (0.0%) | 1 (5.26%) | 7 (10.14%) | <0.001[a] |
| | Good (0.85–0.89) | 5 (19.23%) | 0 (0.0%) | 1 (5.26%) | 6 (8.7%) | |
| | Average (0.9–0.95) | 1 (3.85%) | 0 (0.0%) | 2 (10.53%) | 3 (4.35%) | |
| | At risk (≥0.96) | 14 (53.85%) | 24 (100.0%) | 15 (78.95%) | 53 (76.81%) | |
| | Total | 26 | 24 | 19 | 69 (100%) | |
| Female | Excellent (<0.75) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | <0.099[b] |
| | Good (0.75–0.79) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | |
| | Average (0.8–0.85) | 3 (15.0%) | 0 (0.0%) | 1 (4.76%) | 4 (6.56%) | |
| | At risk (≥0.86) | 17 (85.0%) | 20 (100.0%) | 20 (95.24%) | 57 (93.44%) | |
| | Total | 20 | 20 | 21 | 61 (100%) | |

[a]Kruskal Wallis Test; [b]Fisher's Exact test

**Table 4:** Table showing the association between DR and HbA1c

| *Parameter* | | *DR* | | | *p-value* |
|---|---|---|---|---|---|
| | | *Non-DR (n = 46)* | *NPDR (n = 44)* | *PDR (n = 40)* | |
| HbA1c (%) | Mean (SD) | 6.1 (±0.71) | 7.41 (±0.97) | 8.6 (±1.97) | H statistic: 48.557; *p*-value: <0.001[a] |
| | Median (IQR) | 6.15 (5.52–6.6) | 7.3 (6.68–8.15) | 8.65 (6.65–10.25) | |
| | Min–max | 4.8–7.2 | 6.0–9.2 | 6.2–11.8 | |
| HbA1c | <7% | 41 (58.57%) | 16 (22.86%) | 13 (18.57%) | <0.001[b] |
| | 7–7.9% | 5 (20.83%) | 14 (58.33%) | 5 (20.83%) | |
| | 8–8.9% | 0 (0%) | 10 (71.43%) | 4 (28.57%) | |

[a]Kruskal–Wallis test; [b]Fisher's exact test

Among NPDR patients, 20.45% had normal weight, 79.55% were overweight, and none were obese. In contrast, among those with PDR, only 7.5% had normal weight, 72.5% were overweight, and 20% were obese. These results highlight a clear trend showing that increasing BMI is associated with greater severity of DR.

On evaluation of the relationship between DR and WHR in males, among those with an excellent WHR (<0.85), 23.08% had no DR and 5.26% had PDR. In the good category (0.85–0.89), 19.23% had no DR and 5.26% had PDR. Among those with an average WHR (0.90–0.95), 3.85% had no DR and 10.53% had PDR. In the at-risk group (WHR ≥0.96), 53.85% had no DR, 100.00% had NPDR, and 78.95% had PDR, with $p < 0.001$, showing that male subjects with higher WHR had greater severity of DR.

In females, no subjects fell into the excellent or good WHR categories. Among those with an average ratio (0.80–0.85), 15% had no DR and 4.76% had PDR. In the at-risk group (WHR ≥0.86), 85.00% had no DR, 100.00% had NPDR, and 93.44% had PDR. However, the difference observed among female participants was not statistically significant ($p = 0.099$). These findings suggest a strong association between higher WHR and severity of DR in males but not in females.

Table 4 shows the association between DR and HbA1c. The mean HbA1c level was

**Table 5:** Table showing the association between DR and EFT

| *Parameter* | | *DR* | | | *p-value* |
|---|---|---|---|---|---|
| | | *Non-DR (n = 46)* | *NPDR (n = 44)* | *PDR (n = 40)* | |
| EFT (mm) | Mean (SD) | 3.9 (±0.47) | 6.9 (±0.9) | 7.9 (±1.09) | H statistic: 95.23; *p*-value: <0.001 |
| | Median (IQR) | 3.8 (3.5–4.5) | 6.9 (6.2–7.8) | 8.2 (6.9–9.0) | |
| | Min–max | 3.2–4.5 | 5.4–8.1 | 6.1–9.0 | |
| EFT | <4 mm | 28 (100%) | 0 (0%) | 0 (0%) | <0.001[a] |
| | 4–5.9 mm | 18 (69.23%) | 8 (30.77%) | 0 (0%) | |
| | 6–7.9 mm | 0 (0%) | 28 (60.87%) | 18 (39.13%) | |
| | ≥8 mm | 0 (0%) | 8 (26.67%) | 22 (73.33%) | |

[a]Fisher's exact test

highest in the PDR group at 8.6 ± 1.97%, followed by 7.41 ± 0.97% in the NPDR group and 6.1 ± 0.71% in the non-DR group, with $p < 0.001$. When classified by HbA1c levels, 58.57% ($n = 41$) of patients in the non-DR group had HbA1c <7%, while 22.86% ($n = 16$) in the NPDR group and 18.57% ($n = 13$) in the PDR group had HbA1c <7%. In the 7.0–7.9% HbA1c group, 20.83% ($n = 5$) had no DR, 58.33% ($n = 14$) had NPDR, and 20.83% ($n = 5$) had PDR. Among those with HbA1c between 8.0% and 8.9%, none were without DR, while 71.43% ($n = 10$) had NPDR and 28.57% ($n = 4$) had PDR. The *p*-value of <0.001 indicates a strong correlation between higher HbA1c levels and increased severity of DR.

Table 5 shows the association between DR and EFT. The EFT was highest in the PDR group at 7.9 ± 1.09 mm, followed by 6.9 ± 0.9 mm in the NPDR group and 3.9 ± 0.47 mm in the non-DR group, with a statistically significant difference ($p < 0.001$). When categorized by EFT, among individuals with EFT <4 mm, almost all patients (100%) did not have DR. In the 4.0–5.9 mm category, 69.23% ($n = 18$) did not have DR, 30.77% ($n = 8$) had NPDR, and none had PDR. For those with EFT between 6.0 and 7.9 mm, none were without DR, while 60.87% ($n = 28$) had NPDR and 39.13% ($n = 18$) had PDR. In the ≥8 mm category, none were without DR, 26.67% ($n = 8$) had NPDR, and 73.33% ($n = 22$) had PDR, with $p < 0.001$, indicating a strong correlation between higher EFT and increased severity of DR.

## Discussion

In coming years, it is expected that India will become the new capital of diabetes mellitus patients. This will definitely affect the global health burden of diabetes. In diabetic patients, the major challenge in treatment is the prevention of diabetic complications. Among its many complications, DR remains a major contributing factor to avoidable blindness and is closely related to the duration of the disease. In recent years, obesity, especially the accumulation of central and visceral fat such as epicardial adipose tissue (EAT), has been linked to factors responsible for the onset and progression of DR, likely due to its involvement in systemic inflammation and endothelial dysfunction.

In this study, the prevalence of NDR, NPDR, and DR was relatively similar. This contrasts with findings from several other studies, where NPDR is typically reported to be more common than PDR in individuals with T2DM. A large meta-analysis in Asia reported an overall DR prevalence of 21.7%, with NPDR at 19.9% and PDR at 2.3% among T2DM patients.[10] Another systematic review covering 2.6 million patients across Asia found DR in 28%, with NPDR in 27% and PDR in 6%.[11]

In this study, age did not show any significant association among the 3 groups, with similar mean ages and a similar proportion of participants aged 60 years or older. The association between age and DR remains inconclusive. While Kahn and Bradley[12] also reported similar findings showing a nonsignificant relationship between age and DR in patients with a duration of diabetes of >10 years, Chatziralli et al.[13] reported a positive correlation ($r = 0.4869$, $p < 0.0001$). These variations may be due to differences in study design, diagnostic methods, population characteristics, geographic regions, and confounding factors such as duration of diabetes, glycemic control, and access to health care services.

Gender also did not significantly impact DR, with no major differences between males and females in this study. Evidence regarding gender as a risk factor for DR is inconsistent. Kajiwara et al.[14] observed a higher incidence of retinopathy in females (76.1 vs 51.6 per 1,000 person-years). Conversely, Cherchi et al.[15] found a higher prevalence in males (22.0 vs 19.3%, $p < 0.0001$), while Ozawa et al.[16] showed no significant difference based on gender.

The duration of diabetes showed a positive correlation with DR severity. Patients diagnosed with PDR had a notably longer history of diabetes compared with those with NPDR or no retinopathy. There is strong consensus that longer diabetes duration significantly increases DR risk. Voigt et al.[17] demonstrated a rising DR prevalence with longer diabetes duration, reaching 63% at 30 years. Jerneld and Algvere[18] found duration to be the only significant predictor in multivariate analysis ($p < 0.001$). Raman et al.[19] reported an odds ratio of 6.43 (95% CI, 3.18–12.90) for DR in patients with diabetes >15 years compared with <15 years. These findings imply that duration of diabetes is a critical factor in the progression of DR. Therefore, annual screening for DR is essential, particularly for individuals with a longer duration of diabetes. Early detection through regular eye examinations can help identify retinopathy in its initial stages, allowing timely intervention and significantly improving patient outcomes.

In this study, body mass index (BMI) showed a positive correlation with DR severity, with higher BMI observed in PDR patients. Studies have shown conflicting results regarding the role of obesity in DR. Raman et al.[20] found a higher DR prevalence in individuals with abdominal obesity (26.35 vs 6.08%) and significant associations with increased waist–hip ratio (OR 1.48) and abdominal obesity (OR 2.02). However, other studies have reported an inverse association. Sabanayagam et al.[21] found lower DR odds in obese patients (OR 0.74; 95% CI, 0.59–0.91), and Rooney et al.[22] similarly reported lower odds for overweight and obese individuals compared with those of normal weight. These discrepancies may be due to differences in obesity definitions and measurement methods, variations in study populations such as age, ethnicity, and duration of diabetes, and confounding factors including medications and comorbid illnesses. These findings imply that, in addition to standard treatment, weight management is important. Furthermore, overweight or obese diabetic individuals should undergo more stringent ophthalmological screening for DR.

Waist–hip ratio (WHR) was also positively correlated with DR severity in males, with higher WHR corresponding to more severe DR. The association between central obesity and DR is well documented, although the effect in females was not significant, possibly due to hormonal differences. Fu et al.[23] reported significantly higher WHR values in subjects with DR compared with non-DR. Similarly, Zhang et al.[24] showed that subjects with higher WHR had 3 times higher likelihood of DR compared with those in the lower WHR group, with an odds ratio of 3.327 (95% CI, 2.386–4.638). The discrepancy between males and females may be attributed to hormonal differences. The significant association between high WHR and DR severity in males highlights the importance of measuring and managing abdominal obesity in addition to standard treatment.

HbA1c values showed a strong correlation with DR severity, with more advanced stages of DR observed in patients with higher HbA1c levels. This emphasizes the importance of maintaining good glycemic control to prevent DR progression. These findings indicate that glycemic control is of utmost importance in preventing the progression of DR. Yun[25] demonstrated a significantly increased DR risk in patients in the highest HbA1c quartile (OR 3.46; 95% CI, 1.90–6.30). The DCCT trial[26] demonstrated a 54% reduction in DR incidence and a 76% reduction in progression with intensive glycemic control.

Epicardial fat thickness showed a positive correlation, with higher values observed in patients with DR. EFT acts as a predictive biomarker for DR. Abide et al.[8] reported a positive correlation with PDR ($r = 0.394$, $p < 0.001$) and an independent association on regression analysis (OR 1.643; 95% CI, 1.206–2.237). Similarly, Gameli et al.[27] found elevated EFT in patients with DR and noted its predictive value for NPDR.

## Conclusion

This study highlights the important factors that affect DR severity in subjects with T2DM, notably longer duration of the disease, increased body mass index (BMI), and elevated HbA1c levels. These findings emphasize the need for comprehensive risk assessment in diabetic care. Importantly, this study highlights the importance of 2 novel biomarkers that are also associated with DR, increased EFT and increased monocyte/HDL ratio. These novel biomarkers may aid in early detection of retinopathy cases, leading to improved patient outcomes. However, more extensive studies are necessary to substantiate these conclusions.

### Limitations

This study had several limitations. Its cross-sectional observational study design, with a sample size on the relatively smaller side, limits the strength of the inferences drawn. Additionally, the study was single centric and did not include potential confounding factors. The use of a single-time glycemic measurement may not reflect long-term glycemic control, further limiting the generalizability of the findings to broader populations.

## Recommendations

Future research should focus on large, multicenter, longitudinal studies and risk factor modification. High-resolution data from continuous glucose monitoring and the study of novel biomarkers are recommended. Interventional studies should also evaluate the impact of early risk factor modification on DR progression across diverse populations.

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ORIGINAL ARTICLE

# Educating for Equity: The Role of Educational Intervention in Shaping Health Care Students' Knowledge of Universal Health Coverage and Primary Health Care

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## Abstract

**Background:** Universal health coverage (UHC) and primary health care (PHC) are critical components of equitable health systems. Medical and allied health science students, as future healthcare providers, need to possess knowledge and understanding of these concepts. Educational interventions are pivotal in enhancing this knowledge and preparing students for effective healthcare delivery.

**Objectives:** This study aimed to assess the impact of an educational intervention on the knowledge and perception of UHC and PHC among healthcare students at a private medical university in north Karnataka.

**Methodology:** A quasi-experimental study design was employed involving 300 healthcare students during June–August 2024. The study comprised 3 phases: a pretest to gauge baseline knowledge about UHC and PHC. An educational session focused on UHC and PHC was conducted, and a posttest to evaluate the knowledge acquired was done. The pretest and posttest consisted of a 23-item questionnaire. Statistical analysis comprised the Kruskal–Wallis and Wilcoxon signed ranks tests to compare pre- and postintervention knowledge scores.

**Results:** The pretest results indicated a mean knowledge score of ±8.07. Following the educational intervention, the posttest results revealed a significant increase in knowledge, with a mean score of ±13.8. This positive outcome emphasizes the effectiveness of the educational intervention.

**Conclusion:** The study demonstrates that targeted educational interventions can significantly improve the knowledge of UHC and PHC among healthcare students. Incorporating regular educational programs, including practical seminars on UHC and PHC, in their study curricula is recommended to sustain and enhance this knowledge.


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## Introduction

The World Health Organization describes universal health coverage (UHC) as a system in which all individuals and communities receive the health care they require without experiencing financial hardship. This encompasses a wide range of services, including health promotion, sickness prevention, treatment, rehabilitation, and hospice care. UHC is a critical component of a fair and just society because it assures that everyone, regardless of socioeconomic status, has access to quality health care services without incurring financial hardship. Equality in health care services is a fundamental human right.[1]

The objective of UHC is a global commitment, as evidenced by the United Nations Sustainable Development Goals, particularly Goal 3: "Ensure healthy lives and promote well-being for all at all ages." The 2030 Agenda for Sustainable Development Target 3.8 asks for "achieving UHC, including financial risk protection, access to quality essential health care services, and affordable essential medicines and vaccines for all."[2] Achieving UHC will be essential for improving population health, reducing health disparities, and promoting social and economic development. India's immense and diversified population presents distinct hurdles in achieving UHC. Despite major advances in recent decades, health care access and quality inequities persist.[3] Recognizing the relevance of UHC, India has taken major measures toward its implementation. The National Health Mission and Ayushman Bharat, among other policies and programs, demonstrate the country's commitment to UHC.[2] Ayushman Bharat, launched in 2018, is one of the world's largest government-funded health insurance programs, intending to provide financial protection against catastrophic medical bills.[4]

To achieve UHC and strengthen primary health care (PHC) in India, health care curricula must undergo significant reform. However, current health care curricula often fall short of this need, especially in terms of orienting students about the comprehensive concept of UHC and its components.[5] Students are more focused on specialized hospital individual care rather than preventable and equitable community health care. This knowledge gap limits health care professionals' preparedness to engage with UHC's broad objectives, which include ensuring accessible, quality, and financially protective health care for all. Studies show that while PHC forms the backbone of UHC, many health systems remain under-resourced in PHC, with too few opportunities for health care students to gain practical skills in community-oriented and preventive care.[6] By addressing these gaps, health care students can be better equipped to provide equitable and accessible health care, contributing to the realization of UHC and improving the overall health of the population.

The purpose of this study is to analyze how an educational session affects health care professional students' knowledge and perceptions of UHC and PHC.

## Methodology

### Study Area

The study was conducted at a private medical university in north Karnataka, offering a comprehensive range of courses across all clinical and paraclinical specialties.

### Study Period

The study was conducted for a period of 3 months from June–August 2024.

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### Study Design and Participants

This study is a quasi-experimental study design. It was conducted among 302 third-year MBBS, nursing, and physiotherapy students.

### Inclusion and Exclusion Criteria

All students in the third year across all 3 streams who had consented to participate were included in the study, and those who were absent on that day were excluded.

### Ethical Consideration

The ethical clearance was obtained from the ethical clearance department of the BLDE(DU) Shri B M Patil Medical College Hospital and Research Centre, Vijayapura.

### Data Collection

A pilot study was undertaken to ensure the questionnaire's clarity and feasibility. The questionnaire's reliability was assessed using Cronbach's alpha, which returned a score of 0.814, indicating good reliability. Purposive sampling was used to acquire data from 302 participants. The study was explained to health care students, informed consent was obtained, and data were gathered using a self-administered questionnaire. A pretest was administered first, followed by a 45 minutes educational and interactive session on UHC and PHC, after which a posttest questionnaire was used to assess the students' knowledge and perception of the educational session.

### Data Analysis

The acquired data were analyzed using the Statistical Package for Social Sciences (SPSS), version 26.

## Results

The study participants are enrolled in 3 different programs: MBBS (55.6%), nursing (36.1%), and BPT (8.3%) (Fig. 1).

The age distribution of the participants in the current study shows that the majority (88.7%) are between the ages of 18 and 22, with the remaining 11.3% aged 23–27 years (Fig. 2).

The gender distribution shows a female majority, with 55.0%, and the remaining 45.0% male (Fig. 3).

Table 1 presents the mean scores and standard deviations (SD) for pretest and posttest knowledge assessments across 3 programs (MBBS, nursing, and BPT). MBBS students demonstrated the highest improvement, with their mean score rising from ±9.02 to ±15.82. BPT students also showed significant improvement, with their posttest mean score at 15.20, despite starting with a lower pretest mean of ±4.80. Nursing students improved from ±7.38 to ±10.45. The Kruskal–Wallis test for both pretest and posttest shows significant results ($p < 0.001$), indicating statistically significant differences in knowledge improvement across the 3 programs. Overall, the total mean score increased from ±8.07 (pretest) to ±13.83 (posttest), indicating a general increase in knowledge after the educational session.

Table 2 evaluates changes in participants' perceptions before and after the intervention. MBBS students demonstrated notable improvement, with a pretest mean of ±18.54 and a posttest mean of ±18.92, yielding a significant Wilcoxon signed ranks test result ($p < 0.001$). Nursing and BPT students also improved their perceptions, with nursing showing a significant increase from ±17.87 to ±19.11. The BPT group showed a similar positive shift from ±16.84 to ±18.52. The total perception scores increased from ±18.16 (pretest) to ±18.95 (posttest), indicating an overall improvement in participants' perception following the intervention. The results demonstrate the effectiveness of the educational intervention

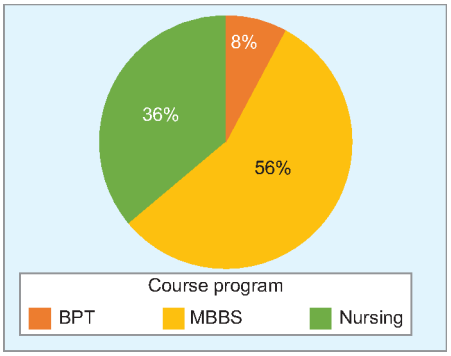


**Fig. 1:** Distribution of participants

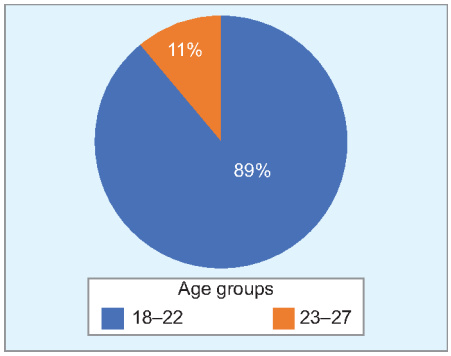


**Fig. 2:** Age distribution among the participants

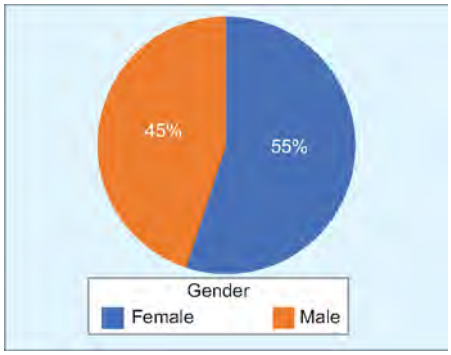


**Fig. 3:** Gender distribution among the participants

**Table 1:** Evaluation of pretest and posttest knowledge

| | | *Pretest* | | | | *Posttest* | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Course* | *No.* | *Mean* | *SD* | *Kruskal–Wallis test* | *p-value* | *Mean* | *SD* | *Kruskal–Wallis test* | *p-value* |
| MBBS | 168 | 9.02 | 3.68 | 30.23 | 0.001* | 15.82 | 1.88 | 110.89 | 0.001* |
| Nursing | 109 | 7.38 | 4.42 | | | 10.45 | 4.03 | | |
| BPT | 25 | 4.80 | 1.84 | | | 15.20 | 1.41 | | |
| Total | 302 | 8.07 | 4.05 | | | 13.83 | 3.80 | | |

*Indicates significance $p < 0.05$

**Table 2:** Evaluation of pretest and posttest perception

| | | *Pretest* | | *Posttest* | | *Pretest vs posttest* | |
|---|---|---|---|---|---|---|---|
| *Course* | *No.* | *Mean* | *SD* | *Mean* | *SD* | *Wilcoxon Signed-Rank test* | *p-value* |
| MBBS | 168 | 18.54 | 2.04 | 18.92 | 2.44 | –3.59 | 0.001* |
| Nursing | 109 | 17.87 | 3.07 | 19.11 | 3.10 | | |
| BPT | 25 | 16.84 | 2.74 | 18.52 | 2.14 | | |
| Total | 302 | 18.16 | 2.56 | 18.95 | 2.67 | | |

*Indicates significance $p < 0.05$

in enhancing both knowledge and perception across all programs, with statistical significance in key areas.

These findings highlight the program's impact on improving understanding and attitudes toward the topics covered, contributing valuable insights into medical education and perception improvement across different student populations.

## Discussion

The results show a clear improvement in both knowledge and perception of UHC and PHC after the interventional session, with MBBS students demonstrating greater gains due to their broader curriculum exposure. However, lower pretest scores suggest a limited understanding of the concepts and components of UHC and PHC before the intervention, even among third-year students of all streams. Minimal exposure to government PHC centers, especially for nursing and physiotherapy students, likely contributed to their lower baseline scores. The improved posttest results highlight the importance of educational interventions and practical exposure to government PHCs in bridging this knowledge gap, emphasizing the need to incorporate such experiences into medical and paramedical syllabi.

Given the evolving needs of the health care industry, it would be highly beneficial for health care education programs to incorporate UHC principles into their curricula. Global health initiatives increasingly emphasize equity, accessibility, and sustainability, embedding these principles into training for future health care professionals. Developing such a curriculum might not only provide students with theoretical knowledge but also integrate practical experiences, such as hands-on exposure at government primary health centers (PHCs).

UHC-focused training needs to be incorporated, as health systems worldwide face challenges from aging populations, pandemics, and rising noncommunicable diseases. Equipping health care students can help build a resilient, inclusive, and sustainable global health workforce.

## Conclusion

The baseline knowledge of UHC and PHC among health care students is below 50%, indicating a critical gap in current curricula. To bridge this, UHC must be systematically integrated into both academic and clinical training, enabling future health care professionals to develop the competencies needed to advance equitable health care access and address health disparities effectively.

## Acknowledgment

The authors would like to express their gratitude to the participants for their support throughout this study.

## Ethical Approval

Ethical clearance was obtained from the ethical clearance department of BLDE(DU) Shri B M Patil Medical College Hospital and Research Centre, Vijayapura. Reference No. (IEC/1101/2024–25).

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ORIGINAL ARTICLE

# Circadian Rhythm Disruption and Osteoporosis in Postmenopausal Women: An Observational Study from a Tertiary Care Center in India

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## Abstract

**Background:** Osteoporosis, a common bone disease among postmenopausal women, where bone is weak by diminished bone mineral density (BMD), increasing the fracture risk. Our body's natural rhythm, called the "circadian rhythm," which is controlled by the brain and body, helps in bone formation and also in breakdown, disruption of this rhythm may affect bone health. This study explores how problems with circadian rhythm might be linked with osteoporosis in postmenopausal women.

**Objective:** To assess the prevalence of osteoporosis among postmenopausal women and to see if it is related to changes in their body's daily sleep–wake cycle, "circadian rhythm," using a composite morningness–eveningness questionnaire (CMEQ) that groups people as morning, evening, or in between types.

**Materials and methods:** This cross-sectional observational study was conducted at Swaroop Rani Hospital, Prayagraj, India, between March 2024 and March 2025. This study included 109 postmenopausal women after applying strict inclusion/exclusion criteria. Each woman underwent clinical evaluation, anthropometric measurements, and biochemical testing. BMD by dual-energy X-ray absorptiometry (DEXA) scan at the lumbar spine with right and left femoral necks. To understand their sleep–wake pattern, "circadian rhythm" participants filled out a special questionnaire called the CMEQ, which groups them as morning, evening, or in between types. Data was analyzed using computer software (SPSS v25.0) to find patterns and differences.

**Results:** The prevalence of osteoporosis was 32.1% (35 among 109 women). Osteoporotic women had significantly lower weight (58.1 ± 11.63 vs 64.3 ± 13.65 kg; $p = 0.023$) and height (149.1 ± 7.12 cm vs 153.0 ± 7.08 cm; $p = 0.008$) compared to nonosteoporotic participants. Body mass index (BMI) was lower in the osteoporotic group (26.3 vs 28.1), though not statistically significant ($p = 0.093$). The mean composite M-E score did not have a significant value between osteoporotic and nonosteoporotic groups (44.8 ± 3.55 vs 44.6 ± 4.23; $p = 0.852$), indicating no significant association between circadian rhythm and osteoporosis.

**Conclusion:** About one-third of postmenopausal women in the study had osteoporosis. Although anthropometric differences were significant, no statistical significance was found between circadian rhythm and BMD. The findings suggest that circadian rhythm may affect bone health, but the questionnaires CMEQ used in this study may not be the best way to measure it. Future studies should use more accurate measures of taste, such as circadian hormone levels, and follow people over time to better understand this relationship.


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## Introduction

Osteoporosis is a skeletal disorder where bones become fragile and break easily because of a low bone mass and microarchitectural deterioration, thus resulting in increased bone fragility and susceptibility to fractures.[1] It is common in postmenopausal women due to estrogen drop, which accelerates bone resorption and compromises bone strength.[2] It is also called "silent disease" because it usually has no symptoms, and a fracture occurs suddenly, even on trivial trauma, most commonly at the vertebrae, hip, or wrist, leading to significant morbidity and mortality.[3] Globally, osteoporosis affects over 200 million people, and it is predicted that about one-third of women over 50 years will experience osteoporotic fractures in their lifetime.[4]

Our bones constantly rebuild through a balanced action of resorption by osteoclasts and formation by osteoblasts, controlled by mechanical, hormonal, and increasingly appreciated circadian rhythm.[5] Our circadian rhythm is an inbuilt 24-hour cycle that controls a range of physiological activities in our body. These rhythms are controlled by a part of the brain, the suprachiasmatic nucleus (SCN) of the hypothalamus, and synchronized with environmental signals such as light and feeding times.[6] At the molecular level, circadian rhythms are governed by transcriptional–translational feedback loops involving clock genes such as *BMAL1* (brain and muscle ARNT-like 1), *CLOCK*, *PER* (period), and *CRY* (cryptochrome). These genes not only maintain systemic circadian timing but are also expressed in osteoblasts, osteoclasts, and osteocytes, indicating that skeletal tissues are under circadian control.[7]

Research shows that bone rebuilding follows the daily pattern. For example, certain markers of resorption, specifically c-terminal telopeptide of type I collagen (*CTX*), peak in the early morning hours.[8] Change in these rhythms is common in shift workers, individuals with irregular sleep patterns, or those exposed to non-natural light at night, which is linked with impaired bone metabolism and risk of osteoporosis.[9]

Hormones such as melatonin, released at night by the brain's pineal gland, play an essential role in circadian rhythm control and have been found to directly support bone health by encouraging bone building and reducing bone breakdown. It increases matrix formation and mineralization. Melatonin enhances osteoblast proliferation and differentiation while inhibiting osteoclastogenesis through modulation of RANKL/OPG signaling. Lower melatonin levels, as seen in individuals with circadian disruption or aging, are associated with increased bone loss. High cortisol value in the early morning exerts catabolic effects on bone by reducing osteoblast activity and promoting osteoclast activity. Chronic circadian impairment, such as in shift workers, exaggerates glucocorticoid exposure and enhances bone resorption.[10] Animal studies have shown that melatonin supplementation improves bone mineral

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density and microarchitecture in rats in which ovaries are removed, mimicking postmenopausal osteoporosis.[11]

Additionally, melatonin levels drop as we age and are even lower in individuals with poor sleep quality or disrupted daily rhythms, both of which correlate with lower bone mineral density (BMD) and increased fracture risk.[12] These findings suggest that a disrupted circadian rhythm is a modifiable risk factor in osteoporosis pathogenesis. Furthermore, giving medications at the right time of day may improve their effectiveness and could offer a new way to treat these diseases.[13]

As people age and more of them experience sleep problems, it becomes important to understand how the daily body rhythm, "circadian rhythm," affects bone health. Understanding and addressing the effect of circadian rhythms on skeletal metabolic processes could help in developing better ways to prevent and treat osteoporosis. Public health efforts and clinical guidelines must evolve to start including the impact of these body rhythms when assessing the risk of osteoporosis and planning treatment.[14]

## Aim and Objectives

### Aim

To study the prevalence of osteoporosis among postmenopausal women and to see if it is related to changes in their body's daily sleep–wake cycle, "circadian rhythm."

### Objective

- To estimate the prevalence of osteoporosis in postmenopausal women using BMD assessment.
- To assess circadian rhythm patterns in postmenopausal women using a composite morningness–eveningness questionnaire (CMEQ).
- To examine the association between circadian rhythm disorganization and osteoporotic status.

## Materials and Methods

### Study Design and Setting

This cross-sectional observational study was carried out in the Department of Medicine at SRN Hospital, affiliated with Moti Lal Nehru Medical College, Prayagraj, Uttar Pradesh (March 2024–March 2025).

### Sample Size

A total of 415 postmenopausal females expressed willingness to participate after providing informed consent. Following the application of exclusion criteria, 269 postmenopausal females were excluded. Thus, 146 postmenopausal females were finally included in the study. Among them, 109 postmenopausal females completed the questionnaire and underwent dual-energy X-ray absorptiometry (DEXA) scanning.

### Inclusion Criteria

Postmenopausal females confirmed by clinical history who were willing to undergo BMD assessment and participate in circadian rhythm evaluation.

### Exclusion Criteria

Patients who had not attained menopause, a history of parathyroid disease or any metabolic bone disease, vitamin D deficiency, liver disease, chronic kidney disease, malignancy, chronic drug use (such as antiepileptic agents, steroids), diagnosed psychiatric illness, or neurodegenerative disorders affecting sleep. Shift workers or those with irregular sleep–wake cycles due to occupational demands or a history of calcium or vitamin D supplementation within 1 year. Patients who were unwilling to undergo study-related diagnostic procedures were excluded from the study.

### Data Collection Tools and Procedures

#### *Clinical Evaluation*

Detailed medical history, including menopausal duration, physical activity, dietary calcium intake, and relevant comorbidities, was recorded.

#### *Anthropometric Measurements*

Measurements such as height, weight, and body mass index (BMI) were obtained using standard protocols.

#### *Bone Mineral Density Assessment*

Bone mineral density was measured using DEXA scan at three anatomical sites as of lumbar spine, the right femur neck, and the left femur neck. WHO T-score criteria were used to classify osteoporosis (T-score ≤ –2.5 SD).

#### *Circadian Rhythm Assessment*

The composite CMEQ was used to assess circadian rhythm. The questionnaire depicted in Table 1 includes 13 items that help in evaluating preferred sleep–wake times, alertness, and diurnal activity levels.[15] Scores were categorized as:

- Morning type with score >44.
- Intermediate type with score 23–43.
- Evening type with score ≤22.

#### *Laboratory Investigations*

Blood samples were collected to measure serum total calcium, serum phosphorus, serum alkaline phosphatase, and 25-hydroxy vitamin D.

#### *Statistical Analysis*

Data were analyzed on a computer using SPSS software (version 25.0). The association between circadian rhythm types and osteoporosis was evaluated using statistical methods such as Chi-square test and analysis of variance (ANOVA) as suitable. A $p$-value < 0.05 is considered statistically significant.

## Results

The above Table 2 and Figure 1 represent the distribution of study participants based on their osteoporotic status. Out of the total 109 participants, 74 individuals (67.9%) were categorized as nonosteoporotic, while 35 individuals (32.1%) were identified as osteoporotic, indicating that approximately one-third of the study population had osteoporosis.

The above Table 3 and Figure 2 represent a comparison of anthropometric parameters age, weight, height, and BMI, between osteoporotic and nonosteoporotic individuals. The mean age of the osteoporotic group was higher (62.7 years) as compared to the nonosteoporotic group (59.9 years), but the difference was not statistically significant ($p$ = 0.153). The distribution of osteoporosis varied markedly with age. Among women aged 41–50 years, osteoporosis was observed in 5 out of 21 cases (23.8%), while osteopenia and normal bone mass were seen equally (38.1%) each. In the 51–60 years age group, osteoporosis was found in 10 out of 37 cases (27%), and osteopenia in 29.7%, indicating that nearly 57% of women already had compromised bone health by this decade. The 61–70 years age group demonstrated the highest burden of osteoporosis, affecting 12 out of 29 women (41.4%), a prevalence equal to the proportion of normal bone density (41.4%), with only 17.2% having osteopenia. In women aged 71–80 years, osteoporosis persisted in 8 out of 20 cases (40%), while normal bone density declined further to 35%, and osteopenia comprised 25%. In the

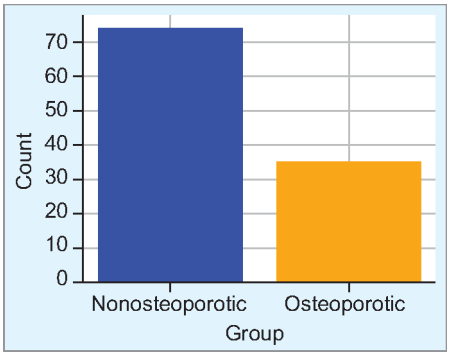


**Fig. 1:** Distribution of study participants based on osteoporotic status

**Table 1:** The composite morningness–eveningness questionnaire

| *Directions: Please check the response for each item that best describes you* |
|---|
| 1. Considering only your own "feeling best" rhythm, at what time would you get up if you were entirely free to plan your day? |
| 5:00 AM–6:30 AM (5) |
| 6:30 AM–7:45 AM (4) |
| 7:45 AM–9:45 AM (3) |
| 9:45 AM–11:00 AM (2) |
| 11:00 AM–12:00 noon (1) |
| 2. Considering your only "feeling best" rhythm at what time would you go to bed if you were entirely free to plan your evening? |
| 8:00 PM–9:00 PM (5) |
| 9:00 PM–10:15 PM (4) |
| 10:15 PM–12:30 AM (3) |
| 12:30 PM–1:45 AM (2) |
| 1:45 AM–3:00 AM (1) |
| 3. Assuming normal circumstances, how easy do you find getting up in the morning? |
| Not at all easy (1) |
| Slightly easy (2) |
| Fairly easy (3) |
| Very easy (4) |
| 4. How alert do you feel during the first half hour after having awakened in the morning? (Check one) |
| Not at all alert (1) |
| Slightly alert (2) |
| Fairly alert (3) |
| Very alert (4) |
| 5. During the first half hour after having awakened in the morning, how tired do you feel? |
| Very tired (1) |
| Fairly tired (2) |
| Fairly refreshed (3) |
| Very refreshed (4) |
| 6. You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week, and the best time for him is 7:00 AM–8:00 AM. Bearing in mind nothing else but your own "feeling best" rhythm, how do you think you would perform? |
| Would be in good form (4) |
| Would be in reasonable form (3) |
| Would find it difficult (2) |
| Would find it very difficult (1) |
| 7. At what time in the evening do you feel tired and, as a result, in need of sleep? |
| 8:00 PM–9:00 PM (5) |
| 9:00 PM–10:15 PM (4) |
| 10:15 PM—12:30 AM (3) |
| 12:30 AM–1:45 AM (2) |
| 1:45 AM–3:00 AM (1) |
| 8. You wish to be at your peak performance for a test, which you know is going to be mentally exhausting and lasting for 2 hours. You are entirely free to plan your day, and considering only your own "feeling best" rhythm, which one of the four testing times would you choose? |
| 8:00 AM–10:00 AM (4) |
| 11:00 AM–1:00 PM (3) |
| 3:00 PM–5:00 PM (2) |
| 7:00 PM–9:00 PM (1) |
| 9. One hears about "morning" and "evening" types of people. Which ONE of these types do you consider yourself to be? |
| Definitely a morning type (4) |
| More of a morning than an evening type (3) |
| More of an evening than a morning type (2) |
| Definitely an evening type (1) |
| 10. When would you prefer to rise (provided you have a full day's work—8 hours) if you were totally free to arrange your time? |
| Before 6:30 AM (4) |
| 6:30 AM–7:30 AM (3) |
| 7:30 AM–8:30 AM (2) |
| 8:30 AM or later (1) |
| 11. If you always had to rise at 6:00 AM, what do you think it would be like? |
| Very difficult and unpleasant (1) |
| Rather difficult and unpleasant (2) |
| A little unpleasant but no great problem (3) |
| Easy and not unpleasant (4) |
| 12. How long, a time, does it usually take before you "recover your senses" in the morning, after rising from a night's sleep? |
| 0–10 minutes (4) |
| 11–20 minutes (3) |
| 21–40 minutes (2) |
| More than 40 minutes (1) |
| 13. Please indicate to what extent you are a morning or evening active individual. |
| Pronounced morning active (morning alert and evening tired) (4) |
| To some extent, morning active (3) |
| To some extent, evening active (2) |
| Pronounced evening active (morning tired and evening alert) (1) |

**Table 2:** Distribution of study participants based on osteoporotic status

| *Group* | *Participants* | *% of total* |
|---|---|---|
| Nonosteoporotic | 74 | 67.9 |
| Osteoporotic | 35 | 32.1 |

**Table 3:** Comparison of anthropometric parameters between osteoporotic and nonosteoporotic individuals

| *Variable (± 2 SD)* | *Nonosteoporotic (N = 74)* | *Osteoporotic (N = 35)* | *p-value* |
|---|---|---|---|
| Age (year) | 59.9 ± 9.9 | 62.7 ± 9.45 | 0.153 |
| Weight (Kg) | 64.3 ± 13.65 | 58.1 ± 11.63 | 0.023 |
| Height (meter) | 1.53 ± 0.0708 | 1.49 ± 0.0712 | 0.008 |
| BMI (kg/m$^2$) | 28.1 ± 5.02 | 26.3 ± 5.59 | 0.093 |

**Table 4:** Comparison of composite M-E scoring between osteoporotic and nonosteoporotic groups

| *Variable* | *Nonosteoporotic (N = 74)* | *Osteoporotic (N = 35)* | *p-value* |
|---|---|---|---|
| Composite M-E scoring | 44.6 ± 4.23 | 44.8 ± 3.55 | 0.852 |

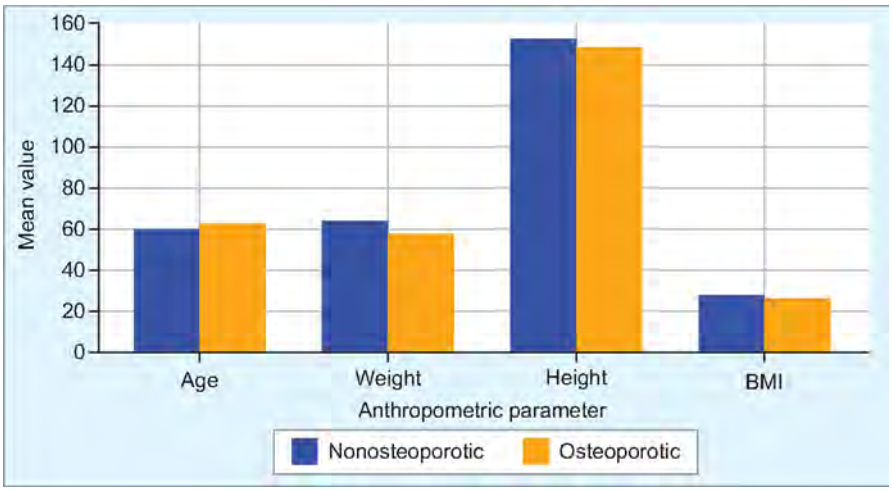


**Fig. 2:** Comparison of anthropometric parameters between osteoporotic and non-osteoporotic individuals

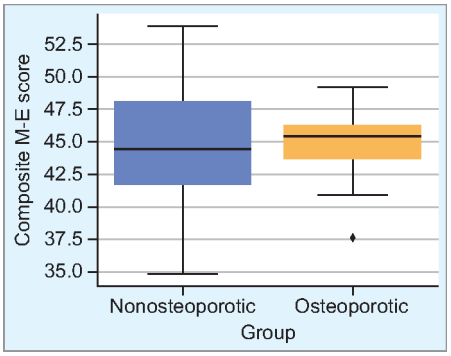


**Fig. 3:** Comparison of composite M-E scoring between osteoporotic and non-osteoporotic groups

81–90 years age group, no women out of two cases had normal bone density, and 100% demonstrated low bone mass (osteopenia 100%, osteoporosis 0%), reflecting advanced skeletal fragility in extreme age. This study shows a significant difference in weight, with the nonosteoporotic group having a higher mean weight (64.3 kg) than the osteoporotic group (58.1 kg), with a *p*-value of 0.023. Similarly, the mean height was statistically significant, with a greater mean height in the nonosteoporotic group (1.53 m) compared to the osteoporotic group (1.49 m), with a *p*-value of 0.008. Although the mean BMI was slightly higher in the nonosteoporotic group (28.1) than in the osteoporotic group (26.3), this was not statistically significant ($p = 0.093$).

The above Table 4 and Figure 3 represent a comparison of composite M-E scoring between osteoporotic and nonosteoporotic groups. The mean score in the nonosteoporotic group ($n = 74$) was 44.6 with a median of 45, standard deviation of 4.23, and standard error of 0.495. In the osteoporotic group ($n = 35$), the mean was 44.8 with a median of 45, standard deviation of 3.55, and standard error of 0.6. The *p*-value of 0.852 suggested that there was no statistically significant correlation in the composite M-E scoring between the osteoporotic and nonosteoporotic groups.

In our study, we correlated the composite morningness–eveningness (CME) score to body measurements such as BMI and blood levels of a few important nutrients, such as vitamin D and serum calcium, using a statistical test called Pearson's correlation coefficient. First, we checked if there is any link between CME score and BMI; the result showed a very weak negative connection ($r = -0.0208$), and the *p*-value (0.830) was quite high, which means this result is not significant, suggesting CME score and BMI do not seem to be related in our study group. Thereafter, we looked at the CME score and serum calcium; the correlation was very weak ($r = 0.0489$), and the *p*-value (0.657) was again high, which is statistically not significant, showing that there is no clear relation between them either. Lastly, we checked the relationship between CME score and vitamin D levels. This showed a slightly better positive correlation ($r = 0.1256$), but still, the *p*-value (0.248) was not statistically significant. Overall, in our Indian postmenopausal female population, no strong or significant connection between CME score and BMI, vitamin D, or serum calcium levels was found which indicated that changes in these values are not clearly linked to changes in CME scores.

## Discussion

In this study, we looked at how common osteoporosis is in postmenopausal women and whether it is related to their daily body rhythm (circadian rhythm) and metabolic factors. We compared our findings with other similar Indian studies to ensure accuracy and relevance.

In this study, as shown in the (Fig. 4) total of 415 postmenopausal females expressed willingness to participate after providing informed consent. Following the application of exclusion criteria, 269 women were excluded due to conditions such as history of parathyroid or metabolic bone disease, vitamin D deficiency, liver disease, chronic kidney disease, malignancy, long-term use of drugs such as antiepileptics or steroids, psychiatric or neurodegenerative illnesses affecting sleep, history of calcium or vitamin D supplementation within the last year, or unwillingness to undergo the required diagnostic procedures. Thus, 146 participants were finally included in the study. Among them, 109 women completed the questionnaire and underwent DEXA scanning, while 37 filled out the questionnaire but declined DEXA scanning at a later stage. Based on DEXA results, 74 participants (67.9%) were categorized as nonosteoporotic, and 35 participants (32.1%) were classified as osteoporotic. Both groups completed the CMEQ, with nonosteoporotic women obtaining a mean score of 44.6 ± 5.55 and osteoporotic women scoring 44.6 ± 4.23.

In this study,109 participants were categorized based on their BMD status as

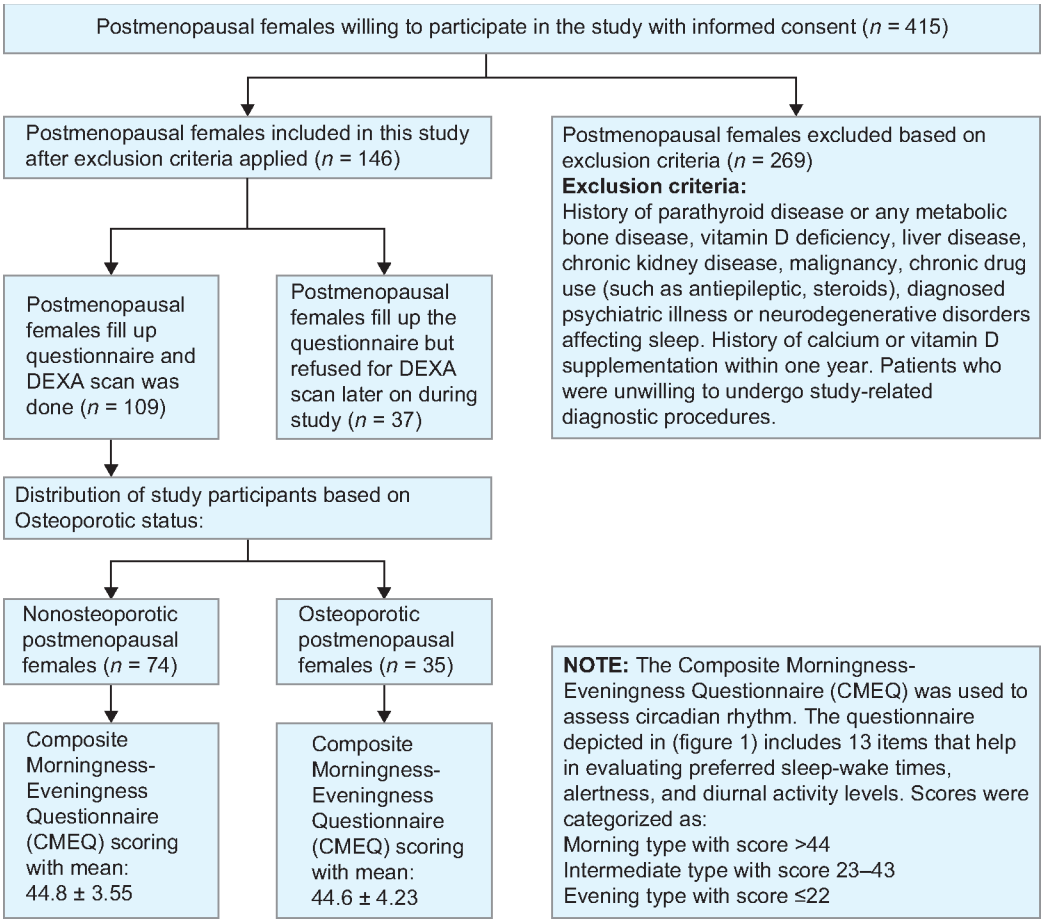


**Fig. 4:** A flowchart detailing recruitment of participants

osteoporotic and nonosteoporotic, out of which approximately one-third of the study population had osteoporosis. This prevalence is consistent with findings from various Indian studies like Sharma et al.,[16] who highlighted that an osteoporosis prevalence of 30–40% among postmenopausal women in North India, while a study by Marwaha et al.[17] also observed a similar trend in urban Indian populations, attributing it to nutritional deficiencies and hormonal changes after menopause. On the international front, the World Health Organization (WHO) estimates that globally one-third of women above the age of 50 years will suffer an osteoporotic fracture during their lifetime.

This study also compared anthropometric parameters between osteoporotic and nonosteoporotic individuals. We observed that the mean age in the osteoporotic group was 62.7 ± 9.45 years, which was higher than the nonosteoporotic group (59.9 ± 9.9 years), but the difference was not statistically significant ($p$ = 0.153). However, weight shows a statistically significant difference (64.3 ± 13.65 kg in nonosteoporotic vs 58.1 ± 11.63 kg in osteoporotic; $p$ = 0.023) and also the height (1.53 ± 0.0708 vs 1.49 ± 0.0712 m; $p$ = 0.008). The BMI was higher in the non-osteoporotic group (28.1 vs 26.3), but the difference was not statistically significant ($p$ = 0.093). These anthropometric associations resonate with findings from the study by Prasad et al.[18] Similarly, Pan et al.[19] reported an average BMI of 26.4 in diabetic osteoporotic women, showing a comparable reduction in BMI, which aligns with our osteoporotic group. The mean age in their cohort was 63.7 years, nearly identical to our osteoporotic mean value. Li et al.[20] reported lower BMI and height in osteoporotic patients without diabetes as well, suggesting anthropometry's universal role irrespective of glycemic status. Lastly, Dimitrova and Hristozov[21] found a mean height of 151.2 cm in osteoporotic postmenopausal women, which approximates our osteoporotic group (149.1 cm), supporting a strong inverse relationship between stature and osteoporosis.

