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Original Research Article

Study of Candida infections in hospitalized COVID-19 patients: An experience at a dedicated COVID hospital in Central India

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Abstract

Background: Candida infections have become increasingly common in patients with COVID-19, particularly in those with comorbidities or requiring prolonged hospital stays. The growing incidence of fungal infections in COVID-19 patients is a cause for concern due to the challenges in timely diagnosis and antifungal resistance. This study aims to evaluate the prevalence, species distribution, and antifungal resistance patterns of Candida isolates from COVID-19 patients.

Materials and Methods: A total of 128 clinical samples were collected from COVID-19 patients admitted to a tertiary care hospital. The samples were subjected to direct microscopy, culture, and species identification using standard microbiological techniques. Antifungal susceptibility testing was performed using the E-test MIC method on RPMI 1640 agar, with fluconazole, amphotericin B, and caspofungin being the primary antifungal agents tested.

Results: Out of 128 samples, 84 (65.62%) were positive for Candida species. The most commonly isolated species were *Candida tropicalis* (40.47%), *Candida albicans* (32.14%), *Candida glabrata* (14.28%), *Candida krusei* (7.14%), and *Candida guilliermondii* (5.95%). Candida infections were more prevalent in male patients (71.62%) and those aged over 60 (87.5%). Comorbid conditions such as diabetes mellitus (65.48%) and hypertension (58.73%) were associated with higher infection rates. Antifungal resistance testing revealed resistance to fluconazole in *Candida glabrata*, *Candida krusei*, and *Candida guilliermondii*, whereas *Candida albicans* and *Candida tropicalis* exhibited moderate resistance. All isolates remained susceptible to amphotericin B and caspofungin.

Conclusion: Candida infections are prevalent among COVID-19 patients, especially in those with comorbidities. Antifungal resistance is emerging, particularly to fluconazole, indicating the need for regular surveillance and tailored antifungal therapy. Early identification and appropriate treatment of Candida infections are crucial to improve patient outcomes in the ongoing pandemic.

Keywords: Candida species, COVID-19, Antifungal resistance, Fluconazole, Candida albicans, Candida tropicalis, Diabetes, Hypertension, Tertiary care hospital.

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1. Introduction

The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, has had a profound impact on global health, healthcare systems, and patient outcomes. Initially recognized for its respiratory manifestations, COVID-19 has revealed a broader spectrum of complications, including secondary infections. Among these, fungal infections, particularly those caused by Candida species, have emerged as significant concerns in hospitalized COVID-19 patients.[1,2]

Candida species are opportunistic pathogens that primarily affect immunocompromised individuals. They are known to cause a range of infections from superficial to systemic, including candidemia, which is associated with high morbidity and mortality rates.[3] In the context of COVID-19, several factors contribute to the heightened risk of Candida infections. Prolonged hospital stays, mechanical ventilation, and the use of broad-spectrum antibiotics and corticosteroids-common in the management of severe COVID-19 cases-create an environment conducive to fungal overgrowth.[4,5]

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The prevalence of Candida infections in COVID-19 patients varies across different geographical regions and healthcare settings. Early studies from Italy and the USA reported a notable increase in Candida infections among critically ill COVID-19 patients.[6,7] In contrast, data from India, while also indicating a rise in Candida infections, highlight regional variations in species distribution and antifungal resistance patterns.[8,9] For instance, *Candida albicans* and *Candida tropicalis* are commonly identified in Indian studies, whereas other regions may report different prevalent species.[10,11]

The rise in Candida infections during the COVID-19 pandemic has been accompanied by concerns about antifungal resistance. The overuse of antifungals, combined with the emergence of resistant Candida strains, poses a significant challenge to effective treatment.[12] In particular, resistance to commonly used antifungals like fluconazole and itraconazole has been documented in various studies, highlighting the need for ongoing surveillance and tailored antifungal therapy.[13,14]

Given the increased incidence of Candida infections and the potential for resistance development, it is crucial to investigate the prevalence, species distribution, and antifungal susceptibility patterns in specific settings. This study aims to address these gaps by providing detailed insights into Candida infections among hospitalized COVID-19 patients at Chirayu Medical College & Hospital in Bhopal, Central India. By focusing on a dedicated COVID-19 hospital, this research seeks to enhance our understanding of current trends and inform effective management strategies for Candida infections in the context of the ongoing pandemic.[15,16]

2. Materials and Methods

2.1. Study design

This retrospective secondary data-based study (Laboratory data) was conducted in Department of Microbiology at Chirayu Medical College & Hospital, Bhopal, from January 2021 to May 2021.

2.2. Study population

All patients admitted to the hospital during the study period with confirmed COVID-19 (RT-PCR positive) and from whom Candida species were isolated were included.

2.3. Sample collection and transport

Clinical samples, including blood, endotracheal aspirates, high vaginal swabs, nasal swabs, penile swabs, pus, sputum, throat swabs, tongue swabs and urine, were collected following standard procedures and transported immediately to the laboratory for processing.

2.4. Processing and identification

Samples were processed using Sabouraud dextrose agar (SDA) with incubation at 25°C and 37°C for up to 4 weeks. Identification of Candida species was based on colony morphology, Gram staining, germ tube formation, Dalmau plate culture on Corn Meal Agar, and sugar assimilation and fermentation tests (Glucose, Maltose, Sucrose, Lactose, Galactose, Mellibiose, Cellobiose, Xylose, Raffinose, Trehalose and Dulcitol sugar disks were obtained from Hi-Media, Mumbai).

2.5. Antifungal sensitivity testing

The minimal inhibitory concentration (MIC) for amphotericin-B, fluconazole, itraconazole, voriconazole, and caspofungin was determined using the E-test method on RPMI 1640 agar plates. The fungal suspensions were adjusted to a 0.5 McFarland standard and inoculated onto the plates before placing the E-strips (procured from Hi-Media, Mumbai). The MIC values obtained from the E-test method were interpreted according to CLSI M27 (4th Edition, 2017) and CLSI M60 (2nd Edition, 2020) guidelines, as well as the EUCAST Antifungal Clinical Breakpoint Table v10.0 (effective from 2020-02-04). Isolates were categorized as Susceptible (S), Susceptible Dose-Dependent (SDD), or Resistant (R) based on MIC breakpoints for each antifungal agent tested.

2.6. Criteria for differentiating candida colonization and infection

To differentiate colonization from true infection, clinical correlation and microbiological criteria were both considered. For sterile body fluids such as blood and deep respiratory specimens (e.g., endotracheal aspirates), any Candida isolation was considered significant in the presence of relevant clinical signs and symptoms (fever, leukocytosis, Leukopenia, or radiological findings).

For non-sterile sites such as urine and sputum, repeat sample testing was performed. Candida was considered pathogenic only when the same species was repeatedly isolated and correlated with persistent or worsening clinical signs (e.g., fever, unresponsive to antibacterial therapy), laboratory abnormalities (elevated CRP or procalcitonin), and response to antifungal therapy. In the absence of such findings or if isolated only once without supportive clinical features, the isolate was considered a colonizer.

2.7. Demographic data

Patient demographic data were extracted from medical records and analyzed.

3. Results

In this study, a total of 128 clinical samples from COVID-19 patients were analyzed to determine the prevalence and

species distribution of Candida infections, as well as their antifungal susceptibility patterns.

Table 1 shows the gender-wise distribution of Candida infections. A total of 128 samples were received, out of which 84 (65.62%) were positive for Candida. The isolation rate was higher in males (71.62%) compared to females (57.41%).

Table 1: Gender-wise distribution of *Candida* Infections

Gender	Samples Received	*Candida Spp.* Isolated	Percentage of *Candida* Isolates
Male	74	53	71.62%
Female	54	31	57.41%

Table 2: Distribution of *candida* infections by age group

Age Group (Years)	Number of Samples Received	Number of Candida Species Isolated	Percentage of Candida Species Isolated (%)
< 1	10	2	20.00%
1-10	15	5	33.33%
11-20	20	16	80.00%
21-30	25	18	72.00%
31-40	20	16	80.00%
41-50	16	9	56.25%
51-60	14	11	78.57%
> 60	8	7	87.50%