In our study, composite M-E scoring was not statistically significant between osteoporotic and nonosteoporotic ($p$ = 0.852), and findings are similar to Prasad et al.[18] who pointed out that standard scoring systems and DEXA parameters underestimate fracture risk. Viggers[22] also pointed out the inadequacy of the composite tool in predicting bone fragility. Dimitrova and Hristozov et al.[21] found that fracture risks are independent of BMD scoring alone, supporting our non-significant M-E scoring difference.

## Conclusion

The osteoporosis prevalence in this study was 32.1%, with 67.9% being nonosteoporotic, indicating that nearly one-third of postmenopausal females had compromised bone health. Participants with osteoporosis were older (mean age: 62.7 years) than nonosteoporotic individuals (mean age: 59.9 years), but the age difference was not statistically significant ($p$ = 0.153). Osteoporotic individuals had significantly lower body weight (58.1 ± 11.63 kg) compared to nonosteoporotic individuals (64.3 ± 13.65 kg), with $p$ = 0.023, highlighting the influence of low body weight on bone density. Mean height was also significantly lower among osteoporotic participants (1.49 m) than their nonosteoporotic counterparts (1.53 m), suggesting a correlation between shorter stature and reduced bone mass ($p$ = 0.008). Composite M-E scoring did not

show statistical significance between the osteoporotic and nonosteoporotic groups ($p$ = 0.852), indicating limited diagnostic value in this study population.

## Limitations

The sample size of 109 participants, which is enough for preliminary analysis, limits the generalizability of the findings to a wider population, especially across different ethnic or regional groups within India. The cross-sectional nature of the study cannot prove cause and effect between osteoporosis and associated risk factors. There was a gender limitation as the study focused only on postmenopausal females, restricting applicability to other populations such as premenopausal women or men. A single-time-point BMD assessment might not reflect longitudinal changes or progression of osteoporosis over time. Variability in BMD measurement may arise due to dependency on a single anatomical site for diagnosis rather than multiple sites. Although some biochemical parameters were compared, markers of bone turnover (e.g., serum osteocalcin, CTX) were not included, limiting a proper understanding of bone metabolism. The study did not control for hormonal status or history of hormone replacement therapy, both of which can impact bone density in postmenopausal women.

## Recommendations

Older age was more frequently associated with osteoporosis, suggesting the need for early age-based screening in postmenopausal females, especially above 60 years. Significantly lower weight (58.1 kg) and height (149.1 cm) were observed in the osteoporotic group, indicating that low body mass and short stature could be potential anthropometric risk markers for osteoporosis and should be considered in routine assessments. BMI, although not statistically significant, was lower in osteoporotic individuals, supporting the role of maintaining a healthy body composition to prevent bone loss. Composite M-E scoring did not significantly differ between osteoporotic and nonosteoporotic individuals, suggesting that subjective or quality-of-life-based assessments may not be sufficient to predict bone health.

## Ethics Approval

Proper Institutional Ethical Committee approval was taken from the institute's Moti Lal Nehru Medical College, Prayagraj (Ethics Committee Registration No. ECR/922/inst/UP/RR-22 issued under New Drugs and Clinical Trial Rules, 2019).

## Written Consent for Publication

We declare that this manuscript is original, has not been published before, and is not currently being considered for publication elsewhere, and we give consent to be published in your journal 'JAPI'.

## Authors' Contributions

AS and PG chose the topic and planned the execution. AKC, AS, PG, and MM wrote the main manuscript. AKC, PG, AS prepared tables and figures, and contributed in discussion. All authors reviewed the manuscript.

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ORIGINAL ARTICLE

# Clinical and Laboratory Profile of Patients with Tropical Coinfections Admitted at a Tertiary Care Center in North India

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## Abstract

**Background:** Tropical coinfections (CI) are the simultaneous occurrence of two or more vector-borne diseases in a single host. The prevalence of such illnesses is not uncommon among tropical and subtropical regions such as India; however, these CIs have not been systematically studied prospectively. Mixed infections can prove potentially detrimental if underdiagnosed or undertreated. We undertook this study to estimate the prevalence and compare the clinical profile, laboratory characteristics, and various outcomes among the patients with tropical CI who presented with acute undifferentiated febrile illness (AUFI).

**Materials and methods:** A prospective, observational study was conducted on adult patients hospitalized with tropical CIs. As per the clinical suspicion, a panel of tests for dengue fever (D), malaria (M), scrub typhus (S), leptospirosis (L), chikungunya (C), and brucella (B) was carried out. Statistical analysis was done using standard methods.

**Results:** The mean age of the population was 39.4 ± 17.3 years. Among 986 patients presenting with AUFI, 8.1% of the patients had CIs. Of these CIs, 95% had dual infections, and 5% had CIs with three tropical pathogens. We observed 17 diverse tropical CI combinations; four predominant being D + L, D + S, D + C, and S + L with a prevalence of 26.2, 25, 15, and 13.8%, respectively. 16.25% of the patients with tropical CIs died, mostly those suffering from D + S and D + L. Coinfection with D + S had predominant acute kidney injury (AKI), whereas acute transaminitis was highest in the D + L category. Acute respiratory distress syndrome (ARDS) was clinically significant in S + L, and multiorgan dysfunction was highest in the D + S combination. Using logistic regression, AKI, hepatitis, ARDS, shock, gastrointestinal bleeding, and myocarditis were independent risk factors for mortality.

**Conclusion:** Our study identified 17 different combinations of CIs. Four groups, i.e., D + L, D + S, D + C, and S + L—accounted for 80% of CIs. Despite significant organ involvement in certain CI combinations, we conclude that a clinical bedside differentiation of tropical CIs from monomicrobial infections is often difficult. Hence, optimal treatment for a possible CI may well be commenced empirically and early, bearing in mind an 8% probability of a concurrent tropical coinfection.


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## Introduction

Tropical infections are commonly encountered entities in tropical and subtropical regions, including India. The presence of two or more infections simultaneously in one host is generally termed a tropical coinfection (CI). This terminology is synonymous with mixed infection, concurrent infections, or polyinfections. Coinfections tend to have more harmful effects on host health than single infections.

Coinfections (CIs) are emerging as a major causative phenomenon among patients of acute undifferentiated febrile illness (AUFI).[1,2] Numerous studies have shown that scrub typhus, malaria, dengue fever, and leptospirosis CIs have a widespread geographical distribution in our country, right from the Himalayan belt to the coastal regions of South India.[3]

The occurrence of the CIs can be postulated by two diverse mechanisms. These can either happen owing to contracting various infections concurrently in a particular time frame or as a consequence of enhanced pathogenicity of a coincident subclinical infection due to altered immune response.[4] It is often seen that when two organisms coexist, they increase each other's penetration and virulence, resulting in more severe outcomes and mortality.

Tropical diseases usually present with similar and nonspecific symptoms such as fever, headache, body aches, and gastrointestinal issues. This overlapping symptomatology makes accurate diagnosis of CIs extremely challenging, leading to diagnostic confusion and delays in appropriate treatment. The lack of distinctive characteristics in the early stages of AUFI/CIs often creates clinical and therapeutic dilemmas for the physicians, especially if the presentation is atypical. Serological cross-reactivity between different pathogens can further complicate diagnostic test interpretation.

Coinfections need to be strongly suspected and substantiated since underdiagnosis and mistreatment may have adverse consequences. Since the clinical features of AUFI widely overlap, the Indian Society of Critical Care Medicine group endorses a 'syndromic approach' for diagnosis and management to help narrow down the possibilities and simplify the treatment.[5] However, CIs may not always follow this syndromic approach, as the clinical presentation may get distorted. From a treatment perspective, CIs complicate drug regimens, risking interactions and reduced efficacy, necessitating integrated therapeutic approaches.

A better understanding of CI epidemiology helps in assessing the true burden of disease and developing comprehensive preventive strategies, guiding public health interventions, resource allocation, and the development of integrated surveillance systems. It promotes a shift from vertical, disease-specific programs to more horizontal and integrated healthcare systems for better management of complex health scenarios. Our study is an attempt to explore and understand these infections when they occur as CIs and emphasize the need for a multidimensional diagnostic approach and treatment.

## Aims and Objectives

- To estimate the prevalence of various tropical CIs among patients presenting with AUFI.
- To compare the clinico-laboratory profile and outcome of these CIs.

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## Materials and Methods

### Study Design and Setting

A prospective and observational study was carried out in the Departments of General Medicine and Microbiology at our hospital, which caters to the northern states of India. The study commenced after due clearance from the Institutional Ethics Committee, GMCH vide letter no. GMC/IEC/2020/570R/226 dated 12.05.2021, and was conducted over 20 months.

### Sample Size

The study population consisted of all patients hospitalized with AUFI who presented to the medicine emergency department during the study period. The sample size was calculated based on prevalence of tropical infection in patients with undifferentiated fever which was found to be 2.1% in the study by Chitkara et al.[6] Assuming a 95% confidence interval and 5% margin of error, the sample size came out to be 30, but keeping the unpredictable nature of outbreaks, the sample size was kept unlocked for the total duration of the study.

### Inclusion Criteria

Patients older than 18 years who were hospitalized with AUFI (a fever ≤14 days) and without evidence of localized infection and diagnosed to have a tropical CIs were included in the study. AUFI was defined as a fever less than 2 weeks in duration with no organ-specific symptom at the onset. Coinfection was defined as simultaneous infections of the host by multiple (two or more) pathogens.[1]

### Exclusion Criteria

Patients having fever with evidence of localized infections, autoimmune diseases, or malignancy, fever of >14 days' duration, prior antibiotic use, or hospitalization were excluded.

### Methodology

Patients with AUFI were enrolled after written informed consent was obtained. All enrolled cases were examined thoroughly and investigated with complete hemogram, ESR, CRP, urine examination, and liver and renal function tests. Simultaneously, a panel of tests for dengue fever, malaria, scrub typhus, leptospirosis, CHIK, and brucella serology and other viral serology such as hepatitis A and E (CTK Biotech, USA) was carried out judiciously as per the clinical suspicion, keeping in mind the diagnostic possibility and available resources.

Malaria (M) was diagnosed based on rapid diagnostic tests for antigen detection (SD biosensor) and peripheral blood smear for malaria parasite (trophozoite of *Plasmodium falciparum*, *Plasmodium vivax,* or mixed). Dengue fever (D) diagnosis was established by detection of dengue NS1 antigen test (TransAsia Bio-Medicals Ltd., India) or by dengue IgM antibody (National Institute Virology, NIV Pune). Leptospirosis (L), scrub typhus (S), brucellosis (B) and chikungunya (C) were established by IgM ELISA for Leptospira organisms (Nova Tec Immundiagnostica, GmBH), *O. tsutsugamushi* (J. Mitra & Co. Pvt Ltd India), brucella IgM (Calbiotech, CA) and anti-CHIKV antibodies, respectively [National Institute of virology (NIV), Pune]. Widal test/typhi dot IgM/blood cultures were carried out for *Salmonella* Typhi. Specific imaging was performed as and when needed. Patients suffering from more than one infectious etiology at the same time were considered CI. The scheme of enrolling the patients is depicted in Figure 1.

For assessing and documenting the complications, the following study definitions were used. Acute liver injury (ALI) was defined according to EASL guidelines as an elevation of liver enzymes 2–3 times the upper normal limit.[7] Acute kidney injury (AKI) was taken into consideration depending upon KDIGO AKI staging.[8] The diagnosis of acute respiratory distress syndrome (ARDS) was made according to Berlin's criteria.[9]

### Statistical Analysis

Statistical analysis was done using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Categorical variables were compared using the chi-square and Fisher's exact test. Continuous variables were analyzed as mean (SD) or median (range) using Student's *t*-test. Logistic regression analyses were performed to find the predictors of mortality/poor outcome. A two-sided $p \leq 0.05$ was considered statistically significant.

## Results

Among 986 patients studied, 8.1% ($n$ = 80) had tropical CIs. Of the 80 patients with CIs, 95% ($n$ = 76) had a dual infection, and 5% ($n$ = 4) had a triple infection. Seventeen different combinations of tropical CIs were obtained with four predominant groups, as shown in Figure 2.

The mean age of our patients was 39.4 ± 17.3 years. 42.5% of patients were aged between 18 and 30 years, 38.8% were between 31 and 50 years, and 18.8% were above 50 years of age. Both genders were equally affected. 62.5% of patients were

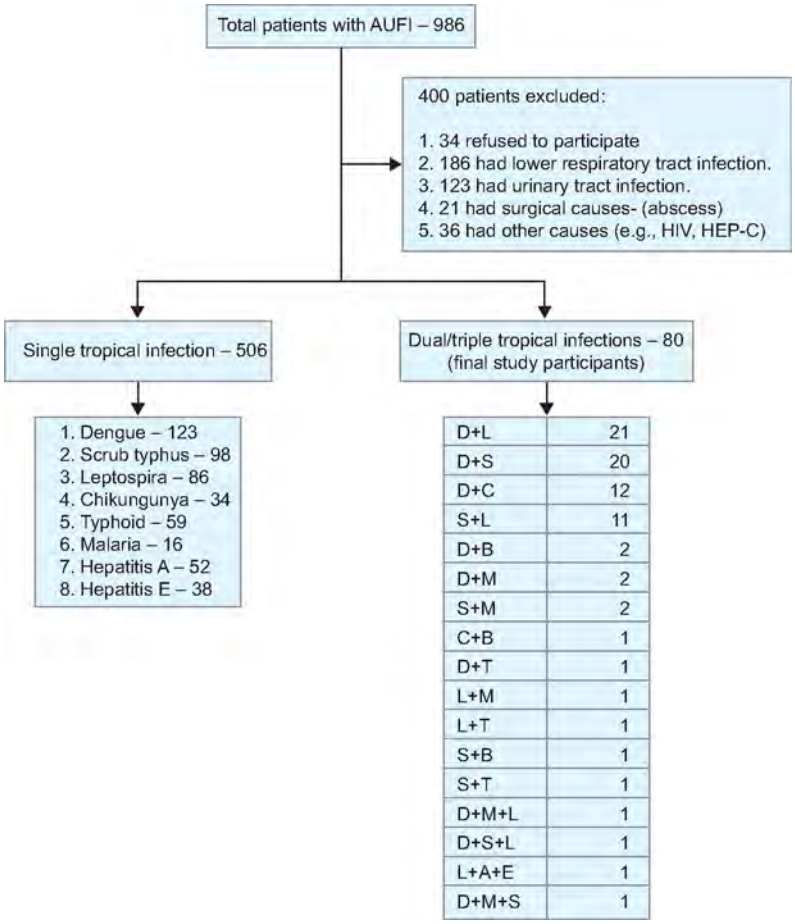


**Fig. 1:** Scheme of enrolling study participants

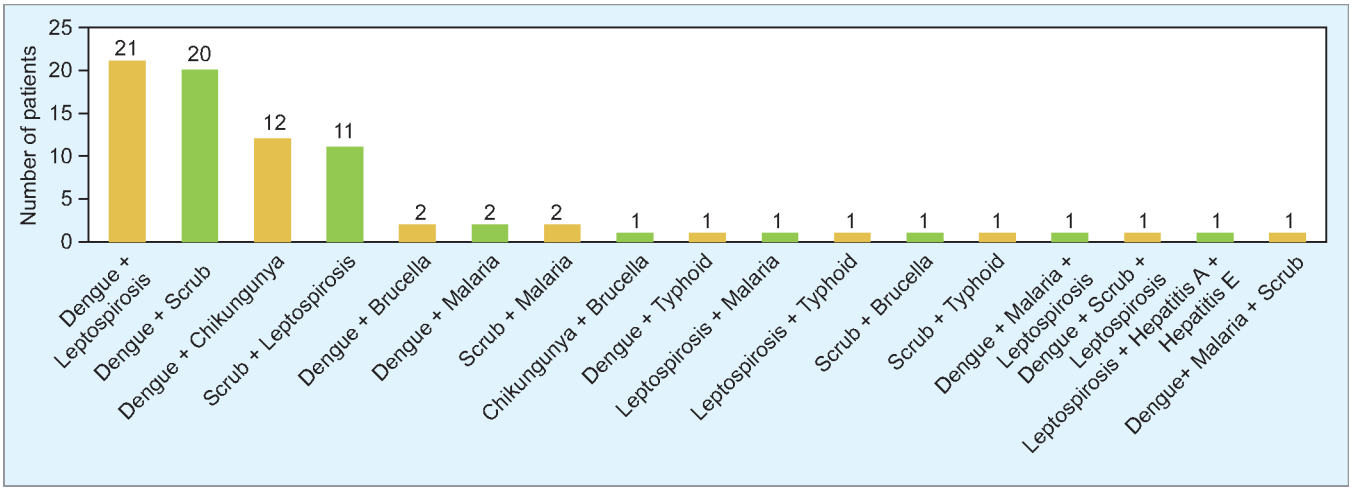


**Fig. 2:** Distribution of tropical coinfections in total study population

**Table 1:** Major laboratory parameters in four major coinfection groups

| *Parameter* | *Total (N = 80)* | *D + L (n = 21)* | *D + S (n = 20)* | *D + C (n = 12)* | *S + L (n = 11)* | *p-value** |
|---|---|---|---|---|---|---|
| Hemoglobin (gm/dL) | 10.86 ± 2.39 | 11.64 ± 2.28 | 10.90 ± 2.09 | 11.13 ± 1.94 | 9.76 ± 2.55 | 0.116 |
| TLC ($\times 10^9$/L) | 9.78 ± 6.43 | 9.23 ± 6.55 | 11.75 ± 5.98 | 6.92 ± 2.62 | 12.78 ± 9.85 | 0.158 |
| Platelet ($\times 10^9$/L) | 76.18 ± 79.36 | 81.90 ± 98.01 | 75.85 ± 59.82 | 81.75 ± 11.08 | 56.72 + 43.84 | 0.672 |
| INR | 1.18 ± 0.27 | 1.13 ± 0.20 | 1.24 ± 0.42 | 1.17 ± 0.26 | 1.13 ± 0.15 | 0.821 |
| Urea (mg/dL) | 69.75 ± 70.14 | 71.95 ± 66.8 | 105.15 ± 102 | 41.25 ± 39.3 | 71 ± 45 | 0.117 |
| Creatinine (mg/dL) | 1.42 ± 1.46 | 1.24 ± 0.66 | 2.30 ± 2.59 | 0.98 ± 0.61 | 1.18 ± 0.49 | 0.261 |
| AST (IU/L) | 769 ± 1659 | 1354.9 ± 2484.7 | 519.5 ± 779.75 | 270.75 ± 516.32 | 471.82 ± 958.46 | 0.253 |
| ALT (IU/L) | 445.16 ± 926.66 | 592.57 ± 918.67 | 381.45 ± 680.10 | 170.33 ± 324.35 | 243.82 ± 413.80 | 0.395 |
| Albumin (gm/dL) | 2.91 ± 0.55 | 3.05 ± 0.62 | 2.91 ± 0.47 | 2.57 ± 0.64 | 2.74 ± 0.44 | 0.093 |
| Alkaline phosphatase (IU/L) | 183.7 ± 132 | 157.62 ± 104.4 | 221.35 ± 143.8 | 171.67 ± 163.6 | 281.45 ± 136.5 | 0.031 |

*Kruskal-Wallis test; D+L: Dengue + Leptospirosis; D + S: Dengue fever + Scrub typhus D + C: Dengue fever + Chikungunya; S + L: Scrub typhus + Leptospirosis; TLC: Total Leucocyte Count; INR: International normalized ratio; AST: Aspartate aminotransferase; ALT: Alanine transaminase; Alkaline phosphatase was the only significant lab parameter consistently elevated in all 4 dual tropical infection groups

from urban backgrounds, and these had predominantly D + C, D + S, and S + L infection. In rural areas, 61.9% had the D + L CIs.

Even in patients with tropical CIs, the most common symptoms were abdominal pain in 36 (45%), followed by shortness of breath in 35 (43.8%), generalized weakness in 28 (35%), and myalgia in 27 (33.8%) patients. Other symptoms were cough and vomiting in 25 (31%) patients each, joint pains in 15 (18.8%), jaundice and diarrhea each in 10%. These symptoms were quite nonspecific and noncontributory. Few symptoms were helpful, for example, abdominal pain, which was predominantly seen in patients with the D + L (57%) CI. Breathlessness was chiefly present in D + S (55%) and D + L (33.3%) CI. However, others, such as mucosal bleeding was observed in all four subgroups: D + S (25%), D + C (33.3%), D + L (14.2%), S + L (27.2%).

Among the physical findings, hepatosplenomegaly was the most consistent sign but had no specific predilection for a particular CI. Nevertheless, pleural effusion seen in 24 (30%) patients did point toward dengue fever, as 54.1% of these had evidence of dengue fever CI (*p*-value 0.002). Eschar was seen exclusively with scrub typhus subgroups. Rash was seen in 90% of patients having dengue fever.

Investigations also helped to narrow down the diagnostic possibilities in AUFI. Aspartate aminotransferase/alanine transaminase (AST/ALT) was found maximally raised in the D + L group. Mild to moderate anemia was part of the spectrum of tropical fevers. Leukocytosis was also noted only in patients having S + L. However, mild to moderate thrombocytopenia was common to all tropical CIs patients. No other parameter showed any significant difference among the CI groups. Table 1 depicts the various laboratory parameters among the four major CI groups in the study population.

Coming to the organ involvement and complications, 86.2% of patients developed at least one complication, namely ALI, AKI, ARDS, pneumonia, myocarditis, or encephalopathy. ALI was seen predominantly in D + S (70%) and D + L (57%) CIs. Similarly, AKI (70%) and ARDS (45%) were predominantly noted in the D + S subgroup. Multiorgan dysfunction was statistically higher in patients with scrub typhus subgroups. On the other hand, polyserositis was noted in the majority having dengue fever as CI. Other complications, such as myocarditis, hemodynamic shock, encephalopathy, and mucosal bleed, were noted only in a small number of patients. Unexpectedly, pneumonia was seen in 85.7% of patients having dengue fever as one of the co-etiologies. The various multiorgan complications in different CI are listed in Table 2. Various syndromic distribution of the four major CIs is shown in Table 3.

Case mortality was seen in 13 (16.25%) patients; the highest among D + L and D + S, resulting in a total of 76.9% deaths. The distribution of mortality among various groups is depicted in Figure 3. We observed that AKI, hepatitis, ARDS, shock, polyserositis, GI bleed, and myocarditis were independent predictors of mortality (Table 4).

## Discussion

Rapid urbanization and immigration without corresponding development of

**Table 2:** Comparison of multiorgan dysfunction in four major coinfection groups

| *Complications* | *D + L (n = 21)* | *D + S (n = 20)* | *D + C (n = 12)* | *S + L (n = 11)* | *p-value** |
|---|---|---|---|---|---|
| Hepatitis + AKI | 11 (52) | 14 (70) | 2 (16.6) | 6 (54.5) | 0.019* |
| Hepatitis + ARDS | 3 (14.2) | 9 (45) | 1 (8.3) | 7 (63.6) | 0.142 |
| Hepatitis + shock | 4 (19) | 3 (15) | 2 (16.6) | 1 (9) | 0.260 |
| Hepatitis + encephalopathy | 4 (19) | 6 (30) | 3 (25) | 1 (9) | 0.679 |
| ARDS + AKI | 3 (14.2) | 9 (45) | 1 (8.3) | 6 (54.5) | 0.010* |
| Encephalopathy + AKI | 4 (19) | 6 (30) | 2 (16.6) | 1 (9) | 0.315 |
| Shock + AKI | 4 (19) | 3 (15) | 2 (16.6) | 1 (9) | 0.190 |
| ARDS + shock | 3 (14.2) | 3 (15) | 1 (8.3) | 1 (9) | 0.037 |
| ARDS + encephalopathy | 3 (14.2) | 6 (30) | 1 (8.3) | 1 (9) | 0.514 |

*Advanced regression analysis; percentage in parenthesis; D + L: Dengue fever + Leptospirosis; D + S: Dengue fever + Scrub typhus; D + C: Dengue fever + Chikungunya; S + L: Scrub typhus + Leptospirosis; AKI: acute kidney injury; ARDS: acute respiratory distress syndrome; Hepatitis in combination with AKI was the most prominent complication seen in the majority followed by ARDS and AKI. These complications were higher in dengue and scrub coinfection

**Table 3:** Syndromic distribution of various tropical coinfections

| *Syndromes* | *N (80)* | *D + L (n = 21)* | *D + S (n = 20)* | *D + C (n = 12)* | *S + L (n = 11)* | *p-value** |
|---|---|---|---|---|---|---|
| Fever with thrombocytopenia | 72 | 16 (76.1) | 18 (90) | 11 (91) | 10 (90.9) | 0.739 |
| Fever with hepatitis | 44 | 12 (57.1) | 14 (70) | 4 (33.3) | 7 (72.7) | 0.327 |
| Fever with renal failure | 36 | 11 (52.3) | 14 (70) | 2 (16.6) | 6 (54.5) | 0.621 |
| Fever with respiratory distress | 22 | 3 (14.2) | 9 (45) | 1 (8.33) | 7 (63.6) | 0.585 |
| Fever with encephalopathy | 14 | 4(19) | 6 (30) | 3 (25) | 1 (9) | 0.186 |

*Advanced regression analysis; percentage in parentheses; D + L: Dengue fever + Leptospirosis; D + S: Dengue fever + Scrub typhus; D + C: Dengue fever + Chikungunya; S + L: Scrub typhus + Leptospirosis; AUFI presented us into five varied syndromes, with majority of the patients presenting with thrombocytopenia. But none of the syndromes was able to predict the presence of mixed tropical infection with a nonsignificant *p*-value

**Table 4:** Logistic regression using complications as independent variables for mortality

| *Complications* | *Mortality* | *p-value #* | *Odds ratio* | *Confidence interval* |
|---|---|---|---|---|
| | *N = 13 (%)* | | | |
| AKI (*n* = 45) | 10 (27.8) | 0.011* | 5.26 | 1.32–20.91 |
| Hepatitis (*n* = 44) | 11 (25) | 0.019* | 5.67 | 1.17–27.54 |
| ARDS (*n* = 22) | 7 (31.8) | 0.037* | 4.04 | 1.18–13.87 |
| Shock (*n* = 13) | 9 (69.2) | 0.001* | 35.44 | 7.51–167.29 |
| Sepsis (*n* = 8) | 1 (12.5) | 1.000 | 0.71 | 0.08–6.35 |
| GI Bleed (*n* = 13) | 5 (38.4) | 0.032* | 2.86 | 0.8–10.3 |
| Encephalopathy (*n* = 14) | 4 (28.5) | 0.227 | 2.53 | 0.65–9.83 |
| Myocarditis (*n* = 2) | 2 (100) | 0.025* | 29.35 | 1.32–651.51 |
| Polyserositis (*n* = 11) | 5 (45.4) | 0.014* | 6.35 | 1.57–25.69 |
| Pneumonia (*n* = 21) | 4 (19) | 0.735 | 1.31 | 0.36–4.8 |
| DIC (*n* = 1) | 1 (100) | 0.162 | 16.2 | 0.62–420.74 |

#Fisher's exact test; percentage in parenthesis; *Indicates a significant predictor of mortality

civic infrastructure have led to increased breeding grounds for arthropod vectors that share common habitats. This leads to ecological co-circulation of vectors and parasites, seasonal epidemics, and co-exposure of pathogens to humans. In recent decades, tropical countries have witnessed an unexpected upsurge of tropical CIs due to these multifactorial reasons.

In hospitalized patients, the clinician is confronted with severe forms of CIs, which can become challenging as the outcomes get compounded due to host–pathogen and pathogen–pathogen interaction. The immune mechanism elicited by one or more pathogens can alter the natural history of an individual disease as well as dysregulate host immunity.

We observed an 8.1% prevalence of CIs in our cases of AUFI. The prevalence of tropical

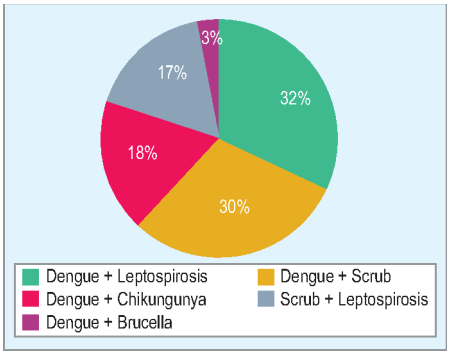


**Fig. 3:** Distribution of mortality among various coinfections

CIs varies tremendously in medical literature. In a Karnataka-based study, 92 (22%) patients had tropical CIs among 420 dengue fever cases, the most common being rickettsia (48.8%) and typhoid fever (22.2%).[10] However, in another study, only 48 (1.9%) patients were found to be suffering from CIs.[11] From western regions of Punjab, of 283 samples tested, 27 sera were positive (9.54%) for dengue and CHIK CIs.[12] A comparative study between mono- and CIs was done by Ahmad et al., which included 233 patients, in which 49 had CIs.[13]

Our study identified 17 different combinations of CIs. Four groups, i.e., D + L, D + S, D + C, and S + L—accounted for 80% CIs. Similar dominant groups were found in a publication by Raina et al.[3] More than 80% of our patients were below 50 years of age, consistent with the demographic profile by Ahmad et al.[13] It is postulated that younger healthy individuals generate aberrant and dysregulated immune responses due to activation of innate immunity and antibody-dependent enhancement.

In the present study, the clinical features across different CI groups were quite nonspecific. Transaminitis was common across all CIs. D+L had the highest AST levels. In 2016, Zubair et al. concluded that severe hepatitis, especially elevated ALT, was a poor prognostic indicator in dengue fever.[14] We noted highest mortality in patients with raised AST levels. Respiratory complications, mainly pneumonia and ARDS, were found to be higher in patients with dengue fever as CI. Secondary bacterial infection in relation to dengue fever has been studied by Thein et al. with similar results.[15] Leptospirosis is another seasonal infection that has outbreaks overlapping with other tropical illnesses.[16] AKI was the only complication which has significant correlation with diagnosis of dual infection, suggesting these patients have more profound kidney injury than monoinfections. Scrub typhus CI with dengue fever and leptospirosis produced the maximum number of complications in our patients. Multiorgan dysfunction was statistically higher in patients with scrub typhus subgroups. Ahmad et al.

also observed a higher incidence of multiorgan dysfunction with D + S CIs.[13]

Mewada et al. conducted a prospective study in Mumbai and suggested a 'syndromic approach' for classifying tropical infections; however, we did not achieve any conclusive differentiation, possibly because the clinical picture becomes transformed in mixed infections.[17] In a recent study conducted on 500 AUFI patients, Kulshrestha et al. stated that CIs are a highly under-recognized entity.[18]

There is no robust data to corroborate the prognosis, survival indices, and mortality rate specifically in patients with tropical CIs. In our study, case fatality was 16.25%, with the D + L and D + S accounting for 76.9% deaths. We have shown that the presence of AKI, hepatitis, ARDS, and shock predicted mortality in the majority of cases.

India, being a vast and diverse country with varying climatic conditions and endemic zones for different pathogens, often requires a region-specific approach to diagnosing and managing tropical CIs. North and Northeastern India have a high prevalence of scrub typhus, dengue, and Japanese encephalitis. Coastal regions have a higher incidence of leptospirosis, especially after floods. In tribal or forested areas, malaria (especially *P. falciparum*) remains a significant concern. In urban centers, dengue and chikungunya are widespread. An algorithmic approach for AUFI often starts with ruling out life-threatening conditions.[19] Based on prevalence in the region and clinical suspicion, specific tests are then ordered in a stepwise manner.[20] If initial tests are negative or the patient does not respond to empirical treatment for common infections, a broader panel of tests for other tropical diseases and CIs is considered. In essence, while formal "region-specific diagnostic protocols" with rigid algorithms for every CI might not be widely published, the Indian medical practice emphasizes a dynamic, syndromic, and context-dependent approach, heavily influenced by the local epidemiology of tropical diseases.

## Limitations

The disease spectrum in our study population was limited to a tertiary hospital setting with a greater number of complicated cases. We could not identify any specific predictors that could guide physicians in formulating the most appropriate diagnostic or management strategy in presence of these CIs. Secondly, in such a clinical setting, the likelihood of cross-reactivity and serological unreliability always remains.[21] Various factors, such as antigenic homology (e.g. viruses with same genus like flaviviruses), original antigenic sin (OAS) mount false positives and mislead diagnosis of CI.[22] Hence, more specific tests such as PCR, paired sera testing, neutralizing tests, and multiplex assays should be incorporated in diagnosis guidelines.

## Conclusion

Coinfections often go unrecognized in community and hospital settings. Through this study, we have ascertained that AUFI is not the domain of a single organism but may host multiple organisms with vectors sharing similar ecological and seasonal disposition. Therefore, we recommend that a thorough exploration of several etiologies must be incorporated into the preliminary diagnostic workup of patients with AUFI. This will ensure that the multiple diagnoses that could contribute to the pathophysiology, manifestations, and complications are not missed. Further research is warranted to better understand how coinfections impact the natural course of individual diseases.

Early recognition, broad-spectrum empirical therapy, and the strategic use of available diagnostic tools are essential, particularly when coinfections are suspected due to atypical clinical features or poor response to treatment. However, the unavailability of reliable point-of-care diagnostics and molecular testing poses a major challenge to evidence-based clinical practice. Until such resources become widely accessible, diagnostic investigations should be guided by local epidemiology and patient presentation. In the interim, an umbrella management covering the spectrum of common tropical fevers, informed by clinical judgment and expert consultation, remains a pragmatic strategy. In addition, there is a paucity of robust, region-specific seroprevalence data on tropical coinfections. Understanding the epidemiology of coinfections is vital for precise disease burden assessment and optimizing public health resource allocation.

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ORIGINAL ARTICLE

# Lipoprotein(a) Augments Coronary Risk Estimation in Type 2 Diabetes: A Cross-sectional Study

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## Abstract

**Objective:** Risk estimation tools have been developed to predict coronary heart disease (CHD) in type 2 diabetes (T2D). To evaluate augmentation following the addition of lipoprotein(a) [Lp(a)] to risk calculation, we performed a pilot study.

**Methods:** A total of 90 successive T2D patients were included. Details of clinical and biochemical features were obtained. Lp(a) was determined using ELISA. CHD risk estimation was performed using Framingham, QRISK-3, SCORE-2D, INTERHEART, and European Atherosclerosis Society (EAS) algorithms with and without Lp(a). Descriptive statistics are reported.

**Results:** Mean age of patients was 55.0 ± 8 years, BP systolic/diastolic 133.7 ± 12 / 95.0 ± 9 mm Hg, body mass index (BMI) 26.0 ± 1.9 kg/m$^2$, waist–hip ratio 0.96 ± 0.08, fasting glucose 198.0 ± 38 mg/dL, HbA1c 9.3 ± 1.3%, total cholesterol 197.0 ± 26 mg/dL, LDL cholesterol 114.2 ± 25 mg/dL, non-HDL cholesterol 153.8 ± 27 mg/dL, and triglycerides 197.8 ± 44 mg/dL. Lp(a) was mean 23.1 ± 9.7 mg/dL and median 22.0 (25–75 IQR 15.9–29.5) mg/dL. Mean risk scores were Framingham 11.2 ± 8.7, QRISK-3 28.6 ± 15.3, INTERHEART 21.0 ± 6.0, SCORE-2D 14.9 ± 8.3, and EAS 29.2 ± 15.2. Patients with raised Lp(a) >30 mg/dL had higher levels of total, LDL, and non-HDL cholesterol and triglycerides ($p < 0.01$). Spearman's correlation of Lp(a) with risk scores was Framingham 0.127, QRISK-3 0.174, INTERHEART 0.137, SCORE-2D 0.050, and EAS 0.320, while EAS-Lp(a) was 0.397. In different risk algorithms, high risk for CHD were: Framingham 14.4%, QRISK-3 64.4%, INTERHEART 45.6%, SCORE-2D 30.0%, EAS 71.1%, and EAS with Lp(a) 74.4%. Area under the curve (AUC) for Lp(a) with various scores were Framingham 0.53 (CI: 0.39–0.68; $p = 0.644$), QRISK-3 0.57 (CI: 0.42–0.71), INTERHEART 0.55 (CI: 0.39–0.69), SCORE-2D 0.47 (CI: 0.32–0.61), EAS 0.65 (CI: 0.50–0.79), and EAS-Lp(a) 0.68 (CI: 0.54–0.83). In addition, adding Lp(a) to the EAS risk calculator increased risk reclassification by a range of 4.6–19.3%.

**Conclusion:** Substantial variation in coronary artery disease (CAD) risk prediction using various clinical algorithms is observed in T2D. The EAS algorithm provides the most robust estimate. The addition of Lp(a) to the risk algorithms augments risk stratification significantly. The results of this pilot study need confirmation with larger prospective studies.


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## Introduction

Lipoprotein(a) [Lp(a)] has emerged as a major coronary artery disease (CAD) risk factor following persuasive data from epidemiological, case–control, and Mendelian randomization studies and clinical trials.[1,2] Studies have also reported that it is an important risk factor for premature CAD in patients with and without type 2 diabetes (T2D).[2–6] Lp(a) consists of apolipoprotein (apo) B100 covalently bound to apo(a).[7] Lp(a) characteristically inherits atherogenicity from both apoB and apo(a), as well as prothrombogenic and proinflammatory traits from apo(a). A major comorbidity of diabetes is CAD and is estimated to affect more than a third to half of all patients with diabetes.[8,9] Many risk scoring algorithms have been developed to predict CAD in T2D, including the Framingham risk score, QRISK-3 from the UK, SCORE of the European Society of Cardiology (SCORE-2D), INTERHEART risk score, and European Atherosclerosis Society (EAS) risk score.[10] Previous studies have reported that most perform suboptimally in non-European populations in general and T2D in particular.[10–13]

Lp(a) and diabetes are both established risk factors for the development of CAD.[13] However, studies trying to link both risk factors find an inverse association between Lp(a) and risk of prevalent and incident diabetes.[4] It is not clear whether this association is causal or whether it is due to Lp(a) itself, the length of the apo(a) isoforms, or both. The results of Mendelian randomization studies are highly heterogeneous.[1,14] Only part of the observed association of Lp(a) with diabetes can be explained by causality. It may also be due to reverse causation, comorbidities, or medications. Previous studies have reported that patients with T2D do not exhibit higher levels of Lp(a).[13] Results from the Biomarker for Cardiovascular Risk Assessment across Europe (BiomarCaRE) consortium indicated that elevated Lp(a) was robustly associated with an increased risk for CAD in individuals with T2D.[14] Therefore, the present study was undertaken to assess (1) the correlation of CAD risk scores with Lp(a) in patients with T2D, and (2) additional CAD risk prediction with Lp(a) using the EAS risk prediction algorithm.

## Methods

This hospital-based cross-sectional study was conducted on 90 successive patients (men 63, women 27) with T2D presenting to the medical outpatient department. The study was approved by the Institutional Ethics Committee. Written informed consent was obtained from all the enrolled individuals. The inclusion criteria were T2D, age 40–70 years, without microvascular or macrovascular complications of diabetes. T2D was diagnosed using the criteria used in recent Indian studies.[15] Participants were excluded if they had a previous history of cardiovascular disease, macrovascular complications of diabetes, were terminally ill, had severe liver or renal insufficiency, type 1 diabetes, cancer, thyroid dysfunction, severe mental illness, pregnancy, peripheral artery disease, hemolytic disease, severe disabilities, or were using drugs interfering with Lp(a) metabolism such as niacin and chronic use of steroids. A detailed questionnaire was obtained for participant information, including demographic characteristics, clinical features, diabetes duration, use of medications, and smoking status. Anthropometric measurements were recorded using standard procedures. Each participant's height and weight were measured, and body mass index (BMI) was calculated by

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dividing the weight in kilograms by height in meters squared. Waist circumference and hip circumference were measured, and the waist–hip ratio was calculated. Blood pressure was measured by a mercury sphygmomanometer. Hypertension was defined as systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg or current use of any treatment with antihypertensive medications.

Blood samples were collected after an overnight fast into appropriate Vacutainer tubes. The serum or plasma was removed within an hour and refrigerated at −80°C for analysis of Lp(a). Measurement of serum glucose, hemoglobin A1C (HbA1C), total cholesterol (TC), triglyceride (TG), and HDL-C was conducted on fresh samples using standard procedures with an automated analyzer (Beckman AU800) and enzymatic assays. Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald formula, except when triglyceride levels were >400 mg/dL. Lp(a) was estimated using a sandwich enzyme-linked immunosorbent assay kit. External validation was performed for Lp(a) estimation, with intra- and inter-assay coefficients of variation of 4.5 and 6.7%, respectively.

### Statistical Analyses

This is an observational pilot study, and the study sample size was determined using previously available studies from India. Cardiovascular risk estimation was performed using multiple algorithms—Framingham risk score (FRS),[16] QRISK-3 (UK),[17] SCORE-2 Diabetes (SCORE-2D) of the European Society of Cardiology,[18] INTERHEART risk score,[19] and European Atherosclerosis Society (EAS) score.[20] All the risk scores were calculated using online calculators.[21] Mean values of risk scores were determined. Participants were stratified into 3 categories according to 10-year coronary heart disease (CHD) risk as low risk (<10%), intermediate risk (10–20%), and high risk (>20%). The Lp(a) distribution was skewed and nonnormal; hence, Spearman's correlation of individual risk scores with serum Lp(a) levels was performed to estimate the variation in risk prediction. Receiver operating curves (ROCs) were plotted for various risk scores with Lp(a) >30 mg/dL, and the area under the curve (AUC) was determined using the SPSS statistical package (version 22.0). Additional discriminant value of Lp(a) was calculated using the EAS risk scoring algorithm with and without Lp(a). The article follows the STROBE guidelines for observational studies. The STROBE checklist is attached as Supplementary File.

## Results

The key characteristics of the study cohort are in Table 1. The mean age of the participants was 55.0 ± 8 years, the majority being men. A high burden of cardiovascular risk factors,

**Table 1:** Clinical and biochemical characteristics of the study cohort

| *Variable* | *Total* | *Lp(a) <30 mg/dL* | *LP(a) >30 mg/dL* | *p-value* |
|---|---|---|---|---|
| Numbers | 90 | 69 | 21 | |
| Men | 63 (70.0) | 48 (69.6) | 15 (71.4) | 0.870 |
| Age years | 54.7 ± 8.0 | 54.6 ± 8.0 | 54.8 ± 8.1 | 0.905 |
| Age-groups | | | | |
| <40 years | 30 (33.3) | 22 (31.9) | 8 (38.1) | 0.712 |
| 40–59 years | 34 (37.8) | 27 (39.1) | 7 (33.3) | 0.744 |
| 60+ | 26 (28.9 | 20 (29.0) | 6 (28.6) | 0.998 |
| Family history of CAD | 37 (41.1) | 27 (39.1) | 10 (47.6) | 0.489 |
| Tobacco use (smoking/smokeless) | 46 (51.1) | 38 (55.1) | 8 (38.1) | 0.173 |
| Alcohol use | 38 (42.2) | 31 (44.9) | 7 (33.3) | 0.346 |
| Hypertension | 47 (52.2) | 36 (52.2) | 11 (52.4) | 0.987 |
| Systolic BP | 133.7 ± 12.0 | 133.6 ± 12.2 | 134.0 ± 11.3 | 0.900 |
| Diastolic BP | 95.0 ± 8.6 | 94.7 ± 8.6 | 95.8 ± 8.9 | 0.627 |
| Waist circumference | 94.9 ± 7.4 | 95.0 ± 7.6 | 94.7 ± 6.8 | 0.892 |
| Waist–hip ratio | 0.96 ± 0.08 | 0.96 ± 0.08 | 0.96 ± 0.08 | 0.921 |
| BMI | 26.0 ± 1.9 | 26.1 ± 2.2 | 25.9 ± 1.8 | 0.680 |
| Diabetes duration | 7.3 ± 5.4 | 7.1 ± 5.3 | 8.2 ± 5.9 | 0.406 |
| Biochemical parameters | | | | |
| HbA1c (mean) | 9.3 ± 1.2 | 9.4 ± 1.2 | 9.0 ± 1.3 | 0.217 |
| HbA1c >7.0% | 90 (100) | 69 (100.0) | 21 (100.0) | 1.00 |
| Total cholesterol, mean | 197.0 ± 26.4 | 191.6 ± 24.2 | 214.6 ± 26.1 | <0.001 |
| High cholesterol >200 mg/dL | 35 (38.9) | 19 (27.5) | 16 (76.2) | <0.001 |
| LDL cholesterol, mean | 114.2 ± 24.7 | 109.7 ± 22.1 | 130.1 ± 26.3 | 0.001 |
| High LDL cholesterol >100 mg/dL | 68 (75.6) | 49 (71.0) | 19 (90.5) | 0.069 |
| Non-HDL cholesterol | 153.8 ± 26.6 | 147.3 ± 23.9 | 175.1 ± 24.0 | <0.001 |
| Non-HDL cholesterol >130 mg/dL | 73 (81.1) | 53 (76.8) | 20 (95.2) | 0.059 |
| Triglycerides | 197.8 ± 44.4 | 189.5 ± 42.7 | 225.0 ± 39.5 | 0.001 |
| High triglycerides >150 mg/dL | 82 (91.1) | 61 (88.4) | 21 (100.0) | 0.102 |
| LDL:HDL ratio | 2.7 ± 0.89 | 2.6 ± 0.80 | 3.4 ± 0.87 | <0.001 |
| Triglyceride:HDL ratio | 4.8 ± 1.6 | 4.4 ± 1.3 | 5.9 ± 1.8 | <0.001 |
| Lipoprotein(a) mean | 23.0 ± 9.7 | 19.0 ± 6.2 | 36.3 ± 6.9 | <0.001 |
| Lipoprotein(a) median, IQR | 22.0 (15.9–29.5) | 22.0 (14.6–24.2) | 33.8 (32.1–39.4) | <0.001 |

family history, hypertension, smoking, or tobacco use is observed. The mean systolic/diastolic BP was 133.7 ± 12/95.0 ± 9 mm Hg, BMI 26.0 ± 1.9 kg/m$^2$, and waist–hip ratio 0.96 ± 0.08. Biochemical analyses showed mean fasting glucose 198.0 ± 38 mg/dL, HbA1c 9.3 ± 1.3%, total cholesterol 197.0 ± 26 mg/dL, LDL cholesterol 114.2 ± 25 mg/dL, non-HDL cholesterol 153.8 ± 27 mg/dL, and triglycerides 197.8 ± 44 mg/dL. A significant proportion of participants had raised levels of total cholesterol (>200 mg/dL, 38.9%), LDL cholesterol (>100 mg/dL, 75.6%), non-HDL cholesterol (>130 mg/dL, 81.1%), and triglycerides (>150 mg/dL, 91.1%). The mean level of Lp(a) was 23.0 mg/dL, with a median value of 22.0 mg/dL (25–75% IQR 15.9–29.5 mg/dL). A total of 21 patients (23.3%) had elevated Lp(a) levels of >30 mg/dL. Clinical and biochemical characteristics of participants with Lp(a) <30 mg/dL ($n$ = 69) were compared with those of participants with raised Lp(a) >30 mg/dL ($n$ = 21). Clinical characteristics and risk factors are similar (Table 1). However, a subgroup of participants exhibiting elevated levels of Lp(a) consistently demonstrates increased levels of total cholesterol, LDL-C, non-HDL-C, and triglycerides ($p$ < 0.05 for all).

Table 2 shows the mean 10-year CHD risk prediction scores using various risk assessment tools. Mean risk scores were the highest for EAS (29.2 ± 15.2) and QRISK-3 (28.6 ± 15.3) risk calculators compared to others. Increased CHD risk (>10%, 10-year risk) using various risk calculators was for Framingham 14.4% ($n$ = 13), QRISK-3 64.4% ($n$ = 58), INTERHEART 45.6% ($n$ = 41), SCORE-2D 30.0% ($n$ = 27), and EAS 71.1% ($n$ = 64). Addition of Lp(a) to the EAS risk calculator enhanced the CHD risk to 74.4% (+4.6%) ($n$ = 67). Correlation between individual CHD risk prediction scores and Lp(a) levels using Spearman's correlation coefficient (rho), as well as parametric (Pearson's $r$), is in Table 3. Weak correlation is observed between Lp(a) levels and Framingham ($p$ = 0.234), INTERHEART ($p$ = 0.197), and SCORE-2D ($p$ = 0.641) risk scores; intermediate for QRISK-3 risk score ($p$ = 0.100); and significant for EAS risk prediction score (rho = 0.320, $p$ = 0.002). The addition of Lp(a) to the EAS risk score further enhances the correlation (rho = 0.397, $p$ < 0.001).

Receiver operating curve (ROC) analyses for risk prediction using various risk scores and Lp(a) levels of >30 mg/dL are in Figure 1. Mean area under the curve (AUC) with various scores were for Framingham 0.53 (CI: 0.39–0.68, $p$ = 0.644), QRISK-3 0.57 (CI: 0.42–0.71, $p$ = 0.347), INTERHEART 0.55 (CI: 0.39–0.69, $p$ = 0.520), SCORE-2D 0.47 (CI: 0.32–0.61, $p$ = 0.654), EAS 0.65 (CI: 0.50–0.79, $p$ = 0.040), and EAS calculator with Lp(a) (>30.0 mg/dL) 0.68 (CI: 0.54–0.83, $p$ = 0.011). The ROC analysis highlights the enhanced risk discrimination of +4.6% when Lp(a) levels were incorporated into the EAS risk calculator and up to 19.6% with other risk estimation algorithms.

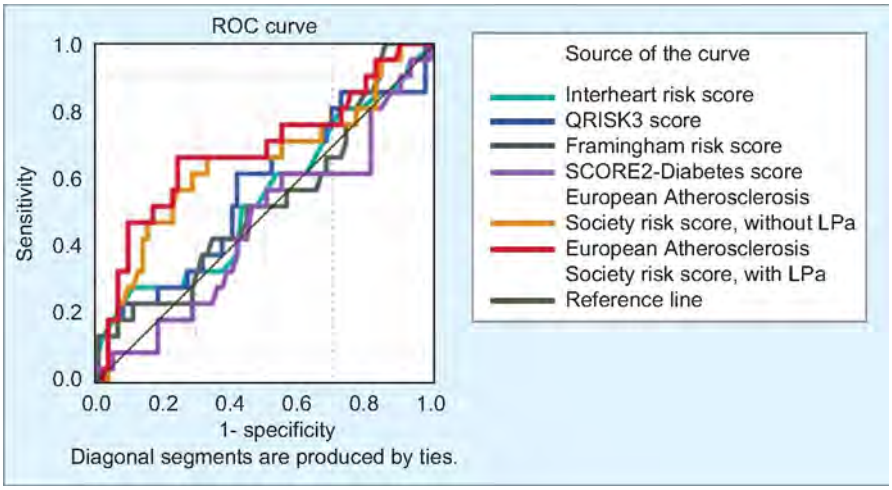


**Fig. 1:** Area-under-the-curve analyses for association of Lp(a) levels (>30 mg/dL) with cardiovascular risk scores among T2D patients using various algorithms. The highest association is with the European Atherosclerosis Society (EAS) risk score incorporating Lp(a)

## Discussion

Coronary heart disease is the most important cause of deaths in T2D.[8] The present study shows that various commonly available risk prediction tools perform suboptimally for CHD risk stratification in diabetes. We also show that the addition of lipoprotein(a) in various risk calculators enhances risk stratification. Recent studies have highlighted the importance of Lp(a) as a coronary risk factor in T2D,[22,23] and the present study, though small, suggests that Lp(a) should be routinely assessed in T2D to estimate CAD risk.

The American College of Endocrinology Consensus (2020) considers T2D as a CHD risk equivalent, and all individuals with it are classified as very high risk.[24] The Framingham risk calculator,[16] American College of Cardiology/American Heart Association (ACC/AHA),[25] and American Diabetes Association (ADA)[26] guidelines have incorporated T2D in risk calculations and do not recommend additional measures for risk estimation. The European Society of Cardiology (ESC) recommends estimation of CAD risk using a diabetes-specific SCORE-2D risk calculator.[18] Our study shows (Table 2) that Framingham and European SCORE-2D risk scores classify only a quarter to half of T2D patients as high risk, highlighting the limitations of the applicability of these risk scores for our patients. Studies have reported that raised Lp(a) levels are associated with a 2–3-fold higher risk.[22,23] In the present study, when Lp(a) is added to the usual risk calculators, there is a 5–20% greater discrimination. The ROC analysis highlights the enhanced risk discrimination when Lp(a) levels were incorporated into the EAS risk algorithm. Figure 1 also demonstrates a sensitivity of the EAS score (with or without Lp(a) inclusion) at 75% even at a specificity of

**Table 2:** Coronary risk scores for the study cohort with high, intermediate, and low cardiovascular risk

| *Risk score* | *Mean ± SD* | *High risk* | *Intermediate* | *Low risk* |
|---|---|---|---|---|
| Framingham risk score | 11.2 ± 8.7 | 13 (14.4) | 35 (38.9) | 42 (46.7) |
| QRISK-3 risk score | 28.6 ± 15.3 | 58 (64.4) | 22 (24.4) | 10 (11.1) |
| INTERHEART risk score | 21.0 ± 6.0 | 41 (45.6) | 44 (48.9) | 5 (5.6) |
| SCORE2-diabetes score | 14.9 ± 8.3 | 27 (30.0) | 46 (51.1) | 17 (18.9) |
| EAS risk score | 29.2 ± 15.2 | 64 (71.1) | 18 (20.0) | 8 (8.9) |
| EAS risk score with Lp(a) | 31.8 ± 16.5 | 67 (74.4) | 17 (18.9) | 6 (6.7) |

EAS European Atherosclerosis Society; INTERHEART Study; QRISK QRESEARCH cardiovascular risk; SCORE2-D Systematic Coronary Risk Evaluation-2 Diabetes

**Table 3:** Nonparametric (Spearman) and parametric (Pearson) correlation of Lp(a) with various risk scores

| | *Spearman's rho* | *Pearson's r* |
|---|---|---|
| Framingham risk score | 0.127 (0.234) | 0.080 (0.446) |
| QRISK-3 risk score | 0.174 (0.100) | 0.136 (0.202) |
| INTERHEART risk score | 0.137 (0.197) | 0.073 (0.495) |
| SCORE2-diabetes | 0.050 (0.641) | 0.021 (0.845) |
| EAS risk score | 0.320 (0.002) | 0.354 (0.001) |
| EAS risk score with Lp(a) | 0.397 (<0.001) | 0.418 (<0.001) |

EAS European Atherosclerosis Society; INTERHEART Study; QRISK-3 cardiovascular risk; SCORE2-D Systematic Coronary Risk Evaluation-2 Diabetes

10–60%. This observation suggests that the addition of Lp(a) to the EAS risk calculator would allow clinicians to identify higher risk in T2D patients earlier and more robustly. Beyond the presentation of statistics, the data indicate that risk reclassification increased by a range of 4.6–19.3%, contingent upon the specific model employed.

Data from the UK Biobank participants ($n$ = 4,60,506) show that the risk of myocardial infarction increases linearly beyond Lp(a) >30 mg/dL and peaks at >150 mg/dL.[27] However, there is no international consensus for the incorporation of Lp(a) in risk assessment scores; the EAS is an exception. The addition of Lp(a) in the risk assessment tool, as done by the EAS, is a step forward. Our study shows that other risk assessment tools should also do likewise. Other risk prediction tools, such as the coronary artery calcium score and raised hsCRP (highly sensitive C-reactive protein), can influence the decision for intensive lipid modifications in T2D patients.[10] It is suggested that a more robust risk assessment tool should be developed for the identification of CAD risk in T2D. Currently, there are no approved medications for lowering Lp(a), although many monoclonal antibodies and small interfering RNA (siRNA) molecules are in the pipeline.[28,29] Presently, guidelines suggest that individuals with raised Lp(a) should be advised to use high-intensity statins to control apolipoprotein B and PCSK9 inhibitors that can reduce Lp(a) levels by 25–30%.[30]

The study has several limitations. In addition to those previously mentioned, these include a small sample size, which limits the robustness of the findings and reduces confidence in the conclusions. Furthermore, the study's single-center design may not adequately represent the broader population of patients with T2D, and its hospital-based cohort design may introduce selection bias and limit generalizability. The use of a sandwich enzyme-linked immunosorbent assay, rather than more robust methods for measuring Lp(a) particle numbers and size, is another limitation. Additionally, the cross-sectional design of the study precludes the establishment of causality and the ability to track changes over time. There are no large epidemiological studies that have measured Lp(a) in the Indian population, and our findings are consistent with smaller studies that report raised Lp(a) in a quarter of the population.[31] However, the median values are lower than those reported from emigrant South Asians.[32,33] Larger studies among diabetics and non-diabetics are required to accurately identify the burden of raised Lp(a) in our country. Due to the absence of long-term follow-up, this study is unable to evaluate potential changes in lipoprotein(a) levels over time or their association with future cardiovascular outcomes. The current findings do not warrant immediate modifications to guidelines or assert causal relationships. Prospective studies or Mendelian randomization studies are needed to definitively identify the role of this lipoprotein in the pathogenesis of CAD in South Asians, a population with one of the highest rates of CAD in the world.[9]

In the present study, the rationale for incorporating Lp(a) is grounded in evidence and characterized by a measured approach. Nevertheless, the establishment of definitive clinical practice requires broader participant enrollment and extended follow-up periods.

## Conclusion

Raised Lp(a) is an important cardiovascular risk factor. The study shows that raised Lp(a) is present in a quarter of patients with T2D. Uniquely, the present study shows that incorporating Lp(a) values into multiple risk scoring algorithms significantly augments the risk and consistently demonstrates enhanced discriminatory power. Larger and prospective cohort studies are required to confirm these findings in our population.

### Ethical Consideration

Institutional ethics committee approval was obtained before the study; therefore, the study was performed according to the ethical standards of the 2013 Declaration of Helsinki. Written informed consent was obtained from each study participant before inclusion. All information collected was kept confidential.

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ORIGINAL ARTICLE

# Drug Resistance in HIV Following First-line ART Failure: Insights from a Cross-sectional Study in India

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## Abstract

**Introduction:** Our study assesses human immunodeficiency virus (HIV) drug resistance (HIVDR) in patients failing first-line (1L) antiretroviral therapy (ART) with dual nucleoside analog reverse transcriptase inhibitor (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) regimens in India.

**Methods:** In this cross-sectional study, consecutive HIV-1–infected patients aged 13 years or older, failing 1L ART after at least 12 months exposure, underwent HIV genotyping and drug resistance testing (DRT) using the ViroSeq™ HIV-1 Genotyping System and the Stanford HIV-1 Database, with HIVDR classification based on a penalty score of ≥30.

**Results:** Among 115 eligible participants, 110 underwent DRT, revealing efavirenz (EFV) or nevirapine (NVP) resistance rates of 85.3% ($n$ = 93/109) and 87.2% ($n$ = 95/109), respectively, and substantial cross-resistance to rilpivirine (RPV) (37.6%, $n$ = 41/109), etravirine (ETV) (30.3%, $n$ = 33/109), and doravirine (DOR) (60.5%, $n$ = 66/109).

The cohort was categorized into 3 groups based on their previous ARV drug exposure: group A (36.4%, $n$ = 40) with prior TA exposure (AZT or d4T) but no TFV exposure; group B (19.1%, $n$ = 21) with prior nonconcomitant exposure to both TAs and TFV; and group C (44.5%, $n$ = 49), exposed to TFV only. Despite group B's 1L ART regimen failure with TFV, the prevalence of AZT resistance was similar (difference in proportions, ΔP: 14.6%, $p$ = 0.277) between group A [57.5% ($n$ = 23/40)] and group B [42.9% ($n$ = 9/21)].

TFV resistance was comparable (ΔP: 0.8%,$p$ = 0.947) between group A (32.5%, $n$ = 13/40) and group B (33.3%, $n$ = 7/21), despite group A's lack of TFV exposure, and was also similar to the TFV-only–exposed group (group C: 38.8%, $n$ = 19/49).

Regarding distinct DRM patterns, the prevalence of K65R DRM was higher (ΔP: 22.4%, $p$ = 0.060) among TFV-only–exposed patients (group C: 36.7%, $n$ = 18/49) compared with PLH exposed to both TAs and TFV (group B: 14.3%, $n$ = 3/21), whereas multiple TAMs occurred at similar rates (ΔP: 12.1%, $p$ = 0.367) among TA-exposed patients [group A: 55.0% ($n$ = 22/40) vs group B: 42.9% ($n$ = 9/21)].

**Conclusion:** The research provides insights into the complexities of HIVDR, emphasizing the interplay of resistance patterns and the role of drug exposure history, especially in the context of resistance to TFV and second-generation NNRTIs.

**Clinical significance:** Ensuring adequate drug exposure history in patients can prevent poor outcomes in PLH being treated with ART due to resistance. Resistance profiling is especially relevant following first-line ART failure.


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## Introduction

A 0.22% prevalence of human immunodeficiency virus (HIV) in India emphasizes the imperative for effective antiretroviral therapy (ART) strategies.[1,2] Dolutegravir (DTG) as a first-line (1L) treatment has enhanced efficacy and reduced resistance,[3] but a considerable number of persons living with HIV (PLH) still use non-nucleoside reverse transcriptase inhibitor (NNRTI) regimens.

NNRTIs, lauded for their affordability and effectiveness, historically formed the cornerstone of HIV elimination efforts.[4,5] Concerns surrounding NNRTI resistance, especially with suboptimal adherence, have underscored the need for alternative approaches.[6] Understanding NNRTI resistance remains relevant, even as their role shifts in primary regimens, as they continue to be part of alternative 1L strategies recommended by the World Health Organization (WHO).[7]

Nucleoside analog reverse transcriptase inhibitors (NRTIs), particularly tenofovir (TFV), remain crucial backbone agents even with DTG-based 1L strategies. Moreover, their enduring relevance, unlike NNRTIs, extends into the realm of second-line (2L) ART, highlighting the complexities of NRTI resistance beyond 1L therapy.[7,8] The potential impact of TFV resistance on future treatment outcomes necessitates thorough evaluation.

The scope of NNRTIs has gained renewed vigor with second-generation NNRTIs, offering the potential for tailored therapies for both ART-naïve PLH and those facing treatment failure across multiple drug classes.[9–12] However, their susceptibility to resistance, including cross-resistance from first-generation NNRTI drug-resistance mutations (DRMs),[10,13,14] is a less explored aspect that is crucial, especially in cohorts heavily exposed to NNRTIs.

Our study comprehensively evaluates HIV drug resistance (HIVDR) in a sizable cohort of PLH who failed on a 1L dual NRTI + NNRTI regimen. Our particular focus is on resistance to TFV and second-generation NNRTIs within this context.

## Methods

### Study Design, Subjects, and Sample Size

This institutional-based cross-sectional study included consecutive HIV-1-infected patients aged ≥13 years, on 1L ART for ≥12 months between July 2019 and May 2021. Inclusion criteria were: (1) PLH on a 1L ART regimen with a dual NRTI backbone [lamivudine (3TC) or emtricitabine (FTC) along with tenofovir (TFV) or zidovudine (AZT)] and a single NNRTI core agent [efavirenz (EFV) or nevirapine (NVP)], (2) failing 1L ART [defined as 2 consecutive viral load (VL) measurements ≥1,000 cp/mL, with 6–8 weeks of enhanced adherence in between], and (3) a most recent VL meeting the threshold for HIV sequencing (VL ≥2,000 cp/mL).

Exclusions comprised: (1) PLH on 2L ART, (2) prior exposure to abacavir (ABC),

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integrase strand transfer inhibitors (INSTI), or boosted protease inhibitors (b/PI), (3) individuals denying consent, and (4) those with sequencing failures. The study patients' HIV-1 management adhered to WHO recommendations from 2016, involving dual NRTI + NNRTI as 1L ART, with EFV as the preferred NNRTI.[15,16] Notably, second-generation NNRTIs were not available in India during the study period. Consequently, none of the patients were exposed to doravirine (DOR), rilpivirine (RPV), or etravirine (ETV). The study received ethical approval from the Institutional Ethics Committee.

According to WHO guidelines, to assess clinic-level HIVDR 12 months after ART initiation at a sentinel site, screening 115 patients, allowing for a 20% attrition rate due to missing data and genotyping failures, effectively results in a sample size of 96 participants consecutively failing 1L ART.[17,18]

### Genotype Sequencing and Sequence Analysis

Samples with VL ≥2,000 cp/mL underwent HIV genotyping and drug resistance testing (DRT) using the ViroSeq™ HIV-1 Genotyping System [reverse transcriptase (RT) gene (codons 1–240), protease gene (codons 1–99), and integrase-encoding regions]. PI, NRTI, and NNRTI DRMs were identified using ViroSeq software™ in conjunction with the updated (version 9.1, update 2022-06-02) Stanford HIV-1 Database (hivdb.stanford.edu).[19]

HIVDR interpretation is based on penalty scores: susceptible (Sus), potential low-level resistance (<15; Pot-LLR), low-level resistance (15–29; LLR), intermediate resistance (30–59; IR), and high-level resistance (≥ 60; HLR).[19] In cases of nucleotide mixtures, these were considered mutant based on their impact on encoded amino acids.[20] Multidrug resistance referred to resistance to ≥2 drugs from the 1L ART regimen.[21,22] To classify patients failing 1L ART as having HIVDR in this study, a penalty score of ≥30 (IR or HLR) was used.

### Data Collection and Statistical Analysis

Demographic and clinical data were collected through interviews and treatment record reviews using semistructured case report forms. Blood samples were collected for plasma VL, CD4+ cell count, and DRT. Screening for opportunistic infections (OIs) and relevant investigations were conducted. All patients provided informed consent and were informed of the results.

As general considerations, categorical variables are presented as percentages with 95% confidence intervals (CI) (1-sample binomial test, Clopper–Pearson exact method). Continuous variables are described with either mean ± standard deviation (SD) or median and interquartile range (IQR), as applicable. Between-group comparisons were made using the Chi-squared test or Fisher exact test, depending on expected counts. Statistical significance was set at $p < 0.05$ (2-tailed), with corrections for multiple comparisons where necessary. Statistical analyses were performed using Excel for Microsoft Office 365 (Microsoft Corporation, Redmond, WA, USA), Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, version 23.0; IBM Corp., Armonk, NY, USA), and Prism GraphPad 8.1 (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

## Results

### Demographic and Baseline Characteristics, and First-line Antiretroviral Therapy Regimens

Out of 115 eligible participants, 110 underwent HIV genotyping and DRT (Fig. 1). The median age of the study participants was 41 (IQR 13; range 13–78) years, with 24.5% ($n$ = 27) females. The median 1L ART duration was 81 (IQR 79; range 12–199) months (Table 1).

DRMs were anticipated to align with the patients' exposure to specific antiretroviral (ARV) agents, leading to 3 groups based on their ARV drug exposure: group A, prior TAs (AZT or d4T) but no TFV exposure (36.4%, $n$ = 40); group B, prior nonconcomitant exposure to TAs and TFV [TA exposure for 40 (median) months followed by TFV exposure for 63 (median) months] (19.1%, $n$ = 21); and group C, TFV-only exposure (44.5%, $n$ = 49). Demographics and baseline characteristics are shown in Table 1.

The predominant HIV-1 genotype was C (genotype C: 99.1%, $n$ = 109; genotype A: $n$ = 1) (Table 1). Overall, 13.8% ($n$ = 15/109) exhibited complete susceptibility across drug classes (NNRTI, NRTI, and PI), with varying percentages across exposure groups: group A, 15.0% ($n$ = 6/40); group B, 9.5% ($n$ = 2/21); and group C, 14.6% ($n$ = 7/48).

### Resistance to Non-nucleoside Reverse Transcriptase Inhibitors

Predicted susceptibility to NNRTIs after 1L ART failure: One (out of 110) sample was excluded due to missing data. NNRTI resistance (IR or HLR to at least 1 NNRTI) was detected in 86.2% [($n$ = 94/109), 95% CI: 78.3–92.1] of PLH failing 1L ART. Overall, 22.9% [($n$ = 25/109), 95% CI: 15.4–31.9] of PLH displayed resistance to all NNRTIs.

Resistance to EFV and NVP: EFV and NVP resistance were observed in 85.3% [($n$ = 93/109), 95% CI: 76.4–90.7] and 87.2% [($n$ = 95/109), 95% CI: 78.5–92.2] of PLH, respectively (Fig. 2).

Resistance to second-generation NNRTIs: Cross-resistance (IR or HLR) to RPV occurred in 37.6% ($n$ = 41/109, 95% CI: 28.5–47.4), to ETV in 30.3% ($n$ = 33/109, 95% CI: 21.8–39.8), and to DOR in 60.5% ($n$ = 66/109, 95% CI: 50.2–69.2) (Fig. 2).

ETV resistance was twice as frequent [difference in proportions (ΔP): 20.7% (95% CI: 0.2–41.3), $p$ = 0.038] in NVP-exposed compared with EFV-exposed patients. A similar trend [ΔP: 29.8% (95% CI: 8.7–50.8), $p$ = 0.005] was observed for RPV resistance in NVP-exposed compared with EFV-exposed patients. In contrast, DOR resistance was markedly more frequent [ΔP: 43.6% (95% CI: 24.5–62.6), $p < 0.001$] in EFV-exposed [74.6% ($n$ = 47/63), 95% CI: 63.9–85.4] than in NVP-exposed [31.0% ($n$ = 9/29), 95% CI: 15.3–50.8] patients (Table S1).

Patterns of NNRTI DRMs: The most common NNRTI DRM was K103N/S [49.5% ($n$ = 54/109), 95% CI: 38.9–58.4], followed by V106M [29.4% ($n$ = 32/109), 95% CI: 20.2–37.9], G190A [16.5% ($n$ = 18/109), 95% CI: 10.1–24.8], and Y181C [13.8% ($n$ = 15/109), 95% CI: 7.9–21.7] (Fig. 3). K103N/S occurred in isolation (absence of major NNRTI DRMs) in only 10.1% ($n$ = 11/109, 95% CI: 5.1–17.3) of PLH.

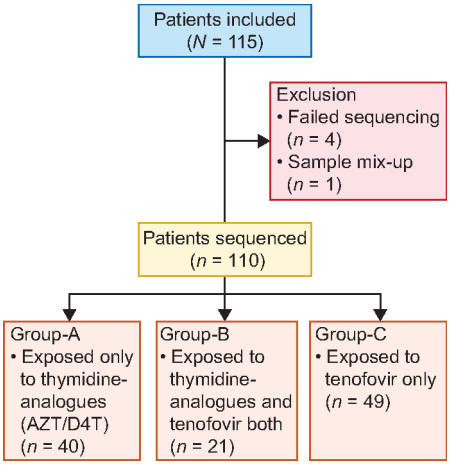


**Fig. 1:** Patient flow. This figure illustrates the flow of patients and their inclusion in the study. Out of the initial 115 people living with HIV (PLH) who met the inclusion criteria, 4 samples failed sequencing, and 1 sample was excluded due to a mix-up. The remaining 110 PLH were categorized into 3 groups: group A, group B, and group C. Within our cohort, 48.2% ($n$ = 53) started 1L-ART before 2014, when AZT was predominantly used in 1L-ART, of which 90.6% ($n$ = 48/53) had been exposed to AZT, and 30.2% ($n$ = 16/53) had received both AZT/D4T (thymidine analogs, TAs) and TFV. The remaining 57 PLH initiated 1L-ART after 2014, with the majority (89.5%, $n$ = 51/57) having received TFV and 8.8% ($n$ = 5/57) with exposure to both TAs and TFV

**Table 1:** Baseline characteristics

| *Characteristics* | *Total (n = 110)* | *Group A (n = 40)* | *Group B (n = 21)* | *Group C (n = 49)* |
|---|---|---|---|---|
| Sex | | | | |
| Male (%, *n*) | 75.5% (83) | 70% (28) | 81% (17) | 77.6% (38) |
| Female (%, *n*) | 24.5% (27) | 30% (12) | 19% (4) | 22.4% (11) |
| Age | | | | |
| Age (median, IQR, range) years | 41 (IQR: 13, range: 13–78) | 45 (IQR: 12, range: 13–62) | 40 (IQR: 14, range: 21–55) | 40 (IQR: 10, range: 26–78) |
| Year of initiation of ART | | | | |
| During or after 2014* (%, *n*) | 51.8% (57) | 15% (6) | 23.8% (5) | 93.9% (46) |
| Before 2014 (%, *n*) | 48.2% (53) | 85% (34) | 76.2% (16) | 6.1% (3) |
| Duration of first-line ART | | | | |
| First-line ART (median, IQR, range) months | – | – | 40 (IQR: 69, range: 8–112) (*n* = 19) | – |
| Alternative first-line ART (median, IQR, range) months | – | – | 63 (IQR: 69, range: 4–137) (*n* = 19) | – |
| Overall (median, IQR, range) months | 81 (IQR: 79, range: 12–199) | 124 (IQR: 62, range: 19–199) | 103 (IQR: 38, range: 59–198) | 35 (IQR: 43, range: 12–120) |
| Cytosine analog exposure | | | | |
| 3TC only (%, *n*) | 97.3% (107) | 100% (40) | 90.5% (19) | 98% (48) |
| FTC only (%, *n*) | 0.9% (1) | 0% (0) | 4.8% (1) | 0% (0) |
| Both 3TC and FTC (%, *n*) | 1.8% (2) | 0% (0) | 4.8% (1) | 2% (1) |
| First-line NNRTI agent | | | | |
| EFV (%, *n*) | 57.3% (63) | 22.5% (9) | 33.3% (7) | 95.9% (47) |
| NVP (%, *n*) | 26.4% (29) | 67.5% (27) | 4.8% (1) | 2% (1) |
| Both EFV and NVP (%, *n*) | 16.4% (18) | 10% (4) | 61.9% (13) | 2% (1) |
| Adherence to regimen prior to diagnosis of first-line ART failure | | | | |
| ≥95% (%, *n*) | 32.3% (30) | 34.4% (11) | 16.7% (3) | 37.2% (16) |
| 85–94% (%, *n*) | 25.8% (24) | 25% (8) | 33.3% (6) | 23.3% (10) |
| <85% (%, *n*) | 41.9% (39) | 40.6% (13) | 50% (9) | 39.5% (17) |
| Not assessed/ doubtful (*n*) | *n* = 17 | *n* = 8 | *n* = 3 | *n* = 6 |
| Immunovirological status | | | | |
| Baseline PVL (median, IQR, range) Log10 Copies/mL | 4.8 (IQR: 1.3, range: 3.3–7.9) | 4.4 (IQR: 1.4, range: 3.3–6.6) | 4.7 (IQR: 1.3, range: 3.7–6.8) | 4.9 (IQR: 1.4, range: 3.3–7.9) |
| PVL 2000–1,00,000 copies/mL (%, *n*) | 53.6% (59) | 55% (22) | 57.1% (12) | 51% (25) |
| PVL 1,00,000–3,00,000 copies/mL (%, *n*) | 21.8% (24) | 22.5% (9) | 28.6% (6) | 18.4% (9) |
| PVL >3,00,000 copies/mL (%, *n*) | 24.5% (27) | 22.5% (9) | 14.3% (3) | 30.6% (15) |
| CD4 (median, IQR, range) cells/mm$^3$ | 169 (IQR: 212, range: 7–892) | 189 (IQR: 259, range: 7–892) | 164 (IQR: 202, range: 11–400) | 150 (IQR: 179, range: 10–779) |
| CD4 200–499 200 cells/mm$^3$ (%, *n*) | 33.6% (37) | 35.0% (14) | 38.09% (8) | 30.6% (15) |
| Advanced HIV (CD4 <200 cells/mm$^3$) (%, *n*) | 53.6% (59) | 47.5% (19) | 57.1% (12) | 57.1% (28) |
| Coinfections | | | | |
| VDRL reactive (*n*) | – | – | – | – |
| HBsAg reactive (*n*) | *n* = 4 | *n* = 1 | *n* = 0 | *n* = 3 |
| Hepatitis-C (n) | *n* = 1 | *n* = 1 | *n* = 0 | *n* = 0 |
| HIV-1 genotype | | | | |
| C (%, *n*) | 99.1% (109) | 100% (40) | 100% (21) | 98% (48) |
| A (%, *n*) | 0.9% (1) | 0% (0) | 0% (0) | 2% (1) |

*2014 marks the year of implementation of TFV in national program; 3TC, lamivudine; ART, antiretroviral therapy; EFV, efavirenz; FTC, emtricitabine; HBsAg, hepatitis-B surface antigen; IQR, interquartile-range; NVP, nevirapine; PVL, plasma viral-load

## Resistance to Nucleoside Reverse Transcriptase Inhibitors

Predicted susceptibility to NRTIs after 1L ART failure: Among the 110 participants, 80.0% [(*n* = 88/110), 95% CI: 71.3–87.0] showed resistance (IR or HLR) to 3TC and FTC due to M184V/I DRMs. TFV and AZT resistance occurred in 35.5% [(*n* = 39/110), 95% CI: 26.6–45.1] and 31.8% [(*n* = 35/110), 95% CI: 23.3–41.4] of PLH, respectively, with no significant difference ($p$ = 0.568). Overall, 16.4% (*n* = 18/110, 95% CI: 9.9–24.6) had dual resistance to TFV and AZT, whereas 49.1% [(*n* = 54/110), 95% CI: 39.4–58.8] remained susceptible to both. TFV susceptibility with

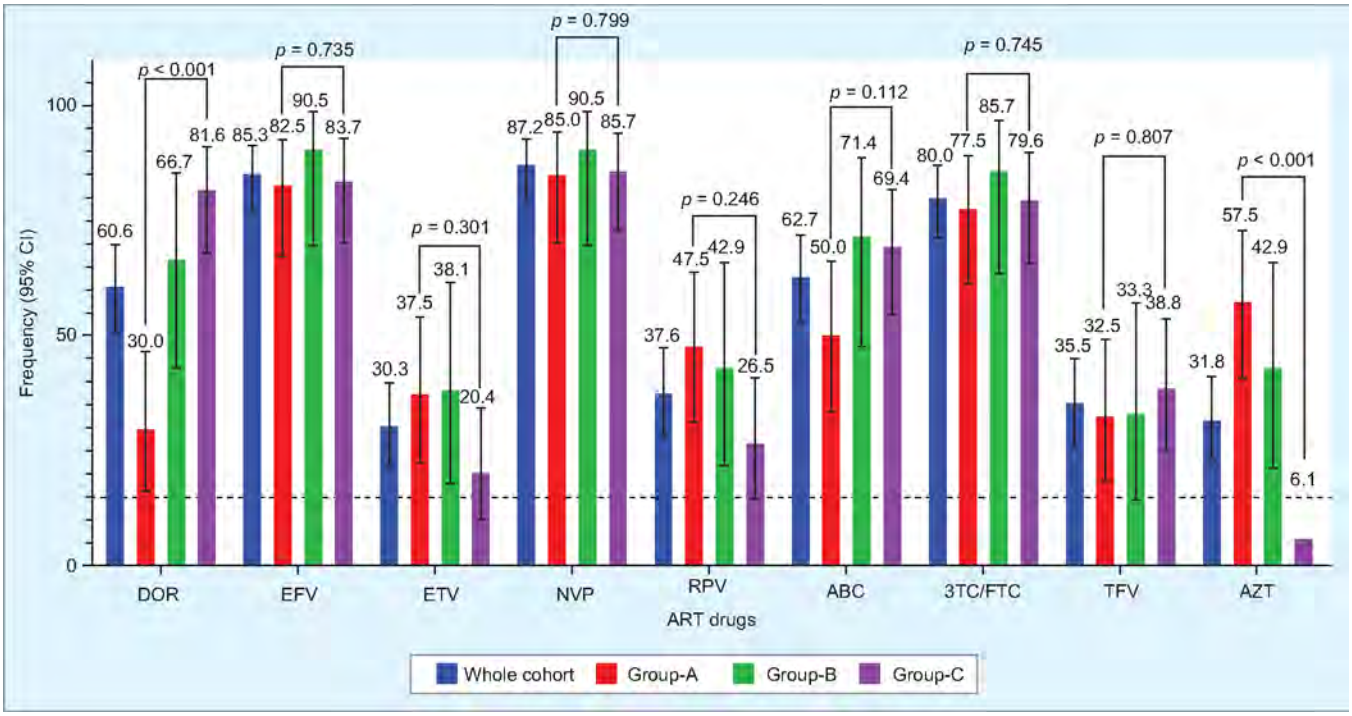


**Fig. 2:** Resistance to NRTI and NNRTI. The figure illustrates the frequency of drug resistance (IR or HLR) to specific antiretroviral agents (ARVs) in the entire cohort as well as in subgroups A, B, and C, denoted by blue, red, green, and purple bars, respectively. Error bars represent the 95% confidence interval (CI), and corresponding *p*-values indicating differences in proportions among groups A to C are provided; 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; AZT, zidovudine; DOR, doravirine; EFV, efavirenz; ETV, etravirine; FTC, emtricitabine; NVP, nevirapine; RPV, rilpivirine

AZT resistance was observed in 48.6% ($n$ = 17/35) of PLH, and AZT susceptibility with TFV resistance in 53.8% ($n$ = 21/39).

Resistance to AZT: AZT resistance was comparable [ΔP: 14.6% (95% CI: −11.5–40.8), $p$ = 0.277] among groups with prior TA exposure [group A: 57.5% ($n$ = 23/40, 95% CI: 40.8–72.9) vs group B: 42.9% ($n$ = 9/21, 95% CI: 21.8–65.9)], despite failure of a TFV-containing 1L ART regimen in group B. Three PLH in group C had AZT resistance (Fig. 2).

Resistance to TFV: TFV resistance was comparable [ΔP: 0.8% (95% CI: −24.0–25.7), $p$ = 0.947] among groups with prior TA exposure [group A: 32.5% ($n$ = 13/40, 95% CI: 18.6–49.1) vs group B: 33.3% ($n$ = 7/21, 95% CI: 14.6–56.9)], despite group A's lack of TFV exposure. TFV resistance among the TFV-only–exposed group [group C: 38.8% ($n$ = 19/49, 95% CI: 25.2–53.7)] was similar to group A [ΔP: 6.2% (95% CI: −13.6–26.2), $p$ = 0.539] and to group B [ΔP: 5.4% (95% CI: −18.9–29.8), $p$ = 0.666] (Fig. 2).

Patterns of NRTI DRMs: The TFV DRM K65R occurred in 19.1% ($n$ = 21/110, 95% CI: 12.2–27.7) of patients, and multiple (≥ 2) thymidine analog mutations (TAMs) occurred in 30.9% ($n$ = 34/110, 95% CI: 22.4–40.4) [TAM-1: 6.4% ($n$ = 7/110), TAM-2: 10.0% ($n$ = 11/110), mixed TAM-1 and TAM-2 pattern: 14.5% ($n$ = 16/110)].

Among TFV-exposed patients, K65R occurred more frequently in the TFV-only–exposed group [group C: 36.7% ($n$ = 18/49, 95% CI: 23.4–51.7)] compared with PLH exposed to both TAs and TFV [group B: 14.3% ($n$ = 3/21, 95% CI: 3.1–36.3)], showing a trend toward statistical significance [ΔP: 22.4% (95% CI: 2.3–42.6), $p$ = 0.060] (Fig. 2).

Multiple TAMs occurred in 55.0% ($n$ = 22/40, 95% CI: 38.5–70.7) of group A and 42.9% ($n$ = 9/21, 95% CI: 21.8–65.9) of group B. This difference was not statistically significant [ΔP: 12.1% (95% CI: −14.0–38.3), $p$ = 0.367]. Multidrug NRTI DRMs, including T69INS and the Q151 complex, were not observed (Fig. 2).

Distinctive patterns of K65R were observed. K65R occurred in isolation or with M184V/I ± other TFV DRMs (K70E or Y115F) in 57.1% ($n$ = 12/21, 95% CI: 34.0–78.1) of PLH harboring K65R. Among the remaining 9 sequences, K65R coexisted with a single TAM (not compromising AZT) in 8 sequences [group C: $n$ = 7/8 (K65R + M41L: $n$ = 5/8; K65R + K219E: $n$ = 2/8); group B: $n$ = 1/8 (K65R + D67N)]. One group B sequence harbored M41L + K65R + M184V + T215YS, compromising both AZT and TFV.

Three patients in group C had multiple TAMs despite no documented TA exposure. Post hoc recategorization into group B yielded similar results (Table S2 and Table S3). Brief results are presented below.

Group A (TA-only exposure) had a higher occurrence of multiple TAMs [55.0% ($n$ = 22/40, 95% CI: 38.5–70.7)] compared with 50.0% ($n$ = 12/24, 95% CI: 29.1–70.9) in group B (TA exposure followed by virological failure on a TFV backbone), but the difference was not statistically significant [ΔP: 5.0% (95% CI: −20.3–30.3), $p$ = 0.698]. Accordingly, AZT resistance was 57.5% ($n$ = 23/40, 95% CI: 40.9–72.9) in group A and 50.0% ($n$ = 12/24, 95% CI: 29.1–70.9) in group B, with no significant difference [ΔP: 7.5% (95% CI: −17.7–32.7), $p$ = 0.560] (Table S2).

K65R occurred more frequently in group C (TFV-only exposure) [39.1% ($n$ = 18/46, 95% CI: 25.1–54.6)] compared with group B [12.5% ($n$ = 3/24, 95% CI: 2.7–32.4)], with a statistically significant difference [ΔP: 26.6% (95% CI: 7.3–45.9), $p$ = 0.021]. However, TFV resistance, although higher in group C [41.3% ($n$ = 19/46, 95% CI: 26.9–56.8)] than in group B [29.2% ($n$ = 7/24, 95% CI: 12.6–51.1)], did not reach statistical significance [ΔP: 12.1% (95% CI: −10.9–35.2), $p$ = 0.318]. As expected, K65R was not observed in group A, yet TFV resistance in group A [32.5% ($n$ = 13/40, 95% CI: 18.6–49.1)] was comparable to group B [ΔP: 3.3% (95% CI: −19.9–26.6), $p$ = 0.781] and group C [ΔP: 8.8% (95% CI: −11.5–29.1), $p$ = 0.399] (Table S2).

### Resistance to Protease Inhibitor

PI resistance was observed in only 6 PLH, with no DRMs detected for commonly used PIs, including atazanavir, darunavir, and lopinavir.

## Discussion

Our study reaffirms the widespread occurrence of NRTI resistance, particularly against TFV, in

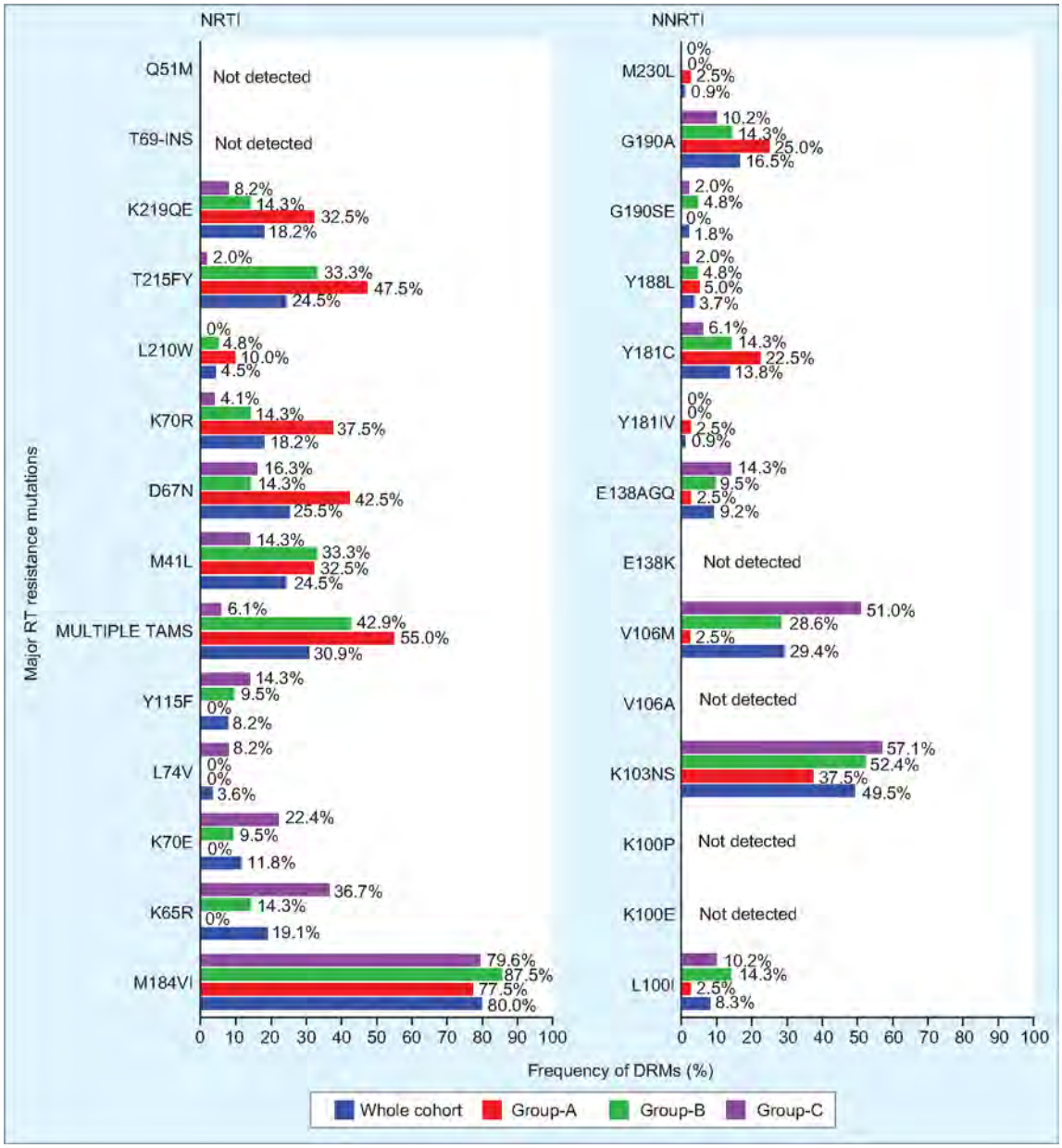


**Fig. 3:** Major RT resistance mutations. This figure displays the frequency of major NRTI and NNRTI drug-resistance mutations (DRMs) in the entire cohort, as well as in subgroups A, B, and C, represented by blue, red, green, and purple bars, respectively; DRMs, drug-resistance mutations; RT, reverse transcriptase

PLH failing 1L ART with dual NRTI + NNRTI, consistent with recent findings highlighting 58–86% TFV resistance, largely associated with the K65R DRM.[23,24] Notably, the international TENORES study, a comprehensive assessment of HIVDR following the scale-up of WHO-recommended TFV-based ART, revealed TFV resistance rates ranging from 20 to 50%, with higher prevalence in low- and middle-income countries (LMICs) across sub-Saharan Africa.[25] In the Indian context, Dinesha et al.'s recent study echoes this trend, revealing TFV resistance linked to the K65R DRM in 28.1% of patients failing TFV-based regimens, with 10.6% of the K65R-negative subgroup exhibiting TFV resistance.[26] Our findings indicate an overall TFV resistance rate of 37.14% among PLH failing a TFV-containing regimen, underscoring the need to address emerging challenges related to TFV resistance, particularly in LMICs where alternative treatment options may be limited.

In our cohort, K65R DRM, a key TFV DRM, was present in nearly 20% of cases. While the escalating prevalence of K65R DRM appears promising for AZT-based 2L ART,[7] the existence of unconventional resistance profiles within a subset of patients complicates matters. This intriguing observation in our study centers on patients who were sequentially exposed to TAs (AZT or d4T), followed by TFV in their 1L ART regimens (group B). While TFV-selected DRMs were expected to be prevalent in this subcohort, we observed a lower prevalence of the K65R DRM (20.4%) and a higher occurrence of multiple TAMs (38.09%). Previous research has documented the occurrence of multiple TAMs in up to 40% of patients failing TFV-based 1L ART, with 13.3–27.6% of such patients not exhibiting susceptibility to AZT.[6,13,23,27,28] Notably, a large secondary analysis of data from the TenoRes study by Gregson et al. indicated that TAMs specifically selected by AZT or d4T were present in approximately 16% of patients failing TFV-based first-line ART.[29] These resistance patterns suggest potential challenges for both TFV and AZT in 2L ART, regardless of the presence of TFV in the failing regimen.

The often understudied phenomenon of cross-resistance to TFV in individuals with no prior TFV exposure and resistance

to AZT in patients failing TFV-based 1L ART holds important implications for future ART regimens for such patients. Given WHO's public health approach of changing the NRTI backbone agent during the transition from first-line to second-line ART[7], addressing and comprehending resistance to both AZT and TFV within this context becomes paramount.

Another intriguing observation in our study is the coexistence of K65R and TAMs in PLH failing TFV-based 1L ART, despite their known antagonism, highlighting diverse coexistence patterns of K65R and TAMs and emphasizing distinct TAM profiles in the presence of K65R.[29,30] Specifically, our findings showed that in 55% of K65R DRM cases, K65R was detected alone or with M184V/I ± other TFV DRMs, such as K70E or Y115F. In 40% of cases, K65R coexisted with a non-AZT-compromising TAM. Notably, 1 sequence displayed M41L + K65R + M184V + T215YS.

In summary, our findings have 3 important implications for TFV resistance. First, virological failure on TFV-containing 1L ART is influenced by both the failing regimen and prior TA exposure, leading to TAM accumulation and impacting AZT efficacy. Second, our precise treatment records enabled categorization of PLH failing 1L ART into 3 different exposure groups, wherein the occurrence of multiple TAMs in PLH sequentially exposed to TAs and TFV reflects mechanisms of TAM emergence in TFV-based 1L ART failure, as outlined by Gregson et al.,[29] including pretreatment resistance, programmatic substitution (occult treatment failure during programmatic substitution to TFV), and undisclosed ART exposure. Third, our observation of nearly 40% AZT resistance in those failing TFV-based 1L ART but with previous exposure to TAs highlights the importance of accurate treatment histories and genotypic drug resistance testing and challenges WHO's 2L ART recommendations[7] for TFV-based 1L ART failures. This is especially relevant for patients with complex treatment backgrounds who initiated ART before TFV inclusion in the national program and experienced interruptions, where substituting TFV with AZT in second-line ART might compromise treatment efficacy.

In the context of NNRTI resistance, it is unsurprising that a high percentage (86.2%) of patients failing 1L ART with dual NRTI + NNRTI exhibited NNRTI resistance, primarily involving EFV or NVP.[6,13,24,26,28,31,32] The prominence of these NNRTIs has historically been central to combination ART, but their susceptibility to resistance due to factors such as low genetic barriers and longer half-lives, especially with suboptimal adherence,[33,34] highlights the need for a critical appraisal of these drugs. As DTG gains prominence as the preferred 1L ART, our findings remain relevant, shedding light on the progression and patterns of DRMs, potentially impacting transmission. A significant aspect of our study focuses on the emergence of resistance to second-generation NNRTIs, an unexplored facet in India.

The primary NNRTI DRM identified in our study was K103N/S (48.6%), followed by V106M (28.4%), G190A (16.5%), and Y181C (13.8%), consistent with findings from broader studies in Brazil,[35] South Africa,[13,27] and China.[36] Notably, Y181C is more prevalent in patients exposed to NVP, while V106M is associated with EFV use.[13] In the Indian context, a systematic review and meta-analysis by Karade et al. documented the relative prevalence of K103N, Y181C, and G190A mutations in Indian PLH.[37] Our findings are in agreement with these studies, confirming the prevalence of specific mutations in Indian PLH. In contrast, a 2017 Indian study by Dutta et al. found Y188L as the most common DRM (18.18%), followed by K103N (6.81%).[38]

The prevalence of the K103N/S DRM in our study is noteworthy, even though it does not directly affect second-generation NNRTIs (ETV and RPV).[27,39] Despite this, our study found substantial resistance rates for both ETV (30.3%) and RPV (37.6%). This trend could be linked to the relatively limited occurrence of K103N/S in isolation, consistent with findings from previous studies.[13,28] The presence of the Y181C mutation, while less frequent than K103N/S, is concerning due to its association with resistance against both ETV and RPV.[40] These findings suggest that >33% of our patients exhibited resistance to second-generation NNRTIs to which they had not been previously exposed.

Our study sets the stage for the introduction of second-generation NNRTIs into India's public health programs. However, concerns arise about their future effectiveness, particularly with approximately 33% of patients on first-line NNRTI-based ART potentially experiencing issues with the RPV + CAB ART regimen, necessitating guided DRT. Similarly, the presence of ETV resistance in a significant proportion of viremic patients on 1L ART suggests that the use of ETV in subsequent ART regimens and tailored therapy, including third-line salvage regimens, should also be guided by DRT.[13,24] Strengthening HIV genotyping and DRT is critical as these second-generation NNRTIs are integrated into India's public health framework.

Regarding DOR, a new-generation NNRTI of interest due to its effectiveness in patients with K103N and G190A DRMs and currently under investigation in combination with islatravir, a nucleoside reverse transcriptase translocation inhibitor,[41,42] our study found a considerable prevalence of DOR resistance (59.6%) in patients failing first-line ART, with V106M emerging as the second most prevalent NNRTI DRM (28.4%). Notably, the prevalence of DOR resistance in our Indian cohort differs from African (84.8%)[10] and European (nearly 20%)[43,44] cohorts, possibly due to variation in the occurrence of the V106M DRM. Regular surveillance is crucial to assess the feasibility of DOR implementation, given the substantial prevalence of DOR resistance in our cohort.

The less frequently examined drug ABC showed substantial resistance at 62.7% in our cohort of patients failing 1L ART, consistent across all subcohorts, suggesting limited efficacy in those unable to tolerate AZT in second-line ART.[13] Fortunately, no PI mutations were identified, representing a favorable outcome.

The findings of our study should be considered within the context of certain limitations. DRT was conducted after prolonged viremia without considering episode durations, limiting insights into the temporal progression of resistance. Additionally, the potential influence of CD4 cell count or viral load on DRMs was not assessed. The lack of pretreatment drug resistance information may have led to an overestimation of resistance levels due to unrecognized transmitted DRMs. Furthermore, the cross-sectional design of our study is inherently prone to bias.

Nonetheless, our study comprehensively analyzes HIV drug resistance after 1L ART failure in India in a sizeable population. Subgrouping by drug exposure offers a distinctive perspective on HIVDR, particularly regarding TFV and AZT, which is crucial for informing ART switch strategies. Our assessment of NNRTI resistance, including second-generation NNRTIs, emphasizes the potential strategic use of DTG to address resistance concerns and ensure sustained efficacy of these agents in future ART regimens.

## Conclusion

Our comprehensive study on HIV drug resistance after 1L ART failure in India emphasizes the importance of TFV and AZT resistance, especially in the context of subsequent ART regimens. Our findings also highlight the resistance profiles of second-generation NNRTIs and suggest that DTG integration could address resistance concerns, ensuring ongoing NNRTI efficacy in future ART regimens.

### Clinical Significance

Ensuring adequate drug exposure history in patients can prevent poor outcomes in PLH being treated with ART due to resistance.

Resistance profiling is especially relevant following first-line ART failure. NNRTIs remain viable ART options in Indian PLH despite the presence of DRMs.