Table 3: Distribution of *candida species* by sample type

Sample Type	No Growth	*C. albicans*	*C. glabrata*	*C. tropicalis*	*C. krusei*	*C. lusitaniae*	*C. guilliermondii*	Total
Blood	0	0	0	1	0	0	1	2
Endotracheal Aspirates	1	0	0	4	0	0	1	6
High Vaginal Swabs	9	3	2	1	0	0	2	17
Nasal Swabs	7	0	0	0	1	0	1	9
Penile Swabs	2	0	0	0	0	0	0	2
Pus	2	0	0	1	0	0	2	5
Sputum	5	1	0	1	0	0	2	9
Throat Swabs	16	8	4	2	1	0	0	31
Tongue Swabs	1	2	0	0	0	0	0	3
Urine	1	13	0	24	1	2	3	44
Total	44	27	6	34	3	2	12	128

Fisher's Exact Test; Fisher's Exact Test value = 99.104; DOF = 54; P Value = 0.0001784

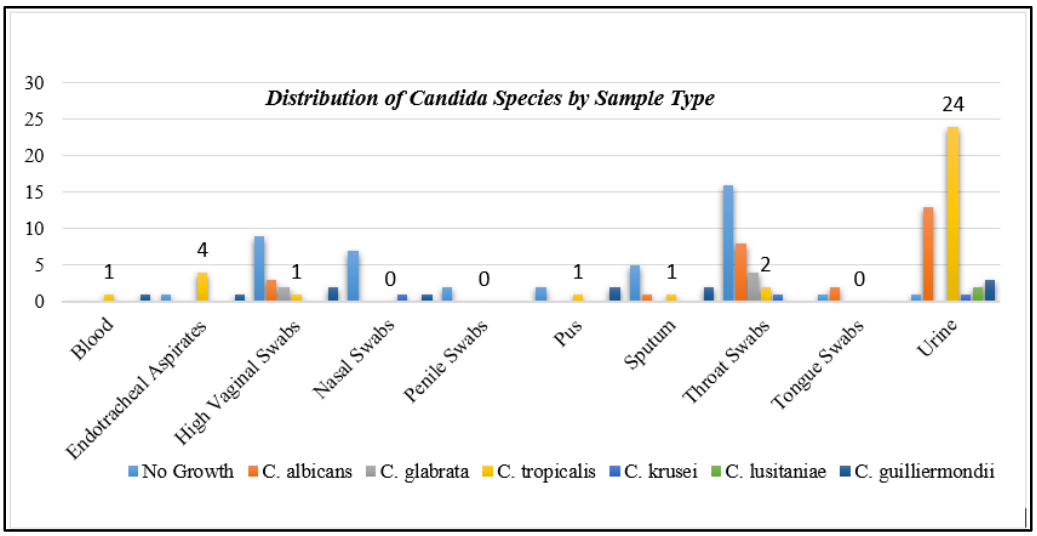
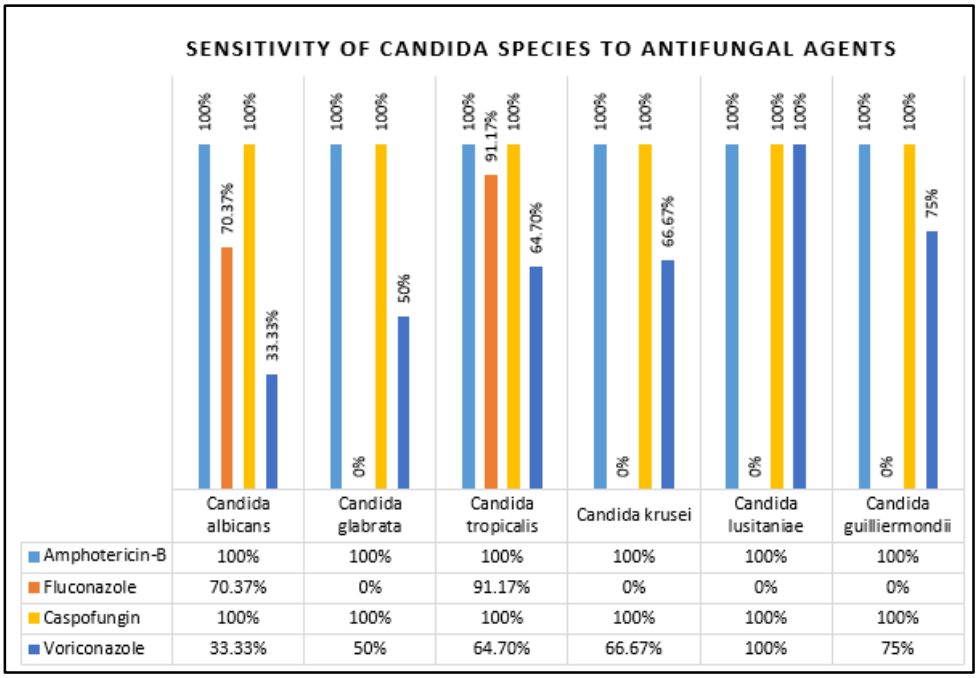