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**Table S1:** Resistance to second generation NNRTIs

| | *EFV in first-line ART (n = 63)* | *NVP in first-line ART (n = 29)* | *Diff. in proportions (95% CI)* | *p* |
|---|---|---|---|---|
| ETV% [(*n*) 95% CI] | 20.6% (*n* = 13/63) 95% CI: 11.5–32.7 | 41.4% (*n* = 12/29) 95% CI: 23.5–61.06 | 20.7% (95% CI: 0.2–41.3) | 0.038 |
| RPV [(*n*) 95% CI] | 25.4% (*n* = 16/63) 95% CI: 15.3–37.9 | 55.2% (*n* = 16/29) 95% CI: 35.7–73.6 | 29.8% (95% CI: 8.7–50.8) | 0.005 |
| DOR [(*n*) 95% CI] | 74.6% (*n* = 47/63) 95% CI: 63.9–85.4 | 31.03% (*n* = 9/29, 95% CI: 15.3–50.8) | 43.6% (95% CI: 24.5–62.6) | <0.001 |

*n* = 18 PLH who were exposed to both EFV and NVP in their 1L ART were excluded

**Table S2:** Drug susceptibility profile and DRMs after recategorizing three patients in group B rather than in group C

| *Characteristics* | *Total (n = 110)* | *Group A (n = 40)* | *Group B (n = 24)* | *Group C (n = 46)* |
|---|---|---|---|---|
| Drug-susceptibility profile | | | | |
| DOR | 60.5 (*n* = 66, 95% CI: 50.2–69.2) | 30 (*n* = 12, 95% CI: 16.6–46.5) | 70.8 (*n* = 17, 95% CI: 48.9–87.4) | 80.4 (*n* = 37, 95% CI: 66.1–90.6) |
| EFV | 85.3 (*n* = 93, 95% CI: 76.4–90.7) | 82.5 (*n* = 33, 95% CI: 67.2–92.7) | 91.7 (*n* = 22, 95% CI: 73–99) | 82.6 (*n* = 38, 95% CI: 71.7–93.5) |
| ETV | 30.3 (*n* = 33, 95% CI: 21.6–39.5) | 37.5 (*n* = 15, 95% CI: 22.7–54.2) | 37.5 (*n* = 9, 95% CI: 18.8–59.4) | 19.6 (*n* = 9, 95% CI: 9.4–33.9) |
| NVP | 87.2 (*n* = 95, 95% CI: 78.5–92.2) | 85 (*n* = 34, 95% CI: 70.2–94.3) | 91.7 (*n* = 22, 95% CI: 73–99) | 84.7 (*n* = 39, 95% CI: 74.5–95.1) |
| RPV | 37.6 (*n* = 41, 95% CI: 28.2–47) | 47.5 (*n* = 19, 95% CI: 31.5–63.9) | 45.8 (*n* = 11, 95% CI: 25.6–67.2) | 23.9 (*n* = 11, 95% CI: 12.6–38.8) |
| ABC | 62.7 (*n* = 69, 95% CI: 53–71.8) | 50 (*n* = 20, 95% CI: 33.8–66.2) | 75 (*n* = 18, 95% CI: 53.3–90.2) | 67.4 (*n* = 31, 95% CI: 52–80.5) |
| 3TC/FTC | 80 (*n* = 88, 95% CI: 71.3–87) | 77.5 (*n* = 31, 95% CI: 61.5–89.2) | 87.5 (*n* = 21, 95% CI: 67.6–97.3) | 78.3 (*n* = 36, 95% CI: 63.6–89.1) |
| Tenofovir | 35.5 (*n* = 39, 95% CI: 26.6–45.1) | 32.5 (*n* = 13, 95% CI: 18.6–49.1) | 29.2 (*n* = 7, 95% CI: 12.6–51.1) | 41.3 (*n* = 19, 95% CI: 27–56.8) |
| AZT | 31.8 (*n* = 35, 95% CI: 23.3–41.4) | 57.5 (*n* = 23, 95% CI: 40.9–73) | 50 (*n* = 12, 95% CI: 29.1–70.9) | 0 (*n* = 0, 95% CI: 0–7.7) |
| NRTI-DRMs | | | | |
| M184VI | 80 (*n* = 88, 95% CI: 71.3–87) | 77.5 (*n* = 31, 95% CI: 61.5–89.2) | 87.5 (*n* = 21, 95% CI: 67.6–97.3) | 78.3 (*n* = 36, 95% CI: 63.6–89.1) |
| K65R | 19.1 (*n* = 21, 95% CI: 12.2–27.7) | 0 (*n* = 0, 95% CI: 0–8.8) | 12.5 (*n* = 3, 95% CI: 2.7–32.4) | 39.1 (*n* = 18, 95% CI: 25.1–54.6) |
| K70E | 11.8 (*n* = 13, 95% CI: 6.4–19.4) | 0 (*n* = 0, 95% CI: 0–8.8) | 12.5 (*n* = 3, 95% CI: 2.7–32.4) | 21.7 (*n* = 10, 95% CI: 10.9–36.4) |
| L74V | 3.6 (*n* = 4, 95% CI: 1–9) | 0 (*n* = 0, 95% CI: 0–8.8) | 4.2 (*n* = 1, 95% CI: 0.1–21.1) | 6.5 (*n* = 3, 95% CI: 1.4–17.9) |
| Y115F | 8.2 (*n* = 9, 95% CI: 3.8–15) | 0 (*n* = 0, 95% CI: 0–8.8) | 8.3 (*n* = 2, 95% CI: 1–27) | 15.2 (*n* = 7, 95% CI: 6.3–28.9) |
| ≥ 2 TAMS | 30.9 (*n* = 34, 95% CI: 22.4–40.4) | 55 (*n* = 22, 95% CI: 38.5–70.7) | 50 (*n* = 12, 95% CI: 29.1–70.9) | 0 (*n* = 0, 95% CI: 0–7.7) |
| M41L | 24.5 (*n* = 27, 95% CI: 16.8–33.7) | 32.5 (*n* = 13, 95% CI: 18.6–49.1) | 41.7 (*n* = 10, 95% CI: 22.1–63.4) | 8.7 (*n* = 4, 95% CI: 2.4–20.8) |
| D67N | 25.5 (*n* = 28, 95% CI: 17.6–34.6) | 42.5 (*n* = 17, 95% CI: 27–59.1) | 20.8 (*n* = 5, 95% CI: 7.1–42.2) | 13 (*n* = 6, 95% CI: 4.9–26.3) |
| K70R | 18.2 (*n* = 20, 95% CI: 11.5–26.7) | 37.5 (*n* = 15, 95% CI: 22.7–54.2) | 16.7 (*n* = 4, 95% CI: 4.7–37.4) | 2.2 (*n* = 1, 95% CI: 0.1–11.5) |
| L210W | 4.5 (*n* = 5, 95% CI: 1.5–10.3) | 10 (*n* = 4, 95% CI: 2.8–23.7) | 4.2 (*n* = 1, 95% CI: 0.1–21.1) | 0 (*n* = 0, 95% CI: 0–7.7) |
| T215FY | 24.5 (*n* = 27, 95% CI: 16.8–33.7) | 47.5 (*n* = 19, 95% CI: 31.5–63.9) | 33.3 (*n* = 8, 95% CI: 15.6–55.3) | 0 (*n* = 0, 95% CI: 0–7.7) |
| K219QE | 18.2 (*n* = 20, 95% CI: 11.5–26.7) | 32.5 (*n* = 13, 95% CI: 18.6–49.1) | 16.7 (*n* = 4, 95% CI: 4.7–37.4) | 6.5 (*n* = 3, 95% CI: 1.4–17.9) |
| T 69 INS | 0 (*n* = 0, 95% CI: 0–3.3) | 0 (*n* = 0, 95% CI: 0–8.8) | 0 (*n* = 0, 95% CI: 0–14.2) | 0 (*n* = 0, 95% CI: 0–7.7) |
| Q51M | 0 (*n* = 0, 95% CI: 0–3.3) | 0 (*n* = 0, 95% CI: 0–8.8) | 0 (*n* = 0, 95% CI: 0–14.2) | 0 (*n* = 0, 95% CI: 0–7.7) |
| NRTI-DRMs (*n* = 109) | | | | |
| L100I | 8.3 (*n* = 9, 95% CI: 3.8–15.1) | 2.5 (*n* = 1, 95% CI: 0.1–13.2) | 16.7 (*n* = 4, 95% CI: 4.7–37.4) | 8.9 (*n* = 4, 95% CI: 2.5–21.2) |
| K100E | 0 (*n* = 0, 95% CI: 0–3.3) | 0 (*n* = 0, 95% CI: 0–8.8) | 0 (*n* = 0, 95% CI: 0–14.2) | 0 (*n* = 0, 95% CI: 0–7.9) |
| K100P | 0 (*n* = 0, 95% CI: 0–3.3) | 0 (*n* = 0, 95% CI: 0–8.8) | 0 (*n* = 0, 95% CI: 0–14.2) | 0 (*n* = 0, 95% CI: 0–7.9) |
| K103NS | 49.5 (*n* = 54, 95% CI: 38.9–58.4) | 37.5 (*n* = 15, 95% CI: 22.7–54.2) | 50 (*n* = 12, 95% CI: 29.1–70.9) | 58.7 (*n* = 27, 95% CI: 44.5–72.9) |
| V106A | 0 (*n* = 0, 95% CI: 0–3.3) | 0 (*n* = 0, 95% CI: 0–8.8) | 0 (*n* = 0, 95% CI: 0–14.2) | 0 (*n* = 0, 95% CI: 0–7.9) |
| V106M | 29.4 (*n* = 32, 95% CI: 20.2–37.9) | 2.5 (*n* = 1, 95% CI: 0.1–13.2) | 33.3 (*n* = 8, 95% CI: 15.6–55.3) | 50.0 (*n* = 23, 95% CI: 35.6–64.4) |
| E138K | 0 (*n* = 0, 95% CI: 0–3.3) | 0 (*n* = 0, 95% CI: 0–8.8) | 0 (*n* = 0, 95% CI: 0–14.2) | 0 (*n* = 0, 95% CI: 0–7.9) |
| E138AGQ | 9.2 (*n* = 10, 95% CI: 4.5–16.2) | 2.5 (*n* = 1, 95% CI: 0.1–13.2) | 16.7 (*n* = 4, 95% CI: 4.7–37.4) | 11.1 (*n* = 5, 95% CI: 3.7–24.1) |
| Y181IV | 0.9 (*n* = 1, 95% CI: 0–5) | 2.5 (*n* = 1, 95% CI: 0.1–13.2) | 0 (*n* = 0, 95% CI: 0–14.2) | 0 (*n* = 0, 95% CI: 0–7.9) |
| Y181C | 13.8 (*n* = 15, 95% CI: 7.9–21.7) | 22.5 (*n* = 9, 95% CI: 10.8–38.5) | 12.5 (*n* = 3, 95% CI: 2.7–32.4) | 6.7 (*n* = 3, 95% CI: 1.4–18.3) |
| Y188L | 3.7 (*n* = 4, 95% CI: 1–9.1) | 5 (*n* = 2, 95% CI: 0.6–16.9) | 4.2 (*n* = 1, 95% CI: 0.1–21.1) | 2.2 (*n* = 1, 95% CI: 0.1–11.8) |
| G190SE | 1.8 (*n* = 2, 95% CI: 0.2–6.5) | 0 (*n* = 0, 95% CI: 0–8.8) | 4.2 (*n* = 1, 95% CI: 0.1–21.1) | 2.2 (*n* = 1, 95% CI: 0.1–11.8) |
| G190A | 16.5 (*n* = 18, 95% CI: 10.1–24.8) | 25 (*n* = 10, 95% CI: 12.7–41.2) | 20.8 (*n* = 5, 95% CI: 7.1–42.2) | 6.7 (*n* = 3, 95% CI: 1.4–18.3) |
| M230L | 0.9 (*n* = 1, 95% CI: 0–5) | 2.5 (*n* = 1, 95% CI: 0.1–13.2) | 0 (*n* = 0, 95% CI: 0–14.2) | 0 (*n* = 0, 95% CI: 0–7.9) |

Patients in group A, who were exposed in their first-line ART to thymidine analogs only, had a discernibly higher occurrence of multiple TAMS, 55.0% (*n* = 22/40; 95% CI: 38.5–70.7), compared to patients in group B, who were exposed to thymidine analogs before failing on a TFV backbone, 50.0% (*n* = 12/24; 95% CI: 29.1–70.9), the difference not being remarkably significant [difference in proportions: 5.0% (95% CI: −20.3 to 30.3); *p* = 0.698]. This corresponded with AZT resistance being detected with a frequency of 57.5% (*n* = 23/40; 95% CI: 40.9–72.9) in group A and 50.0% (*n* = 12/24; 95% CI: 29.1–70.9) in group B, the difference not being statistically significant [difference in proportions: 7.5% (95% CI: −17.7 to 32.7); *p* = 0.560].

The incidence of K65R among patients in group C, who were exposed to TFV only, was 39.1% (*n* = 18/46; 95% CI: 25.09–54.6), and among those in group B, who were exposed to thymidine analogs before failing on a TFV backbone, was 12.5% (*n* = 3/24; 95% CI: 2.7–32.4), the difference being evidently significant [difference in proportions: 26.6% (95% CI: 7.3–45.9); *p* = 0.021]. Yet TFV resistance, though distinctly higher in group C, 41.3% (*n* = 19/46; 95% CI: 26.9–56.8), compared to group B, 29.2% (*n* = 7/24; 95% CI: 12.6–51.09), was not large enough to attain statistical significance [difference in proportions: 12.1% (95% CI: −10.9 to 35.2); *p* = 0.318]. As expected, K65R was not observed in patients in group A, who had no TFV exposure, yet the incidence of TFV resistance in group A, 32.5% (*n* = 13/40; 95% CI: 18.6–49.1), was comparable to that in group B (difference in proportions: 3.3% [95% CI: −19.9 to 26.6]; *p* = 0.781) and group C [difference in proportions: 8.8% (95% CI: −11.5 to 29.1); *p* = 0.399].

**Table S3:** Drug susceptibility profile and DRMs after recategorizing three patients in group B rather than in group C and sensitivity analysis of key results

| *Scenario* | *Comparison* | *Group A (%)* | *Group B (%)* | *ΔP (%)* | *p-value* | *Statistical significance* |
|---|---|---|---|---|---|---|
| Scenario 1 | Multiple TAMs | 55.0% (22/40) | 42.9% (9/21) | 12.1 | 0.367 | Not significant |
| | AZT-resistance rates | 57.5% (23/40) | 50.0% (12/24) | 7.5 | 0.560 | Not significant |
| Scenario 2 | Multiple TAMs | 55.0% (22/40) | 50.0% (12/24) | 5.0 | 0.698 | Not significant |
| | AZT-resistance rates | 57.5% (23/40) | 50.0% (12/24) | 7.5 | 0.560 | Not significant |

Interpretation

Scenario 1 represents original handling of data, and scenario 2 represents *post hoc* recategorization of 3 patients in group B rather than in group C based on drug resistance testing results.

In both scenarios:

Multiple TAMs: There is no statistically significant difference between group A and group B.

AZT resistance rates: There is no statistically significant difference between group A and group B.

These findings suggest that the presence of multiple TAMs and AZT resistance rates do not significantly differ between group A (TAs-only exposure) and group B (TAs exposure and subsequent virological failure on a TFV backbone) across both scenarios.

ORIGINAL ARTICLE

# Profile of Acute Kidney Injury in Patients Undergoing Cardiac Surgery with Use of Cardiopulmonary Bypass Machine

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## Abstract

**Introduction:** Acute kidney injury (AKI) is a well-known serious complication of cardiopulmonary bypass (CPB) surgery and one of the significant risk factors for mortality, prolonged hospital stay, and additional cost. Patients having preexisting kidney dysfunction are more likely to develop AKI in the perioperative period. The complexity of CPB surgery often leads to AKI. Mechanisms of AKI include kidney hypoperfusion due to low-pressure blood flow. The nonpulsatile perfusion of the kidney, hypothermia, and inflammatory milieu, which causes afferent arteriolar constriction, contribute to AKI. The early postoperative period is characterized by a low cardiac output state, which gradually surpasses kidney compensatory mechanisms and filtration reserve. Various indigenous and infused vasopressors cause markedly elevated afferent arteriolar resistance, leading to a drop in glomerular filtration rate (GFR). Several studies have assessed the value of risk factors and their association with AKI after cardiac surgery. The evidence was mixed, with some showing a positive association. With an aim to clarify this relationship further, especially in the Indian population, we tried to study the incidence and clinical profile of AKI and its correlation with functional and clinical outcomes. We also tried to look for any diagnostic markers of AKI in the setting of cardiac surgery.

**Methodology:** The study was conducted among patients attending the Department of General Medicine and Cardiology at a tertiary care hospital in Delhi. It was a prospective longitudinal observational study conducted between March 2022 and February 2024.

Around 200 patients underwent cardiac surgery using a cardiopulmonary bypass machine at the study center during the study period. History, including comorbidities such as transient ischemic attacks, previous stroke, coronary artery disease, diabetes mellitus, hypertension, chronic obstructive pulmonary disease (COPD), and complete physical examination, were recorded. Patients were followed up preoperatively and postoperatively up to day 28. Preoperative details such as hemoglobin, serum creatinine, blood transfusion, and urine output were recorded. Intraoperative details such as duration of surgery, ACC (aortic cross-clamp) duration, hypotension, vasopressor use, and re-exploration were recorded. Postoperative findings such as urine output and serial kidney function tests on day 3, day 7, and day 28 were documented.

**Results:** Among 200 subjects, 99 patients had hypertension, and 70 patients developed AKI. Older age (>60 years) was significantly associated with AKI ($p$-value 0.04367). Comorbid conditions such as T2DM, hypertension, dyslipidemia, and COPD were significantly associated with AKI as compared to those without comorbidities (Chi-squared test, $p$-value < 0.0001). In the study, there was no association between the type of surgery and the development of AKI (Chi-squared test, $p$-value 0.07). There was no relationship between AKI severity and cardiopulmonary bypass (CPB) duration. Similarly, there was no association between the severity of AKI and ACC duration. Intraoperative hypotension was significantly associated with AKI. About 53% of hypotensive patients developed AKI during surgery as compared to 19.44% of normotensive patients ($p$-value < 0.0001, Chi-squared test). AKI was linked with a significantly prolonged hospital stay. A prolonged stay of >3 weeks was seen in 8.5% (6 out of 70) of patients who developed AKI as compared to 2.3% (3 out of 130) of patients without AKI. Most patients with AKI (57%) recovered within 1 week, and 24.28% recovered between 1 and 4 weeks. In the study, 8 patients (11.2%) developed acute kidney disease (AKD), and 5 patients (7%) died.

**Conclusion:** This prospective study concluded that AKI is a common complication in the perioperative period of cardiopulmonary bypass surgery. Older age, comorbid conditions, and intraoperative hypotension were significantly associated with AKI. AKI was linked with extended hospital stay and longer recovery times. Severe grades of AKI were associated with progression to AKD, need for dialysis, and higher mortality. It is imperative to focus on interventions to minimize and address the risk factors to reduce morbidity and mortality associated with AKI in CPB surgery.

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## Introduction

Acute kidney injury (AKI) is regarded as a very important complication of cardiac surgery and one of the important predictors of mortality. In cardiopulmonary bypass (CPB) surgery, it is a frequently seen complication, and severe AKI increases mortality 3–8-fold, leading to increased hospital stay and costs of care.[1,2] Perioperative AKI is the most significant factor for immediate and delayed mortality after CPB surgery.[3,4] Preexisting renal dysfunction predisposes patients to AKI and poor outcomes.[5,6] A rise in serum creatinine by 50% in 7 days, >0.3 mg/dL in 48 hours, or oliguria qualifies as AKI as per KDIGO (Kidney Disease Improving Global Outcome). Depending upon the definition of AKI used, the incidence of cardiac surgery-associated AKI (CSA-AKI) varies. The CSA-AKI may be asymptomatic or severe enough to require renal replacement therapy (RRT), which increases operative mortality and length of stay in the intensive care unit and hospital. In cardiac surgery, approximately 20% of patients develop AKI, 1% require RRT, and 8% die within 90 days. The complexity of CPB surgery often leads to AKI. AKI is characterized by low-pressure blood flow leading to renal hypoperfusion. The nonpulsatile perfusion of the kidney, hypothermia, and inflammatory milieu cause afferent arteriolar constriction

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and contribute to AKI. The early postoperative period is also characterized by a low cardiac output state.[7] When hypotension persists, it gradually surpasses kidney compensatory mechanisms and glomerular filtration reserve. Various endogenous and infused vasopressors cause markedly elevated afferent arteriolar resistance, leading to a drop in glomerular filtration rate (GFR). This may lead to prerenal azotemia with or without oliguria, but tubular function may still be preserved. Persistent ischemia results in structural tubular alterations, causing tubular cell disruption and obstruction of the tubular lumen with back leakage. This is accompanied by oxidative and inflammatory injury, causing worsening of hypoperfusion and further damage to tubular cells. Certain factors may predispose patients to AKI, including age, sex, preexisting cardiac or renal dysfunction, past cardiac surgery, or comorbidities such as obstructive pulmonary disease or poorly controlled diabetes mellitus.[8] Nephrotoxic drugs may further contribute to AKI. Of particular interest are commonly used medications, for example, painkillers; drugs acting on the renin–angiotensin–aldosterone system; and diuretics, which may impair glomerular afferent and efferent arteriolar autoregulation.[9] In the setting of normal baseline renal function and uneventful CPB surgery with stable perioperative hemodynamics, the risk of AKI is minimal (<2%). However, the risk of postoperative AKI and overall mortality rises significantly with preexisting renal dysfunction.

A rise in serum creatinine >1.5 mg/dL qualifies as AKI. However, serum creatinine presents several limitations when used as a marker of kidney function. Serum creatinine–based equations are inaccurate at GFR values above 60 mL/minute. In other words, serum creatinine does not rise until >50% of GFR is lost. A better measure of renal function is creatinine clearance, which more accurately predicts glomerular function.[10] A GFR <60 mL/minute/1.73 $m^2$ is a better marker of renal dysfunction, and the risk of worsening AKI increases significantly below this level.[4]

The chronic kidney disease epidemiology collaboration (CKD-EPI) equation for estimation of GFR is more accurate at near-normal GFR. It provides better risk stratification of AKI for patients with near-normal renal function undergoing high-risk surgery than the MDRD equation. Even mild renal dysfunction is often linked with adverse renal outcomes and mortality. The risk of AKI increases 4.8 times with each 1 mg/dL increase in serum creatinine. Although severe AKI requiring RRT is infrequent (1–4%), it results in a significant increase in operative mortality, ranging from 40 to 80%.

## Rationale of the Study

It is important to implement all preventive strategies possible to preserve kidney function in the setting of CSA-AKI. Any preexisting renal impairment should therefore alert the physician to look for reversible factors to prevent AKI. Identification and correction of potential risk factors in the perioperative period will optimize renal perfusion and can prevent the complications and mortality associated with AKI following cardiac surgery.

In spite of many previously done studies, there still remains doubt about the predictors of AKI in post-CPB surgery patients. Recent studies showed promising, though not conclusive, evidence of risk factors for AKI as outcome-predictive markers. Several studies have assessed the value of risk factors and their association in the early phases of AKI after cardiac surgery, but not many evaluated the relationship between prognostic markers and functional outcomes.

The findings of most studies were inconclusive, with some showing a positive association. To clarify this issue, especially in the Indian population, we conducted this study to find the incidence and clinical profile of AKI, determine its association and correlation with functional and clinical outcomes, and also look for any prognostic marker.

## Methodology

This study aimed to describe the incidence and clinical profile of AKI in post-CPB surgery with the use of a cardiopulmonary bypass machine and its association with clinical outcomes. Secondary objectives also included the study of risk factors for AKI after cardiac surgery. The study was conducted among patients attending the Departments of General Medicine and Cardiology at a tertiary care hospital in Delhi. It was a prospective longitudinal observational study conducted between March 2022 and February 2024. The study was approved by the institutional ethics committee, IEC registration number 38/1022, dated 22nd July 2022. Informed written consent was obtained from all subjects. Patients older than 12 years of age who underwent cardiac surgery using a cardiopulmonary bypass machine were included in the study. Kidney transplant recipients, donors, patients with end-stage kidney disease requiring maintenance hemodialysis or chronic peritoneal dialysis support, post-CPR patients without immediate return to a communicative state, HIV infection, body weight <35 kg, nephrotic syndrome with anasarca, long-term corticosteroid therapy, and decompensated liver disease were excluded from the study.

### Sample Size

Sample size was calculated for determining the proportion of patients who develop AKI after cardiac surgery by use of cardiopulmonary bypass with a 95% confidence level and 10% precision. Assuming 30% of patients develop AKI after cardiac surgery and a 15% lost-to-follow-up rate, the sample size required for the study was calculated at 96 patients.

Around 200 patients underwent surgery using a cardiopulmonary bypass machine at this study center during the study period. The subjects meeting the study criteria were explained the nature and purpose of the study, and informed consent was obtained. History, including comorbidities such as transient ischemic attack, previous stroke, coronary artery disease, diabetes mellitus, hypertension, and complete physical examination, was recorded. Patients were followed up preoperatively and postoperatively up to day 28. Preoperative details such as hemoglobin, serum creatinine, blood transfusion, and urine output were recorded. Intraoperative details such as duration of surgery, aortic cross-clamp (ACC) duration, hypotension, vasopressor use, and re-exploration were recorded. Postoperative findings such as urine output and serial kidney function tests on day 3, day 7, and day 28 were documented.

### Statistical Analysis

Data was analyzed using SPSS version 26. Data was expressed as percentages for categorical variables. The Chi-square test, independent *t*-test, and paired *t*-test were used for analysis. A *p*-value of 0.05 was considered statistically significant for all statistical tests performed.

## Results

In this study, 200 subjects underwent cardiac surgery. Baseline characteristics of subjects are shown in Table 1. 70 subjects (35%) developed AKI. The mean age of the study population was 55.2 years, and in those with AKI it was 57 years. Table 2 describes various factors associated with AKI. About 42% of 200 subjects were above 60 years of age. Older age was significantly associated with AKI (*p*-value 0.043). About 48.5% of patients with AKI were older than 60 years, 37.1% of patients were in the 45–59 year age-group, and 11.4% were in the <44 year age-group. About 74.5% of subjects in the study population were males and 25.5% were

females. Among patients who developed AKI, 78.5% were males and 21.4% were females. There was no significant difference in the incidence of AKI between males and females (*p*-value 0.332).

Comorbid conditions such as T2DM, hypertension, dyslipidemia, and COPD were significantly associated with AKI as compared to those without comorbidities, as shown in Table 2 (*p*-value < 0.0001, Chi-squared test). Out of 200 patients, 99 (49.5%) had hypertension, 83 (41.5%) had dyslipidemia, 59 (29.5%) had diabetes mellitus, 13 (6.5%) had chronic obstructive pulmonary disease (COPD), and 47 (23.5%) did not have any underlying comorbidity (Fig. 1). In the AKI group, these figures were 74.2, 65.7, 65.7, 5.7, and 5.7%, respectively.

In the study, there was no association between the type of surgery and the development of AKI (*p*-value 0.07, Chi-squared test). There was no relationship between hemoglobin level and the incidence of AKI (*p*-value 0.49, Chi-squared test for linear trend) when AKI incidence was analyzed in 3 hemoglobin categories: <11 gm/dL, 11–14.9 gm/dL, and >15 gm/dL.

Intraoperative hypotension was significantly associated with AKI. About 70% of patients who developed AKI had a history of hypotension during surgery as compared to 30% of AKI patients who were normotensive (Chi-squared test, *p*-value < 0.0001). CSA-AKI was linked with a significantly increased hospital stay, as shown in Table 2. The duration of hospitalization among all patients was between 1 and 7 days in 94 (72.3%), 8–14 days in 29 (22.3%), 15–21 days in 4 (3%), and 21–28 days in 3 (2.3%) patients, as compared to 18 (25.7%), 37 (52.8%), 9 (12.8%), and 6 (8%) patients, respectively, in the AKI group. Prolonged stays of 8–14 days, 15–21 days, and 21–28 days were seen in 52.8, 12.8, and 8% of the AKI group as compared to 22.3, 3, and 2.3% in the non-AKI group, respectively (*p*-value 0.00001).

There was no relationship between CPB duration and the severity of AKI. The mean duration of CPB surgery was 190.6 minutes in those who developed stage 1 AKI as compared to 233 minutes in those who developed stage 2 and stage 3 AKI, but this difference was not significant (Table 3, *p*-value 0.117, Chi-squared test). Similarly, there was no relationship between the severity of AKI and aortic cross-clamp duration. The mean ACC duration was 118.7 minutes in those who developed stage 1 AKI and 157.8 minutes in those who developed stage 2 and stage 3 AKI (Table 3, Chi-squared test, *p*-value 0.067).

The recovery time among the AKI patients is shown in Table 4. The duration of recovery in the majority of the 70 AKI patients was between 4 and 28 days. In the study, 8 patients developed acute kidney disease (AKD). The overall mortality rate was 6%. The mortality rate was 7.1% among AKI patients and 5.6% among non-AKI patients. Most patients with CSA-AKI (57%) recovered within 1 week. A total of 17 patients (24.28%) recovered between 1 and 4 weeks. In the study, 8 patients (11.2%) developed AKD, and 5 patients (7%) died.

In the study, 70 subjects with AKI were classified according to KDIGO staging. 60 patients developed stage 1, 6 developed stage 2, and 4 developed stage 3 AKI, as shown in Table 5. AKI resolved in most patients with stage 1 AKI (91.6%, 55 out of 60) as compared to stage 2 AKI (33.3%, 2 out of 6). None of the patients with stage 3 AKI recovered

**Table 1:** Preop baseline characteristics

| | *Mean* | *SD* |
|---|---|---|
| Age (Yr) | 55.5 | 12.5 |
| Weight (kg) | 65.29 | 11.55 |
| Hb (gm/dL) | 13.20 | 1.55 |
| Platelet (1000/mm$^3$) | 222.79 | 76.21 |
| Urine output (mL/min) | 73.77 | 11.65 |
| Serum creatinine (mg/dL) | 0.84 | 0.24 |
| Blood urea nitrogen (mg/dL) | 14.36 | 6.23 |

**Table 2:** Distribution of AKI according to type of surgery

| *Type of surgery* | *Frequency (n = 200)* | *AKI (n = 70)* |
|---|---|---|
| CABG | 114 (57%) | 48 (42.10%) |
| CABG + valve surgery | 13 (6.6) | 5 (38.46%) |
| Valve surgery | 44 (22%) | 10 (22.72%) |
| Others | 29 (14.5%) | 7 (24.13%) |
| Total | 200 | 70 |

**Table 3:** Duration of hospitalization

| *Duration (days)* | *AKI (n = 70)* | *Non-AKI (n = 130)* |
|---|---|---|
| 1–7 days | 18 | 94 |
| 8–14 days | 37 | 29 |
| 15–21 days | 9 | 4 |
| 21–28 days | 6 | 3 |
| Total | 70 | 130 |

**Table 4:** Recovery time among the AKI patients (*n* = 70)

| *Recovery time (days)* | *Frequency* |
|---|---|
| 1–3 days | 6 (8.5%) |
| 4–7 days | 34 (48.57%) |
| 8–28 days | 17 (24.28%) |
| AKD | 8 (11.42%) |
| Died | 5 (7%) |

**Table 5:** Staging of AKI according to KDIGO among the AKI patients (*n* = 70)

| | *Outcome at 28 days* | | | | |
|---|---|---|---|---|---|
| *KDIGO staging of AKI* | *AKI (n = 70)* | *AKI resolved (n = 57)* | *AKI to AKD (n = 8)* | *Renal replacement therapy requirement (n = 2)* | *Mortality (n = 5)* |
| Stage 1 | 60 | 55 | 2 | 0 | 2 |
| Stage 2 | 6 | 2 | 5 | 0 | 1 |
| Stage 3 | 4 | 0 | 1 | 2 | 2 |
| Total | 70 | 57 | 8 | 2 | 5 |

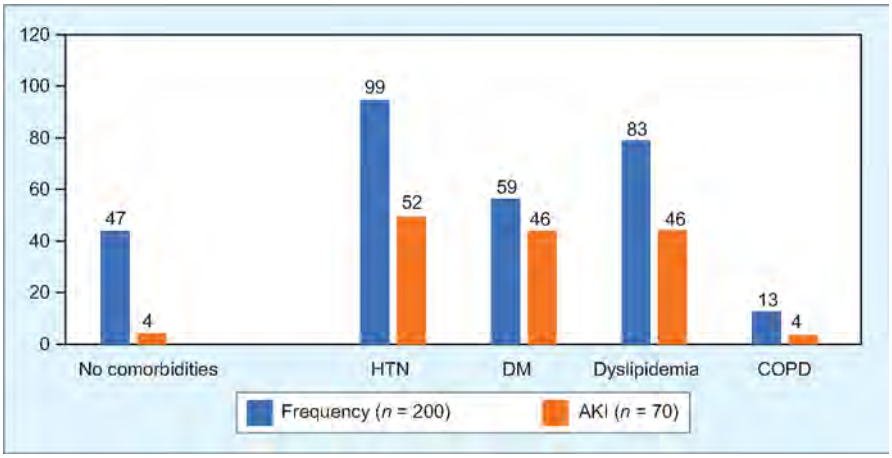


**Fig. 1:** Column chart showing comorbidity among the AKI patients

renal function (2 died, and 2 required renal replacement therapy).

## Discussion

This study describes the incidence and clinical profile of AKI in cardiac surgery from an Indian perspective. CSA-AKI is a common complication following cardiac surgery, including CABG and valve replacement. Previous studies have documented the incidence of AKI in the CABG setting in the range of 7.6–48.5%.[11] However, most of these studies were retrospective and used only a rise in serum creatinine rather than a fall in urine output.[12] Prospective data are scarce, particularly from the Indian setting. In this study, 70 subjects (35%) were diagnosed with AKI. The mean age of the study population was 55.2 years, and in those with AKI it was 57 years. Age >60 years was significantly associated with AKI. However, male or female gender was not associated with AKI. In a study by Wittlinger et al.,[13] the median age in the AKI group was 68 years, 71% were males, and 29% were females. In another study by Karim et al.,[14] the mean age of subjects was 37.01 ± 12.28 years, and female preponderance (57%) was noted. In another study by Hung et al.,[15] 66.2% were males, 33.8% were females, and the mean age of study subjects was 68.6 ± 9.2 years.

The various comorbidities in the study subjects were hypertension, diabetes, dyslipidemia, and COPD. The most common comorbidities associated with AKI in our study were hypertension, diabetes mellitus, and dyslipidemia. The present study findings have similarity with the study by Clough et al.,[16] in which the most common comorbidities among patients undergoing cardiopulmonary bypass were diabetes, hypertension, obesity, COPD, vascular disease, and liver disease. In a study by Patra et al.,[17] common comorbidities were diabetes, hypertension, asthma, and COPD. Oliveira et al.[18] also found that the majority of patients had at least 2 comorbidities, that is, diabetes and hypertension.

In our study, the incidence of AKI was not related to the type of surgery, that is, CABG with or without valve surgery. In a study by Priyanka et al.,[19] 16% of patients with AKI had undergone CABG plus valvular surgery as compared to 1.8% of all patients with or without AKI. This study showed that CABG plus valvular surgery resulted in more AKI as compared to CABG alone. In a study by Gangadharan et al.,[20] CABG with valve repair was the most common procedure, followed by valve replacement and repair, in both AKI and non-AKI patients.

In our study, the severity of AKI was not dependent on the duration of CPB or ACC. In a study by Karim et al.,[14] cross-clamp time among non-AKI patients was 81.44 ± 30.99 minutes and among AKI patients was 64.49 ± 40.24 minutes. However, in a study by Zeng et al.,[21] the type of cardiac surgery, CPB duration, and ACC duration were all significantly associated with AKI. In a study by Pontillo et al.,[22] compared to patients with CPB duration above the median, patients with CPB duration below the median had a significantly lower incidence of AKI.

Intraoperative hypotension was the most important factor associated with the development of AKI in our study. 53% of patients having intraoperative hypotension developed AKI. Intraoperative hypotension is common in cardiac surgery and is a main contributor to the development of AKI. In a study by Patra et al.,[17] 29.8% had intraoperative hypotension. In a study by Jung et al.,[23] the cumulative duration of intraoperative hypotension had a significant influence on the incidence of AKI (OR 1.004; 95% confidence interval (CI) 1.003–1.005; $p$-value < 0.001).

AKI was linked with extended hospital stay. A prolonged hospital stay of >3 weeks was seen in 8.5% (6 out of 70) of patients who developed AKI as compared to 2.3% (3 out of 130) of patients without AKI. In a study by Cheruku et al.,[24] AKI was also associated with a significantly higher length of stay in the intensive care unit (5.4 vs 2.2 days) and hospital (15.0 vs 10.5 days).

The mild form of AKI (grade I) recovered in the majority of patients. Severe AKI (grade II and grade III) was associated with progression to AKD, need for dialysis, and increased mortality. Most patients with CSA-AKI (57%) recovered within 1 week. About 24.28% recovered between 1 and 4 weeks. In the study, 8 patients (11.2%) developed acute kidney disease (AKD), and 5 patients (7%) died. A study by Jung et al.[23] showed that the incidence of AKI was 22.3% and the mortality rate was 10.7%. The mean duration of hospital stay was 15 days. Another study by Wittlinger et al.[13] showed that the incidence of AKI among cardiopulmonary bypass patients was 7% and the mortality rate was 6%. In a study by Thakar et al.,[25] postoperative mortality was 5.9% among patients who developed AKI. Mortality across various studies, including our study, was significant and comparable.

## Conclusion

This prospective study concluded that AKI is common during CPB surgery with a cardiopulmonary bypass machine. Older age, comorbid conditions, and intraoperative hypotension were significantly associated with AKI. AKI was linked with extended hospital stay and longer recovery times. Severe grades of AKI were associated with progression to AKD, need for dialysis, and higher mortality. It is imperative to focus on interventions to reduce risk factors to minimize morbidity and mortality linked with AKI in cardiac surgery.

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ORIGINAL ARTICLE

# Different Methods of Low-density Lipoprotein Cholesterol Estimation and the Impact on Lipid-lowering Therapy in Patients with Coronary Artery Disease

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## Abstract

**Background:** Indirect estimation of low-density lipoprotein cholesterol (LDL-C) is a common clinical practice. The Friedewald equation is used most often but has inherent limitations. Clinical implications of such a practice have not been well defined, especially in the current era of targeting low (<50–70 mg/dL) or ultralow (<30–40 mg/dL) LDL-C levels.

**Methods:** Overall, 3,028 consecutive subjects with coronary artery disease (CAD) undergoing coronary revascularization were included. Four methods of LDL-C estimation were compared: direct estimation, the Friedewald, Martin, and Sampson equations.

**Results:** The mean age of the subjects was 61.3 ± 10.2 years, and 2,525 (83.4%) were men. Mean direct LDL-C was 78.9 ± 32.9 mg/dL. Compared with the direct estimation, all three indirect methods significantly underestimated LDL-C, but the Martin equation had the least bias (mean differences of −10.5 ± 9.7 mg/dL, −5.2 ± 7.6 mg/dL, and −7.2 ± 8.3 mg/dL with the Friedewald, Martin, and Sampson equations, respectively; *p*-values <0.001 for all the comparisons). Among patients with LDL-C >70 mg/dL and >50 mg/dL, the Friedewald equation erroneously classified 24.6% and 19.9%, respectively, as having LDL-C below these thresholds. This error increased with increasing triglyceride levels. The Martin equation was the most accurate, whereas the Sampson equation had intermediate accuracy.

**Conclusion:** Our study shows that the Friedewald equation underestimates LDL-C and can potentially result in significant undertreatment in patients with CAD in whom aggressive LDL-C lowering is crucial. Direct estimation is the preferred method, but the Martin equation could be a reasonable alternative if the direct estimation is not feasible due to logistical constraints.

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## Introduction

Dyslipidemia is among the most important risk factors for atherosclerotic cardiovascular disease (ASCVD). Low-density lipoprotein cholesterol (LDL-C) is the main culprit and hence, the primary target for therapy.[1] A meta-analysis of several randomized trials has shown that for every 1 mmol/L reduction in LDL-C, there is an approximately 21% relative reduction in the risk of ASCVD events.[2] The patients with the highest baseline ASCVD risk benefit the most. Accordingly, all guidelines recommend aggressive LDL-C lowering to <50–70 mg/dL in patients with established ASCVD and to even lower levels (<30–40 mg/dL) in those with recurrent vascular events, polyvascular disease, or other high-risk features.[3–5]

Accurate estimation of LDL-C is crucial for properly guiding lipid-lowering therapy, especially in patients in whom aggressive LDL-C reduction is required. Direct estimation using enzymatic assays is considered the most accurate method. However, due to cost constraints and other logistical issues, many laboratories worldwide, and especially in India, continue to estimate LDL-C using indirect methods. The Friedewald equation[6] is the most commonly used method for this purpose, but it has inherent limitations. To overcome these limitations, many different equations have been proposed, which have variable accuracy.[7,8] Of all these methods, the Martin equation[9] and the Sampson equation[10] appear to be the most accurate.[7,8,11] Newer machine learning methods are also being explored, but the data are limited at present.[12,13]

Although several studies have compared the accuracy of the different methods of LDL-C estimation,[7,8,11,14] only a few have evaluated the potential impact of the measurement error on the therapeutic decision-making.[15–17] Hence, we sought this study to assess the potential impact of indirect LDL-C estimation on the lipid-lowering therapy in patients with established coronary artery disease (CAD) in whom this issue would be of the greatest relevance. We selected the three clinically most relevant, indirect methods of LDL-C estimation—the Friedewald equation, Martin equation, and Sampson equation.

## Methods

This was a retrospective study conducted at a premier, tertiary care center in North India. All subjects with newly diagnosed CAD who had undergone coronary revascularization at our center during the period 1 January 2023 to 31 December 2023 were included. The patients in whom a lipid profile was not done during the index hospitalization were excluded. If a patient had had multiple coronary interventions during this 1-year period, only the first hospitalization was considered. Based on these criteria, 3,064 subjects were found to be eligible for inclusion in this study. Of these, 36 subjects (1.2% of all) with serum triglycerides (TG) >400 mg/dL were also excluded because both the Friedewald and the Martin equations are not applicable if TG is above 400 mg/dL.

For all subjects, baseline lipid profile findings, along with relevant clinical and biochemical details, were retrieved from the hospital medical records. As per the hospital policy, all patients had undergone fasting lipid profile estimation within the first 24 hours of their presentation. Enzymatic assays on the Vitros Dry Chemistry Autoanalyzer were used for measuring total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), and TG. The LDL-C estimation was done using the two-step cholesterol esterase/

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cholesterol oxidase/peroxidase and catalase method. LDL-C was also estimated indirectly using the following three methods:

- *Friedewald equation*:[6] LDL-C = TC–HDL-C–TG/5 (all values in mg/dL)
- *Martin equation*: The Martin equation is almost identical to the Friedewald equation, except that the fixed factor '5' is replaced with an adjustable factor. This adjustable factor is derived from a 180-cell table that takes into consideration the patient's TG and non-HDL-C.[9] Batch calculation of LDL-C can be performed using an Excel spreadsheet, which can be downloaded from www.ldlcalculator.com. We used the 5,000-row version of the spreadsheet to match our sample size.
- *Sampson equation*: The following equation was used[10] [LDL-C = TC/0.948−HDL-C/0.971−(TG/8.56+TG×Non-HDL-C/2140−$TG^2$/16100)−9.44]

For all the above methods, the proportions of the patients having LDL-C <70 mg/dL and <50 mg/dL were calculated. This calculation was done for the entire study population as well as for the groups based on TG levels, as follows:

- 0–99 mg/dL,
- 100–199 mg/dL,
- 200–299 mg/dL, and
- 300–399 mg/dL

We also calculated non-HDL-C by subtracting HDL-C from TC.

The study was approved by the Institutional Review Board and the Independent Ethics Committee [MICR 1722/2024 (academic)]. The requirement for patient consent was waived because of the retrospective nature of the study.

### Statistical Analysis

The baseline characteristics and other descriptive variables were summarized using standard statistical tools such as mean ± standard deviation or counts and proportions as appropriate. The repeated measures analysis of variance with the Bonferroni method for *post hoc* comparison was used for comparing the LDL-C values derived using the different methods. The correlations among the different LDL-C values were assessed using Pearson's correlation coefficients, and Fisher's *z*-transformation was used for comparing the strengths of different correlations (https://www.psychometrica.de/correlation.html). A two-sided *p*-value <0.05 was considered statistically significant. All the analyses were performed using SPSS version 20.0.

## Results

A total of 3,028 subjects were included in this study. The mean age of the study subjects was 61.3 ± 10.2 years, and 2,525 (83.4%) were men.

### Baseline Characteristics

Table 1 summarizes clinical and laboratory characteristics of the study subjects. Diabetes mellitus was present in 1,583 (52.5%) subjects, and hypertension in 1,901 (62.8%). Acute coronary syndrome was the presentation in 1,185 (39.1%) subjects, and the majority (1,838; 60.7%) had undergone percutaneous coronary intervention.

Table 2 summarizes the lipid parameters in the study population. The mean LDL-C using the direct method was 78.9 ± 32.9 mg/dL. Low HDL-C was present in 71.6% patients. Mean TG was 144.4 ± 57.8 mg/dL, with the vast majority (85.7%) having TG <200 mg/dL.

### Low-density Lipoprotein Cholesterol Using the Different Methods

Compared with the direct method, all three indirect methods significantly underestimated the LDL-C (Table 3). The Friedewald equation provided the lowest values, whereas the Martin equation had the least negative bias. Despite this systematic underestimation, there was a significant correlation among the different methods for LDL-C estimation. However, the Martin equation had the strongest correlation with direct LDL-C as compared to the other two indirect methods (Table 4).

### Attainment of the Low-density Lipoprotein Cholesterol Goals

The direct LDL-C was >70 mg/dL in 1,616 (53.4%) subjects (Table 5). The Friedewald equation categorized 24.6% of these as having LDL-C <70 mg/dL (Fig. 1). This error increased with increasing TG levels ($p <$

**Table 1:** Clinical and laboratory characteristics of the study population

| *Parameter* | *Overall (n = 3,028)* |
|---|---|
| Age, years | 61.3 ± 10.2 |
| Male gender | 2,525 (83.4) |
| Hypertension | 1,901 (62.8) |
| Diabetes mellitus | 1,583 (52.3) |
| Hypothyroidism | 311 (10.3) |
| Systolic blood pressure, mm Hg | 129 ± 15 |
| Diastolic blood pressure, mm Hg | 78 ± 9 |
| Hemoglobin, g/dL | 13.0 ± 1.7 |
| Glycosylated hemoglobin, % | 7.0 ± 1.7 |
| Blood urea, mg/dL | 41.3 ± 23.2 |
| Serum creatinine, mg/dL | 1.1 ± 0.7 |
| Serum uric acid, mg/dL | 6.1 ± 1.8 |
| Acute coronary syndrome | 1,185 (39.1) |
| Procedure | |
| Percutaneous coronary angioplasty | 1,838 (60.7) |
| Coronary artery bypass surgery | 1,190 (39.3) |

Continuous values are reported as mean ± standard deviation and categorical values as actual numbers with percentages in parentheses

**Table 2:** Lipid parameters in the study population

| *Parameter* | *Overall (n = 3,064)* |
|---|---|
| Total cholesterol, mg/dL | 134.0 ± 40.1 |
| Direct LDL-C, mg/dL | 78.9 ± 32.9 |
| HDL-C, mg/dL | 36.7 ± 10.0 |
| Low HDL-C (<40 in men, <50 in women) | 2,167 (71.6) |
| Triglycerides, mg/dL | 144.4 ± 57.8 |
| 0–99 mg/dL | 636 (21.0) |
| 100–199 mg/dL | 1,959 (64.7) |
| 200–299 mg/dL | 357 (11.8) |
| 300–399 mg/dL | 76 (2.5) |
| Non-HDL-C, mg/dL | 97.2 ± 37.8 |

Continuous values are reported as mean ± standard deviation and categorical values as actual numbers with percentages in parentheses; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol

**Table 3:** Low-density lipoprotein cholesterol estimated using different methods

| *Parameter* | *n = 3,028* |
|---|---|
| Direct measurement, mg/dL | 78.9 ± 32.9 |
| *Friedewald equation* | |
| LDL-C, mg/dL | 68.3 ± 34.4 |
| Difference, mg/dL | −10.5 ± 9.7 |
| *Martin equation* | |
| LDL-C, mg/dL | 73.6 ± 34.0 |
| Difference, mg/dL | −5.2 ± 7.6 |
| *Sampson equation* | |
| LDL-C, mg/dL | 71.6 ± 34.4 |
| Difference, mg/dL | −7.2 ± 8.3 |

All values are reported as mean ± standard deviation. The LDL-C values derived using all four methods differed significantly from each other, with a *p*-value <0.001; LDL-C, low-density lipoprotein cholesterol

0.001). The corresponding proportions were much lower for the Martin and the Sampson equations, with the Martin equation being the most accurate. Neither of these equations was affected by the TG category ($p > 0.05$).

Similar findings were seen in patients with direct LDL-C >50 mg/dL (Table 5). However, for this group, the accuracy of the Martin equation improved with increasing TG levels.

A significantly higher proportion of males achieved the LDL-C goals (both <50 mg/dL and <70 mg/dL) as compared to females, regardless of the measurement method (Table 6).

**Table 4:** Correlation among different methods for low-density lipoprotein cholesterol estimation (*n* = 3,028)

| | *Pearson's correlation coefficients* | | | |
|---|---|---|---|---|
| | *Direct measurement* | *Friedewald equation* | *Martin equation* | *Sampson equation* |
| Direct measurement | 1 | 0.959* | 0.975* | 0.971* |
| Friedewald equation | 0.959 | 1 | 0.986 | 0.996 |
| Martin equation | 0.975 | 0.986 | 1 | 0.997 |
| Sampson equation | 0.971 | 0.996 | 0.997 | 1 |

All correlations had *p*-values <0.001; *The Martin equation had a much stronger correlation with the direct measurement as compared to the other two indirect methods (*p*-value 0.017 for comparison with the Sampson equation and <0.001 for comparison with Friedewald equation)

**Table 5:** Attainment of low-density lipoprotein cholesterol goals according to the different estimation methods and the serum triglycerides categories

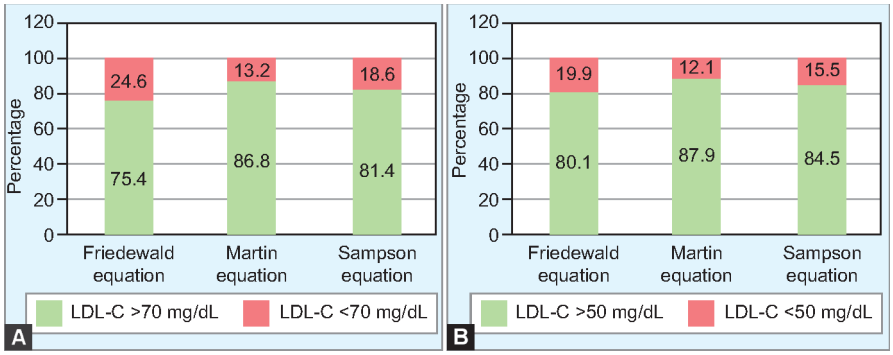
| *Direct LDL-C >70 mg/dL (n = 1,616, 53.4% of all)* | | | | | | |
|---|---|---|---|---|---|---|
| *TG category ↓* | *Friedewald equation* | | *Martin equation* | | *Sampson equation* | |
| | *<70 mg/dL* | *>70 mg/dL* | *<70 mg/dL* | *>70 mg/dL* | *<70 mg/dL* | *>70 mg/dL* |
| Overall | 398 (24.6) | 1,218 (75.4) | 214 (13.2) | 1,402 (86.8) | 300 (18.6) | 1,316 (81.4) |
| 0–99 mg/dL (*n* = 216) | 34 (15.7) | 182 (84.3) | 34 (15.7) | 182 (84.3) | 33 (15.3) | 183 (84.7) |
| 100–199 mg/dL (*n* = 1078) | 263 (24.4) | 815 (75.6) | 144 (13.4) | 934 (86.6) | 200 (18.6) | 878 (81.4) |
| 200–299 mg/dL (*n* = 260) | 77 (29.6) | 183 (70.4) | 32 (12.3) | 228 (87.7) | 53 (20.6) | 207 (79.6) |
| 300–399 mg/dL (*n* = 62) | 24 (38.7) | 38 (61.3) | 4 (6.5) | 58 (93.5) | 14 (22.6) | 48 (77.4) |
| *p*-value for the trend | <0.001 | | 0.276 | | 0.428 | |
| *Direct LDL-C >50 mg/dL (n = 2,512, 83.0% of all)* | | | | | | |
| *TG category ↓* | *Friedewald equation* | | *Martin equation* | | *Sampson equation* | |
| | *<50 mg/dL* | *>50 mg/dL* | *<50 mg/dL* | *>50 mg/dL* | *<50 mg/dL* | *>50 mg/dL* |
| Overall | 500 (19.9) | 2,012 (80.1) | 304 (12.1) | 2,208 (87.9) | 389 (15.5) | 2,123 (84.5) |
| 0–99 mg/dL (*n* = 450) | 69 (15.3) | 381 (84.7) | 76 (16.9) | 374 (83.1) | 74 (16.4) | 376 (83.6) |
| 100–199 mg/dL (*n* = 1656) | 334 (20.2) | 1322 (79.8) | 199 (12.0) | 1,457 (88.0) | 251 (15.2) | 1,405 (84.8) |
| 200–299 mg/dL (*n* = 335) | 79 (23.6) | 256 (76.4) | 26 (7.8) | 309 (92.2) | 54 (16.1) | 281 (83.9) |
| 300–399 mg/dL (*n* = 71) | 18 (25.4) | 53 (74.6) | 3 (4.2) | 68 (95.8) | 10 (14.1) | 61 (85.9) |
| *p*-value for the trend | 0.017 | | <0.001 | | 0.882 | |

All values are actual numbers with percentages in parentheses; LDL-C, low-density lipoprotein cholesterol; TG, serum triglycerides

**Table 6:** Attainment of low-density lipoprotein cholesterol goals in males and females according to the different estimation methods

| | *LDL-C <50 mg/dL* | | | | *LDL-C <70 mg/dL* | | | |
|---|---|---|---|---|---|---|---|---|
| | *Overall (n = 3,028)* | *Males (n = 2,525)* | *Females (n = 503)* | *p-value* | *Overall (n = 3,028)* | *Males (n = 2,525)* | *Females (n = 503)* | *p-value* |
| Direct method | 516 (17.0) | 442 (17.5) | 74 (14.7) | 0.128 | 1,412 (46.6) | 1,219 (48.3) | 193 (38.4) | <0.001 |
| Friedewald equation | 1003 (33.1) | 877 (34.7) | 126 (25.0) | <0.001 | 1789 (59.1) | 1527 (60.5) | 262 (52.1) | <0.001 |
| Martin equation | 796 (26.3) | 699 (27.7) | 97 (19.3) | <0.001 | 1585 (52.3) | 1369 (54.2) | 216 (42.9) | <0.001 |
| Sampson equation | 887 (29.3) | 779 (30.9) | 108 (21.5) | <0.001 | 1683 (55.6) | 1442 (57.1) | 241 (47.9) | <0.001 |

All values are actual numbers with percentages in parentheses; LDL-C, low-density lipoprotein cholesterol




**Figs 1A and B:** Categorization of the patients above or below the specific low-density lipoprotein cholesterol (LDL-C) thresholds according to the different methods for indirect LDL-C estimation. (A) Patients with direct LDL-C >70 mg/dL (*n* = 1,616). (B) The patients with direct LDL-C >50 mg/dL (*n* = 2,512)

## Discussion

Ours is the first study to provide a direct perspective on the treatment implications of the LDL-C estimation methods in contemporary clinical practice. The study shows that in patients with CAD, indirect LDL-C estimation using the Friedewald equation can potentially lead to undertreatment in approximately 25% of the subjects if the LDL-C target is <70 mg/dL and in 20% subjects if the target is <50 mg/dL. This inaccuracy increases with increasing TG levels. The Martin equation and the Sampson equation provide a more accurate assessment, with the former being the most reliable.

### Need for Aggressive Low-density Lipoprotein Cholesterol Reduction

Atherosclerotic cardiovascular disease is the leading killer in the world, accounting for more than 25% of all deaths.[18] The Indian population is worse affected with not only a higher prevalence of the disease, but also more premature onset and a higher case fatality rate.[19] Aggressive LDL-C lowering is one of the most effective strategies to reduce the mortality associated with ASCVD. The trials with proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have shown that there is an incremental reduction in the ASCVD risk at ultralow LDL-C levels with no apparent threshold below which the benefit ceases to exist.[20,21] There are also no apparent safety concerns even at very low LDL-C levels.[22–24] Accordingly, all the leading societies recommend lowering LDL-C to at least <50–70 mg/dL in all patients with ASCVD.[3–5] Even lower targets (<30–40 mg/dL) have been recommended for patients with recurrent vascular events, polyvascular disease, and other high-risk features.[4,5]

### Low-density Lipoprotein Cholesterol Estimation

Accurate LDL-C estimation is crucial for proper guidance of lipid-lowering therapy, especially in the current era of achieving very low LDL-C levels. The direct estimation of LDL-C using one of the enzymatic methods is considered the most accurate method for LDL-C estimation. However, due to the cost constraints and other logistical reasons, many laboratories worldwide continue to use indirect methods for estimating LDL-C. There are also issues with the standardization of the analytic methodology used for direct LDL-C estimation.

The Friedewald equation is the most popular method for indirect estimation. Proposed by Friedewald in 1972, the equation requires TC, HDL-C, and TG to estimate LDL-C.[6] This method is applicable only if TG is <400 mg/dL. Although this equation works well for day-to-day clinical needs, it has several limitations that are well recognized. First, the equation is not applicable if TG is >400 mg/dL. Second, the accuracy of this method is compromised as the TG increases above 150 mg/dL or when LDL-C is very low, the latter being very relevant to the current clinical practice. And third, a fasting blood sample is required for minimizing errors, which presents a major practical challenge as most of the current guidelines nowadays recommend non-fasting sampling for initial LDL-C estimation.[3–5]

Several different equations have been proposed to overcome various limitations inherent to the Friedewald equation.[7,8] More than 25 such equations are currently available, which have been compared in many different studies. These studies have shown inconsistent accuracy of these equations, which varies according to the population studied.[7,8,11,14] The two equations, however, appear to be the most robust–the Martin equation and the Sampson equation.[7,8,11,14] The former is applicable up to TG <400 mg/dL whereas the latter can be used even in patients with TG up to 800 mg/dL. The Martin equation has been shown to have very good accuracy even at very low LDL-C levels, as seen in the trials with the PCSK9 inhibitors.[25,26]

The accuracy of the Friedewald, Martin, and Sampson equations has been compared in several studies. Most of these studies have shown that all three equations lead to underestimation of LDL-C, but the Martin equation has the least bias.[7,8,11,14] Furthermore, whereas the error with the Friedewald equation increases with increasing TG levels, the accuracy of the Martin equation remains stable.[27] In our study, we also found exactly similar results.

Although numerous studies have tested the accuracy of various methods for LDL-C estimation, very few have evaluated the impact of the measurement error on treatment decisions, especially in patients with established ASCVD in whom achieving low/very low LDL-C is crucial.[15–17] Shi et al. studied 30,349 individuals with angiographically proven CAD. LDL-C was underestimated by all the three methods (Friedewald, Martin, and Sampson) in comparison to the direct estimation. The underestimation increased when TG was >150 mg/dL. Among patients with LDL-C 70–100 mg/dL, the three equations categorized 37.7, 19.2, and 26% of the subjects, respectively, as having LDL-C <70 mg/dL. The corresponding figures for patients with LDL-C 55–70 mg/dL, categorized as having LDL-C <55 mg/dL, were 53.6, 29, and 39.8%, respectively.[15] These findings are very similar to ours, except that the proportional underestimation was much larger in the study by Shi et al. because only the patients within a specific LDL-C category were used as the denominator and not all those above a certain LDL-C threshold. Another analysis by the same group involving patients in their percutaneous coronary intervention registry also reported similar findings.[16] Zafrir et al. also, in a study of 10,009 individuals undergoing coronary angiography, showed significant misclassification of individuals into lower LDL-C categories using the Friedewald equation compared with the Martin and Sampson equations. However, the direct LDL-C was not measured in this study.[14] Incorrect classification into lower LDL-C categories has been reported in a few other studies as well.[17]

These studies collectively show that indirect estimation of LDL-C has significant potential to lead to undertreatment of individuals. However, the impact of such underestimation on the long-term outcome has not been studied. This issue is relevant because in many of the large-scale trials, which form the basis for the current guidelines, the LDL-C was measured using the Friedewald equation and not the direct methods. Unfortunately, no randomized controlled study

comparing treatment strategies based on the different methods of LDL-estimation is feasible due to ethical reasons. However, a recent study has shown that the 10- or 20-year ASCVD prediction models incorporating LDL-C values of the Martin equation have superior predictive ability than the models based on the Friedewald or Sampson equation.[28] Furthermore, since there is clear evidence to show that reduction of LDL-C to lower levels is associated with a lower risk of ASCVD events, it can be safely concluded that leaving the patients at a higher LDL-C level will be deleterious.

The major lipid guidelines have acknowledged these issues. The 2018 American College of Cardiology/American Heart Association guidelines mention that the Friedewald method is inaccurate when LDL-C is <70 mg/dL and instead recommend using either direct method or the Martin equation (class IIa).[3] Similarly, the National Lipid Association in 2021 has advocated using the Martin equation instead of the Friedewald equation in patients with LDL-C <100 mg/dL or TG 150–400 mg/dL.[29] The 2019 European Society of Cardiology guidelines have also highlighted the concerns about the suboptimal accuracy of the Friedewald method at very low LDL-C levels or when TG is >177 mg/dL. However, no clear recommendation is made for an alternative option.[4]

## Limitations

Our study had a few limitations that need to be discussed. First, being a single-center study, our findings may not be generalizable. However, we wish to emphasize that in our study, the mean TG was 144.4 ± 57.8 mg/dL, which was in the normal range. Hence, the error in the LDL estimation observed with the Friedewald equation in our study is likely to be an underestimation only. Second, due to the cross-sectional and retrospective nature of the study, the long-term impact of treatment guidance using the different LDL estimation methods on clinical outcomes could not be assessed. However, as already mentioned, a prospective trial to answer this question is not feasible due to ethical reasons.

## Conclusion

In conclusion, our study shows that the Friedewald equation underestimates LDL-C and can potentially result in significant undertreatment in patients with CAD in whom aggressive LDL-C lowering is crucial. Direct estimation is the preferred method, but the Martin equation could be a reasonable alternative if the direct estimation is not feasible due to logistical constraints.

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ORIGINAL ARTICLE

# Exploring Hypovitaminosis B12 in New Onset Type 2 Diabetes Mellitus and Prediabetes

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## Abstract

**Background:** Diabetics often develop vitamin B12 deficiencies, which are crucial for blood, nerve, cognitive, and cardiovascular functions. The impact of metformin on vitamin B12 levels, leading to complications such as peripheral neuropathy and anemia, is well-known; yet no studies focus on deficiency status at diabetes diagnosis or the start of treatment.

**Methods:** A cross-sectional study was conducted at 2 tertiary care institutions in India, Command Hospital (Western Command), Haryana, and Civil Hospital in Sirsi, Karnataka, from July 2022 to November 2023. The study included 326 newly diagnosed type II diabetes mellitus (DM) patients and prediabetes individuals attending outpatient and inpatient departments, collecting data on substance use, dietary practices, fasting blood sugar, random blood sugar, HbA1c, and vitamin B12 levels (CLIA method).

**Results:** The study population of 326 individuals showed significant regional differences in mean age, gender distribution, and dietary preferences. Vitamin B12 deficiency (<200 pg/mL) was prevalent in 43.4% of prediabetic and 51.9% of type II DM patients. Significant differences in fasting blood sugar, postprandial blood sugar, and HbA1c levels were observed between regions. However, no significant correlation was found between vitamin B12 levels and HbA1c, age, or fasting glucose levels. Vegetarian individuals exhibited significantly higher vitamin B12 deficiency.

**Conclusion:** This study revealed a high prevalence of vitamin B12 deficiency in newly diagnosed diabetes patients, emphasizing the need for early identification and treatment to prevent complications such as neuropathy. The study recommends incorporating initial vitamin B12 assessment into the diagnosis protocol for newly detected diabetes patients to improve patient care and prevent complications in the Indian population.


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## Introduction

Diabetes is a major public health problem around the globe and in India.[1] These patients with diabetes develop multiple micronutrient deficiencies.[2] One of the important micronutrients is vitamin B12, which is important for blood function, nerve function, cognitive function, and cardiovascular functioning.[3] It is also known that metformin, the most common and most effective oral hypoglycemic drug used, causes vitamin B12 deficiency over a period of use.[4] According to the latest statements released in January 2019 by the American Association of Clinical Endocrinologists and the American College of Endocrinology, it has good antihyperglycemic efficacy at doses of 1,000–2,000 mg/day. However, in 16% of metformin users, metformin is responsible for vitamin B12 malabsorption and/or deficiency.[5,6] A review of existing literature has shed light on the impact of metformin and other OHAs on vitamin B12 levels.[7] Vitamin B12 malabsorption may lead to deficiency, which could be a causal factor in the development of peripheral neuropathy and anemia, further worsening the chronic disease.[8] It usually takes 4–6 months to start depletion of vitamin B12 and 5–10 years to show clinically the signs and symptoms of deficiency. Hence, there have been recommendations for annual screening for vitamin B12. However, there are no studies focusing on the deficiency status at the start of diagnosis or treatment.[9]

Hence, timely identification and treatment of the same are very essential. However, vitamin deficiency is very high in the general population itself,[10] hence patients are at risk of having vitamin B12 deficiency even before the start of antidiabetic treatment in those with new-onset diabetes. In such scenarios, the preexisting deficiency or insufficiency can add insult to injury and cause rapid progression of neuropathy or other symptoms of deficiency. This leads to severe damage earlier than expected. Hence, it is further important to identify and treat early so that complications such as neuropathy and worsening of overall health can be prevented. The preexisting vitamin B12 deficiency is high due to various reasons such as high PPI use and the increasing prevalence of NASH in the population.[11,12]

Thus, with this background, this study was conducted to assess the prevalence of low vitamin B12 in newly diagnosed type II diabetes mellitus (DM) patients.

## Methods

This cross-sectional study was conducted at 2 tertiary care institutions, 1 North Indian center, Command Hospital (Western Command), Chandimandir, Haryana, and another in the South Indian population, the trust-run TSS Hospital in Sirsi, Karnataka. The study was conducted among new-onset or newly diagnosed type II DM patients or prediabetes patients attending OPD and IPD during the period from July 2022 to November 2023. Informed consent was obtained from all participants after explaining the study in detail in the local language.

### Sample Size and Sampling

Sample size was calculated assuming the proportion of low serum vitamin B12 levels as 39.5% as per the study by Shahwan et al.[13] The other parameters considered for sample size calculation were 5.5% absolute precision and 95% confidence level. The following formula was used for sample size as per the study by Daniel and Cross.[14] The required sample size as per the abovementioned calculation was 303. However, 326 subjects were collected to increase precision and confidence in the findings. All newly diagnosed type II DM patients and prediabetes (IGT) subjects attending the OPD and IPD were included in the study, and those who were known cases of diabetes (chronic type II DM patients) were excluded. After informed consent, demographic details, substance use, and dietary practices were documented along with the FBS, RBS, and HbA1c. Vitamin B12 estimation was done

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by CLIA (chemiluminescence immunoassay) method using the Beckman Coulter machine.

### Ethical and Informed Consent

Ethical approval was obtained from the Institutional Review Board (ref: IHEC: IEC/2022–23/002) of the center concerned. Informed written consent was obtained before the study started, and confidentiality was maintained throughout.

### Statistical Analysis

Descriptive analysis was carried out using frequency and proportion for categorical variables. Continuous variables were presented as mean ± SD for normally distributed variables and median (IQR) for nonnormally distributed variables. The Chi-squared test was used to test the statistical significance of crosstabulation between categorical variables. The independent *t*-test was used to compare the mean ± SD of normally distributed continuous variables. Pearson correlation was used to assess the linear relationship between continuous variables. *p*-value < 0.05 was considered statistically significant. coGuide v 1.0.3 was used for statistical analysis.

## Results

The demographic characteristics of a study population comprising 326 individuals from South and North areas were analyzed. The mean age exhibited a significant difference between the 2 regions ($p < 0.001$), with South area participants being older (52.4 ± 13.5) than those from the North area (42.9 ± 9.9). No significant differences in mean body mass index (BMI) were observed between the areas ($p = 0.841$). Gender distribution showed a substantial discrepancy between the South and North areas ($p < 0.001$), with a higher proportion of females in the South (52.5%) compared to the North (10.8%). Marital status exhibited no significant difference between the regions ($p = 0.196$), and the majority of participants were married (97.2%). Religious affiliation varied significantly ($p = 0.02$), with Hinduism being the predominant religion across both areas and combined (92.6%). Dietary preferences differed significantly between areas ($p < 0.001$), with a higher prevalence of nonvegetarian diets in the North (76.5%) compared to the South (55.6%). Smoking habits and alcohol consumption showed no significant differences between areas, with a predominant nonsmoking population (81.3%) and varying alcohol consumption patterns across regions (Table 1).

In the comparison of past medical history across 326 individuals from the South and North areas, no significant differences were observed in the prevalence of chronic liver disease, coronary artery disease (CAD), hypothyroidism, and old stroke between the 2 regions. For chronic liver disease, the positive cases were minimal and identical in both the South and North areas (1.2%, $p = 1.000$). Similarly, the occurrence of CAD did not significantly differ, with 4.4% in the South and 3.0% in the North, resulting in a combined rate of 3.7% ($p = 0.719$). However, differences emerged in the prevalence of hypertension (HTN), showing a higher proportion in the North (57.2%) compared to the South (43.1%), with a combined prevalence of 50.3% ($p = 0.015$). Hypothyroidism displayed no significant regional variance, with a combined prevalence of 8.6% ($p = 0.623$). Old stroke cases were minimal and comparable across regions, with a combined prevalence of 1.5% ($p = 1.000$). These findings suggest that while certain medical histories exhibit regional consistency, the prevalence of hypertension differs between the South and North areas in the study population (Table 2).

In the descriptive analysis of laboratory parameters for a study population of 326 individuals from the South and North areas, significant differences were observed across regions for fasting blood sugar ($p < 0.001$), postprandial blood sugar ($p < 0.001$), and HbA1c levels ($p < 0.001$). Participants from the South area exhibited higher mean levels of fasting blood sugar (178.3 ± 64.9) compared to those from the North area (125.4 ± 30.1), resulting in a combined mean of 143.9 ± 51.8. The mean postprandial blood sugar was higher in the South (264.9 ± 95.4) compared to the North (172.1 ± 50.1), yielding a combined mean of 204.0 ± 81.8. The HbA1c levels were higher in the South (8.3 ± 1.7) than in the North (6.5 ± 1.1), leading to a combined mean of 7.4 ± 1.7. One of the reasons for the

**Table 1:** Descriptive analysis of demographic characteristics across the area in the study population (*N* = 326)

| *Parameter* | *Area* | | | *Combined (n = 326)* | *p-value* |
|---|---|---|---|---|---|
| | *Level* | *South (n = 160)* | *North (n = 166)* | | |
| Age | Mean ± SD | 52.4 ± 13.5 | 42.9 ± 9.9 | 47.6 ± 12.7 | <0.001 |
| Body mass index (in kg/m$^2$) | Mean ± SD | 25.8 ± 3.3 | 25.9 ± 3 | 25.9 ± 3.2 | 0.841 |
| Gender | Female | 84 (52.5%) | 18 (10.8%) | 102 (31.3%) | <0.001 |
| | Male | 76 (47.5%) | 148 (89.2%) | 224 (68.7%) | |
| Marital status | Married | 156 (97.5%) | 161 (97.0%) | 317 (97.2%) | 0.196 |
| | Single | 2 (1.2%) | 5 (3.0%) | 7 (2.1%) | |
| | Unmarried | 2 (1.2%) | 0 (0.0%) | 2 (0.6%) | |
| Religion | Hindu | 150 (93.8%) | 152 (91.6%) | 302 (92.6%) | 0.02 |
| | Muslim | 9 (5.6%) | 4 (2.4%) | 13 (4.0%) | |
| | Christian | 1 (0.6%) | 3 (1.8%) | 4 (1.2%) | |
| | Sikh | 0 (0.0%) | 7 (4.2%) | 7 (2.1%) | |
| Diet | Veg | 71 (44.4%) | 39 (23.5%) | 110 (33.7%) | <0.001 |
| | Nonveg | 89 (55.6%) | 127 (76.5%) | 216 (66.3%) | |
| Smoker | Current | 17 (10.6%) | 19 (11.4%) | 36 (11.0%) | 0.935 |
| | Past | 13 (8.1%) | 12 (7.2%) | 25 (7.7%) | |
| | Never | 130 (81.2%) | 135 (81.3%) | 265 (81.3%) | |
| Alcoholic | Current | 20 (12.5%) | 34 (20.5%) | 54 (16.6%) | 0.028 |
| | Past | 12 (7.5%) | 21 (12.7%) | 33 (10.1%) | |
| | Never | 128 (80.0%) | 111 (66.9%) | 239 (73.3%) | |

**Table 2:** Comparison of past history across area (*N* = 326)

| *Parameter* | *Level* | *Area* | | *Combined (n = 326)* | *p-value* |
|---|---|---|---|---|---|
| | | *South (n = 160)* | *North (n = 166)* | | |
| Chronic liver disease | Positive | 2 (1.2%) | 2 (1.2%) | 4 (1.2%) | 1.000 |
| | Negative | 158 (98.8%) | 164 (98.8%) | 322 (98.8%) | |
| Coronary artery disease | Yes | 7 (4.4%) | 5 (3.0%) | 12 (3.7%) | 0.719 |
| | No | 153 (95.6%) | 161 (97.0%) | 314 (96.3%) | |
| Hypertension (HTN) | Yes | 69 (43.1%) | 95 (57.2%) | 164 (50.3%) | 0.015 |
| | No | 91 (56.9%) | 71 (42.8%) | 162 (49.7%) | |
| Hypothyroidism | Yes | 12 (7.5%) | 16 (9.6%) | 28 (8.6%) | 0.623 |
| | No | 148 (92.5%) | 150 (90.4%) | 298 (91.4%) | |
| Old stroke | Yes | 2 (1.2%) | 3 (1.8%) | 5 (1.5%) | 1 |
| | No | 158 (98.8%) | 163 (98.2%) | 321 (98.5%) | |

**Table 3:** Comparison of vitamin B12 category between types of diabetes in north and south populations

| *Vitamin B12 category* | *North (160)* | | *Total* | *South (166)* | | *Total* |
|---|---|---|---|---|---|---|
| | *Type of diabetes* | | | *Type of diabetes* | | |
| | *Prediabetes (N = 117)* | *Type 2 diabetes (N = 49)* | | *Prediabetes (N = 14)* | *Type 2 diabetes (N = 146)* | |
| <200 | 50 (42.74%) | 22 (44.9%) | 72 (43.4%) | 12 (85.71%) | 71 (48.6%) | 83 (51.9%) |
| 200–300 | 37 (31.62%) | 15 (30.61%) | 52 (31.3%) | 1 (7.14%) | 26 (17.8%) | 27 (16.9%) |
| >300 | 30 (25.64%) | 12 (24.49%) | 42 (25.3%) | 1 (7.14%) | 49 (33.6%) | 50 (31.25%) |

**Table 4:** Comparison of vitamin B12 category according to comorbidities (*N* = 326)

| *Comorbidities* | *Vitamin B12 category* | | | *p-value* |
|---|---|---|---|---|
| | *<200* | *200–300* | *>300* | |
| Chronic liver disease | | | | |
| Negative (*n* = 322) | 154 (47.83%) | 77 (23.91%) | 91 (28.26%) | 0.459 |
| Positive (*n* = 4) | 1 (25%) | 2 (50%) | 1 (25%) | |
| Coronary artery disease | | | | |
| No (*n* = 314) | 148 (47.13%) | 78 (24.84%) | 88 (28.03%) | 0.423 |
| Yes (*n* = 12) | 7 (58.33%) | 1 (8.33%) | 4 (33.33%) | |
| Hypertension | | | | |
| No (*n* = 162) | 79 (48.77%) | 39 (24.07%) | 44 (27.16%) | 0.890 |
| Yes (*n* = 164) | 76 (46.34%) | 40 (24.39%) | 48 (29.27%) | |
| Hypothyroidism | | | | |
| No (*n* = 298) | 147 (49.33%) | 68 (22.82%) | 83 (27.85%) | 0.069 |
| Yes (*n* = 28) | 8 (28.57%) | 11 (39.29%) | 9 (32.14%) | |
| Old stroke | | | | |
| No (*n* = 321) | 154 (47.98%) | 77 (23.99%) | 90 (28.04%) | 0.454 |
| Yes (*n* = 5) | 1 (20%) | 2 (40%) | 2 (40%) | |

above difference in the results is the higher proportion of prediabetes in the North population compared to diabetes as per ADA guidelines.

The table presents a comparison of vitamin B12 categories between individuals classified with prediabetes (*N* = 117) and those diagnosed with type 2 diabetes (*N* = 49) within the North population (total *N* = 166). The distribution of vitamin B12 levels between the 2 diabetes types showed no statistically significant difference ($p = 0.967$). This suggests a comparable distribution of vitamin B12 levels in individuals with prediabetes and type 2 diabetes in the North population. Among individuals in the South population, with a total sample size of 160, differentiated by prediabetes (*N* = 14) and type 2 diabetes (*N* = 146), the results revealed a significant distinction in vitamin B12 levels ($p = 0.029$). A higher percentage of individuals with prediabetes fell into the <200 category compared to type 2 diabetes (85.71 vs 48.63%). Overall, there was a significant difference ($p = 0.01$) in vitamin B12 categories between North and South participants. There was no significant correlation between vitamin B12 and HbA1c ($r = 0.005$, $p = 0.923$), age ($r = -0.0039$, $p = 0.48$), or fasting glucose levels ($r = 0.017$, $p = 0.79$) (Table 3).

There was no significant relationship between various comorbidities such as chronic liver disease, CAD, hypertension, hypothyroidism, and old stroke and vitamin B12 deficiency (Table 4).

**Table 5:** Comparison of vitamin B12 category according to area and type of diabetes ($N = 326$)

| *Parameters* | *Vitamin B12 category* | | | *p-value* |
|---|---|---|---|---|
| | *<200* | *200–300* | *>300* | |
| Alcoholic | | | | |
| Current ($n = 54$) | 22 (40.74%) | 15 (27.78%) | 17 (31.48%) | 0.583 |
| Never ($n = 239$) | 115 (48.12%) | 55 (23.01%) | 69 (28.87%) | |
| Past ($n = 33$) | 18 (54.55%) | 9 (27.27%) | 6 (18.18%) | |
| Smoker | | | | |
| Current ($n = 36$) | 14 (38.89%) | 9 (25%) | 13 (36.11%) | 0.669 |
| Never ($n = 265$) | 127 (47.92%) | 64 (24.15%) | 74 (27.92%) | |
| Past ($n = 25$) | 14 (56%) | 6 (24%) | 5 (20%) | |
| Type of diabetes | | | | |
| Prediabetes ($n = 131$) | 62 (47.33%) | 38 (29.01%) | 31 (23.66%) | 0.159 |
| Type 2 diabetes ($n = 195$) | 93 (47.69%) | 41 (21.03%) | 61 (31.28%) | |
| Diet type | | | | |
| Nonveg ($n = 216$) | 87 (40.28%) | 60 (27.78%) | 69 (31.94%) | <0.001 |
| Veg ($n = 110$) | 68 (61.82%) | 19 (17.27%) | 23 (20.91%) | |

Irrespective of smoking status, alcohol use, or prediabetes or newly diagnosed diabetes status, there was no difference in vitamin B12 level status. Significantly higher levels of insufficiency and deficiency of vitamin B12 were observed among vegetarians (Table 5).

There were significantly more prediabetics from the North side than the South side (70.48 vs 8.75%, $p < 0.001$) and more alcohol users, either current or past, in the North side than the South side (33 vs 20%, $p = 0.028$).

## Discussion

This study revealed a very high prevalence of vitamin B12 deficiency (43.8–51.9%) during prediabetes or new-onset diabetes when metformin is not yet started. This is almost every alternate person having a deficiency of vitamin B12. Diabetes patients are known to develop neuropathy after 10–20 years, despite reasonable glycemic control, and sheer due to long-term dysglycemia. Thus, this preexisting deficiency adds insult to injury and further adds to the misery of diabetes patients. Studies on levels of vitamin B12 in the general population also indicate high levels of vitamin B12 deficiency.[10,15] One of the reasons for high levels of deficiency is increasing use of PPIs, worsening eating habits leading to frequent gastritis, increasing levels of alcoholism, and increasing NASH.[12,16,17]

Although it cannot be recommended to have a nonvegetarian diet, which is rich in vitamin B12, the area (North site) had higher nonvegetarians and lower deficiency of vitamin B12. However, animals are the only source of vitamin B12.[3,18] However, dietary practice alone may not determine B12 deficiency, as chronic gastritis can lead to intrinsic factor deficiency and hence decreased absorption of B12, eventually leading to B12 deficiency.[3]

This directs B12 assessment and treatment from the time of diagnosis itself to prevent or worsen neuropathy symptoms and prevent the early onset of neuropathy or anemia. Also, whether vitamin B12 deficiency is the cause or effect of NASH is still being investigated.[16] Similarly, preexisting vitamin B12 deficiency raises the question of whether it is bidirectional, both cause and effect, in diabetes. This leads to recommendations for meta-analysis and larger community-based studies to test the above hypothesis.

The prevalence of vitamin B12 deficiency (<200 pg/mL) in newly diagnosed diabetics (44%) is comparable to the general population of North India (47%), as reported by Singla et al.[10] Although the Standards of Medical Care in Diabetes—2022 state that there is no clear evidence that dietary supplements with vitamins, minerals, herbs, or spices can help with the management of diabetes,[19] deficiency requires management. The study recommends modifying existing guidelines by recommending initial assessment of vitamin B12 among newly detected diabetes patients and treating accordingly, so that patient care improves and complications, especially neuropathy, are reduced by diagnosing and treating as early as the time of diagnosis. Currently, although guidelines suggest annual screening for vitamin B12 deficiency, evidence reports that this is not being followed even in developed countries.[20,21] However, in a country like India, where high vitamin B12 deficiency exists,[15,18,22] there is a dire need for guidelines to be modified.

## Orcid

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ORIGINAL ARTICLE

# To Determine Vitamin B12 Deficiency in Type 2 Diabetes Mellitus Patients on Metformin Therapy

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## Abstract

**Introduction:** India harbors the second-largest population with diabetes, with over 100 million, and type 2 diabetes mellitus (T2DM) constitutes the major share. Metformin remains the first-line pharmacotherapy for T2DM due to its safety profile, cost-effectiveness, and beneficial metabolic effects.

**Materials and methods:** The aim of the study was to assess the frequency of vitamin B12 deficiency in patients with T2DM on metformin therapy and compare it with their cohabiting family members who are not on metformin but share similar dietary habits.

**Results:** This study included 180 participants with 90 cases and controls each, and we enrolled 89 females (49.4%) and 91 males (50.6%). The mean age was 57 (± 4.88) years, and overall gender distribution and dietary pattern were nearly balanced among cases and controls. The mean duration of diabetes among cases was 7.69 ± 4.35 years, and duration of metformin use was 5.22 ± 3.77 years, ranging from 1–16 years. The mean daily dose of metformin was 1238.89 ± 586.50 mg/day, with a median dose of 1000 mg/day. The mean serum vitamin B12 level in metformin users was significantly lower than in controls (206.66 ± 59.09 pg/mL vs 301.44 ± 72.28 pg/mL, $p < 0.001$). Vitamin B12 deficiency was present in 40.0% of metformin users versus 11.1% of controls, yielding an odds ratio of 5.33 (95% CI: 2.44–11.65), which was a highly significant difference between the two groups ($t = -9.631$, $p < 0.001$), strongly suggesting an association between metformin use and reduced B12 levels. Neurological symptoms were observed in 14.4% of cases (OR 4.896, 95% CI: 1.345–17.827; $p = 0.009$).

**Conclusion:** Long-term metformin use in T2DM patients is strongly associated with both biochemical vitamin B12 deficiency and an increased likelihood of neurological symptoms.


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## Introduction

Diabetes mellitus (DM) is a long-term metabolic disorder with rapidly mounting global burden.[1] Globally, India ranks second in terms of the diabetic population, with over 100 million, and type 2 diabetes mellitus (T2DM) constitutes the major portion.[2] Metformin mainly acts by suppressing gluconeogenesis in the liver and improving insulin sensitivity.[3] According to prevailing guidelines, metformin is recommended as the initial agent for most T2DM patients, especially those who are overweight or obese with preserved renal function.[1–4] However, prolonged metformin therapy has been linked to vitamin B12 (cobalamin, VB12) deficiency. VB12 is a vital water-soluble nutrient involved in DNA synthesis, erythropoiesis, and neuroprotection. The absorption of vitamin B12 occurs in the terminal ileum after forming a complex with intrinsic factor (IF). Metformin may interfere with this absorption process by impairing the calcium-dependent mechanism that facilitates the uptake of the IF–B12 complex in the ileum and by altering intestinal motility.[5,6] Vitamin B12 deficiency can cause megaloblastic anemia and neurological manifestations, including neuropathy, cognitive decline, and subacute combined degeneration of the spinal cord. Symptoms such as fatigue, memory loss, depression, and glossitis may also occur.[7,8] In addition to worsening diabetic neuropathy, B12 deficiency can exacerbate cardiovascular risk through elevated homocysteine levels. Hence, awareness, timely identification, and correction are of paramount importance. Recent studies have quantified this risk of VB12 deficiency and have estimated the prevalence in metformin users ranging from 8 to 30%.[5–9] In South Asia, widespread vegetarian dietary patterns further contribute to reduced vitamin B12 intake, amplifying the risk of deficiency. However, routine assessment and management may be inadequate, particularly in a resource-constrained and predominantly vegetarian population. The true burden of this deficiency in real-world Indian diabetic cohorts and its differentiation from dietary or other environmental causes remains poorly characterized.

Hence, we planned this study to bridge this gap and to isolate the pharmacological impact of metformin from dietary and genetic confounders. We aimed to evaluate the frequency of Vitamin B12 deficiency among individuals with T2DM who are undergoing metformin treatment and to compare it with their cohabiting family members not on metformin but sharing similar dietary habits. The primary objective was to determine the prevalence of vitamin B12 deficiency in T2DM patients who have been receiving metformin either as monotherapy or alongside other antidiabetic medications for a duration exceeding one year. The secondary objective was to compare the vitamin B12 status of T2DM patients with their household family members who share similar dietary practices but are not exposed to metformin.

## Materials and Methods

This was designed as a case-control, observational study following approval from the institutes' ethics committee for academic research projects vide approval number ECARP/2024/25, dated 25/09/2024 at a tertiary care teaching public hospital. The total study duration was 18 months, commencing from 01/10/2024. The objective of the study was to determine the prevalence of vitamin B12 deficiency in T2DM patients who have been receiving metformin either as monotherapy or alongside other antidiabetic medications for a duration exceeding 1 year and compare it with their cohabiting family members who are not on metformin but share similar dietary habits. All the patients of T2DM on metformin therapy attending the outpatient department (OPD) of a public hospital with their cohabiting family member during the study period were screened and enrolled in the study. Written informed

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consent was obtained from all participating patients and their respective family members after explaining the purpose, procedure, and confidentiality of the study. We included adult patients 18 years and above of either sex. For cases, patients of T2DM on metformin for more than 1 year as monotherapy or in combination with other drugs were enrolled. For controls, family members of the above patients, who were nondiabetic and not on metformin, residing in the same household and sharing similar dietary habits, were selected. We excluded type 1 DM, patients diagnosed with inflammatory bowel disease, those with a history of gastrectomy or colectomy, chronic liver or kidney disorders, malignancies, long-term alcohol use, or any other condition known to influence vitamin B12 levels. Patients on vitamin B12 supplementation were also excluded from the study.

After thorough screening according to the predetermined inclusion and exclusion criteria, participants received a thorough explanation of the study protocol, followed by the acquisition of written informed consent. Each participant's involvement in the study was limited to a single outpatient visit. This included the informed consent process, detailed history, examination of the patients, collection of demographic and clinical data, and blood sampling for hemogram and serum vitamin B12 analysis. For the cases group, blood sampling was done as a standard of care, and for the control group, it was done only for the study purpose. Data gathered from each participant encompassed their demographic characteristics, comprehensive clinical history, comorbidities, duration of T2DM, duration and dose of metformin, general and detailed multisystem examination, including neuropathy evaluation. Blood sampling was done following the standard aseptic procedure and sent to the laboratory for hemogram, including hemoglobin (Hb), mean corpuscular volume (MCV), and serum vitamin B12 levels. A threshold of <200 pg/mL, consistent with established diagnostic criteria, was used to define vitamin B12 deficiency.

The sample size was determined based on prevalence data reported by Kumar et al., applying the following formula.[10]

$n = Z^2 \times P \times (1-P)/E^2$ (where: $Z = 1.96$, $P = 0.2733$, $1-P = 0.7267$, $E = 0.10$ with acceptable margin of error) = 0.7627. Thus, sample size ($n$) = 76.27. Adjusting for a 10% dropout rate: $n\_adj = n/(1-d) = 76.27/(1-0.10) = 76.27/0.90 \approx 85$. Thus, the final sample size was rounded to 90 participants per group, making a total of 180 participants to ensure adequate power and accommodate potential dropouts.

Vitamin B12 was assessed at a single point in time among cases and controls rather than following individuals who were initially free of deficiency to see who later developed it; prevalence is the appropriate measure. Categorical variables were described using frequencies and percentages, whereas continuous variables were presented as mean ± standard deviation or median with interquartile range (IQR). Within the qualitative data, nominal variables comprised group classification (case/control), participant sex, metformin usage, and dietary habits (vegetarian/mixed), vitamin B12 deficiency (yes/no), and neurological symptoms. Associations between qualitative variables were evaluated using the Chi-square test, incorporating continuity correction for all 2 × 2 contingency tables. In cases where expected cell counts were too low to validate the Chi-square test, Fisher's exact test was employed for 2 × 2 tables. For tables with more than two rows and insufficient cell counts, adjacent rows were combined, and the Chi-square test was reapplied. Continuity correction was also applied to all 2 × 2 tables following data pooling—for instance, when examining the relationship between dietary patterns (vegetarian/ mixed) and group category (case/control). Quantitative variables were presented as mean ± standard deviation (SD) or as median with IQR. These included measures such as age, duration of diabetes mellitus in cases, metformin dose, duration of metformin use in cases, VB12 level, MCV, and hemoglobin level. Quantitative data were compared across the binary qualitative variable representing group classification (case/control) using the unpaired *t*-test if the data passed the "Shapiro–Wilk normality test," or the Mann–Whitney U test if the data failed the "normality" test. [e.g., comparison of vitamin B12 Level (pg/mL) between cases and controls]. Graphical representations were employed where appropriate to enhance the presentation of results. Statistical analyses were conducted using suitable software tools, including but not limited to MS Excel, Microsoft Office 365, and PSPP (version 2.0.1, released on 21 March 2024). A *p*-value threshold of ≤0.05 was considered indicative of statistical significance.

## Results

This study included 180 participants with 90 cases and controls each. Overall, the study population was almost equally distributed with 89 females (49.4%) and 91 males (50.6%). The mean age of participants was 57 years (SD ± 4.88). The normality of age distribution was evaluated by applying the Shapiro–Wilk test ($p = 0.456$ for cases and $p = 0.142$ for controls; $p > 0.05$ for both) and an independent samples *t*-test assuming equal variances ($t = -1.158$, $p = 0.248$) revealed no statistically significant difference between the groups, confirming they were age-matched (Table 1). Dietary patterns were matched between the two groups, with 41 out of 90 participants (45.6%) in each group following a vegetarian diet and 49 out of 90 participants (54.4%) in each group having a mixed diet. The case and control groups were comparable in terms of age, sex, and dietary patterns, with no statistically significant differences observed between them.

Duration of DM (years), metformin dosage, and duration of metformin use (years) were assessed only in the case group ($n$ = 90), since all individuals in the control group were nondiabetics by study design. The mean (SD) duration of DM among cases was 7.69 ± 4.35 years. The mean (SD) daily dose of metformin was 1238.89 ± 586.50 mg/day, with a median dose of 1000 mg/day. The average duration of metformin use was 5.22 years (SD ± 3.77), with a range spanning 1–16 years and an IQR of 6 years (Fig. 1). Assessment of normality using the Shapiro–Wilk test indicated that the data were not normally distributed for duration of diabetes ($p = 0.00805$, $p < 0.05$), metformin dose ($p < 0.00000001$), and duration of metformin ($p = 0.0000083$), respectively, indicating that the data is right-skewed. The mean MCV and Hb were 90.26 ± 4.24 fL, and 13.13 ± 1.19 gm/dL among the 90 cases and 89.35 ± 3.91 fL. and 13.40 ± 1.05 gm/dL in the 90 controls, respectively. The Shapiro–Wilk test demonstrated that MCV and Hb values were normally distributed in both groups. Various variables of both groups are compared in Table 1.

The mean serum vitamin B12 level was 206.66±59.09 pg/mL with an IQR of 90.47 pg/mL among the 90 cases, while controls had a value of 301.44 ± 72.28 pg/mL, with an IQR of 96.08 pg/mL (Fig. 2) The Shapiro–Wilk test for normality revealed that vitamin B12 levels were not normally distributed in the case group

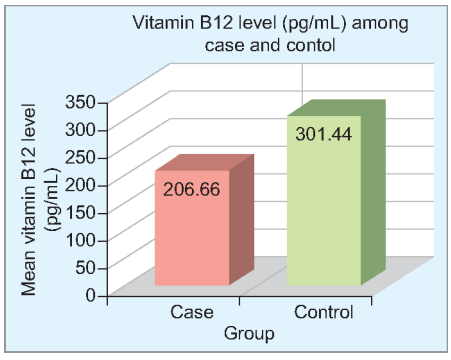


**Fig. 1:** Mean vitamin B12 values in cases and controls

**Table 1:** Various variables amongst cases and controls

| | *Groups* | *Statistic* | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Mean* | *Std. deviation* | *Median* | *Interquartile range* | *Minimum* | *Maximum* |
| Age (years) | Case | 56.956 | 4.690 | 57.000 | 6.000 | 44.000 | 67.000 |
| | Control | 57.778 | 4.834 | 58.000 | 6.250 | 48.000 | 71.000 |
| Duration of diabetes mellitus (years) | Case | 7.689 | 4.352 | 8.000 | 6.000 | 1.000 | 18.000 |
| Metformin dose (mg/day) | Case | 1238.889 | 586.495 | 1000.000 | 1500.000 | 500.000 | 2000.000 |
| Duration of metformin use (years) | Case | 5.222 | 3.768 | 4.500 | 6.000 | 1.000 | 16.000 |
| Vitamin B12 level (pg/mL) | Case | 206.658 | 59.086 | 213.387 | 90.471 | 110.550 | 330.656 |
| | Control | 301.439 | 72.283 | 308.900 | 96.075 | 134.000 | 459.300 |
| MCV (fL) | Case | 90.264 | 4.242 | 90.300 | 6.075 | 81.500 | 98.800 |
| | Control | 89.353 | 3.906 | 89.050 | 5.625 | 80.800 | 102.300 |
| Hemoglobin (gm/dL) | Case | 13.130 | 1.195 | 13.250 | 1.500 | 10.000 | 15.900 |
| | Control | 13.397 | 1.049 | 13.300 | 1.325 | 10.800 | 16.100 |

| | *Groups* | *Shapiro–Wilk* | | |
|---|---|---|---|---|
| | | *Statistic* | *Significance* | *df* |
| Age (years) | Case | 0.986 | 0.456 | 90 |
| | Control | 0.979 | 0.142 | 90 |
| Duration of diabetes mellitus (years) | Case | 0.961 | 0.00805 | 90 |
| Metformin dose (mg/day) | Case | 0.829 | 8.4911E-009 | 90 |
| Duration of metformin use (years) | Case | 0.907 | 8.3351E-006 | 90 |
| Vitamin B12 level (pg/mL) | Case | 0.961 | 0.00831 | 90 |
| | Control | 0.980 | 0.169 | 90 |
| MCV (fL) | Case | 0.986 | 0.451 | 90 |
| | Control | 0.978 | 0.134 | 90 |
| Hemoglobin (gm/dL) | Case | 0.992 | 0.837 | 90 |
| | Control | 0.989 | 0.646 | 90 |

*t*-test

| | *Groups* | *N* | *Mean* | *Std deviation* | *Std error mean* |
|---|---|---|---|---|---|
| Age (years) | Case | 90 | 56.96 | 4.69 | 0.49 |
| | Control | 90 | 57.78 | 4.83 | 0.51 |
| Vitamin B12 level (pg/mL) | Case | 90 | 206.66 | 59.09 | 6.23 |
| | Control | 90 | 301.44 | 72.28 | 7.62 |
| MCV (fL) | Case | 90 | 90.26 | 4.24 | 0.45 |
| | Control | 90 | 89.35 | 3.91 | 0.41 |
| Hemoglobin (gm/dL) | Case | 90 | 13.13 | 1.19 | 0.13 |
| | Control | 90 | 13.40 | 1.05 | 0.11 |

| | | *t-test for equality of means* | |
|---|---|---|---|
| | | *t* | *Significance (2-tailed)* |
| Age (years) | Equal variances assumed | −1.158 | 0.248 |
| Vitamin B12 level (pg/mL) | Equal variances assumed | −9.631 | 6.18E-018 |
| MCV (fL) | Equal variances assumed | 1.499 | 0.136 |
| Hemoglobin (gm/dL) | Equal variances assumed | −1.591 | 0.113 |

($p = 0.00831$) but were normally distributed in the control group ($p = 0.169$). However, given the large sample size, the central limit theorem supported the use of a parametric test. An independent samples *t*-test, conducted under the assumption of equal variances, revealed a highly significant difference in serum vitamin B12 levels between the two groups ($t = -9.631$, $p < 0.001$), indicating a strong association between prolonged metformin therapy and reduced vitamin B12 concentrations (Table 1). Vitamin B12 deficiency was identified in 36 participants from the case group (40.0%) and in 10 individuals from the control group (11.1%) (Fig. 3). This indicates that the prevalence of deficiency among cases was over threefold higher than that in controls. Statistical evaluation using Pearson's Chi-square test revealed a highly significant difference between the groups ($\chi^2 = 19.740$, $df = 1$, $p < 0.001$), which was further substantiated by Fisher's exact test ($p = 1.31 \times 10^{-5}$). The calculated odds ratio exhibited that the odds of having vitamin B12 deficiency were 5.333 times higher in participants with T2DM on metformin therapy compared to their matched household controls (95% CI: 2.442–11.647) (Table 2). Neurological

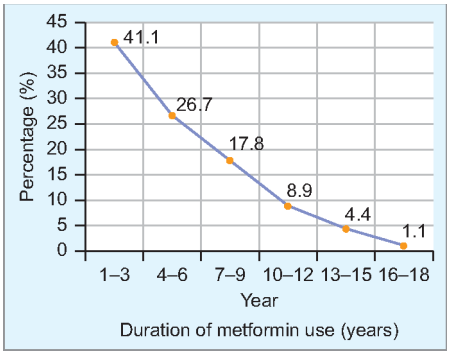


**Fig. 2:** Duration of metformin use (years) among cases

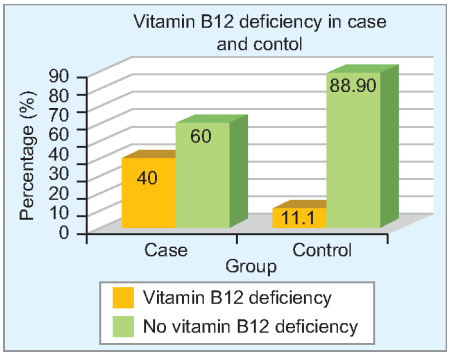


**Fig. 3:** Frequency of vitamin B12 deficiency among cases and controls

symptoms suggestive of B12 deficiency, including paresthesia, numbness, and balance disturbances, were noted in 14.4% of cases compared to 3.3% of controls (OR 4.896, 95% CI: 1.345–17.827; $p = 0.009$), underscoring the clinical impact of deficiency. The difference between the two groups was statistically significant (Pearson's Chi-square = 6.860, $df = 1$, $p = 0.009$; Fisher's exact test = 0.016) (Table 3). The odds of having neurological symptoms were 4.896 times higher in the cases compared to the controls (95% CI: 1.345–17.827), highlighting a significant correlation between metformin

**Table 2:** Vitamin B12 deficiency among study participants

| | | | Group | | Total |
|---|---|---|---|---|---|
| | | | Case | Control | |
| Vitamin B12 deficiency | Yes | Count | 36 | 10 | 46 |
| | | % within group | 40.0% | 11.1% | 25.6% |
| | No | Count | 54 | 80 | 134 |
| | | % within group | 60.0% | 88.9% | 74.4% |
| | Total | Count | 90 | 90 | 180 |
| | | % within group | 100.0% | 100.0% | 100.0% |

| | Value | df | Asymptotic significance (2-sided) | Exact significance (2-sided) | Exact significance (1-sided) |
|---|---|---|---|---|---|
| Pearson Chi-square | 19.740(b) | 1 | 8.87E-006 | | |
| Continuity correction(a) | 18.251 | 1 | 1.94E-005 | | |
| Likelihood ratio | 20.677 | 1 | 5.44E-006 | | |
| Fisher's exact test | | | | 1.31E-005 | 6.55E-006 |
| *n* of valid cases | 180 | | | | |

| | Value | 95% confidence interval | |
|---|---|---|---|
| | | Lower | Upper |
| Odds ratio for group (case/control) | 5.333 | 2.442 | 11.647 |
| For cohort vitamin B12 deficiency = Yes | 3.600 | 1.904 | 6.805 |
| For cohort Vitamin B12 Deficiency = No | 0.675 | 0.562 | 0.811 |
| *N* of valid cases | 180 | | |

**Table 3:** Neurological symptoms among study participants

| | | | Group | | Total |
|---|---|---|---|---|---|
| | | | Case | Control | |
| Neurological symptoms | Yes | Count | 13 | 3 | 16 |
| | | % within group | 14.4% | 3.3% | 8.9% |
| | No | Count | 77 | 87 | 164 |
| | | % within group | 85.6% | 96.7% | 91.1% |
| Total | | Count | 90 | 90 | 180 |
| | | % within group | 100.0% | 100.0% | 100.0% |

| | Value | df | Asymptotic significance (2-sided) | Exact significance (2-sided) | Exact significance (1-sided) |
|---|---|---|---|---|---|
| Pearson Chi-square | 6.860(b) | 1 | 0.00882 | | |
| Continuity correction(a) | 5.556 | 1 | 0.01841 | | |
| Likelihood ratio | 7.348 | 1 | 0.00671 | | |
| Fisher's exact test | | | | 0.016 | 0.008 |
| *N* of valid cases | 180 | | | | |

| | Value | 95% confidence interval | |
|---|---|---|---|
| | | Lower | Upper |
| Odds ratio for group (case/control) | 4.896 | 1.345 | 17.827 |
| For cohort neurological symptoms = Yes | 4.333 | 1.278 | 14.691 |
| For cohort neurological symptoms = No | 0.885 | 0.806 | 0.971 |
| *N* of valid cases | 180 | | |

therapy and the occurrence of neurological symptoms.