Figure 1: The bar chart shows the distribution of *Candida* species and no-growth outcomes across sample types, with *C. tropicalis* dominating in urine samples and *C. albicans* prevalent in throat swabs.



	Candida albicans	Candida glabrata	Candida tropicalis	Candida krusei	Candida lusitaniae	Candida guilliermondii
Amphotericin-B	100%	100%	100%	100%	100%	100%
Fluconazole	70.37%	0%	91.17%	0%	0%	0%
Caspofungin	100%	100%	100%	100%	100%	100%
Voriconazole	33.33%	50%	64.70%	66.67%	100%	75%

Figure 2: The Bar chart illustrates that all species exhibited 100% sensitivity to Amphotericin-B and Caspofungin, confirming these as the most effective agents. *Candida albicans* showed moderate sensitivity to Fluconazole (70.37%) and lower responses to Itraconazole (40.74%) and Voriconazole (33.33%). *C. tropicalis* demonstrated high sensitivity to Fluconazole (91.17%) and moderate sensitivity to Itraconazole (61.76%) and Voriconazole (64.70%). In contrast, *C. glabrata*, *C. krusei*, *C. lusitaniae*, and *C. guilliermondii* were completely resistant to Fluconazole with variable sensitivity to Voriconazole and Itraconazole.

Table 2 shows the age-wise distribution of Candida infections, with the number of samples received, the number of Candida species isolated, and the percentage of isolation for each age group: < 1 year (20.00%), 1-10 years (33.33%), 11-20 years (80.00%), 21-30 years (72.00%), 31-40 years (80.00%), 41-50 years (56.25%), 51-60 years (78.57%), and > 60 years (87.50%).

Table 3 shows the distribution of *Candida* species and No growth outcomes across clinical sample types. Out of 128 samples, 44 showed no growth, while the remaining 84 yielded *Candida* isolates. Among the identified species, *Candida tropicalis* was the most common, comprising 34 isolates (primarily from urine samples, with 24 isolates). *Candida albicans* followed with 27 isolates, predominantly found in throat swabs (8 isolates). Less frequently identified species included *Candida glabrata* (6 isolates), *Candida krusei* (3 isolates), and others. This analysis highlights the importance of recognizing the diversity of *Candida* species in clinical samples to ensure effective treatment strategies.

4. Discussion

The present study presents rich insights into the prevalence, species distribution and antifungal resistance patterns of Candida infections in hospitalized COVID-19 patients Central India. These findings are consistent with global and regional work, and point to some challenges for species diversity and antifungal susceptibility of critically ill patients with Candida infections. The most frequent isolated species was Candida tropicalis (40.47%) and Candida albicans (32.14%). This differs from previous studies such as by Niyas et al. (2021) in Kerala, South India, where the Candida albicans was the most prevalent species. Ours may be explainable by regional distribution of species or differences in patient demographics, treatment protocols, and hospital environment, which may increase the incidence of Candida tropicalis. Furthermore, candidemia with Candida glabrata and Candida krusei are intrinsically resistant to fluconazole, identifies the concern of fluconazole resistance in our area.[2]

Our results are consistent with a study by Rovina et al. (2022) in Europe, which reported high prevalence rates of Candida infections in critically ill COVID-19 patients. However, the European cohort exhibited a lower incidence of *Candida glabrata* and *Candida krusei* infections compared to our findings, suggesting possible regional variations influenced by local healthcare practices, antibiotic stewardship, and infection control measures. The global rise in Candida infections, particularly during the COVID-19 pandemic, has been attributed to factors such as prolonged hospitalization, the use of broad-spectrum antibiotics, and corticosteroids, which create favorable conditions for fungal overgrowth.[5]