## Discussion

This case-control study was conducted at a tertiary care hospital to determine the prevalence of vitamin B12 deficiency among patients with T2DM undergoing metformin therapy, in comparison to their nondiabetic household members who shared similar dietary patterns. The study enrolled a total of 180 participants, comprising 90 T2DM patients who had been on metformin for over 1 year and 90 matched controls without diabetes and not receiving metformin. The average age of participants was 57 years, with a SD of ±4.88, and overall gender distribution and dietary pattern were nearly balanced among cases and controls. The two groups were matched for age, sex, dietary habits, and living environment, thereby minimizing potential confounding factors. Demographic parameters were comparable with prior studies.[11,12] This middle-aged cohort likely reflects both the typical age of T2DM onset and the time required for chronic metformin exposure to result in clinically detectable vitamin B12 deficiency. However, dietary patterns from other studies show variability. Bora et al observed that the majority of their participants were nonvegetarians, while Agarwal et al. reported a predominance of vegetarian participants.[9,12] These discrepancies highlight the diversity influencing dietary practices across study populations. Animal-derived foods serve as the principal dietary source of vitamin B12, and it is essential to meticulously match dietary factors that strengthen the internal validity of the present study.

The mean (SD) duration of DM among cases was 7.69 ± 4.35 years, and duration of metformin use was 5.22 ± 3.77 years, ranging from 1–16 years. The mean (SD) daily dose of metformin was 1238.89 ± 586.50 mg/day, with a median dose of 1000 mg/day. Studies have reported consistent duration of DM and comparable mean daily dose.[13–16] The mean MCV and Hb were comparable among cases and controls. Majority studies mention similar findings; however, a few did observe a high proportion of cases with low MCV, indicating possible coexisting iron deficiency and higher anemia prevalence among metformin users.[9,17–19] Together, these findings from the present study and comparable literature underscore that while vitamin B12 deficiency frequently occurs in patients on metformin therapy, macrocytosis and anemia are not consistent or sensitive markers, particularly in populations with coexisting nutritional deficiencies or chronic disease, biochemical screening to detect early deficiency is necessary.

The mean serum vitamin B12 level was 206.66 ± 59.09 pg/mL among the 90 cases, while controls had a value of 301.44 ± 72.28 pg/mL. Vitamin B12 deficiency was observed in 36 cases (40.0%) and in 10 controls (11.1%). The likelihood of vitamin B12 deficiency was 5.333 times greater among cases compared to controls. The present study captures the cumulative metabolic impact of both diabetes and metformin therapy, which may partly explain the higher prevalence of B12 deficiency observed. Deficiency is affected by duration of exposure, dose, and individual metabolic factors. A recent study reiterates that higher doses and longer durations of metformin was linked with lower vitamin B12 concentrations, and vitamin B12 screening is highly recommended.[20] Overall, the consistency between our results and other long-term metformin user cohorts suggests that our mean duration of ~5 years is representative of real-world chronic T2DM management, and cumulative duration, rather than just dose, serves as a key contributing factor in the vitamin B12 deficiency. B12 deficiency may remain clinically silent in terms of hematologic manifestations. Findings from the current study demonstrate a clear, statistically robust association between prolonged metformin therapy and decreased serum vitamin B12 concentration, both in absolute values and in prevalence of deficiency. The large effect size (OR > 5) is noteworthy and exceeds that reported in several previous studies, likely due to the careful matching of dietary patterns and living environments between cases and controls, which removed major confounders such as meat intake variability. Comparable trends have been observed in previous studies with variation in mean B12 levels due to diet patterns, duration, and dose of metformin, etc.[20–25] Neurological symptoms suggestive of B12 deficiency, including paresthesia, numbness, and balance disturbances, were observed in 14.4% of cases, and the odds of having neurological symptoms were 4.896 times higher in the cases. Therefore, individuals with T2DM undergoing prolonged metformin treatment are at increased risk of developing vitamin B12 deficiency and are more prone to manifest neurological symptoms. Because fewer participants exhibited neurological symptoms than biochemical deficiency, this may imply that neuropathic manifestations progress over time rather than appearing immediately. Multiple studies have consistently demonstrated a link between metformin therapy, vitamin B12 deficiency, and the emergence of neuropathic symptoms, though reported prevalence rates vary.[26–28] Overall, the combined evidence supports that metformin-associated B12 deficiency increases the likelihood of neuropathy, reinforcing the need for early detection and preventive supplementation. Current management involves oral or intramuscular replacement with cyanocobalamin or methylcobalamin. Comparison with literature demonstrated consistent trends linking metformin with lower B12 levels and neuropathy, though the effect varied depending on population characteristics, dietary background, and study design.[28]

A key strength of this study is its robust case-control design, incorporating meticulous matching of cases and controls, which enhances the validity of the observed associations. By selecting cohabiting family members as controls, the study effectively minimized variability in dietary vitamin B12 intake, environmental exposures, and socioeconomic factors, thereby isolating the effect of extended metformin use on serum vitamin B12 levels. The relatively large sample size for a single-center study provided adequate statistical power. The study has certain limitations. Being a single-center, observational case-control design and cross-sectional data, causal inference cannot be firmly established, and residual confounding from unmeasured variables, such as differences in physical activity, undiagnosed comorbidities, cannot be excluded. Neurological symptoms were assessed using clinical history without electrophysiological confirmation, which might underestimate subclinical neuropathy prevalence.

From this case-control study of 180 participants, we conclude that patients with T2DM receiving long-term metformin therapy exhibited a substantially higher prevalence of vitamin B12 deficiency than their matched household controls who shared comparable dietary habits. The two groups were comparable in age, sex, and dietary pattern, and the odds of developing vitamin B12 deficiency were 5.333 times greater among cases than controls. The study revealed a highly significant association between extended metformin therapy and diminished serum vitamin B12 levels, both in terms of absolute values and deficiency prevalence. The diabetic cohort was characterized by a long history of diabetes and varying doses and durations of metformin, suggesting cumulative exposure, and B12 deficiency was present without any hematological alterations. Odds of having

neurological symptoms consistent with vitamin B12 deficiency were 4.896 times higher in the cases, highlighting its clinical relevance. Hence, we may recommend that routine monitoring of vitamin B12 levels should be integrated into the standard management protocol for patients with T2DM receiving long-term metformin therapy.

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ORIGINAL ARTICLE

# Molecular Identification of *Mycobacterium bovis* in Human Pulmonary Tuberculosis: Insights from a Tertiary Care Hospital in Gujarat, India

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## Abstract

**Background:** *Mycobacterium bovis*, the causative agent of bovine tuberculosis, is a zoonotic pathogen capable of infecting cattle and humans. Human contraction of bovine tuberculosis, particularly pulmonary infection, remains a significant public health concern. The differentiation between *Mycobacterium bovis* and *Mycobacterium tuberculosis* is challenging due to limitations in conventional diagnostic methods, leading to an underestimated burden of *M. bovis* in human population. This study focuses on the prevalence of *M. bovis* in cases of pulmonary tuberculosis in a tertiary care teaching hospital located in Karamsad, Anand, a rural district of Gujarat.

**Methods:** In this cross-sectional study, 1,000 sputum samples from patients clinically suspected of having pulmonary tuberculosis were collected at the Department of Respiratory Medicine from November 2017 to June 2018. All samples underwent Ziehl–Neelsen staining for Acid Fast Bacilli detection, followed by molecular testing using primers targeting the *HupB* gene (a histone-like protein), to differentiate between *M. tuberculosis* and *M. bovis*.

**Results:** Of the 1,000 sputum samples, 100 (10%) tested positive for Acid Fast Bacilli. Molecular analysis revealed that 90% of these positive samples were *M. tuberculosis*. Among the remaining samples, 4% were positive for *M. bovis*, and 6% indicated a mixed infection with both *M. tuberculosis* and *M. bovis*.

**Conclusion:** The study found the prevalence of *M. bovis* in 10% cases of pulmonary tuberculosis in the Anand district of Gujarat. The findings highlight the limitations of conventional diagnostic methods in identifying *M. bovis* infections and demonstrate the efficacy of molecular techniques, explicitly targeting the *HupB* gene, for accurate detection and differentiation of *M. tuberculosis* and *M. bovis*. The evidence of coinfection in 6% patients further emphasizes the complexity of tuberculosis diagnosis in endemic areas.


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## Introduction

Tuberculosis, a global health crisis, continues to affect millions every year. A significant contributor to this burden is *Mycobacterium bovis*, the causative agent of bovine tuberculosis in humans and animals. This form of tuberculosis is especially prevalent in developing countries, where there is a high degree of interaction between humans and animals in rural areas, raising public health concerns and causing economic losses.[1]

In 2019, the World Health Organization estimated that *M. bovis* was responsible for approximately 143,000 new tuberculosis cases and 12,300 deaths.[1] However, the reporting and surveillance of *M. bovis*-related tuberculosis are not uniform across countries. This inconsistency is evident as only 16 countries provided specific data on this issue. The estimated number of cases varied widely, ranging from 71,000 to 240,000, accounting for about 1.4% of the 10 million global pulmonary tuberculosis cases.[2] A focused study in Mexico City from 2000 to 2014 showed that *M. bovis* represented 26% of isolates from all sites and 16% of pulmonary samples, indicating its significant regional impact.[3]

The transmission of bovine tuberculosis to humans typically occurs through inhalation of aerosols containing the bacteria, direct contact with infected animals, or consumption of contaminated products.[4,5] The severity of lung involvement in pulmonary tuberculosis varies, influenced by several factors, including the bacterial strain, the host's immune response, and the timeliness of diagnosis and treatment.[5,6]

Clinically, *M. bovis* cannot be easily distinguished from *M. tuberculosis* in patients, as they present similarly radiologically and pathologically. Most conventional microbiological techniques designed for diagnosing tuberculosis identify members of the Mycobacterium tuberculosis complex but do not differentiate between specific species, including *M. bovis*. The introduction of nucleic acid-based methods, particularly polymerase chain reaction (PCR), has greatly improved the ability to identify and differentiate mycobacterial species, a vital step in cases where *M. bovis* infection is suspected.[7–9]

In resource-limited settings, obtaining comprehensive national data on the frequency of human tuberculosis caused by *M. bovis* is challenging.[10] Consequently, the full extent of *M. bovis*-related tuberculosis in certain regions remains largely unknown. In Gujarat, for instance, the burden of *M. bovis* is not well-documented, often leading to misdiagnosis.[11] Therefore, our study focuses on determining the prevalence of *M. bovis* in human pulmonary tuberculosis cases. We selected a tertiary care teaching hospital as our study site, where we collected sputum samples from patients exhibiting tuberculosis symptoms. These samples were subjected to molecular techniques, primarily nucleic acid-based methods, to identify and differentiate the *Mycobacterium* species present.

By conducting this study, we aim to find the prevalence of *M. bovis* in pulmonary tuberculosis cases in this region of Gujarat.

The findings could provide valuable insights into the regional impact of this disease and aid in developing targeted strategies for diagnosis, treatment, and prevention.

## Methods

### Ethics Statement

This study was conducted with the approval of the Institutional Ethics Committee of Shree Krishna Hospital, Karamsad, Gujarat, under the

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HREC clearance number HMPCMCE/HREC/14/SESSION-2/12. Adherence to ethical standards was a priority; thus, written informed consent was obtained from all participants, ensuring their autonomy and confidentiality. The research team took great care to ensure that no personal information of the patients was disclosed during or after the study.

### Study Design and Participants

The research design was a prospective cross-sectional study carried out from November 2017 to June 2018. The participants included patients who exhibited clinical symptoms suggestive of pulmonary tuberculosis. These individuals sought medical attention at the Outpatient Department of Respiratory Medicine at Shree Krishna Hospital, Karamsad. However, patients diagnosed with extrapulmonary tuberculosis were excluded from the study. Comprehensive demographic data and other relevant information, such as occupation, animal-handling habits, and consumption of raw, unpasteurized milk, were meticulously gathered from each participant using a structured proforma.

### Sample Collection and Processing

In this study, 1,000 patients suspected of having pulmonary tuberculosis were enrolled. The collection of sputum samples involved obtaining preferably one early morning specimen and two spot specimens. These were collected in sterile, leak-proof containers, with patients receiving detailed instructions on the collection process. The collected specimens were then transported to the microbiology laboratory for further processing.

For the detection of acid-fast bacilli (AFB), the Ziehl–Neelsen staining method was utilized. Sputum smears, prepared from the purulent part of the sample, were stained and examined under an oil immersion lens of a light microscope. The presence and quantity of AFB were graded following the Revised National Tuberculosis Control Programme (RNTCP) guidelines.[12] The sputum samples underwent a decontamination process, were homogenized, and liquefied using the modified Petroff's concentration method to effectively release the bacilli trapped in the mucus. The sputum sample was stored at –20°C for molecular procedure.

### Identification of *Mycobacterium bovis* using Molecular Technique

DNA extraction from the concentrated sputum samples was performed using the QIAGEN DNA mini kit.

### Polymerase Chain Reaction Amplification

The study utilized specific primers targeting the *HupB* gene (coding for histone-like protein), as detailed in Table 1.[11,13] The primer sequences were validated using the primer-BLAST database of NCBI and synthesized by Eurofin Genomics India Pvt. Ltd. Each PCR mix, with a total volume of 25.0 µL, included 12.50 µL of Emerald master mix, 7.50 µL of PCR water, 1.00 µL each of reverse and forward primers, and 3.00 µL of the DNA template.

The PCR protocol for amplifying the *hupB* gene target included an initial denaturation at 95°C for 10 minutes, followed by 35 cycles comprising denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 1 minute, with a final extension phase at 72°C for 7 minutes. Postamplification, 10 µL of the amplicon was mixed with 3 µL of DNA loading dye and loading buffer for electrophoresis on a 2% agarose gel prepared with ethidium bromide. The results were visualized using a Gel Doc system.

The data was collected in a predesigned proforma and entered into an Excel sheet. The Statistical Package of Social Sciences (SPSS), IBM version 22, was used for the statistical analysis of data. Categorical measurements were summarized as the number and percentage, and descriptive statistics were used for numerical variables wherever applicable.

## Results

In this study involving 1,000 enrolled patients, we identified 100 individuals who tested positive for AFB, as detailed in Table 2. These positive cases exhibited varying AFB presence according to the RNTCP guidelines. Notably, most of these cases (41 out of 100) demonstrated a 1 + grade level of AFB in their sputum samples. On the lower end of the spectrum, 10 patients showed only scanty amounts of AFB.

Demographically, as illustrated in Table 3, the predominant age group affected was between 26 and 40 years, accounting for 29% of the cases. Individuals above the age of 70 closely followed this. The mean age across the patient cohort was 17.18 years, with a standard deviation of ±9.27 years, encompassing a wide age range of 10–85 years. Regarding gender distribution, the study observed a higher prevalence in males, with 73 male patients compared to 27 females.

Further insights into the clinical profile and risk factors of these patients are presented in Table 4. A common characteristic among the participants was the duration of respiratory symptoms, which, in all cases, had persisted for over a month, indicative of active tuberculosis. The aspect of this study was the high percentage of patients engaged in certain occupations or activities that could potentially increase their risk of TB exposure. A significant 61% of the patients were involved in animal handling, while 40% had a history of contact with known TB cases. Additionally, lifestyle factors such as dietary habits were also notable; 45% of the patients reported consuming raw milk, and 30% were employed in farming.

This demographic and clinical data provide valuable insights into the patterns and risk factors associated with tuberculosis in this patient population, offering crucial information for targeted public health interventions and further research.

### Detection and Identification of *Mycobacterium tuberculosis* and *Mycobacterium bovis* in Sputum Samples by PCR

In this study, DNA was extracted from the sputum samples of 100 patients and subsequently subjected to PCR analysis. This technique was employed to ascertain the specific species of mycobacteria in each sample. The determination of the

**Table 2:** RNTCP grade in AFB-positive sputum samples ($n$ = 100)

| *RNTCP grade (AFB)* | *No. of sputum samples* |
|---|---|
| Scanty | 10 |
| 1+ | 41 |
| 2+ | 29 |
| 3+ | 20 |
| Total | 100 |

**Table 1:** Characteristics of the primer used for detection of *M. bovis*

| *Target genes* | *Primers* | *Primer sequence* | *Product size (bp)* | *Reference* |
|---|---|---|---|---|
| *HupB* | *HupB* (F)<br>*HupB* (R) | 5′GCAGCCAAGAAGGTAGCGAA-3′<br>5′GTATCCGTGTGTCTTGACCTATTTG-3′ | 336<br>*M. tuberculosis* and 309 *M. bovis* | Prabhakar et al.[13] |

**Table 3:** Age and sex distribution of participants ($n = 100$)

| *Age (in year)* | *Male* | *Female* | *No. of participants (%)* | *Mean age (years)* | *Std dev ± 2* |
|---|---|---|---|---|---|
| 10–25 | 15 | 08 | 23 (23) | 17.18 | 9.27 |
| 26–40 | 23 | 06 | 29 (29) | | |
| 41–55 | 17 | 08 | 25 (25) | | |
| 56–70 | 15 | 03 | 18 (18) | | |
| 70 and above | 03 | 02 | 5 (5) | | |
| Total | 73 | 27 | 100 (100) | | |

**Table 4:** Clinical profile and risk factors in pulmonary tuberculosis patients ($N = 100$)

| *Characteristics* | *Mycobacterium tuberculosis (n = 90)* | *Mycobacterium bovis (n = 4)* | *Mixed (n = 6) (Mycobacterium tuberculosis and Mycobacterium bovis)* | *N = 100* |
|---|---|---|---|---|
| Fever | 82 (91%) | 3 (75%) | 2 (33%) | 87 |
| Cough | 87 (97%) | 3 (75%) | 6 (100%) | 96 |
| Weight loss | 66 (73%) | 2 (50%) | 5 (83%) | 73 |
| Hemoptysis | 23 (26%) | 2 (50%) | 2 (83%) | 30 |
| HIV | 2 (2.2%) | 0 | 0 | 2 |
| Previous contact with a TB case | 34 (38%) | 4 (100%) | 4 (67%) | 40 |
| Raw milk consumption | 51 (57%) | 4 (100%) | 6 (100%) | 45 |
| Animal handler | 36 (40%) | 4 (100%) | 5 (83%) | 61 |
| Farmer | 24 (27%) | 2 (50%) | 4 (67%) | 30 |

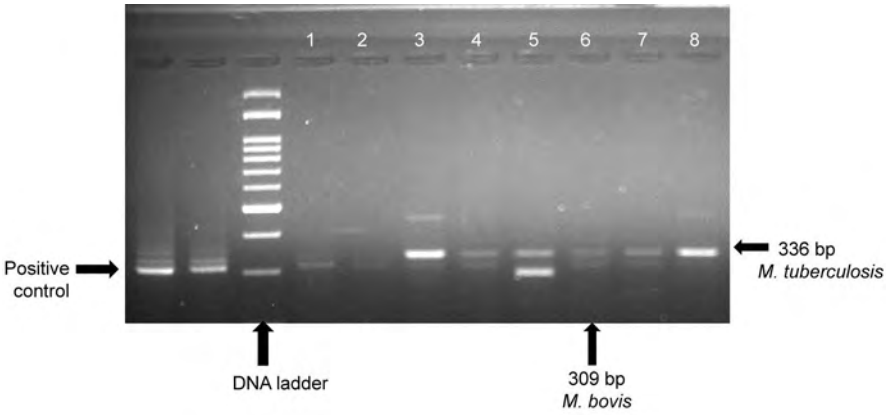


**Fig. 1:** PCR result for the identification of *Mycobacterium tuberculosis* and *Mycobacterium bovis*.

mycobacterial species was achieved by analyzing the molecular size of the PCR products. These products were then subjected to electrophoresis on a 2% agarose gel alongside appropriate controls to ensure the accuracy and reliability of the results.

The findings of the PCR assays are detailed in Figure 1. Most of the samples, accounting for 90%, were identified as containing *M. tuberculosis*, the primary causative agent of tuberculosis in humans. In addition, *M. bovis*, a species more commonly associated with tuberculosis in cattle but capable of infecting humans, was detected in 4% of the samples. Interestingly, a small percentage of the samples, amounting to 6%, contained both *M. tuberculosis* and *M. bovis*.

These results highlight the prevalence of *M. tuberculosis* in this patient cohort while also pointing to the presence of *M. bovis* in a notable fraction of cases. Detecting both species in some samples underscores the complexity of tuberculosis infections and the necessity for accurate species-level identification for effective diagnosis and treatment.

## Discussion

The earliest recorded case of bovine pulmonary tuberculosis in a human, identified through bacteriological testing, was reported in 1909. This landmark discovery opened avenues for extensive research over the subsequent decades, revealing that *M. bovis* was the causative agent in 1–3% of human cases of pulmonary tuberculosis.[14] The current study aimed to assess the prevalence of pulmonary tuberculosis due to *M. bovis* in a tertiary care teaching hospital in Gujarat, India, a region where tuberculosis remains a significant public health challenge.

In this study, we collected 1,000 sputum samples from patients suspected of having pulmonary tuberculosis and conducted tests for AFB. Of these, 100 samples were found to be positive for pulmonary tuberculosis. This 10% positivity rate is in line with the findings from Peshawar, Pakistan,[8] where a similar study reported that 100% of the samples tested were positive for pulmonary tuberculosis, underscoring the persistent prevalence of the disease in South Asia.

Of the 100 positive cases in our study, 73 (73%) were male. This gender disparity echoes the findings of Tchatchouang et al.,[15] who observed a higher infection rate in men (53.16%) than women (46.84%). Similarly, Bapat et al.[9]noted that the disease primarily affects men aged between 20 and 45 years. These observations suggest that occupational factors, possibly linked to increased exposure risks in certain professions predominantly held by men, may play a role in disease distribution.

To enhance the specificity of our diagnostic approach, we employed PCR, a technique known for its rapidity and sensitivity, particularly compared with traditional methods such as Ziehl–Neelsen staining and culture.[8] For this purpose, purified genomic DNA from the sputum samples was subjected to PCR using sequence-specific primers targeting the *HupB* gene. The resulting PCR products were 309 bp (corresponding to *M. bovis*) and 336 bp (corresponding to *M. tuberculosis*), facilitating

the differentiation between these closely related mycobacterial species.

*Mycobacterium tuberculosis*'s histone-like protein gene (*HupB* [*Rv2986c*]) has been recognized as a critical marker for distinguishing *M. tuberculosis* and *M. bovis* within the MTB complex via a PCR assay. In our analysis of the 100 samples, 90 (90%) were positive for *M. tuberculosis*, 4 (4%) for *M. bovis*, and 6 (6%) indicated coinfection with both species. These findings are consistent with those reported by Prasad,[10] highlighting the presence of both *Mycobacterium* species in pulmonary cases.

The study by Bapat et al.[9] obtained 32 culture isolates from 347 samples using traditional culture techniques. While effective in growing mycobacteria, these methods lacked the specificity required for species-level identification. To overcome this, PCR assays were applied to the same samples, revealing 9 cases (2.59%) of *M. bovis* and 60 cases (17.29%) of *M. tuberculosis*. These results demonstrate the enhanced accuracy of PCR in identifying members of the *Mycobacterium tuberculosis* complex (MTC).

This pattern was further evidenced in the study by Nawaz,[16] where 100 sputum samples examined using PCR identified 37 (37%) as positive for *M. tuberculosis* and 5 (5%) for *M. bovis*. The present study's findings align with these results, confirming the coexistence of both *M. tuberculosis* and *M. bovis* in the sputum samples identified through the advanced molecular technique of PCR.

In our research, 6 (6%) patients presented with coinfections by *M. tuberculosis* and *M. bovis*, a scenario similar to the findings of Silva,[17] who reported a 1.6% rate of *M. bovis–M. tuberculosis* coinfections. The application of the *HupB* gene as a PCR target was instrumental in these identifications. The study also analyzed 100 mycobacterial strains, finding the *HupB* gene-specific primers suitable for distinguishing MTB complex members. This technique aligns with the discoveries made by Prabhakar et al.,[13] who observed variations in *HupB* gene product sizes between *M. tuberculosis* (645 bp) and *M. bovis* (618 bp).

Our study revealed that the *HupB* gene was found in 6% of mixed infections by both species, 4% of *M. bovis*, and 90% of *M. tuberculosis* cases. Multiple copies of the target gene in the mycobacteria genome likely account for the high PCR activity observed. However, integrating additional markers such as IS6110, oxyR, and rpoB in future studies could provide further confirmation and insight into the genomic complexity of these pathogens.[15]

It is noteworthy that reported incidence rates of zoonotic TB in developed regions such as Europe, the United States, Australia, and New Zealand have consistently remained below 1 per 100,000 population per year.[18,19] In contrast, the incidence rates in several other regions, including parts of Asia and Africa, are not as well documented. In the Indian context, studies like those conducted by Shah et al.[20] and Prasad et al.[10] have provided crucial insights into the occurrence of *M. bovis* in humans and cattle. These studies and the present research underscore the importance of comprehensive screening and accurate differentiation of *M. tuberculosis* complex members in humans and livestock, as Mittal et al. emphasized.[21]

In summary, this study successfully demonstrates the presence of pulmonary infections caused by *M. bovis* and *M. tuberculosis* in a tertiary care hospital in Anand, Gujarat. The results, confirmed via advanced molecular techniques, highlight the utility and necessity of PCR in differentiating *M. tuberculosis* and *M. bovis*. This distinction is crucial for appropriate disease management and the formulation of targeted public health strategies. Our findings contribute to the growing body of evidence on the prevalence and characterization of tuberculosis-causing mycobacteria, reinforcing the need for continued research and surveillance in this critical area of public health.

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ORIGINAL ARTICLE

# Prevalence of Vitamin D Deficiency in Pulmonary Tuberculosis: A Prospective Cross-sectional Study

Vijaya Sekhar

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## Abstract

**Objective:** The current cross-sectional study examined the extent of vitamin D (Vit-D) deficiency among pulmonary tuberculosis (TB)–affected patients and explored the potential associations of demographic factors with Vit-D status.

**Methodology:** Conducted from 1st August 2014, to 1st February 2016, at a tertiary care center, the study included patients aged 18–60 years. Ethical approval was obtained, and exclusion criteria such as category II or multidrug-resistant TB, secondary immunodeficiency states, and extrapulmonary TB were applied. Clinical and laboratory data, including Vit-D levels, were collected. Statistical studies employed ANOVA, Chi-squared tests, and one-sample *t*-tests.

**Results:** Among the 72 patients with TB, the majority were aged 50 years and above, with male preponderance (62%). Fifty-two (75%) TB patients had Vit-D deficiency, with an average Vit-D level of 16.68 ng/mL. The prevalence of Vit-D deficiency was significantly higher in women compared to men (92.6 vs 64.4%; $p = 0.026$). All patients with bilateral lung lesions had Vit-D deficiency compared to 59.3% in unilateral lung lesion patients ($p = 0.002$). Sputum microscopy and culture contributed to 65.28% of TB diagnoses. Vit-D deficiency prevalence was 75%, with an average Vit-D level of 16.68 ng/mL.

**Conclusion:** The study highlights gender- and lesion-associated vulnerabilities to Vit-D deficiency among pulmonary tuberculosis patients. Despite limitations, the findings suggest the need for Vit-D screening in TB care and further clinical trials to explore the role of Vit-D levels in management.


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## Introduction

Tuberculosis (TB) continues to pose a significant global threat, surpassing human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) as the primary cause of death from infectious diseases.[1,2] With over 25% of the global population affected,[3] the impact of TB is particularly pronounced in Southeast Asia, the Western Pacific, and Africa.[4] Major contributors to the TB epidemic include poverty, drug resistance, and HIV, predominantly affecting developing countries (95% of cases).[5] While global TB incidence has gradually declined since 2003, with an approximate 2% annual deceleration, significant challenges persist.[6]

Despite the World Health Organization (WHO) setting ambitious targets to end the TB epidemic by 2030,[1] the challenges in achieving these targets have been exacerbated by the COVID-19 pandemic, the HIV epidemic, and the emergence of extensively drug-resistant (XDR) and multidrug-resistant (MDR) TB strains. This increases infection rates and also contributes to increased morbidity and mortality among TB patients.

The rate of recovery for TB patients affected by drug-susceptible strains can exceed 90% with proper infrastructure for diagnosis and treatment. MDR-TB cure rates are notably lower, reaching, at best, 57%, according to the latest WHO estimates.[7] Factors influencing TB incidence and treatment outcomes include socioeconomic conditions, healthcare access, adherence to medication, and the emergence of drug-resistant strains.[7] Examining supplementary treatment approaches becomes essential to improve clinical outcomes for tuberculosis cases that are drug-resistant as well as drug-susceptible.

Vitamin D (Vit-D) levels are among the major factors known to influence the incidence and progression of TB.[8–10] In addition to its important role in bone metabolism, Vit-D also plays a crucial role in preventing infections.[11] As early as the 1930s, Vit-D obtained from cod liver oil was used in the treatment of TB. However, the advent of anti-infective chemotherapy in the 1950s supplanted the use of Vit-D in TB.[12] Recent epidemiological research has indicated associations between reduced Vit-D levels and an increased risk of infections, including septic shock,[13] influenza,[14,15] and respiratory infections.[14] Numerous studies highlight that the binding of the biologically active form of Vit-D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), to the Vit-D receptor (VDR) activates signaling that induces antimicrobial responses. This stimulates autophagy, activates antimicrobial peptides, and intracellular killing of TB bacteria[16,17] (Fig. 1).

Vit-D has a vital role in the maintenance of calcium homeostasis and bone metabolism. During prolonged or severe shortage due to reduced intestinal absorption of calcium and phosphorus, there is increased susceptibility to hypocalcemia and secondary hyperparathyroidism. The secondary hyperparathyroidism-induced phosphaturia further aggravates bone demineralization. The cascading effects of this process may lead to conditions such as osteomalacia and osteoporosis in adults and osteomalacia and rickets in children.[18]

While previous studies have emphasized the significance of Vit-D in preventing various infections, its specific influence on pulmonary TB remains less explored. In this context, the aim of the current cross-sectional study is to explore the occurrence of Vit-D deficiency among patients diagnosed with pulmonary tuberculosis and investigate its potential role in the context of TB.

## Methodology

### Study Duration

This cross-sectional study was conducted from 1st August 2014, to 1st February 2016, at a tertiary care center.

### Study Population

The study included patients who were seen in the outpatient and inpatient departments of the department of general medicine and respiratory medicine.

### Inclusion Criteria

Patients of either sex with pulmonary TB aged between 18 and 60 years.

### Exclusion Criteria

Patients excluded from this cross-sectional study were those diagnosed with category II or multidrug-resistant TB (MDR-TB). Furthermore,

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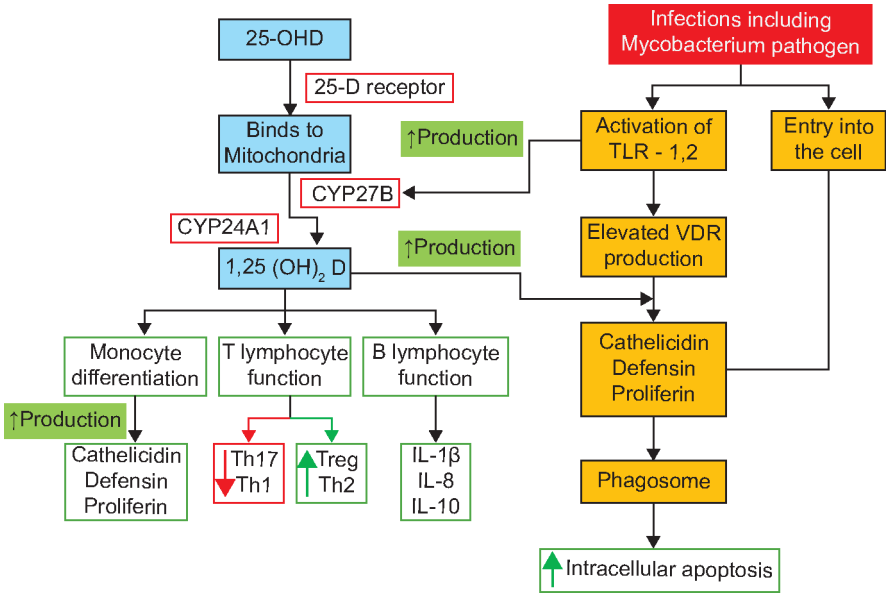


**Fig. 1:** 25-OHD, 25-hydroxyvitamin D; 1,25-dihydroxyvitamin D; TLR, toll-like receptors

**Table 1:** Distribution of age and gender in the study population

| *Age-group/gender* | *Number (n)/percentage* |
|---|---|
| <30 | 9 |
| 31–40 | 13 |
| 41–50 | 10 |
| >51 | 40 |
| Total | 72 |
| Male | 62% |
| Female | 38% |

**Table 2:** Relation between age, gender, and Vit-D levels

| *Age-group* | *Total* | *Deficient* | *Insufficient* | *Sufficient* | *p-value* |
|---|---|---|---|---|---|
| ≤30 | 9 | 5 | 4 | 0 | 0.473 |
| 31–40 | 13 | 9 | 3 | 1 | |
| 41–50 | 10 | 9 | 1 | 0 | |
| >51 | 40 | 31 | 6 | 3 | |
| *Gender* | *Total* | *Deficient* | *Insufficient* | *Sufficient* | *p-value* |
| Male | 45 | 29 | 12 | 4 | 0.026 |
| Female | 27 | 25 | 2 | 0 | |

individuals with secondary immunodeficiency conditions such as HIV, organ transplantation, malignancy, corticosteroid treatment, hepatitis B or C positivity, extrapulmonary TB, or those requiring surgical intervention were not included. Exclusion criteria also encompassed patients currently or recently (within the last 3 months) undergoing cytotoxic therapy, pregnant or lactating individuals, those with a known seizure disorder, symptomatic cardiac disease, abnormal renal function, hepatic dysfunction, hematological abnormalities, serious illness, or those unable to comply with the treatment regimen. Furthermore, individuals with a history of alcohol or drug abuse were excluded.

## Sample Size Determination

Based on a study by Talat et al.,[19] the sample size was determined to be 72 using the formula $n > z^2\ pq\ /\ d^2$, with a confidence coefficient ($z$) of 1.96, error of estimate ($d$) of 10%, and an incidence rate ($p$) of 76%.

## Ethical Approval and Informed Consent

The Institutional Ethics Committee approved the trial, and the patients' next of kin gave their informed permission.

## Data Collection

Simple random sampling was used to recruit patients. Clinical details were recorded in a well-designed proforma, including sociodemographic data, medical and drug history, and laboratory variables. Criteria such as the presence of acid-fast bacilli in sputum smears, culture positivity for Mycobacterium tuberculosis, TB PCR positivity, or evidence of persistent caseating granulomatous inflammation were used to confirm the diagnosis of tuberculosis.

## Laboratory Measurements

Blood samples were collected for a range of assessments, including complete blood count, serum 25(OH)D, albumin, and calcium levels. Additionally, renal function tests, including serum creatinine and urea, and liver function parameters, including ALP and ALT, were estimated. The Elecsys Vit-$D_3$ assay was utilized to determine serum 25(OH)D concentrations.

## Vitamin D Status Definition

Vitamin D status was categorized based on the Endocrine Society clinical practice recommendations.[20] Baseline serum 25(OH)D values of ≥30 ng/mL, 21–29 ng/mL, and ≤20 ng/mL were used to define normal, insufficiency, and deficiency of Vit-D, respectively.

## Statistical Analysis

Continuous and categorical measurements were subjected to descriptive statistics, while significance testing utilized analysis of variance (ANOVA) and the Chi-squared test. One-sample *t*-test was used for analyzing Vit-D deficiency. Confidence intervals and significance levels were determined, and statistical software such as IBM SPSS version 20 and Microsoft Word and Excel were utilized for data analysis and presentation.

# Results

The cross-sectional study included 72 pulmonary tuberculosis patients selected from the department of pulmonary and general medicine. The study population had a majority of individuals belonging to the >51 age-group, with a distribution of 62% males and 38% females (Table 1).

The incidence of Vit-D deficiency was notably higher in the >51 age-group ($p = 0.473$), and a substantial association was found between gender and Vit-D deficiency, with females exhibiting a higher prevalence ($p = 0.026$) (Table 2).

The study also identified a noteworthy association between the site of lung lesion, area of residence, and Vit-D level (Table 3). Vit-D deficiency showed a clear association with unilateral or bilateral lung lesions ($p = 0.002$), but there was no statistically significant

**Table 3:** Association between site of lung lesion, area of residence, and Vit-D level

| *Parameter* | *Vit-D level* | | | *p-value* |
|---|---|---|---|---|
| | *Deficient* | *Insufficient* | *Sufficient* | |
| UL | 32 (59.3%) | 14 (100%) | 4 (100%) | 0.002 |
| BIL | 22 (40.7%) | 0 (0%) | 0 (0%) | |
| *N* = 22 | 22 (100%) | 0 (0%) | 0 (0%) | |
| Rural | 29 (53.7%) | 7 (50%) | 3 (75%) | 0.829 |
| Urban | 25 (46.3%) | 7 (50%) | 1 (25%) | |

**Table 4:** Prevalence of Vit-D deficiency in study population

| *Vit-D status* | *Frequency* | *Percentage* |
|---|---|---|
| Deficient | 54 | 75% |
| Insufficient | 14 | 19.44% |
| Sufficient | 4 | 5.56% |

correlation between Vit-D deficiency and the place of residence ($p$= 0.829).

Regarding the diagnostic method, sputum microscopy and culture contributed to 65.28% of TB diagnoses, while BACTEC culture played a role in 34.72%. The average Vit-D level observed in the study population was 16.68 ng/mL. The incidence of Vit-D deficiency in the study population was 75%. Additionally, 19.4% had insufficient Vit-D, with only 5.6% having normal levels of Vit-D (Table 4).

## Discussion

This study aims to investigate the prevalence of Vit-D deficiency among pulmonary tuberculosis (TB) patients, assessing various demographic factors and their potential correlations with Vit-D status. This study, conducted from 1st August 2014, to 1st February 2016, involved 72 randomly selected patients.

Our findings revealed a notable age skew, with a majority of patients falling into the >51 age-group. This observation aligns with the known demographic vulnerability of older individuals to TB. Moreover, the study highlighted a significant gender disparity, with females exhibiting an elevated incidence of Vit-D deficiency in comparison to males ($p$ = 0.026). The prevalence analysis revealed a substantial burden of Vit-D deficiency among the study population, with 75% of participants exhibiting deficiency, 19.44% having insufficient levels, and only 5.56% being sufficient ($p$ < 0.001).

The examination of Vit-D levels and age-groups showed an interesting pattern, with those in the >51 cohort showing the highest frequency of insufficiency, although statistical significance was not reached ($p$ = 0.473). This observation aligns with existing literature that suggests a potential age-related susceptibility to Vit-D deficiency.[21] The study by Giustina et al. recommends combining Vit-D with calcium to reduce fractures, establishing a goal of 25(OH)D > 50 nmol/L. It favors daily low-dose regimens for fall prevention in the elderly and emphasizes Vit-D supplementation effectiveness over alternative strategies for achieving sufficiency in the aging population.[21]

Vit-D deficiency was observed to be higher among women in the current study. About 93% of women had Vit-D deficiency compared to 64.45% of men ($p$ = 0.026). A study revealed a strong correlation between Vit-D levels and gender, particularly highlighting a substantial prevalence (71.2%) in women aged 19–39 years.[22] This discrepancy can be attributed to reduced sunlight exposure due to veiling, insufficient intake of dietary Vit-D, residing in urban areas, and parity in women, as reported in different studies. Factors associated with hypovitaminosis D include covering arms from sunlight, inadequate Vit-D supplementation in postmenopausal women, elevated BMI levels, and low education levels. In contrast, season, sun exposure, and dietary Vit-D were not identified as significant predictors.[23]

A notable discovery in this study arose regarding the association between the site of lung lesions and Vit-D deficiency. Patients with unilateral or bilateral lung lesions exhibited a significantly higher prevalence of deficiency ($p$ = 0.002). In a prospective study, it was found that Vit-D deficit was linked to an augmented risk of total and respiratory mortality, highlighting its potential role in influencing health outcomes in older men with varying lung function statuses over a 20-year period. The underlying mechanisms linking lung lesions and Vit-D deficiency were not explored in this study and warrant further investigation.[24]

The results of this investigation did not show a statistically significant correlation between Vit-D insufficiency and residential area (rural vs urban). Rural regions had a higher prevalence of Vit-D insufficiency (53.7%) than urban areas (46.3%), although the difference was not statistically significant ($p$ = 0.829). Marzban et al. also observed a high prevalence of Vit-D deficiency in their study conducted among rural populations in the province of Bushehr.[20]

The mean Vit-D level observed in the population was 16.68 ng/mL, shedding light on the overall Vit-D status among tuberculosis patients. To facilitate further comparison and contextualize the findings, the widely recognized categorization of Vit-D status as defined by the Endocrine Society guidelines was adopted. Normal Vit-D levels are considered sufficient if 25(OH)D ≥30 ng/mL, insufficiency as 20–29 ng/mL, and deficiency as <20 ng/mL.[25] This average provides a quantitative measure that can serve as a reference for healthcare providers in assessing and managing Vit-D deficiency in this patient group.

This research echoes existing evidence on the beneficial effect of Vit-D supplementation in the treatment of pulmonary tuberculosis. The study population's significant incidence of Vit-D insufficiency is consistent with other findings, highlighting the need to address Vit-D status in tuberculosis therapy. The positive clinical outcomes observed in TB-infected children in a study[13] further support the potential of Vit-D as an adjunctive therapeutic strategy. The results highlight the necessity for additional clinical studies to ascertain the optimal role and dose of Vit-D in tuberculosis treatment. In the context of providing holistic care to TB patients, routine testing and adjustment of Vit-D levels may be considered, which might improve treatment response and overall outcomes.

### Limitations

One of the study's drawbacks is that it included only 72 individuals with pulmonary TB, which may have limited the generalizability of the results. Furthermore, creating a CONSORT flow diagram was not possible because of the absence of information on the patients who were omitted.

## Conclusion

In conclusion, the study emphasizes the imperative for further clinical trials aimed at delineating the optimal role and dosage of

Vit-D in tuberculosis therapy. Considered as an essential part of the comprehensive care given to patients with TB, frequent testing and adjustment of Vit-D levels may improve treatment responses and general health.

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ORIGINAL ARTICLE

# A Study of Role of Bronchoscopy in Intensive Care Units

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## Abstract

**Objectives:** (1) To know the contributions of bronchoscopy in intensive care units (ICUs) in terms of therapeutic benefits and diagnostic purposes. (2) To know the safety of the bronchoscopy procedure in ICUs in critically ill patients.

**Materials and methods:** This is a retrospective observational study that included 41 patients who underwent bronchoscopy in the ICU of a tertiary care center. Data collected included the patient's clinical profile, vitals, cause of ICU admission, indication for bronchoscopy, and complications.

**Results:** There were 41 ICU patients who required and underwent bronchoscopy. A number of 15 patients (36.5%) were on mechanical ventilation, and 10 patients (24.3%) were on noninvasive ventilation (NIV) support. The most common indication was lung collapse in 23 (56%) patients. Out of 41 patients who underwent the procedure, 28 patients (68.2%) showed postprocedure improvement, which shows the utility of the procedure. Minor complications occurred in 18 patients (43%) and included hypoxia, bleeding, and bronchospasm. Zero mortality was reported during or after the procedure.

**Conclusion:** Bronchoscopy provides excellent diagnostic yield and therapeutic benefits in ICU patients with respiratory conditions, and it is relatively safe even in high-risk patients when done by trained consultants.


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## Introduction

Bronchoscopy is a procedure to look inside the airway with the help of a camera located at the end of the scope for various diagnostic and therapeutic purposes. The advantage of maneuverability, feasibility of a wide spectrum of diagnosis and therapeutics, patient comfort, and ease of conscious sedation has made bronchoscopy an important tool in the pulmonary medicine field. Its use is increasing in critical care due to its extended diagnostic and therapeutic benefits.

Since the introduction of bronchoscopy in the 20th century, bronchoscopy has played an important role in the pulmonary medicine field. Apart from various diagnostic uses, it is now also used for therapeutic purposes in different clinical scenarios. Bronchoscopy is done in stable patients as well as in critically ill patients, as its portability and flexibility in use allow various interventions at the bedside.[1]

Patients in intensive care units (ICUs) often have various comorbidities and high-risk clinical conditions, which make them high risk for bronchoscopy; therefore, the risk of the procedure must be weighed against the need and benefit from it.[2] In spite of the high risk of the procedure in critically ill patients, bronchoscopy is relatively safe in trained and experienced hands.[3,4]

This study highlights the importance of bronchoscopy in critical care settings and focuses on indications for its use and complications associated with it.

Bronchoscopy indications in the intensive care setup are mentioned below[5,6]:

Diagnostic indications:
- Pneumonia.
- Hemoptysis.
- Tracheal stenosis.
- Inhalation injury/burns.
- Upper airway and vocal cord assessment.

Therapeutic indications:
- Mucus plugging/lung collapse.
- Foreign body removal.
- Difficult endotracheal intubation.
- Intrabronchial instillation of antibiotics/medications.
- Placement of amplatzer device in bleeding segment.
- Bronchopleural fistula closure.
- Debulking of endobronchial tumor.
- Tracheal or bronchial stenting.

### Objectives of Study

- To know the contributions of bronchoscopy in ICUs in terms of therapeutic benefits and diagnostic purposes.
- To know the safety of bronchoscopy in ICUs in critically ill patients.

## Materials and Methods

Patients in ICU with various respiratory conditions required bronchoscopy for diagnostic and therapeutic purposes.

Calls were received from the ICU for the management of lung collapse, pulmonary infiltrates for diagnosis of offending organisms, retained secretions, suspected foreign body aspiration, and difficult intubation.

Patients admitted to the ICU who gave consent and underwent bronchoscopy were included in my study.