Our study shows a complex landscape of resistance of Candida species in the context of the COVID-19 pandemic, with the antifungal susceptibility profiles observed in the study presenting an urgent need for an individualized treatment protocol that is based on species-specific susceptibility testing. An azole class antifungal, fluconazole has served as first line treatment for Candida infections for

over two decades due to its broad spectrum of activity and oral availability. Nevertheless, the increased and prolonged use of fluconazole, especially in critically ill patients, has led to the observed rise in resistance of several Candida species. Additionally, in our study, Candida glabrata, Candida krusei, and Candida guilliermondii exhibited complete resistance to fluconazole, which are significant concerns, since these species are frequently implicated in bloodstream infections (candidemia) and invasive candidiasis.

The increasing recognition of *Candida glabrata* for its multidrug resistance (MDR) traits has raised alarms in clinical settings. This species exhibits reduced susceptibility to fluconazole due to alterations in the ergosterol biosynthesis pathway, the target of azoles. Our cohort's complete resistance of *Candida glabrata* to fluconazole aligns with existing literature that underscores the necessity for vigilant susceptibility testing and the exploration of alternative therapies for effective management. *Candida krusei* is intrinsically resistant to fluconazole due to a naturally occurring mutation in the gene encoding the target enzyme (lanosterol 14α-demethylase). The presence of *C. krusei* in our study highlights the importance of recognizing its natural resistance, prompting clinicians to consider alternative antifungal agents early in treatment. Notably, *Candida guilliermondii* also exhibited resistance to fluconazole, a trend consistent with global observations indicating increasing fluconazole resistance in this species. The resistance mechanisms may include the overexpression of efflux pumps and mutations in ergosterol biosynthesis genes, complicating the treatment landscape, particularly in regions where fluconazole remains a commonly prescribed antifungal agent.[1,5]

Moreover, our study identified Candida lusitaniae, which demonstrated resistance to both fluconazole and itraconazole. Traditionally considered sensitive, this emerging non-albicans species poses significant clinical challenges, particularly among immunocompromised patients. The resistance in *C. lusitaniae* is attributable to mechanisms such as overexpression of efflux pumps and mutations in the ERG11 gene, mirroring resistance patterns observed in other Candida species. In contrast to the growing resistance to azoles, our study found that echinocandins (such as caspofungin) and amphotericin B remain highly effective against most Candida species, with 100% susceptibility across all isolates. Echinocandins inhibit β-1,3-glucan synthesis, a crucial component of the fungal cell wall, and have become the recommended first-line therapy for invasive candidiasis, especially for species like *Candida glabrata* and *Candida krusei*, which demonstrate resistance to fluconazole. Despite the increasing reports of reduced susceptibility to echinocandins, particularly in *Candida glabrata* due to mutations in the FKS1 and FKS2 genes, our cohort showed no resistance to caspofungin, highlighting the continued utility of echinocandins as a cornerstone of antifungal therapy in critically ill patients.[1]

Amphotericin B, a polyene antifungal that disrupts the fungal cell membrane by binding to ergosterol, also demonstrated complete efficacy in our study. Despite its associated nephrotoxicity, amphotericin B remains crucial for treating resistant Candida infections, especially in resource-limited settings where echinocandins may not be readily available. Our findings align with global data that demonstrate the broad efficacy of amphotericin B against various Candida species, including those resistant to azoles. The antifungal resistance patterns observed in our study highlight the pressing need for robust antifungal stewardship programs. Misuse or overuse of antifungal agents, especially azoles, exacerbates resistance trends, complicating treatment and increasing morbidity and mortality in critically ill patients.

The impact of COVID-19 on *Candida* infections, particularly in patients without prior immunosuppression, has been a point of concern. Studies like that of Arastehfar et al. (2020) emphasize that severe COVID-19, with its prolonged hospitalizations and the extensive use of invasive medical devices, creates a unique environment conducive to fungal infections. Our findings echo this, as we identified *Candida* infections in patients who were not traditionally immunocompromised, suggesting that COVID-19 itself may be a risk factor for fungal infections. This points to the need for heightened vigilance and early detection in all critically ill COVID-19 patients, regardless of their immunological status.[3]

Candida infections were more common in males (71.62%) than females (57.41%). This trend contrasts with some studies where females are more susceptible, but may reflect gender differences in COVID-19 severity, as males are generally at higher risk for severe outcomes and secondary infections.