Data of the patients were collected from inpatient files, from hospital information system databases, and from the relatives. Data consisted of age, gender, clinical features, cause of ICU admission, comorbidity, significant past history, mode of ventilation, laboratory investigations, and radiological investigations.

Bronchoscopy was done in 41 patients after analyzing the general condition of the patients and obtaining informed written consent.

### Procedure

Bronchoscopy was done according to British Thoracic Society (BTS) guidelines.

Procedure performed in the presence of an anesthesia team under continuous cardiac monitoring.

Procedure done *via* the nasal route in conscious and cooperative patients who were on room air or oxygen support by mask or bilevel positive airway pressure (BiPAP). In intubated patients, the procedure was performed through the endotracheal tube (ET).

A T-tube adapter was used in patients on ventilator support as it allowed bronchoscopy without discontinuation of the ventilator.

Bronchoalveolar lavage (BAL) was taken in all patients, and transbronchial lung biopsy (TBLB) was also taken in selected patients.

One patient had difficulty in intubation due to reduced mouth opening; intubation was done successfully using a flexible bronchoscope.

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**Table 1:** Mode of ventilation in study subjects

| *Mode of ventilation* | *Numbers (n = 41)* | *Percentage (%)* |
|---|---|---|
| Mechanical ventilation | 15 | 36.5 |
| NIV support | 10 | 24.3 |
| Oxygen *via* face mask/nasal prongs | 16 | 39 |

**Table 2:** Indication of bronchoscopy in study subjects

| *Indication* | *Numbers (n = 41)* | *Percentage (%)* |
|---|---|---|
| Lung collapse | 23 | 56 |
| Consolidation | 13 | 31.7 |
| Foreign body | 1 | 2.4 |
| Lung mass/lesion | 2 | 4.8 |
| Difficult intubation | 1 | 2.4 |
| Intrabronchial instillation of antibiotics | 1 | 2.4 |

**Table 3:** Postprocedure clinical–radiological improvement

| *Air entry and CXR improved* | *Numbers (n = 41)* | *Percentage (%)* |
|---|---|---|
| Improved | 28 | 68.2 |
| No improvement | 13 | 31.7 |

**Table 4:** Organisms isolated in BAL

| *Organism isolated* | *Numbers (n)* | *Percentage (%)* |
|---|---|---|
| *M. tuberculosis* | 3 | 7.5 |
| *A. baumannii* | 11 | 27.5 |
| *Streptococcus pneumoniae* | 1 | 2.5 |
| *Klebsiella pneumoniae* | 2 | 5 |
| *E. coli* | 1 | 2.5 |
| *P. aeruginosa* | 4 | 10 |
| *Candida* | 1 | 2.5 |
| No organism isolated | 17 | 42.5 |

### Study Design

Retrospective observational study.

### Study Place

Tertiary care center in Ahmedabad.

### Study Period

One year (February 2023 to February 2024).

## Results

Total 41 patients in ICU underwent bronchoscopy for various respiratory conditions. All bronchoscopies were performed by the Respiratory Medicine Department, and all procedures were supervised, and sedation was given by the anesthesia team.

In my present study, the maximum age of the subjects was 84 years and the minimum age was 15 years. Out of 41 patients, 21 (51.3%) were males and 20 (48.7%) were females.

Table 1 shows that 15 (36.5%) patients were on mechanical ventilation, in whom the procedure was done *via* ET or tracheostomy tube, 10 (24.3%) patients were on noninvasive ventilation (NIV) support, and 16 (39%) patients were on oxygen support *via* face mask or nasal prongs.

Table 2 shows that the most common indication for which bronchoscopy was performed was lung collapse. About 23 patients out of 41 (56%) had lung collapse. A total of 13 patients (31.7%) had consolidation. One patient who underwent bronchoscopy for suspected collapse or consolidation had a foreign body, which was removed successfully. One patient was found to have an endobronchial growth in the left lower lobe bronchus; an endobronchial biopsy was taken, which turned out to be poorly differentiated nonsmall cell carcinoma. One patient had a lesion in the upper trachea from which a biopsy was taken, which on histopathological examination (HPE) turned out to be mucormycosis. One patient was found to have extensive endobronchial infection, for which intrabronchial instillation of antibiotics was done. One patient was referred for difficult intubation due to reduced mouth opening, in which intubation was successfully done by flexible bronchoscope.

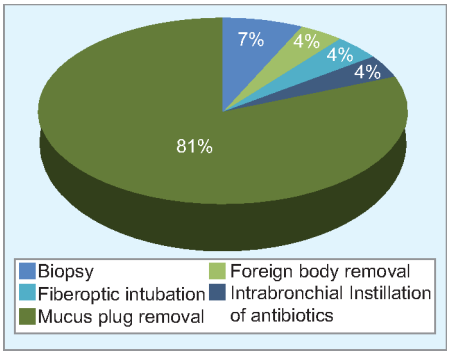


**Fig. 1:** Bronchoscopy procedures done in study subjects

Table 3 shows that postprocedure clinical and radiological improvement in terms of air entry and chest X-ray findings was observed in 28 (68.2%) out of 41 patients in whom bronchoscopy was done for various above listed conditions.

As shown in Table 4, BAL was done in 40 patients, out of whom 23 patients (57.5%) showed growth in the culture. *Acinetobacter baumannii* was the most common organism isolated in BAL culture, in 11 (27.5%) patients. Three patients (7.5%) showed growth of *Mycobacterium tuberculosis*. Four (10%) patients showed growth of *Pseudomonas aeruginosa*. One patient showed mixed growth of *Escherichia coli* and *Candida*.

As demonstrated in Fig. 1, among bronchoscopy procedures, BAL was taken in 40 patients, as BAL helps in collecting samples for microbiological diagnosis. Mucus plug removal done in 21 patients offered therapeutic benefit to the patients. Biopsy was taken from a growth in two patients, foreign body removal in one patient, intrabronchial instillation of antibiotics in one patient, and fiberoptic intubation was done in one patient.

### Complications

Complications associated with the procedure occurred in a total of 18 (43.9%) patients out of 41, including hypoxia ($SpO_2$ <90% during or after the procedure), bleeding, and bronchospasm. Ten patients out of 41 (24.3%) developed hypoxia, which was resolved upon giving high-flow oxygen for a few minutes. Bronchospasm was observed in five patients (12.1%), which was resolved upon nebulization with bronchodilators. Minor bleeding was observed in three (7.3%) patients. No death occurred during or immediately after the procedure.

## Discussion

Bronchoscopy is a widely used procedure in various respiratory conditions. It has become an important tool in ICUs for its therapeutic

and diagnostic benefits. Benefits and safety of the procedure are two key features that have led to its increasing use in ICUs. Patients in ICUs suffer from different types of critical care illness and are likely to develop various respiratory complications like lung collapse and severe pneumonia.[7]

In my present study, lung collapse (56%) is the most common indication for bronchoscopy, where mucus plug removal resulted in significant therapeutic benefit. In a study conducted over 3 years by Krishna et al.,[8] the most common indication was consolidation, which comprised 67.9% of the total patients who underwent bronchoscopy in ICUs.

In a study by Turner et al.,[9] a total of 147 bronchoscopies were performed; 37 were for therapeutic purposes, of which 28 were done for lung collapse. After the procedure, full lung expansion was observed in 20 patients (71%), which is comparable to my study, in which 16 patients out of 23 patients with lung collapse (69.5%) showed significant lung expansion.

Overall efficacy of bronchoscopy in ICU in my current study is 68.2%, which is comparable with the study by Estella (71%)[10] and Patel and Udwadia (75%).[11]

In my study, 23 out of 40 patients (57.5%) had shown growth in their BAL culture, which is comparable with a study by Woske et al.,[12] which yielded a 56% culture positivity rate.

Bronchoscopy is a relatively safe procedure if it is done with basic precautions even in critical patients. The major complication risk is 0.08–2%, and the mortality rate is extremely low (0.01–0.05%).[13]

Most common complication observed in our study was hypoxia in 10 patients out of 41 (24.3%), which improved and resolved with high-flow oxygen support. Minor bleeding was observed in three patients (7.3%), and bronchospasm was observed in five patients (12.1%), which resolved with nebulization. No bronchoscopy-related death occurred in our study.

Dang et al.[14] in his study prospectively evaluated the patients undergoing bronchoscopy over a 12-month period. A total of 558 bronchoscopies were performed. In 216 procedures, transbronchial biopsy was also taken. They found that only 2.2% major complications occurred in the first 4 hours of bronchoscopy, and the complications that occurred also did not require any major intervention.

Olopade and Prakash[15] in their study of 804 bronchoscopies in the ICU did not observe any bronchoscopy-related death.

Major limitation of our current study is that it was carried out at a single tertiary care facility. Ideally, this study should be expanded to multiple centers for better analysis. Another limitation is its small sample size, which makes generalization difficult.

## Conclusion

Bronchoscopy in the ICU provides important diagnostic information and therapeutic utility required in patients with various respiratory conditions. Nowadays, bronchoscopy has become an important part of the everyday life of a pulmonologist as well as critical care physicians. Safety and benefits are key features for its use, and it can be performed safely in patients with the above-listed indications when done by trained pulmonologists under basic precautions.

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ORIGINAL ARTICLE

# Interobserver Variability of Both Glasgow Coma Scale and Full Outline of Unresponsiveness Scores in Forecasting the Results of Critically Ill Patients with Altered Sensorium

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## Abstract

**Background:** Altered mental status (AMS) refers to changes in cognitive function or consciousness, encompassing cognitive, attention, arousal, and consciousness disorders. The Glasgow Coma Scale (GCS) and full outline of unresponsiveness (FOUR) score are tools used to evaluate patients with altered consciousness. Few studies have compared the interobserver reliability of these scales. This study aimed to assess interobserver variability between GCS and FOUR scores in predicting outcomes of critically ill patients with altered sensorium.

**Methodology:** This hospital-based forecasting experimental study included 200 patients who were admitted to the critical care unit at King Edward Memorial (KEM) Hospital, Pune. Patients were randomly selected and scored once within 24 hours of admission using both GCS and FOUR scores by two independent observers, a critical care resident (CCR) and a critical care consultant (CCC), with a 5-minute interval between assessments. Interrater reliability was measured using kappa values, with outcomes focused on agreement within ±1 score point for both scales. Statistical analysis was conducted using Epi Info.

**Results:** Demographics showed males (62%) outnumbered females (38%). The largest age-group was 51–70 years (38 %). GCS and FOUR scores showed no significant differences between CCR and CCC in mean GCS (CCR: 8.2 ± 2.9; CCC: 8.5 ± 3.0; $p = 0.249$) or FOUR score (CCR: 10.74 ± 3.2; CCC: 10.9 ± 3.1; $p = 0.6118$). A close to borderline difference was observed in GCS for females ($p = 0.0423$). Interrater agreement showed kappa values for GCS components eye-openings (0.78291), verbal responses (0.64858), and motor responses (0.38867). For FOUR scores, kappa values were eye-openings (0.81014), motor responses (0.77721), brainstem reflexes (0.89801), and respirations (0.91623).

**Conclusion:** The study found very good interobserver reliability for GCS eye and verbal components but poor agreement for motor responses due to confusion with localization and abnormal movements. The FOUR score demonstrated good to excellent reliability across all components and provided more detailed neurologic assessments, especially in intubated patients and those with brainstem dysfunction. It is more efficient in predicting outcomes, making it a preferred tool in intensive care units (ICUs). Larger studies are recommended to incorporate the FOUR score as a standard neuromonitoring tool in the intensive care unit.


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## Introduction

Altered sensorium or altered mental status (AMS) refers to changes in cognitive function or self-consciousness, and undifferentiated AMS is a common reason for emergency visits and hospitalizations, especially in the elderly. It includes symptoms like coma, drowsiness, confusion, irritability, and abnormal behavior. Causes of AMS vary by age, with cerebrovascular disorders, systemic failure, and infections being primary causes in the elderly, while drugs, toxic factors, and metabolic issues are more common in younger patients.[1]

The reported causes of altered sensorium include vascular events such as ischemic stroke, intracerebral hemorrhage, and subarachnoid hemorrhage. Infectious causes include urinary tract infections, pneumonia, septic conditions, viral encephalitis, and meningitis. Toxic effects include intoxication and overdose, with withdrawal of substances like alcohol, barbiturates, heroin, benzodiazepines, and drug use (prescribed, herbal preparations, or over-the-counter). Traumatic causes include concussion and subdural hematoma. Autoimmune causes are neuropsychiatric lupus, Behçet syndrome, vasculitis, and disseminated acute encephalomyelitis. Neoplastic causes include large brain tumors and carcinomatous meningitis. Seizure-related causes include postictal states, nonconvulsive status epilepticus, and epilepticus. Degenerative causes include dementia with Lewy bodies and prion disease.[2] Risk factors for altered sensorium include age over 65, anesthesia, preexisting cognitive impairment, environmental changes, constipation or urinary retention, dehydration, depression, alcohol abuse history, previous delirium episodes, intensive care unit stays, malnutrition, medical conditions (such as heart, lung, liver, or kidney disease), polypharmacy, sleep deprivation, social isolation, visual or hearing impairments, and the presence of medical devices like urinary catheters or intravenous cannulae.[3] Definitive care involves supportive measures, patient monitoring, and transfer to the emergency department (ED) for further evaluation. Treatment options for altered sensorium include intubation, external pacing, volume resuscitation, glucose administration, neurological interventions, antibiotics, psychological support, noise reduction, and patient mobilization.[4] Clinical assessment of neurological condition is essential for decision-making, outcome prediction, and communication among healthcare providers. The Glasgow Coma Scale (GCS) is mostly used to measure the level of consciousness but presents challenges, particularly in intubated patients; to reduce these limitations, the full outline of unresponsiveness (FOUR) score was introduced in 2005.[5,6] The FOUR score assesses responsiveness of eyes, sensory motor responses, brainstem reflexes, and respiratory patterns, providing a more comprehensive evaluation. Although the GCS remains popular, the FOUR score is considered more effective for assessing

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intubated patients and offers better insights into brainstem function.[7–9] While several studies have compared the precision of the GCS and FOUR scores, the FOUR score has shown advantages in certain aspects. However, few studies have focused on comparing the interobserver reliability of these two scoring systems. The objective of this study is to evaluate and compare the interobserver variability of both the GCS and FOUR scores in evaluating outcomes for severely ill patients with altered sensorium.

## Resources and Tools

### Study Map

It was a forecasting experimental study undertaken in which the GCS and FOUR scores were compared.

### Study Site

The study was executed in the Department of Critical Care Medicine, King Edward Memorial (KEM) Hospital, Pune, India, 550+ bed multispecialty tertiary care center.

### Study Duration

The study period was from June 2022 to March 2023.

### Study Population

All cases who were admitted to the Department of Critical Care Medicine, KEM Hospital, Pune, with altered sensorium and assessed upon arrival to the Critical Care Unit during the period of study, fulfilling the study criteria below.

### Inclusion Criteria

All patients who were admitted with altered sensorium and were above 18 years of age.

### Exclusion Criteria

Patient relatives refusing consent for this study. Patients receiving neuromuscular blocking agents or heavy sedation were excluded.

The number of samples was estimated with the software Epi Info™, created by the Centers for Disease Control and Prevention (CDC). Based on the study by Suresh et al., the agreement for the eye-opening component of the GCS was 65.8%, thus $p = 0.658$ was used for this study. The required sample size was determined to be approximately 200 subjects, with a power of 89.8% (~90%). The formula used for sample size estimation was $n = 4pq/(L^2)$, where $n$ = required number of samples, $p = 0.658$ (based on Suresh et al.'s study), $q = 1 - p = 0.342$, L = information loss = 10.2%.

The total sample size was 200. Patients were randomly selected from critically ill cases admitted to the intensive care unit (ICU) with altered sensorium, using random numbers for selection.

### Sampling Technique

Patients were chosen at random using random numbers generated from the "Kevin Conroy: 5120 Random Numbers" (a JavaScript pseudorandom number generator).

### Definition

Consciousness is stated as the condition of being aware of oneself and the environment. Altered sensorium refers to a disturbance in this awareness, covering a spectrum of brain dysfunctions such as confusion, clouded consciousness, disorientation, inattention, behavioral changes, and drowsiness.

The ethical committee's approval was obtained from the Board of Institutional Ethics and Research Committee.

### Subject Confidentiality and Informed Consent

Data collection was conducted on paper. Patients were screened for eligibility, and those who qualified for the inclusion criteria were enrolled in the study. Only study personnel contacted the enrolled patients. All patient-related data was handled with strict confidentiality. Informed consent was obtained from all participants, with the consent form providing detailed information to ensure patients understood their role in the study. The form was written in a language accessible to the study population.

### Data Collection

A self-administered and predesigned pro forma was prepared to keep the objectives of the study at the center point. The motive of this study was briefed to the patient, and informed consent was obtained. Patients were chosen for the study as per inclusion criteria. In the preparation of the pro forma, every aspect desired to be studied was incorporated. Scoring was done at the first possible occasion within 24 hours of hospital admission, and each patient was scored only once. The patient was scored by two independent observers [critical care resident (CCR) and critical care consultant (CCC)] within a time interval of 5 minutes. Each patient was scored using GCS and FOUR score separately. The observers remained constant throughout the study. GCS scoring was recorded on a one-sided form having written instructions. In the case of intubated patients, the score of the speech response of the GCS was considered to be 1, whereas in the case of FOUR scoring, the observers utilized a form with both written as well as visual instructions. The written instructions were from the original guidelines to follow from the Mayo Clinic, while the visual instructions were a color reproduction of the 2005 published version, resized to fit the scoring form.

Statistical analysis was performed using the software Epi Info™, developed by the CDC. The interrater accuracy of both the GCS and FOUR scores was determined using the kappa statistics. A kappa value of 0.4 or below indicated low agreement, values between 0.4 and 0.6 represented medium agreement, values between 0.6 and 0.8 indicated high agreement, and values above 0.8 were considered excellent.

The primary outcome measured was interrater agreement within ±1 point for both the GCS and FOUR scores. Another outcome included exact interrater agreement and an assessment of the individual subcomponents of both scoring systems.

The kappa statistic ($k$) was utilized to assess agreement, while internal consistency for both the GCS and FOUR scores was evaluated with the help of Cronbach's α and Spearman's correlation coefficient. Internal consistency values of 0.5 or below were regarded as unacceptable, values between 0.5 and 0.6 as poor, values between 0.6 and 0.7 as questionable, values between 0.7 and 0.8 as acceptable, values between 0.8 and 0.9 as good, and values above 0.9 as excellent. A $p$-value of <0.05 was considered statistically significant.

### Key Outcome

Around 200 patients took part in this experiment from the Department of Critical Care Medicine, KEM Hospital, Pune, India, a 550+ bedded multispecialty tertiary care hospital, between June 2022 and March 2023. Age and gender distribution is shown in Table 1 and Table 2.

The GCS parameters included were explained along with the scoring pattern in Table 3. The levels of responses in the components of the GCS are scored from 1 for no response up to normal values of 4 (eye-opening response), 5 (verbal response), and 6 (motor response). Further, GCS and FOUR scores recorded by CCRs and CCCs with gender distribution were depicted in Table 4 and Table 5.

The mean GCS score evaluated by CCRs was 8.2 ± 2.9 and by CCCs was 8.5 ± 3.0, with no statistically notable difference between the two groups ($p = 0.249$). There was no statistically significant difference in the mean FOUR score assessed by residents (10.74 ± 3.2) and consultants (10.9 ± 3.1) ($p = 0.6118$).

The mean GCS assessed by residents and consultants in males was 8.42 ± 3.1

**Table 1:** Gender allocation for subjects enrolled

| *Gender* | *N* | *%* |
|---|---|---|
| Men | 124 | 62 |
| Women | 76 | 38 |
| Total | 200 | 100 |

As the above table depicts that, males (62%) outnumbered the female population (38%)

**Table 2:** Age-group distribution of the subjects enrolled

| *Age (years)* | *N* | *%* |
|---|---|---|
| 10–30 | 19 | 9.5 |
| 31–50 | 51 | 25.5 |
| 51–70 | 76 | 38 |
| >70 | 54 | 27 |
| Total | 200 | 100 |

The majority 38% of the study population belonged to the age-group 51–70 years; followed by beyond >70 years (27%); 31–50 years (25.5%) and 10–30 years (9.5%)

**Table 3:** GCS parameters and their scoring pattern

| | |
|---|---|
| Best eye reaction (4) | • Do not open eyes<br>• Does open eyes to pain stimuli<br>• Does open eyes to sounds<br>• Spontaneous eyes-opening |
| Best verbal reaction (5) | • Zero verbal response<br>• Mumbling sounds<br>• Not suitable words<br>• Perplexed<br>• Familiarized |
| Best motor reaction (6) | • No motor response<br>• Abnormal extension to pain<br>• Abnormal flexion to pain<br>• Withdrawal from pain<br>• Localizing pain |

**Table 4:** GCS and FOUR scores recorded by CCR and CCC

| *Scores* | *Group* | *Mean ± SD* | *p-value* |
|---|---|---|---|
| GCS | CCR | 8.2 ± 2.9 | $p = 0.3099$ |
| | CCC | 8.5 ± 3.0 | |
| FOUR | CCR | 10.7 ± 3.2 | $p = 0.5259$ |
| | CCC | 10.9 ± 3.1 | |

**Table 5:** GCS scores recorded by CCRs and CCCs

| *Gender* | *GCS–CCR Mean ± SD* | *GCS–CCS Mean ± SD* | *p-value* |
|---|---|---|---|
| Men | 8.42 ± 3.1 | 8.59 ± 3.2 | 0.5898 |
| Women | 7.78 ± 2.7 | 8.31 ± 2.5 | 0.0423 |

**Table 6:** FOUR scores recorded by CCR and CCC

| *Gender* | *FOUR score–CCR Mean ± SD* | *FOUR score–CCS Mean ± SD* | *p-value* |
|---|---|---|---|
| Men | 10.86 ± 3.3 | 10.87 ± 3.4 | 0.9762 |
| Women | 10.55 ± 2.9 | 10.93 ± 2.6 | 0.1684 |

**Table 7:** Interrater agreement between CCR and CCC about GCS score

| *GCS* | *Kappa value* | *95% CI* |
|---|---|---|
| Eye opening | 0.78291 | 0.71421–0.85162 |
| Verbal response | 0.64858 | 0.56631–0.73084 |
| Motor response | 0.38867 | 0.30114–0.47620 |

**Table 8:** Interrater agreement between CCR and CCC in regard to FOUR score

| *FOUR scale* | *Kappa value* | *95% CI* |
|---|---|---|
| Eye response | 0.81014 | 0.74653–0.87375 |
| Motor response | 0.77721 | 0.70472–0.84970 |
| Brainstem reflexes | 0.89801 | 0.81153–0.98449 |
| Respiration | 0.91623 | 0.86631–0.96615 |

and 8.59 ± 3.2, respectively, without any significant difference ($p = 0.5898$). There exists a borderline significant difference in the mean GCS score assessed by residents (7.78 ± 2.7) and consultants (8.31 ± 2.5) in females ($p = 0.0423$).

The mean FOUR score assessed by residents and consultants in males was almost similar, with a value of 10.86 ± 3.3. The mean FOUR score in females assessed by consultants and residents was 10.55 ± 2.9 and 10.93 ± 2.6, respectively. There was no significant difference in the FOUR scores assessed by residents and consultants, as shown in Table 6.

With regard to interrater agreement between CCR and CCC in individual parameters of GCS scoring, the kappa value for eye-opening, verbal responses, and motor response was 0.78291, 0.64858, and 0.38867, respectively, as shown in Table 7. Distribution of the interrater agreement between CCR and CCC regarding FOUR score is shown in Table 8. With regard to interrater agreement between CCR and CCC in individual parameters of FOUR scoring, the kappa value for eye-opening, motor response, brainstem reflexes, and respiration was 0.81014, 0.77721, 0.89801, and 0.91623, respectively.

## Discussion

Altered sensorium, or AMS, is a collection of clinical symptoms involving reduced consciousness, impaired attention, and cognitive dysfunction.[10,11] Patients with undifferentiated AMS, where the exact cause is not known, frequently present in departments of emergency and ICU. These patients often exhibit vague symptoms, making their evaluation and management particularly challenging for ICU physicians.[12–14] The GCS is the preferred tool to assess consciousness, while the FOUR score is a newer, validated alternative to the GCS.[15–18] Few studies in India have compared the interobserver variability in GCS and FOUR score assessments by resident doctors to predict outcomes in AMS patients. This study aimed to assess the interobserver agreement and variability within CCR and CCC in predicting outcomes for critically ill patients with altered sensorium.

This prospective observational study included 200 patients, with a male-to-female ratio of 1.6:1, consistent with other studies by Suresh et al., Haldar et al., and Iyer et al., which reported male-to-female ratios of 2.5:1, 1.6:1, and 1.2:1, respectively. The mean age of participants was 56.22 ± 18.2 years, higher than the 40.1 ± 17.6 years in Suresh et al.'s study, but comparable to the 58-year median in Haldar et al.'s study[19] and the 63.0 ± 18.4 years in Iyer et al.'s study.

An ideal scale for a coma must be reliable, valid, linear, and easy to refer. The GCS evaluates three components—eye-opening, motor response, and verbal response, which assess

the cerebral cortex, reticular activating system, and upper brainstem.[20,21] However, it has several limitations. Its verbal component is often questioned for its usefulness in assessing consciousness, and the GCS does not account for brainstem reflexes, rapid eye movements, or complex sensory-motor responses. Additionally, it tends to be skewed toward motor responses. While many other scales have been developed to address these shortcomings, none have gained widespread acceptance as a replacement for the GCS.[18] The FOUR score, however, aims to overcome such limitations by incorporating four equally weighted components—rapid eye response, sensory and motor response, brainstem reflexes, and respiration pattern. This scale is easy to remember and provides a comprehensive neurological assessment, particularly useful for patients with metabolic derangements, septic shock, or nonstructural brain injuries.

In this study, the interobserver variability between CCR and CCC in GCS and FOUR scores was compared. The kappa score for eye-opening in GCS showed good agreement between CCR and CCC ($k$ = 0.7829), while the FOUR score showed very good agreement ($k$ = 0.8101). Most eye responses in GCS were on the lower end (E1/E2: no eye-opening or response of eye to pain), while in the FOUR score, responses ranged from E0 to E3 (eyes closed with pain, eyes open but not tracking). The additional subscore in the rapid eye responses component of the FOUR score, which enhanced the total responses to 5, improved interobserver agreement and added clinical value. Heron et al. also found high interrater reliability for eye response in GCS.

For the motor component, GCS showed only fair agreement ($k$ = 0.38867), while the FOUR score demonstrated good agreement ($k$ = 0.7772). Most motor responses in GCS were withdrawal from or localization to pain (M4/M5), while the FOUR score ranged from M2 to M3 (flexion response to pain and localization to pain). The addition of specific motor responses, such as "thumbs-up" or "peace sign," to the FOUR score's M4 subscore was well-received by observers. The GCS motor component had the lowest interobserver reliability, a finding supported by Heron et al., who also noted significant interrater disagreement for motor responses in GCS.[21]

The verbal components of the GCS, often difficult to assess in intubated patients, showed good agreement between CCR and CCC ($k$ = 0.64858). Most verbal responses fell on the lower end of the scale (V1/V2/V3: no verbal response, incomprehensible sounds, and inappropriate words). Holdgate et al.[22] reported excellent interrater agreement for GCS verbal scores between nurses and senior physicians in the ED, with intermediate reliability for motor and eye scores.

In this study, brainstem reflexes in the FOUR score were well distributed, with most patients displaying pupil and corneal reflexes. The kappa score for brainstem reflexes showed very good agreement between CCR and CCC ($k$ = 0.89801), suggesting these components should be incorporated in future coma evaluations. The respiratory component also showed very good agreement ($k$ = 0.91623), though it should be interpreted cautiously due to the limited evaluation of breathing patterns. Most patients in this study were not intubated and had regular breathing patterns, making assessment straightforward.

Previous studies, such as those by Wijdicks et al. and Wolfe and Brown, found excellent reliability for both the GCS ($k$ = 0.82) and FOUR score. Similarly, Stead et al. reported excellent interrater reliability for both scales ($k$ = 0.88 for FOUR and $k$ = 0.86 for GCS).[6,8]

This study has several limitations. The raters were not blinded to the case diagnoses, which may have introduced bias into clinical assessments. A fundamental limitation in validating coma scales is the lack of an objective measure for the level of coma. Therefore, better interrater accuracy does not necessarily equate to greater accuracy. A follow-up study to evaluate patient outcomes using the FOUR score was not conducted. The study may lack external validation for surgical patients, as the sample primarily consisted of medical patients with fewer surgical cases. Since the raters assessed both the GCS and FOUR score simultaneously, any real-time changes in consciousness levels cannot account for the noted contrasts in interrater agreement of the two scoring systems.

## Conclusion

This study found that the interobserver reliability for the eye and verbal components of the GCS was very good, but the motor component had the lowest reliability. Raters experienced confusion due to differences in localization, abnormal flexion, and extension responses, which were major sources of disagreement. In contrast, the FOUR score demonstrated good to excellent interobserver reliability across all components. Since the verbal component of the GCS cannot be used in intubated patients, it may be more appropriate for nonintubated, less critical patients without brainstem dysfunction.

The FOUR score has several key advantages, as it includes detailed assessments of brainstem reflexes and eye movements, which the GCS does not provide. It can further distinguish a GCS score of 3 and is more accurate in assessing a patient's consciousness level and predicting disease outcomes. This makes the FOUR score a more dependable score for patients in medical ICUs. Given the level of disagreement observed in GCS scoring, it should not be solely relied upon for clinical decisions and must be interpreted alongside other clinical data. Additionally, this research should encourage larger studies to consider using the FOUR score as a valuable neuromonitoring tool in all ICU.

## Orcid

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# OBITUARY

## Padma Shri Prof Dr Alaka Deshpande

(1946 – 2025)

The medical fraternity mourns the passing of Prof Dr Alaka Deshpande, an eminent physician, revered teacher and a compassionate healer, whose life was dedicated to the service of medicine and humanity.

Dr Deshpande Madam served as the Head of the Department of Internal Medicine at Sir J.J. Hospital and Grant Medical College, Mumbai, where she played a transformative role in strengthening academic medicine and patient care. A visionary leader, she was instrumental in starting the first HIV OPD at J.J. Hospital at a time when the disease was burdened with stigma and fear, thereby bringing science, dignity and structured care to countless patients. She also established the Medical ICU at J.J. Hospital, significantly enhancing critical care services and saving innumerable lives.

In recognition of her exceptional contributions to medicine and public health, she was conferred the Padma Shri Award by the Government of India, one of the nation's highest civilian honors.

An outstanding academician, Dr Deshpande Madam was a gifted teacher and mentor who taught and inspired generations of undergraduate and postgraduate students. Her clarity of thought, clinical acumen and insistence on ethical, patient-centered practice left an indelible mark on all who trained under her. She was a respected examiner in Internal Medicine, a sought-after speaker who delivered numerous orations at national and international conferences of medicine and an author of medical books that continue to guide learners and practitioners alike.

Beyond her formidable professional achievements, Prof Dr Deshpande was, above all, a deeply humane and empathetic physician.

On a personal note, she was the treating physician of my father, late Prof Dr Ashok Saraf, when I was just 11 years old. This gave me the unique and lasting privilege of witnessing her compassion up close, not merely as a celebrated clinician and teacher, but as a doctor who listened, cared, and stood by her patients and their families with grace and kindness. Eventually, I had the privilege of becoming her student and one of the unique things Madam used always in her clinics was "Remember students, the best diagnostic tool is…. (she used to point to her hand)"! Her diagnostic tools always were 'Touch, Feel and Talk to your patient'. I also had the very unique opportunity to become a colleague examiner with Madam for an MD Medicine practical exam. During the exam, I noticed that unlike most of the examiners, Madam used to correct the wrong answers given by students, even during his/her exam! Her point being that if I don't correct mistakes there and then, they may be carried on forever in the budding doctors' professional life. These moments revealed the true essence of her greatness.

Padma Shri Prof Dr Alaka Deshpande leaves behind a rich legacy of institutions strengthened, minds shaped, patients healed, and values upheld. Her influence will live on through her students, her writings, and the countless lives she touched.

She will be remembered with deep respect, gratitude and affection.

May her soul rest in everlasting peace.

**Prof Dr Amit Saraf**
MD FRCP (London, Edinburgh, Glasgow) FACP (Philadelphia) FICP FCPS
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ORIGINAL ARTICLE

# Bedaquiline-related QTc Prolongation in Multidrug Resistant Tuberculosis Patients: A Prospective Study

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## Abstract

**Introduction:** Bedaquiline (BDQ) has revolutionized multidrug-resistant tuberculosis (MDR-TB) management in the Indian population with a high MDR-TB burden. However, its potential cardiotoxicity in the form of QTc prolongation warrants careful monitoring. This study aims to evaluate the prevalence, severity, and risk factors of BDQ-related QTc prolongation in MDR/rifampicin-resistant (RR)-TB patients. Given the genetic variability and diverse environmental factors, extrapolating foreign data to Indian patients is challenging; thus, local evidence is crucial.

**Methods:** A prospective analytical study was conducted over a period of 18 months on 55 adult patients with RR or MDR pulmonary or extrapulmonary TB initiated on BDQ-containing regimens. Electrocardiograms (ECGs) were performed at baseline, 1, 3, and 6 months. QTc intervals were calculated using Fridericia's formula at each time interval. Prevalence and severity of QTc prolongation were documented. Significant prolongation, defined as an absolute QTcF value ≥500 ms or a change from baseline of ≥60 ms, was also noted.

**Results:** The overall prevalence of QTc prolongation was 37.25%, with 13.7% of patients experiencing significant prolongation. The highest proportion of moderate to severe cases occurred at 3 months. Male gender and body mass index (BMI) >18.5 kg/m$^2$ were identified as statistically significant risk factors. All patients with significant QTc prolongation were under 60 years old, contrasting with prior research. Temporary withdrawal of BDQ was required in 1.96% of patients due to severe QTc prolongation, but no serious cardiac events were observed, consistent with previous studies.

**Conclusion:** This prospective study highlights that while QTc prolongation is a frequent occurrence in MDR/RR-TB patients receiving BDQ, severe cases necessitating treatment modification remain uncommon. These findings reaffirm the critical role of BDQ in MDR-TB management while emphasizing the necessity of stringent cardiac monitoring, particularly during the initial 3 months of therapy.

**Limitations:** The study's small sample size and concomitant use of other QTc-prolonging medications may have influenced the results. Further large-scale studies are needed to confirm these findings and explore additional risk factors.


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## Introduction

Tuberculosis (TB) is an extremely infectious bacterial disease that is caused by *Mycobacterium tuberculosis*. It affects the pulmonary system but can also impact various other organ systems. TB is still a worldwide health concern, particularly in developing nations that have limited resources for prevention, diagnosis, and treatment.[1] Drug-sensitive TB, also known as drug-susceptible TB, refers to TB caused by strains that are susceptible to first-line antitubercular drugs, such as isoniazid, rifampicin, ethambutol, and pyrazinamide. These cases can be effectively treated with standard anti-TB regimens.[2]

The strains of *M. tuberculosis* that are resistant to rifampicin cause rifampicin-resistant TB (RR-TB). Rifampicin resistance is a significant concern, as it often indicates resistance to other first-line drugs as well.[3]

Multidrug-resistant TB (MDR-TB) is a more severe form caused by strains that are resistant to both isoniazid and rifampicin. MDR-TB is more difficult to treat and requires a longer treatment duration with second-line antitubercular drugs that are more toxic, more expensive, but often less efficacious.[2]

Both rifampicin-resistant and MDR-TB pose significant challenges in the global fight against TB. Effective management of these resistant forms requires prompt diagnosis, appropriate treatment regimens, strict adherence to treatment, and robust infection control measures to prevent further transmission.

The highest burden of TB cases worldwide is in India, with an ever-increasing proportion of drug-resistant TB (DR-TB) cases. 48,332 MDR/RR-TB cases were diagnosed in 2021, and 43,380 (90%) were started on treatment containing oral bedaquiline (BDQ) in the regimen.[4]

The evidence on incorporating a shorter oral BDQ-containing regimen with phasing out injectables is largely based on data from South African countries, which were studied by the World Health Organization (WHO). Initial studies done in South Africa show reduced time to culture conversion and improved cure rates with this drug.[5]

Since being approved by the US Food and Drug Administration (FDA), BDQ has expanded the limited range of treatment options available for the management of MDR-TB. However, the Center for Disease Control and Prevention (CDC) encourages expert consultation with regional health authorities prior to the use of BDQ due to its potential to cause serious adverse events.[6] Serious adverse events are categorized into grade III (severe), grade IV (disabling or life-threatening), and grade V (death related to adverse events).[7]

Bedaquiline has a favorable safety profile, but possible adverse reactions of BDQ include QT prolongation, which can lead to fatal arrhythmias like torsades de pointes, ventricular tachycardia, and ventricular arrhythmias, along with sudden cardiac death (for which a black box warning has been issued by the US FDA). Instances of hepatitis, jaundice, and increased serum transaminases have also been reported.[8] Hence, monitoring of electrocardiogram (ECG) and serum electrolytes (Na, K, Cl, Ca, and Mg) may be needed along with regular liver function test (LFT) monitoring.[8]

Sanctioned by the WHO, "Guidelines for Programmatic Management of Drug-resistant TB—PMDT in India—2021" were published.[4]

Bedaquiline is now incorporated in both the short and longer oral regimens for MDR-TB treatment for the initial 6 months under the programmatic management of drug-resistant tuberculosis (PMDT) guidelines.[4]

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There are some published data on the cardiotoxic effects of BDQ; however, the consensus is that the benefits of the drug outweigh the potential risks of cardiotoxicity.[9]

This study was planned to determine the proportion of Indian patients with RR/MDR-TB who develop QTc prolongation related to the use of BDQ at the end of 6 months of therapy and to grade the severity of this adverse effect. It will also aid in identifying some of the possible risk factors that may be contributing to its cardiotoxicity.

The evidence on oral BDQ-containing regimen is largely based on data from South Africa. However, most of these studies, which establish the effectiveness and safety profile of BDQ, have been done outside of India, mainly in South Africa, and a few in Europe, China, and California.[6]

Hence, very little evidence exists in Indian MDR-TB patients, and considering the differences in genetic makeup and environmental factors, it becomes difficult to extrapolate data from other countries to the Indian population.

Keeping in mind that India has umpteen numbers of TB patients and increasing numbers of DR-TB, it becomes important to address these knowledge gaps. The current guidelines also emphasize that further data are needed, especially from resource-limited countries with rampant TB cases.

## Methods

This prospective analytical study was conducted over 18 months from August 2022 to February 2024. The study population included Indian adult patients (>18 years) diagnosed with RR or MDR pulmonary or extrapulmonary TB who were initiated on treatment at Kasturba Medical College, Mangaluru teaching hospitals. Patients with baseline QTcF >500 ms, preexisting cardiac conduction abnormalities, or those not receiving BDQ were excluded.

Data collection was performed through direct interviews using a pretested, semi-structured questionnaire. Demographic data, clinical history, and baseline serum electrolytes were recorded. Baseline ECGs were taken as part of the pretreatment evaluation, and the QTc was calculated using Fridericia's formula. Follow-up ECGs were obtained at 1, 3, and 6 months after initiation of BDQ-containing treatment.

The primary outcomes were the number and proportion of subjects who developed an absolute QTcF value ≥500 ms or a change in QTc interval ≥60 ms from baseline after 6 months of BDQ therapy. The severity of QTc prolongation was graded according to PMDT guidelines.

Continuous variables were stated as mean ± standard deviation, and categorical variables as proportions and numbers. For comparison of the data, Chi-square tests were utilized, and $p < 0.05$ was considered significant. The calculated sample size for the study was 55 patients.

## Results

In this prospective analytical study, data was collected from 55 adult patients with RR or MDR-TB who were initiated on BDQ-containing regimens. The population had a mean age of 40.9 years, with males constituting 61.8% of participants. Patient demographics, clinical characteristics, and ECG measurements were recorded at baseline and follow-up intervals of 1, 3, and 6 months. QTc intervals were calculated using Fridericia's formula, with significant prolongation defined as an absolute QTcF value ≥500 ms or a change from baseline ≥60 ms.

Table 1 outlines the baseline demographics and characteristics of the study population, highlighting that 58.2% of participants were underweight and the majority (63.6%) had RR-pulmonary TB. The distribution between long and short MDR regimens was 32.7% and 67.3%, respectively, with only 3.6% of patients being HIV positive.

Table 2 presents the key outcomes regarding QTc prolongation. Four patients were not included, as one patient was lost to follow-up and three patients died during the course of treatment. Data from 51 patients was analyzed. It revealed an overall prevalence of 37.25% for QTc prolongation. Notably, 13.73% of patients experienced significant QTc prolongation, with 11.76% showing a change in QTcF ≥60 ms from baseline and 1.96% reaching an absolute QTcF ≥500 ms.

The progression of QTc prolongation over time is detailed in Table 3, demonstrating that the peak of severity occurred at the 3-month interval. At this time point, 11.3% of patients experienced mild prolongation, 3.8% had moderate prolongation, and 1.9% had severe prolongation. By the 6-month mark, there was a noticeable improvement, with no severe cases and only 1.9% moderate cases remaining.

Table 4 analyzes the association between various clinical factors and significant QTc prolongation. The analysis identified male gender and body mass index (BMI) ≥18.5 as statistically significant risk factors (with $p < 0.001$ and $p = 0.005$, respectively). Interestingly, all patients who experienced significant QTc prolongation were under 60 years of age, although this was not statistically significant ($p = 0.43$). All patients with significant prolongation had normal calcium, potassium, and magnesium levels.

## Discussion

Multidrug-resistant TB continues to be a major global health challenge, with BDQ emerging

**Table 1:** Patient demographics and characteristics

| *Characteristic* | | *Frequency (value)* | *Percentage (%)* |
|---|---|---|---|
| Mean age | | 40.9 ± 15.4 years | |
| Gender | Males | 34 | 61.8 |
| | Females | 21 | 38.2 |
| BMI | Underweight (<18.5) | 32 | 58.2 |
| | Normal (18.5–24.9) | 23 | 41.8 |
| Diagnosis | RR-pulmonary TB | 35 | 63.6 |
| | MDR-pulmonary TB | 19 | 34.5 |
| | Extrapulmonary RR-TB | 1 | 1.8 |
| HIV status | Positive | 2 | 3.6 |
| | Negative | 53 | 96.4 |
| MDR regimen | Long | 18 | 32.7 |
| | Short | 37 | 67.3 |

**Table 2:** QTc prolongation prevalence and significance

| *QTc prolongation* | *Prevalence* |
|---|---|
| Overall QTc prolongation | 37.25% (19/51) |
| Significant QTc prolongation (≥500 ms absolute value or ≥60 ms change from baseline) | 13.73% (7/51) |
| Absolute QTcF ≥500 ms | 1.96% (1/51) |
| Change in QTcF ≥60 ms from baseline | 11.76% (6/51) |

**Table 3:** Severity of QTc prolongation over time

| *Time point* | *Mild (%)* | *Moderate (%)* | *Severe (%)* |
|---|---|---|---|
| 1 month | 7.5 | 5.7 | 0 |
| 3 months | 11.3 | 3.8 | 1.9 |
| 6 months | 11.3 | 1.9 | 0 |

**Table 4:** Association of significant QTc prolongation with risk factors

| *Risk factor* | *Significant QTc prolongation (%)* | *p-value* |
|---|---|---|
| Age ≤60 years | 100 | 0.43 |
| Male gender | 71.4 | <0.001 |
| BMI ≥18.5 | 71.4 | 0.005 |
| HbA1c >6.5 | 14.3 | 0.72 |
| Calcium ≥8 mg/dL | 100 | – |
| Potassium 3.5–5 mEq/L | 100 | – |
| Magnesium 1.7–2.2 mEq/L | 100 | – |

as a promising treatment option. However, its use has been associated with QTc interval prolongation, a potentially life-threatening adverse effect.[10–12] Our study aimed to detect the proportion of MDR/RR-TB patients developing QTc prolongation after 6 months of BDQ therapy and assess its severity and risk factors.

Our findings revealed a total QTc prolongation prevalence of 37.25%, with 13.7% experiencing significant prolongation. This prevalence is lower compared to some previous studies.[13–16] The severity of QTc prolongation varied over time, with the highest proportion of moderate and severe cases observed at the 3-month interval. Most patients with significant QTc prolongation were males and had a BMI >18.5 kg/m$^2$, both statistically significant risk factors. Interestingly, all patients with significant QTc prolongation were under 60 years old, contrasting with some previous research findings.[17]

While 1.96% of patients required temporary withdrawal of BDQ due to severe QTc prolongation, no serious cardiac events were observed. This aligns with other studies reporting low rates of therapy discontinuation due to QTc prolongation.[6,14,18,19]

However, the study's limitations, including a small sample size and concomitant use of other QTc-prolonging medications, necessitate further research to substantiate these findings and explore additional risk factors.

## Conclusion

This prospective study demonstrates that while QTc prolongation is a frequent occurrence in patients receiving BDQ-containing regimens for MDR/RR-TB, severe prolongation necessitating treatment modification is rare.

The study identified male gender and BMI ≥18.5 as significant risk factors and observed that QTc prolongation typically peaks at 3 months before showing improvement.

Despite these cardiac effects, the absence of serious cardiac events suggests that BDQ can be safely administered with vigilant ECG surveillance and judicious patient selection.

These findings reaffirm the critical role of BDQ in MDR-TB management while emphasizing the necessity of stringent cardiac monitoring, particularly during the initial 3 months of therapy.

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ORIGINAL ARTICLE

# Study of Platelet Indices as Markers of Retinopathy in Patients with Diabetes Mellitus

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## Abstract

**Background:** Diabetes mellitus poses a substantial global health burden, with diabetic retinopathy (DR) being a prevalent and potentially devastating microvascular complication. Platelet activation has been implicated in the pathogenesis of DR, suggesting platelet indices such as mean platelet volume (MPV), platelet distribution width (PDW), platelet-large cell ratio (P-LCR), and plateletcrit (PCT) as potential noninvasive markers for predicting its onset.

**Materials and Methods:** We conducted a cross-sectional study involving 300 patients diagnosed with type 2 diabetes mellitus (T2DM) attending a tertiary care center. Demographic data, duration of diabetes, and HbA1c levels were recorded. Platelet indices were measured using complete blood counts, and DR was diagnosed based on fundus examination findings.

**Results:** Among the study participants, group B ($n$ = 140) comprising patients with DR had significantly higher levels of MPV (13.28 ± 2.14 fL), PDW (14.56 ± 2.37), P-LCR (29.59 ± 6.018%), and PCT (0.29 ± 0.06) compared to group A ($n$ = 160) without DR (MPV: 9.99 ± 1.64 fL, PDW: 12.81 ± 2.28, P-LCR: 27.64 ± 8.36%, PCT: 0.26 ± 0.09) ($p$ < 0.001 for all comparisons). Subgroup analysis within poorly controlled diabetics (HbA1c > 7%) also showed significantly higher platelet indices in those with DR compared to those without.

**Conclusion:** Our findings underscore a significant association between elevated platelet indices and the presence of DR in patients with T2DM, independent of glycemic control status. These indices could serve as valuable surrogate markers for identifying individuals at risk of developing DR, facilitating early intervention strategies in clinical practice.

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## Introduction

Diabetes mellitus is a complex disease that is considered to be a global pandemic. Due to its expanding population, India is on the way to becoming the diabetes capital of the world. At present, India has the world's second-largest population with diabetes. In the tenth edition of the diabetes atlas, the global prevalence of diabetes is estimated as 537 million (10.5%), which is expected to rise to 783 million by 2045. In India, there are approximately 74.2 million people with diabetes, and the number is expected to cross 124.87 million by 2045.[1]

Diabetic retinopathy is a prevalent and potentially severe complication in the natural history of diabetes mellitus. The onset of diabetic retinopathy serves as a critical warning for treating physicians, as it is often one of the earliest indicators of microvascular damage and signals the need for a more intensive approach to achieving optimal glycemic control. Therefore, early identification of diabetic retinopathy is crucial for physicians to address and manage this condition effectively.

Abnormally increased platelet activation is thought to be the central dogma in the pathophysiology of diabetic retinopathy.[2] Platelet activation can be noninvasively studied by analyzing the platelet indices, which include mean platelet volume (MPV), platelet distribution width (PDW), platelet-large cell ratio (P-LCR), and plateletcrit (PCT). These may be used as predictors of the onset of diabetic retinopathy.

Distorted platelet morphology and their abnormal functions are reported in patients with uncontrolled diabetes,[3] and the association between platelet indices and diabetes was most obvious in those with poorly controlled diabetes.[4] The effect of hyperglycemia on platelet indices has been studied less. Also, the relationship between platelet indices and diabetic retinopathy remains to be clarified.

The present study aimed to evaluate the relationship between HbA1c, which is a parameter used to define uncontrolled diabetes, and platelet indices, and to evaluate the relationship between these variables and diabetic retinopathy.

## Materials and Methods

### Study Design

This was an observational, descriptive, cross-sectional study conducted at the Department of Medicine, Jawahar Lal Nehru Medical College and Associated Hospitals, Ajmer, Rajasthan. The study duration spanned over 1 year and included both inpatients and outpatients diagnosed with type 2 diabetes mellitus.

### Ethical Considerations

The study was initiated only after obtaining ethical clearance from the Institutional Ethics Committee (IEC). Written informed consent was obtained from all the participants prior to inclusion in the study.

### Study Population and Sampling

The sample size was calculated using the formula for comparing two proportions, assuming a power of 80%, alpha of 5%, and an expected difference of 20% in abnormal platelet indices between patients with and without diabetic retinopathy. Based on prior literature, the estimated proportions were 30% in nonretinopathy group and 50% in the retinopathy groups. The minimum required sample size was 91 patients per group (~182 total). Adjusting for a 10% nonresponse rate, the sample size was revised to ~200–210. We included 300 participants in our study to enhance the robustness and validity of results. Patients with type 2 diabetes mellitus (T2DM) on treatment with oral hypoglycemic agents and/or insulin therapy were included using consecutive sampling over the study

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period. These patients were enrolled from medical wards and outpatient services.

### Inclusion Criteria

- Patients with a confirmed diagnosis of type 2 diabetes mellitus.
- Patients on treatment with oral hypoglycemic agents and/or insulin.
- Patients are willing to give informed consent.

### Exclusion Criteria

- Patients with type 1 diabetes mellitus.
- Patients on antiplatelet drugs (e.g., aspirin, clopidogrel).
- Patients with severe anemia (Hb < 6 gm/dL).
- Patients with recent febrile or viral illnesses.
- Patients with hematologic disorders affecting bone marrow (e.g., aplastic anemia, leukemia).
- Patients with preexisting nondiabetic retinal disorders.

### Data Collection

Participants were interviewed to obtain detailed demographic and clinical data, including age, sex, duration of diabetes, treatment history, and family history. Anthropometric measurements and blood pressure were recorded.

### Laboratory Investigations

Complete blood count and platelet indices: Blood samples were collected in EDTA vials and analyzed using the SYSMEX XP-100 hematology analyzer based on flow cytometry and electrical impedance.

Platelet indices measured included:

- Mean platelet volume (MPV).
- Platelet distribution width (PDW).
- Plateletcrit (PCT).
- Platelet-large cell ratio (P-LCR).
- Glycated hemoglobin (HbA1c): Estimated using a latex agglutination inhibition assay with reagents provided by the institutional central laboratory. HbA1c values were categorized as:
  - Good glycemic control: HbA1c ≤7%.
  - Poor glycemic control: HbA1c >7%.

### Ophthalmologic Evaluation

The initial fundoscopic examination was performed by the primary investigator. All findings were subsequently reviewed and confirmed by a senior ophthalmologist with over a decade of clinical experience in diagnosing diabetic retinopathy. This two-step approach was implemented to ensure diagnostic accuracy and consistency across all study participants.

Diabetic retinopathy was diagnosed based on presence of at least two microaneurysms and/or retinal hemorrhages, exudates, and other retinal lesions consistent with DR.

### Statistical Analysis

Data was analyzed using IBM SPSS Statistics v24.0. Continuous variables were expressed as mean ± standard deviation, and categorical variables as percentages. Comparisons between groups were performed using unpaired *t*-tests or chi-square tests as appropriate. A *p*-value <0.05 was considered statistically significant.

## Results

A total of 300 patients ($N = 300$) were included in study, considering the inclusion and exclusion criteria. After the baseline investigations, the study participants were categorized according to presence of diabetic retinopathy.

Group A ($n = 160$) comprised of patients without diabetic retinopathy, and group B ($n = 140$) comprised of patients with diabetic retinopathy. In group A, 71 patients had HbA1c ≤7 (labeled as group A1) and 89 patients had HbA1c >7 (labeled as group A2), while in group B, only 10 patients (labeled as group B1) had good control of diabetes and 130 patients had HbA1c > 7 (labeled as group B2) ($p < 0.001$). Platelet indices were calculated and compared among these groups.

The data analysis revealed that group B contained older-aged (61.84 ± 11.07 years) patients as compared to group A (53.64 ± 9.67 years), having a *p*-value of 0.03, which was significant. Females outnumbered males in both study groups, but this was not statistically significant ($p = 0.15$).

Group B patients had a longer duration of diabetes (10.66 ± 5.04 years) as compared to group A (6.44 ± 3.69 years), and this difference was statistically significant($p = 0.03$).

### Comparison of HbA1c Among Study Groups (Table 1)

The mean HbA1C of study participants in group B was more (9.08 ± 1.74 gm%) when compared with group A (7.405 ± 1.87 gm%), having a *p*-value <0.001, which is statistically significant.

The mean HbA1C of group A2(study population without retinopathy and HbA1C >7) was 8.15 ± 2.05, and mean HbA1C of group B2 (patients with retinopathy and HbA1C >7) was 9.26 ± 1.67. The difference observed was significant ($p < 0.001$) (Table 2).

### Comparison of Platelet Indices Among Study Groups

- All the studied platelet indices, viz PDW, MPV, P-LCR and PCT, were higher in group B patients as compared to group A patients (Table 3).

**Table 1:** HbA1C values in the study groups A and B

| *Groups* | *Mean HbA1c* | *Std deviation* | *p-value* |
|---|---|---|---|
| Group A | 7.405 | 1.87 | <0.001 (S) |
| Group B | 9.08 | 1.74 | |

**Table 2:** HbA1C values in the study groups A2 and B2

| *Groups* | *Mean HbA1c* | *Std deviation* | *p-value* |
|---|---|---|---|
| Group A2 | 8.15 | 2.05 | <0.001 (S) |
| Group B2 | 9.26 | 1.67 | |

**Table 3:** Comparison of platelet indices between study groups

| *Index* | *Group A (without retinopathy)* | *Group B (with retinopathy)* | *p-value* |
|---|---|---|---|
| PDW | 12.81 ± 2.28 | 14.56 ± 2.37 | <0.001 (S) |
| MPV | 9.99 ± 1.64 | 13.28 ± 2.14 | <0.001 (S) |
| P-LCR | 27.64 ± 8.36 | 29.59 ± 6.018 | 0.02 (S) |
| PCT | 0.26 ± 0.09 | 0.29 ± 0.06 | 0.002 (S) |

**Table 4:** Comparison of Platelet indices between Group A2 and Group B2

| | *Group A2 [without retinopathy (n = 89)]* | *Group B2 [with retinopathy (n = 130)]* | *p-value* |
|---|---|---|---|
| PDW | 12.8 ± 2.25 | 14.56 ± 2.37 | <0.01 |
| MPV | 10.05 ± 1.67 | 13.28 ± 2.14 | <0.01 |
| LPCR | 27.62 ± 8.12 | 29.59 ± 6.01 | 0.01 |
| PCT | 0.26 ± 0.08 | 0.29 ± 0.06 | 0.003 |

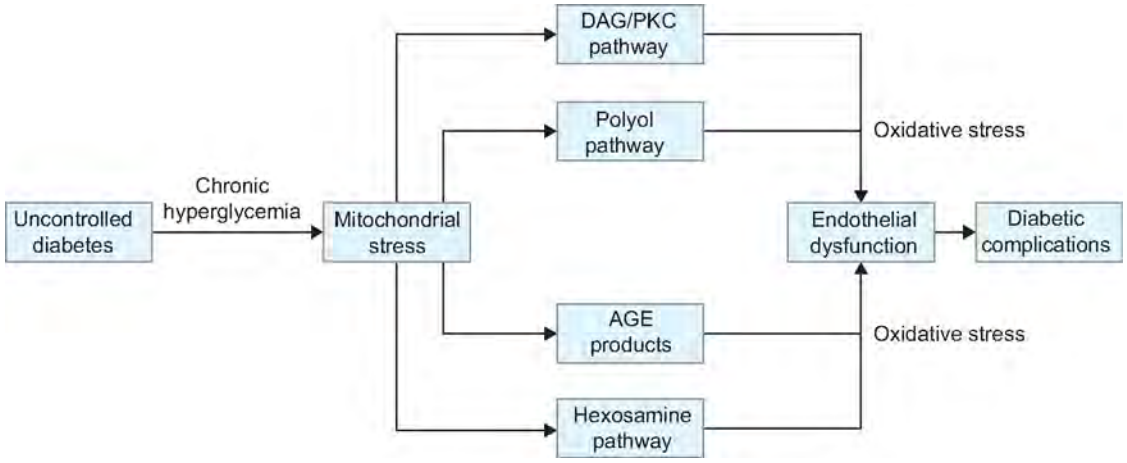


**Fig. 1:** Endothelial dysfunction in diabetes mellitus

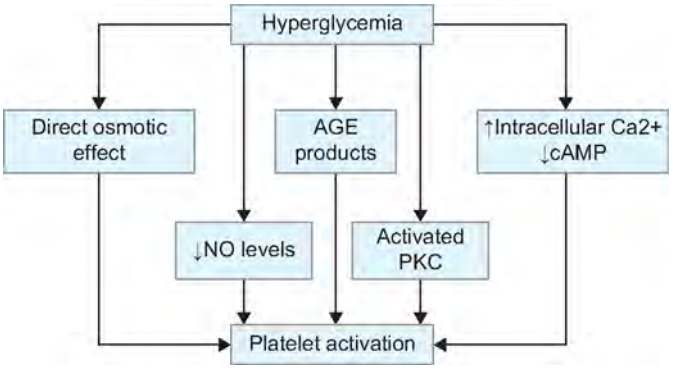


**Fig. 2:** Effect of hyperglycemia on platelets

- Comparison of platelet indices between uncontrolled diabetics (HbA1 C >7) without retinopathy (group A2) and with retinopathy (group B2) (Table 4).
- This showed that, although in both groups HbA1C was >7, PDW, MPV, L-PCR, and PCT were elevated in patients with retinopathy, and this difference was significant, as shown in the table.

## Discussion

Diabetes mellitus encompasses a range of prevalent metabolic disorders characterized by chronically elevated blood glucose levels. A shared characteristic among the long-term complications of diabetes is damage to the vascular system, including both microvascular and macrovascular issues. These events are marked by the progressive narrowing of blood vessel lumens and abnormal protein permeability.[5] Platelets are instrumental in the development of diabetic complications, with various abnormalities in platelet function observed both *in vitro* and *in vivo*.[6]

Chronic hyperglycemia can lead to several complications, broadly categorized into:

- Microvascular complications: These include diabetic retinopathy, neuropathy, and nephropathy.
- Macrovascular complications: These encompass coronary artery disease (CAD), cerebrovascular stroke, and peripheral artery disease (PAD).

While chronic hyperglycemia is a significant contributor to the development of these complications, genetic factors may also predispose individuals to specific issues. Extensive randomized clinical trials, which included type-1 and type-2 diabetes patients, have shown that reducing chronic hyperglycemia can delay or prevent nephropathy, neuropathy, and retinopathy. Endothelial dysfunction is identified as a key mechanism driving both microvascular and macrovascular complications of diabetes mellitus. Endothelial dysfunction results from Chronic hyperglycemia, leading to mitochondrial stress (Fig. 1).

### Effects of Hyperglycemia on Platelet Function (Fig. 2)

Both acute and chronic hyperglycemia cause the activation of protein kinase C (PKC), which plays an important role in mediating several proaggregatory platelet signals.[7] Persistent and recurrent hyperglycemia induces nonenzymatic glycation reactions between reducing sugars and the primary amino branches of proteins, resulting in the production of advanced glycation end products (AGEs).[8] These AGEs promote the externalization of phosphatidylserine on the thrombocyte membrane, which activates surface clotting factors and directly increases the prothrombotic state.[9]

Chronic hyperglycemia also causes the release of large-sized platelets with reduced levels of cyclic adenosine monophosphate (cAMP). In patients with chronic diabetes, platelets exhibit elevated intracellular calcium levels. The combination of higher calcium levels and decreased cAMP makes these platelets more prone to activation and aggregation even at lower stimuli.

The interaction between glucose and lipids results in the formation of glycated

low-density lipoprotein, which adversely affects nitric oxide (NO) formation. A low nitric oxide level leads to increased platelet activity.

In our study, platelet indices, namely MPV, PDW, P-LCR, and PCT were compared between patients with diabetic retinopathy and without retinopathy. Mean platelet volume serves as an indicator of function as well as activation of platelets. Similarly, PDW reflects the variation in size of platelets and is also considered another marker of platelet activation.

### Mean Platelet Volume

In the present study, the mean platelet volume values were elevated in patients with diabetic retinopathy (13.28 ± 2.14 fL) when compared to those without diabetic retinopathy (9.99 ± 1.64 fL), with $p < 0.001$. Also, the MPV values were elevated in patients with poorly controlled diabetes (12.09 ± 2.52 fL) when compared to patients with good control of diabetes (10.124 ± 1.76 fL), and this was statistically significant ($p = 0.002$). Comparable findings were observed in the studies conducted by Bhattacharjee et al.[10] and Dermitas et al.[11] Additionally, Kodiatte et al.[12] concluded that MPV is significantly increased in diabetic patients with microvascular complications.

### Platelet Distribution Width

Platelet distribution width was elevated in diabetic patients with retinopathy (14.56 ± 2.37) when compared to those patients without retinopathy (12.81 ± 2.28), and this difference was statistically significant ($p < 0.001$). Also, the platelet distribution width values were elevated in patients with poorly controlled diabetes (13.88 ± 2.505) as compared to patients with well-controlled diabetes (12.92 ± 2.22), which was statistically significant ($p = 0.003$). These results were comparable to the results of studies published by Dermitas et al.,[11] Bhattacharjee et al.[10]

Buch et al.[13] conducted a study to evaluate platelet volume indices as predictive biomarkers for complications in patients with type 2 diabetes mellitus. A study showed that platelet volume markers (MPV and PDW) are predictive biomarkers for diabetic microvascular complications.

### Platelet-large Cell Ratio

Platelet-large cell ratio was higher in diabetic patients with retinopathy (29.59 ± 6.018) when compared to those patients without retinopathy (27.64 ± 8.36) and this difference was statistically significant ($p < 0.001$).

Also, the P-LCR values were elevated in patients with poorly controlled diabetes (29.16 ± 7.42) as compared to patients with well controlled diabetes (27.80 ± 7.21), which was statistically not significant ($p = 0.15$). This might be explained by the fact that there was large difference between number of patients with poorly controlled diabetes ($n = 219$) and number of patients with good control of diabetes ($n = 81$).

### Plateletcrit

Plateletcrit levels were higher in diabetic patients with retinopathy (0.29 ± 0.06) when compared to those without retinopathy (0.26 ± 0.08). This difference was statistically significant ($p < 0.001$).

Also, the plateletcrit values were higher in patients with poorly controlled diabetes (0.28 ± 0.077) as compared to patients with a good control of diabetes (0.27 ± 0.092), which was statistically not significant ($p = 0.14$). This might be due to large difference between number of patients with poorly controlled diabetes ($n = 219$) and number of patients with good control of diabetes ($n = 81$).

Dermitas et al.[11] conducted a study to evaluate the association between hematological indices and diabetes, impaired glucose regulation, and microvascular complications of diabetes. They concluded that platelet indices were inexpensive and easily accessible markers of inflammation and tendency of coagulation, and microvascular complications.

In our study, when platelet indices were compared between uncontrolled diabetics without retinopathy and those with retinopathy, all the indices were significantly higher among those with retinopathy, showing that the presence of retinopathy had a significant effect on the platelet indices, irrespective of HbA1c levels.

## Conclusion

Our findings reveal that elevated platelet indices are associated with higher HbA1C levels, suggesting that poor glycemic control significantly impacts platelet activation. Additionally, our study also demonstrated that platelet indices were notably elevated in patients with diabetic retinopathy compared to those without, with this difference reaching statistical significance.

These results underscore the potential of using platelet indices as surrogate markers for the onset of retinopathy in diabetic patients, highlighting their importance in monitoring and managing diabetic complications.

## Orcid

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REVIEW ARTICLE

# Catheter-induced Left Main Dissection: A Minefield in Interventional Cardiology

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## Abstract

Coronary angiography is an invasive diagnostic procedure used to assess the coronary anatomy. Although rare, iatrogenic coronary artery dissection during coronary catheterization is a dreaded complication. Here we report the case of an 89-year-old patient diagnosed with acute coronary syndrome—Non-ST-segment elevation myocardial infarction, who underwent coronary angiography. During the coronary angiogram, he sustained a fatal left main coronary artery dissection. Here we discuss the interventions attempted to tackle the situation and a review of the approach to managing iatrogenic left main dissections.


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## Introduction

One known side effect of cardiac catheterization is iatrogenic catheter-induced dissection of the left major coronary artery, which can cause sudden vascular closure, myocardial infarction, and death. A diagnostic or guiding catheter, as well as the insertion of interventional hardware, may result in an iatrogenic LM dissection. The process is thought to be brought on by damage to the artery wall during stenting, balloon dilatation, contrast media injection, or catheter or wire advancement.[1]

Emergent intervention is necessary for the majority of patients with this condition. Prior to 1993, when the first successful percutaneous bailout left major coronary artery (LMCA) stenting procedure was carried out, the preferred course of treatment was urgent coronary artery bypass surgery (CABG).

Since it can result in an abrupt stoppage of blood flow to a significant portion of the myocardium supplied by the left anterior descending (LAD) and left circumflex (LCx) arteries, many patients with left main dissection have a rapid decline in condition, and most of them worsen even before being moved for CABG. Heart arrest and pump failure follow from this. As shown in our example, prompt percutaneous coronary intervention (PCI) appears to be a suitable and practical substitute if carried out by a skilled interventionalist. There may be terrible repercussions if mechanical support is not received right away.

The major problem faced with iatrogenic artery dissection during diagnostic coronary angiograms is wiring the true lumen of the dissected arteries for stenting and the increased risk of perforation. Prevention is better than a cure. Therefore, taking the right measures before inserting the diagnostic/guide catheter into the coronary artery ostium helps avoid such disastrous outcomes.[2]

## Case Description

An 89-year-old man arrived at the emergency room 2 hours prior to presentation, complaining of chest trouble. He was a known case of dilated cardiomyopathy with left bundle branch block (LBBB) who had undergone cardiac resynchronization therapy with defibrillator implantation in 2008, with pulse generator change twice in 2012 and 2018 because of the elective replacement indicator (ERI) of the pulse generator. On arrival, cardiac enzymes were elevated, and the ejection fraction was noted to be 35% with global hypokinesia of the left ventricle on echocardiography.

A coronary angiography was recommended for him, and a right radial access was acquired. The patient was diagnosed with arteria lusoria during the procedure, which made it difficult to enter the ascending aorta. Given the anatomical difficulty, the femoral approach was considered. Judkins right (JR) was used to cannulate the RCA, which was normal. The LMCA, which was cannulated, was accessed via the Judkins left (JL) route. After confirming the absence of dampening, contrast was injected. Unfortunately, the injection revealed contrast hanging in the LMCA with TIMI 0 flow to the LAD and TIMI 1 flow in LCx with a dissection flap from LMCA to LCx, suggesting coronary artery dissection in the LMCA occluding the LAD and spiral dissection in LCx (Fig. 1).

The patient complained of worsening chest pain, following which the ECG on the monitor showed ST elevations along with hemodynamic collapse. Immediately, the patient was put on inotropic, mechanical ventilatory, and intra-aortic balloon counterpulsation support. The wiring of the LCx and LAD true lumens was accomplished effectively. XIENCE Xpedition 3.5 × 48 mm was deployed in LCx, and SYNERGY stent 3.5 × 48 mm was deployed from the left main coronary artery to mid-LAD (Fig. 2).

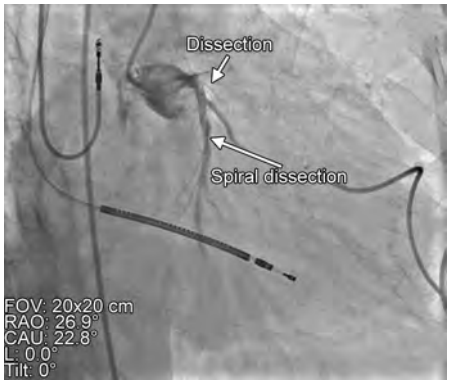


**Fig. 1:** RAO caudal view showing dissection of left main coronary artery (grade F) and spiral dissection (grade D) of left circumflex artery after contrast injection

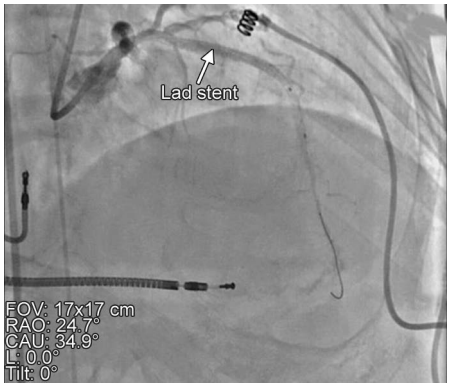


**Fig. 2:** RAO cranial view showing stent from left main coronary artery to mid Left anterior descending artery [LAD] and from mid to distal LAD

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**Table 1:** Studies on iatrogenic coronary artery dissection

| *Study* | *No. of patients* | *Incidence (%)* | *Percutaneous coronary intervention (%)* | *CABG (%)* | *Conservative (%)* | *Mortality (%)* |
|---|---|---|---|---|---|---|
| Dunning et al. 2000[3] | 9 out of 43,143 | 0.02 | 100 | | | Nil |
| Lee et al. 2004[4] | 10 out of 34,190 | 0.03 | 100 | | | Nil |
| Eshtehardi et al. 2010[5] | 38 out of 51,452 | 0.07 | 37 | 45 | 15 | 3 |
| Cheng et al. 2008[6] | 13 out of 18,400 | 0.071 | 84 | 7 | 9 | |
| B Gryko A et al. 2021[7] | 74 patients | 0.06 | 100 | | | |
| Raj et al. 2023[8] | 3 | | IVUS guided PTCA 100 | | | |
| Sumiyoshi et al. 2021[9] | 1 | | Straw Technique | | | |

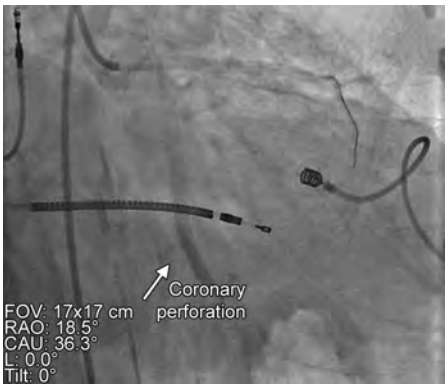


**Fig. 3:** RAO caudal view showing perforation of left circumflex artery (grade IV Ellis) after stenting of the left circumflex artery

However, stenting of the LCx led to a grade IV Ellis coronary artery perforation that led to pericardial tamponade and worsening of hemodynamic instability (Fig. 3). The patient underwent emergency pericardiocentesis, and 250 mL of hemorrhagic fluid was drained. A covered stent of size 4.0 × 19 mm was placed in the LCx that sealed off the perforation. Despite the above measures, the patient persisted to be in shock. He could not be resuscitated and succumbed shortly after the procedure.

## Discussion

It has been estimated that the clinical incidence of coronary artery dissection caused by catheterization is less than 0.1%. It can be retrograde or antegrade. Risk factors include left main disease and calcification, use of an Amplatz catheter, catheter manipulation, vigorous contrast injection, deep intubation of the catheter, dilated aorta (too short Judkins), variant anatomy of the coronary ostium, and vigorous deep inspiration. The catheter should be appropriately sized, positioned, and coaxially aligned. It can be prevented by properly training the operator to look for pressure dampening before contrast injection and to avoid roofing the catheter into the vessel wall. Regarding the choice of arterial access site for cardiac catheterization, there is no difference between the transradial and transfemoral approaches. The right coronary artery has catheter-induced dissections 50% more often than the left major artery (45%). This is because type I collagen makes up the LMCA's sinotubular junction, whereas type III collagen makes up the RCA's. The tensile strength of type I collagen is higher than that of type III collagen. Variation in the angle of origin of the RCA from the aorta can also contribute to frequent dissection in the right coronary artery. Because it threatens a wide area downstream from the injury, iatrogenic LMCA dissection is an emergency, and its treatment is contingent upon the distal vessel's patency and the dissection's extent of propagation. PCI is currently the most common treatment method for iatrogenic LMCA dissection that results in ischemia. PCI avoids the CABG-related delays and can restore coronary patency, preventing prolonged ischemia, which is associated with a higher risk of myocardial infarction and death. This is especially crucial for patients who are hemodynamically unstable.[10]

Three forms of iatrogenic aortocoronary dissection (IACD) are included in Eshtehardi's simplified classification based on the extension of left main (LM) ostial dissection: type I—a localized dissection in the LM ostium, type II—extension of the dissection from the LM into the LAD artery or LCx artery, and type III—extension of the dissection flap into the aortic root. In this case series, hemodynamic instability was noted in 21 individuals with type I dissection (Table 1). Nevertheless, seven of the 17 patients with type II or III dissections (41%) experienced hemodynamic instability, and five of them (29%) required cardiopulmonary resuscitation.[5]

In catheter-induced coronary dissection, blood flows into the artery's subendothelial layers when the endothelial cell layer is disrupted, which is the process of damage. It causes an intramural hematoma to form in the false lumen, which can compress the actual lumen and result in ischemia. The magnitude of the dissection and the degree of antegrade blood flow determine the prognosis in these situations. With the exception of those with left main coronary artery stenosis of greater than 50% and impaired TIMI flow in vessels larger than 2.5 mm, minor dissections are treated conservatively. Stopping the contrast injection and thinking about IABP implantation is advised if dissection is identified. A soft-tipped wire should be advanced into the actual lumen as far as possible. Emergency CABG must be taken into consideration if many attempts fail to access the actual lumen. Since it helps us detect the actual lumen and the dissection flap in patients who are hemodynamically stable, intravascular ultrasound (IVUS) has been used extensively in coronary intervention and will play a significant role in managing this problem.[8]

Stenting distal to the proximal vessel must be taken into consideration when the actual lumen has been accessed. If there is retrograde propagation into the ascending aorta with hemodynamic instability, the patient should be posted for emergency CABG. If the patient is hemodynamically stable, ancillary imaging, such as computerized tomography, transesophageal echocardiography, and magnetic resonance imaging to define the extent of the dissection, must be considered, based on which the decision for either surgery or conservative management can be taken. Stenting dissected vessels carries a high risk of propagation of intramural hematoma and stent malapposition. During stenting, less aggressive pre- and postdilation must be considered in view of hematoma propagation, longer stents must be considered, and care must be taken to avoid oversizing the stents. Two methods can be followed if the true lumen cannot be wired in hemodynamically stable patients: the STRAW and STAR-R techniques. If accessing the actual lumen is challenging, the subintimal transcatheter withdrawal technique, or STRAW technique, is employed. The parallel wire and STRAW technique can be used with the help of a microcatheter in the false lumen to aspirate intramural hematoma and subsequently wire the true lumen.[9]

STAR-R (subintimal tracking and re-entry technique)—contrast-guided STAR-R is a feasible option for vessel revascularization. This method was previously applied to chronic complete occlusion. This has the disadvantage of loss of side branches. In order to produce tubular dissection and establish a link between the true and false lumen, this involves injecting contrast into the dissection plane (hydrodynamic recanalization). In case of failure of hydrodynamic recanalization, a guidewire can be advanced into the true lumen to create mechanical recanalization to facilitate wiring from the false to true lumen.[11]

Iatrogenic aorta coronary dissection is the term used to describe retrograde dissection of the aorta. Its incidence ranges from 0.04 to 0.12%. To better direct the treatment of this rare condition, Dunning et al.[3] created three categories of aortocoronary dissection in 2000: class I for focal dissection limited to the sinus of Valsalva; class II for dissection that propagated less than 40 mm to the ascending aorta; and class III for dissection extending 40 mm or more to the ascending aorta. These authors suggested surgical therapy for class III dissections and stenting of the dissection entrance point for class I and class II dissections. Nevertheless, there have been a few recent reports of class III IACD cases—including those involving the aortic arch—being effectively treated with early detection and quick coronary stenting to seal the entrance point.[12]

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# A Young Male with Five Kidneys

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A 36-year-old male with chronic kidney disease (CKD) V (bilateral small-sized kidney) underwent ABO compatible first renal transplant from a maternal uncle in 1994 elsewhere. As a result of chronic allograft nephropathy (CAN), he lost his graft and reached end-stage kidney disease (ESKD) in 2001. He had no family history of kidney disease, and the native kidney disease is unknown.

After a short time on dialysis, he was grafted an ABO-compatible kidney with donor being maternal cousin in another hospital. He took triple immunosuppressants including cyclosporine A, mycophenolate mofetil, and prednisolone. The second allograft failed in 2021, and after a brief period on hemodialysis, he underwent a third transplant from a first cousin with us in July 2021. The graft functioned immediately, though it was a technical challenge to find a suitable vascular anastomosis. The allograft single renal artery was anastomosed at the origin of the right common iliac artery (Fig. 1).

The complement-dependent cytotoxicity (CDC), flow cytometry crossmatch, and donor-specific antibodies (DSA) were negative between donor and recipient, implying zero sensitization. He was inducted with a single dose of thymoglobulin 75 mg and other immunosuppressants such as prednisolone, tacrolimus, and mycophenolate mofetil. His discharge serum creatinine was 1.1 mg/dL, and urine examination was normal. In July 2024, serum creatinine was 0.84 mg/dL with normal routine urine examination. For immunological reasons, the first and second grafts were not removed to prevent sensitization.

A retransplant of a kidney becomes necessary when the previous graft fails. Most of the time, graft failure is due to recurrence of original disease such as IgA nephropathy, dense deposit disease, primary hyperoxaluria, calcineurin inhibitor (CNI) toxicity, noncompliance with medications, familial diseases, or *de novo* glomerulonephritis. The half-life of a graft is estimated depending upon human leukocyte antigen (HLA) matching,[1] compliance, and monitoring pharmacokinetic and pharmacodynamic properties of immunosuppressive agents. Lower urinary tract obstructions due to neurogenic bladder, transplant ureteric strictures and benign prostatic hypertrophy, or congenital abnormalities of the lower urinary tract such as urethral narrowing are not uncommon. As our patient had no sensitization issues from the previous transplants, the immunological risk was very low.

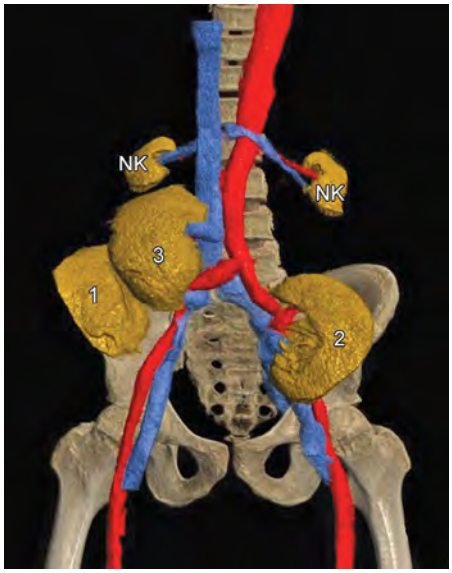


**Fig. 1:** Native kidneys (NK): 1, 2, and 3 are the transplanted kidneys, with 3 being the latest

Factors contributing to higher immunological risk in retransplantation are prior sensitization, HLA mismatch, acute rejection, mixed rejection, delayed graft function, positive crossmatch, and previous rejection episodes.

A pretransplant computed tomography (CT) angiogram of the lower abdomen and iliac vessels is important in choosing the site for vascular anastomosis and space for the allograft as in our case.[2]

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# "The Cancer that Carried the Chalk"—NXP2+ Paraneoplastic Dermatomyositis Unleashing Calcinosis Cutis and Peripheral Neuropathy

Jayaram Saibaba[1*], Nidhish Chandra[2], Deepak Amalnath[3], DKS Subrahmanyam[4]

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Paraneoplastic dermatomyositis (DM) is a rare but significant manifestation of underlying malignancies, often presenting with unique clinical challenges. Idiopathic inflammatory myopathies are a heterogeneous group of disorders that cause muscle weakness and multiorgan involvement, affecting the skin, heart, lungs, and joints.[1] Dystrophic calcification causing calcinosis cutis occurs in 20–40% of juvenile DM cases.[2]

A 36-year-old female diagnosed with mucinous cystadenocarcinoma of the ovary and treated with surgery and chemotherapy 1 year prior presented with a 3-month history of dysphagia, generalized weakness, and inability to walk. She had proximal muscle (shoulder and hip girdle) weakness with Medical Research Council (MRC) grade 2/5 power and bulbar weakness. On examination, she exhibited neck flexor and proximal muscle weakness, heliotrope rash (Fig. 1A), and a holster sign (Fig. 1B) on the bilateral proximal thighs, leading to a clinical suspicion of DM. Baseline hemogram, serum electrolytes, and CA-125 levels were normal, but erythrocyte sedimentation rate (ESR), C-reactive protein, and creatine kinase levels were elevated (1046 IU/L; reference range <145 IU/L). A multiplex line blot immunoassay tested positive for anti-NXP2 and anti-Ro52 antibodies, suggesting paraneoplastic DM. The patient was treated with immunosuppression and showed improvement.

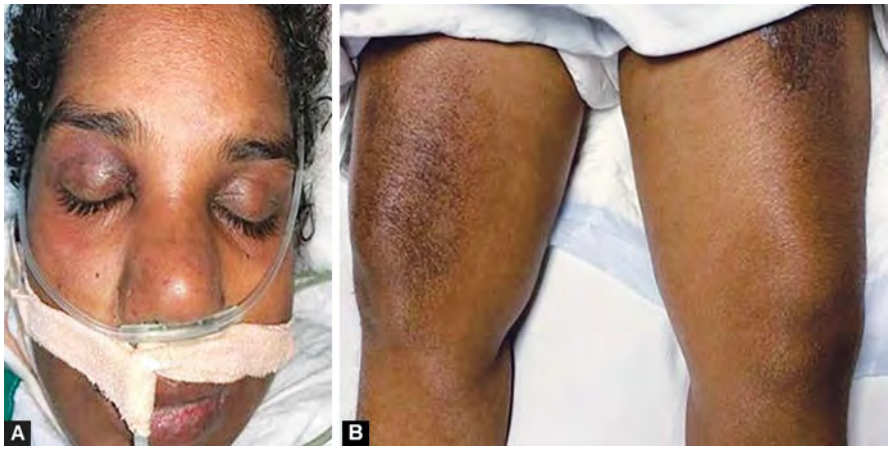
Ten months later, she presented with new-onset swellings in both arms, numbness in the medial two fingers of both hands, and anasarca of 3 months' duration. Local examination revealed 4 × 4 cm hard, painless subcutaneous swellings on both arms, mobile in all directions, with similar lesions on the abdomen and bilateral thighs. Bilateral Froment's sign and the card test were positive, but the median nerve was intact. Nerve conduction study revealed bilateral ulnar sensorimotor axonal peripheral neuropathy. The coexistence of DM and axonal peripheral neuropathy may be termed Neuromyositis. Chest X-ray PA view showed calcification in the right axilla (Fig. 2A). X-rays of the lower abdomen and thigh showed calcifications, and noncontrast computed tomography (CT) of the abdomen confirmed diffuse subcutaneous calcifications (Figs 2B and C). Fine needle aspiration cytology (FNAC) from the lesion showed the presence of calcium deposits consistent with calcinosis cutis, which was confirmed by Giemsa and von Kossa staining (Figs 2D and E). Myoblot testing was positive for anti-NXP2 and anti-Ro52.

The patient was treated with intravenous (IV) methylprednisolone pulse therapy (15 mg/kg/day × 5 days) followed by oral steroids and IV immunoglobulin 2 gm/kg given over 5 days. Her muscle power improved to MRC grade 4/5, and she was able to walk with support. During follow-up, a steroid-sparing agent (azathioprine) was added, and steroid dosage was gradually tapered.

Anti-NXP2 and anti-TIF1γ antibodies are linked to the presence of underlying malignancies. Dystrophic calcinosis typically occurs in collagen vascular diseases despite normal calcium and phosphate metabolism. Proposed mechanisms include tissue damage, inflammation, or necrosis, which trigger alkaline phosphatase release from damaged lysosomes. However, the exact etiology of dystrophic calcinosis remains unclear.[3]

Concomitant involvement of the peripheral nervous system in DM, termed "Neuromyositis", was first introduced by Senator in 1893.[4] Peripheral neuropathy may be one of the important extramuscular manifestations in patients with DM. The association between inflammatory myopathy and peripheral neuropathy is unclear. Matsui et al. reported two adult DM patients with polyneuropathy, showing vasculitis and vascular endothelial growth factor (VEGF) overexpression in muscle, skin, and nerve tissues. Other VEGF-associated factors related to vasculitis or capillary endothelial lesions may play a significant role in cutaneous and neurological manifestations of DM.[5]

In summary, DM can rarely lead to calcinosis cutis due to dystrophic calcification. The coexistence of DM and peripheral




**Figs 1A and B:** Heliotrope rash (A) and holster sign in the anterolateral aspect of the thigh (B)

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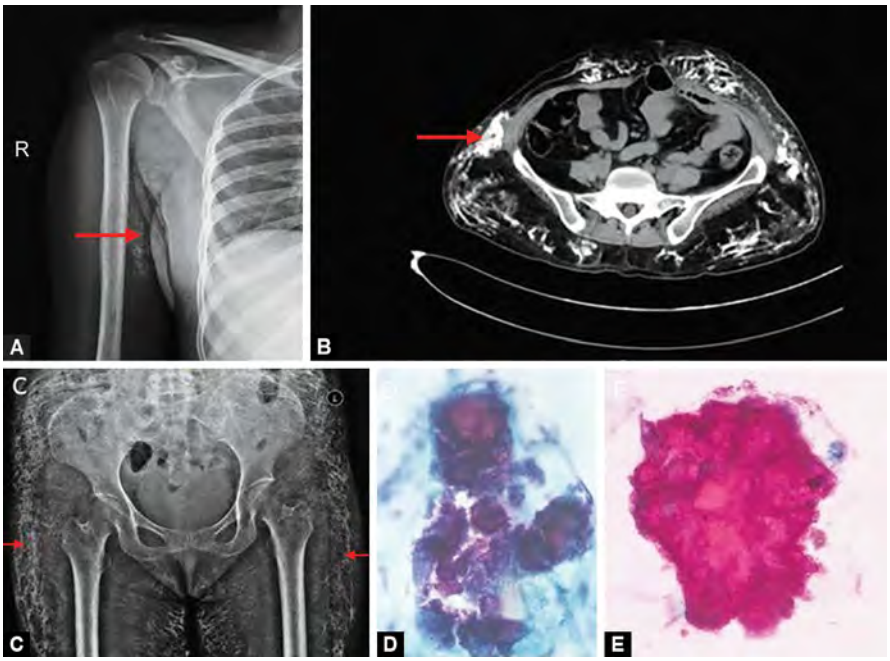


**Figs 2A to E:** Chest X-ray posteroanterior (PA) view showing calcification in the right axilla (A); contrast CT of the abdomen showing diffuse calcification in subcutaneous plane (B) (marked by arrow); X-ray of the pelvis and thigh showing calcinosis cutis (C) (marked by arrow); FNAC of the right axilla showing amorphous calcium deposition in Giemsa stain (D); and von Kossa stain (E)

neuropathy (neuromyositis) responded well to steroids, IV immunoglobulin, and immunosuppressive therapy. This case highlights that late and rare presentations of paraneoplastic DM can occur even after the successful treatment of primary ovarian cancer. A high level of clinical suspicion and prompt treatment are essential for the optimal management of these patients.

## Authors' Contributions

Jayaram Saibaba, Nidhish Chandra, Deepak Amalnath, and DKS Subrahmanyam were involved in the conception, organization, and execution of the research project. All authors participated in writing the first draft of the manuscript as well as its review and critique.

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## Conflict of Interest

None.

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# Black Pleura Sign in Silicosis

Ramakant Dixit

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Black pleura sign is a radiological sign seen on chest skiagram or high-resolution computed tomography (HRCT) of chest where a strip of peripheral black line all around the lung periphery is seen just beneath the pleura separating the bony rib cage and the high-density calcified lesions in the lung parenchyma. This sign is a classic feature in patients with pulmonary alveolar microlithiasis (PAM), appearing as a strip of peripheral lucency (darker area) that runs tangentially beneath the ribs, contrasting sharply with the adjacent dense, calcified lung tissue.[1] Despite its designation, the "black pleura" sign actually represents the subpleural sparing of underlying diffuse pulmonary calcification that occurs centrally within the alveoli of the secondary pulmonary lobules. First described on plain radiographs as a means of identifying alveolar microlithiasis, the sign is also demonstrable on chest HRCT.

Pulmonary alveolar microlithiasis is a rare, chronic disease of unknown or poorly understood origin, characterized by a discrepancy between clinical symptoms and radiological findings. The condition involves the intra-alveolar accumulation of innumerable diffuse calcium phosphate microliths (calcospherites) throughout the lung parenchyma, with a predilection for the lower and mid zones, manifesting radiologically as dense micronodular opacities with a characteristic "sandstorm" appearance. The "black pleura sign" manifests as a vertical linear radiolucency between the ribs and lung parenchyma, typically indicating subpleural cystic changes identifiable on HRCT or pathological assessment.[2]

A 23-year-old unmarried female was admitted with complaints of shortness of breath and cough for the last 6 months and low-grade fever for last 15 days. These symptoms were gradual in onset and progressively increasing over time. For the last 3 years, she worked in a stone-grinding factory where white stone was processed, with her daily shift lasting 8 hours. On physical examination, she had anemia, but no clubbing, lymphadenopathy. Respiratory system examination revealed increased respiratory rate (22 per minute) and normal breath sounds. Her chest X-ray revealed bilateral diffuse nodular shadows. Her induced sputum was negative for acid-fast bacilli. Routine investigations of blood and urine were also normal. CT scan of the chest revealed bilateral nodular lesions at both lung fields along with areas of ground-glass haziness (Fig. 1). A thin black rim at the peripheral lung fields was clearly evident suggesting "black pleura sign" (Fig. 2). Based on above features, she was diagnosed with acute silicosis based on occupational history and suggestive radiological findings. She received symptomatic treatment with partial improvement in symptoms.

Occupational exposure to silica dust leads to silicosis, an incurable chronic lung disease characterized by widespread small, calcifying fibrous nodules, predominantly found in the upper lung zones. Eggshell calcification and pleural plaques are other findings. Development of emphysema and bullae is also described in silicosis patients, which is independent of smoking and increases the risk of pneumothorax. Para-septal emphysema is classically subpleural in location and has also been demonstrated in silicosis.[3]

Subpleural sparing in HRCT chest is described as pathological lesions affecting lung that spares the extreme peripheral margins abutting the pleura or chest wall on cross-sectional imaging. This finding has a variety of causes, including idiopathic, inflammatory, infectious, inhalational, cardiac,

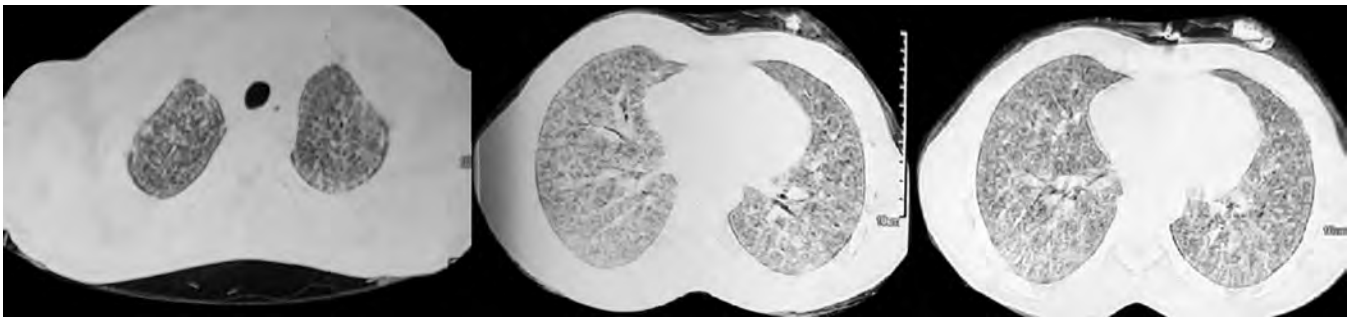

**Fig. 1:** CT scan chest showing bilateral nodular lesions with areas of ground-glass haziness

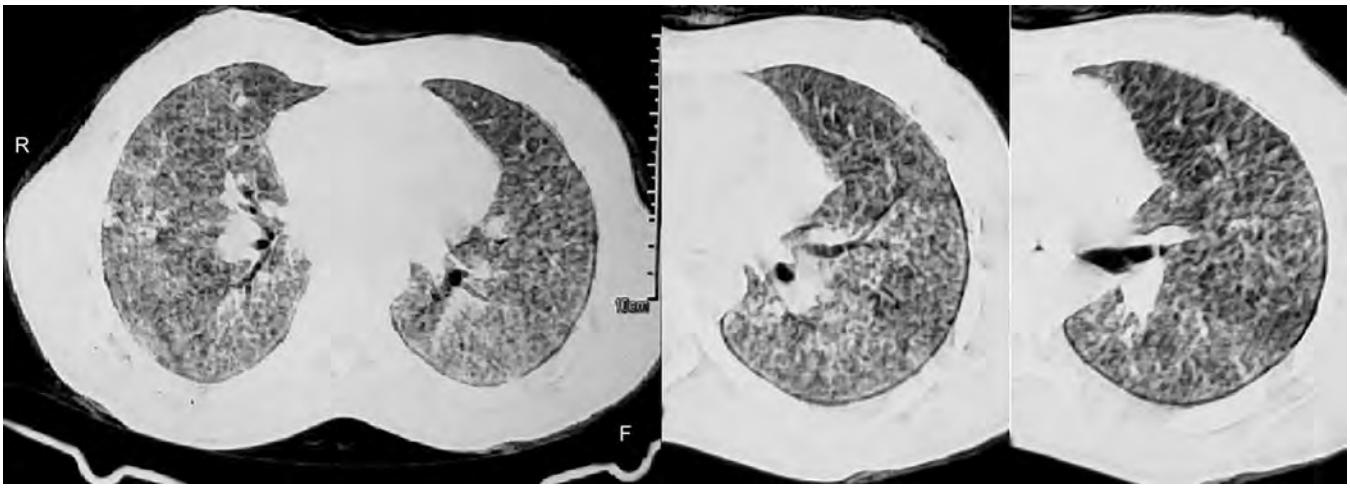


**Fig. 2:** CT scan chest showing peripheral subpleural linear sparing of lesions producing "black pleura sign"

traumatic, and bleeding disorders. Among the specific conditions, subpleural sparing (peripheral sparing) may be seen in nonspecific interstitial pneumonia, organizing pneumonia, pulmonary alveolar proteinosis, diffuse alveolar hemorrhage, vaping-associated lung injury, cracked lung, pulmonary edema, *Pneumocystis jirovecii* pneumonia, pulmonary contusion, and COVID-19 pneumonia.[4]

Subpleural sparing in PAM is notable to cause black pleura sign because of innumerable calcified nodules, making a striking density difference at the lung periphery. Since silicosis is also characterized by high-density pulmonary nodules and emphysematous changes, subpleural sparing in this condition may also produce "black pleura sign" as seen in the present case. Clinical history, including occupation, clinical presentation, radiological pattern, and distribution of lesions and other findings, usually helps to suggest the appropriate diagnosis.

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# Can the Dying Clinical Medicine be Resuscitated?


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To the Editor,

With great interest, we have read the editorial titled "Is Clinical Medicine Dying?" by Dr Bhagwat.[1] We agree that the sociocultural environment has changed. The investigations, laboratory, and/or imaging are usually complementary to the clinical judgment to reach a diagnosis. They are valuable to confirm the diagnosis in differential scenarios. We want to share our responses and perspectives regarding the current "dying art of clinical medicine."

## Additional Barriers

### Infotechnological Boom

Rampant availability and accessibility of medical knowledge, media connectivity, and a comparative mentality among patients have led to many misconceptions and misinterpretations about the rationale behind investigations. Our experience suggests that in many instances, despite adequate counseling and disclosure of clinical information, patients, rather than their relatives, demand additional investigations based on "their" experiences and knowledge from internet sources.

### Resources Utilized or Misused

Various schemes, periodic health check-ups, and health-care policies offer a package of various investigations. Various associations and health-care institutions conduct various investigational camps. Yet again, the patient as a "whole" is seldom evaluated. The rationale and need for investigations are often misaligned and misdirected. Is the human body a car or a machine that would need a routine/frequent servicing irrespective of ailment? Furthermore, even the minor variations in these reports are treated or further investigated for the life.[2] Many patients themselves feel that the cycle of demand and supply is better than clinical correlation. Workload and level of experience, and confidence, also contribute to the burden of unnecessary investigations and admissions.[3] Treatment protocols are also guided by reports rather than clinical judgment. The scenario is the same at many hospitals during the preoperative evaluation of a patient.[4]

### Supervested Interests

"Age over Beauty" is a lost concept. The amount of work and income generated is considered more important than the quality of work and appropriate health care delivery. This may occur due to the changing perspectives of administrative authorities filled with freshly passed MBAs or similar.

### Great Need for Good "Clinical Teachers"

The race of good teachers are thinning out. The new generation is preoccupied with other commitments/interests of life and is less experienced, so the concept of peer learning is also ignored. There are fewer role models for the current generation of medical students to look up to and grow.

## Steps to Improve the Scenario

As rightly mentioned by Dr Bhagwat, communication plays a vital role in managing any patient.

The National Medical Commission has implemented competency-based medical education (CBME). The improved focus on actual skill training across the cognitive, psychomotor, and affective domains is a boon. Programs like skill training, AETCOM, clinical clerkship, family adoption, district residency program, etc., are aimed at training the Indian medical graduates (IMGs) in real-life scenarios. Formative assessments and reflective writing will add to change the outlook of students as mentioned in the Editorial. The CBME also focuses on improving the inter-doctor (peer) relationships, which may improve the learning and application of appropriate clinical knowledge.[5]

### Documentation

Litigations and strained doctor–patient relationships are to some extent inevitable in this era of rat-race and media boom. Habit of documentation (all aspects, adequate and relevant history, past history, current clinical findings, correlation with investigations, and appropriate proposed plan of action) is an equally important indicator that a competent IMG has well-applied his/her knowledge and skills.

Small steps together can make this journey achievable. We hope the following steps can help us achieve the vision proposed by the NMC:

- Adequate time, effort, manpower, and management.
- Paradigm shift in perceptions.
- Adequate engagement by all stakeholders; most importantly the students, parents and patients.
- Faculty development aligned with the changing sociocultural environment of medical education.

## Author Contributions

AS Rayate and BS Nagoba contributed to the idea behind the manuscript, literature search, collection, writing the paper, modification of content and final approval of the draft.

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