The highest *Candida* isolation rate was observed in the elderly (>60 years) group (87.5%), followed by 11-20 and 31-40 years (80%). Younger age groups, such as under 1 year (20%) and 1-10 years (33.33%), showed significantly lower infection rates, consistent with more robust immune responses in children. The higher rates in adults could be linked to the severity of COVID-19 and the use of immunosuppressive treatments.

Given the variability in species prevalence and resistance patterns, it is essential to perform routine antifungal susceptibility testing for all Candida isolates to guide appropriate therapy. Prioritizing species identification in clinical settings can enhance treatment efficacy; for example, *Candida glabrata* and *Candida krusei* infections should prompt immediate consideration of echinocandins or amphotericin B. The rise in antifungal resistance necessitates stringent antifungal stewardship programs that monitor prescribing practices, ensure judicious use of antifungals, and promote education regarding emerging resistance patterns. The growing resistance to existing antifungals underscores

the need for developing novel agents with new mechanisms of action. Emerging drugs, such as ibrexafungerp (an oral glucan synthase inhibitor), show promise in early trials and may offer alternative treatment options.

In summary, the increasing incidence of *Candida* infections, the presence of fluconazole-resistant species like *Candida glabrata* and *Candida krusei*, and the potential for antifungal resistance in COVID-19 patients represent significant challenges for clinicians. Early diagnosis and tailored antifungal therapy are essential in improving patient outcomes. Our study adds to the growing evidence that calls for enhanced vigilance, continued research, and the development of effective management strategies for fungal infections in the COVID-19 Pandemic.

5. Conclusion

In conclusion, our study highlights the critical and often overlooked issue of *Candida* infections in COVID-19 patients, particularly those in intensive care settings. The rising incidence of these infections, varying species distribution, and the emergence of antifungal resistance present significant challenges for healthcare providers. While our findings mirror those of studies from around the world, regional differences in species prevalence and drug sensitivity underline the need for localized approaches to treatment and prevention.

The younger age group affected in our study, along with the predominance of male patients, suggests that fungal infections are not merely confined to older or more traditionally at-risk populations. This calls for a broader awareness of the risk factors and early identification of *Candida* infections in all critically ill COVID-19 patients.

As the pandemic continues to evolve, so must our understanding and response to its complications. Vigilant monitoring, judicious use of antifungal medications, and collaboration between regions will be essential to managing these infections effectively and improving patient outcomes. Our study contributes to this growing knowledge and reinforces the need for continued research and attention to fungal infections in the context of COVID-19.

5.1. Key considerations for management

1. Routine Antifungal Susceptibility Testing: For practical reasons though, it is essential to perform routine Candida antifungal susceptibility testing for all Candida isolates to decide on the optimal therapy.
2. Species-Specific Treatment: Identification of species is prioritized in clinical settings, to improve efficacy of treatment. For example, infections with Candida glabrata or Candida krusei should promptly lead to discussion on echinocandins or amphotericin B.
3. Stewardship Practices: Antifungal resistance, especially the increasing resistance to azoles, and other antifungals demands very strict antifungal stewardship programs, that monitor prescribing practices and the use of antifungals and educate the health care providers on the new emerging resistance patterns.
4. Development of New Antifungals: In light of increasing resistance to existing antifungals, it becomes increasingly important to develop new agents with novel mechanisms of action. Early trial results of emerging drugs, including Ibrexafungerp (an oral glucan synthase inhibitor) may provide alternative treatment options.

6. Declaration

This study was conducted using retrospective data derived exclusively from laboratory records and patient files, without any direct patient interaction or intervention. Ethical approval was obtained from the Institutional Human Ethics Committee of Chirayu Medical College & Hospital (Ref No.: CMCH/EC/2021/29.1 dated 27.09.2021). Since the study involved analysis of already existing and anonymized data from January 2021 to May 2021, the ethics committee permitted a retrospective approval in accordance with the ICMR National Ethical Guidelines (2017) for Biomedical and Health Research Involving Human Participants.

7. Source of Funding

None.

8. Conflict of Interest

The authors declare that there is no conflict of interest related to this study.

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