Original Article

Clinico-Mycological Study of Onychomycosis in Indian Diabetic Patients

Sonia Agrawal, Archana Singal, Chander Grover, Shukla Das[1], S. V. Madhu[2]

Departments of Dermatology and STD, [1]Microbiology, and [2]Endocrinology, University College of Medical Sciences and GTB Hospital, Delhi, India

Abstract

Background: Onychomycosis (OM) is the most common nail disorder accounting for 40–50% of all onychopathies. Onychomycosis is caused by dermatophytes in majority, mostly *Trichophyton* (*T.*) *rubrum* followed by *T. mentragrophytes var. interdigitale*. However, there is a variation in the etiological profile with the subset of population, time, and geographical location. In immunocompromised hosts, non-dermatophytic molds (NDMs) and yeasts like *Candida albicans* and *Candida parapsilosis* are the main causative agents. Diabetes mellitus (DM) is a well-established risk factor for OM. **Aim and Objectives:** This study was conducted to determine the clinical and mycological characteristics of OM in diabetic patients and to evaluate the clinico-etiological correlation, if any. **Materials and Methods:** Three hundred consecutive diabetic patients were screened, of whom 102 (34%) patients were diagnosed with OM based on clinical, mycological, dermoscopic, and histological criteria. **Results:** Distal lateral subungual onychomycosis was the most common clinical variant seen in 80 (78.43%) patients. Fungal culture was positive in 57 (55.88%) of which NDMs constituted approximately half (47.61%) of the isolates, followed by *Candida* species (30.15%) and dermatophytes (22.22%). The clinico-mycological correlation was performed to look for the association of various fungi with the clinical type of OM. Distal lateral subungual onychomycosis was majorly caused by NDMs (51.02%), followed by *Candida* species (28.57%), and dermatophytes (20.40%). **Conclusion:** Non-dermatophytic molds are increasingly incriminated as the causative organisms for OM in DM and must be considered as potential pathogens in the present scenario, thus necessitating the change in the treatment options accordingly.

Keywords: *Candida, dermatophytes, diabetes, non-dermatophyte molds, onychomycosis*

Introduction

Onychomycosis (OM) is derived from the Greek words "όνυξ" which means nail and "μύκης" meaning fungus.[1] Onychomycosis has a worldwide prevalence of 0.5–5% and accounts for 40–50% of all onychopathies.[2,3] Risk factors for OM include elderly age, diabetes mellitus (DM), peripheral arterial disease, immune-compromised state, presence of tinea pedis, smoking, recurrent trauma, and sharing of public bathing facilities, etc.[4] Diabetics are more prone to develop OM due to multiple reasons including associated microangiopathy leading to peripheral vascular compromise and neuropathy. In addition, there is a compromise in the anti-oxidant function and poor neutrophil activity.[5] OM may serve as a potential portal of entry for bacteria resulting in recurrent bacterial infections. Sometimes, serious limb-threatening infections may ensue like cellulitis and osteomyelitis.[5] There is limited data regarding the prevalence of OM in patients with DM from across the world[6-8] and India.[9,10]

The clinical presentations of OM are variable and depend on the site of fungal invasion: distal lateral subungual OM (DLSO), proximal subungual OM (PSO), superficial OM (SO), endonyx, and total dystrophic OM (TDO). Dermatophytes like *Trichophyton rubrum* followed by *T. Mentragrophytes/interdigitale complex* are the chief etiological agents in general population.[11] In immune-compromised hosts, the causative organisms include non-dermatophytic molds (NDMs) and yeasts like *Candida albicans* and *Candida parapsilosis* as well.[12] In recent times, NDMs and yeasts have been increasingly incriminated in the causation of OM.[3]

We carried out this study to determine the clinical and mycological characteristics

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Address for correspondence:
Dr. Archana Singal, Department of Dermatology and STD, University College of Medical Sciences and GTB Hospital, Dilshad Garden, Delhi - 110 095, India.
E-mail: archanasingal@gmail.com

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of OM in diabetic patients and to evaluate the clinico-etiological correlation if any.

Materials and Methods

This cross-sectional analytical study was conducted on 300 consecutive diabetic patients attending the outpatient departments of dermatology and endocrinology, after approval from the Institutional Ethics Committee.

Patients were explained the study protocol and written informed consent was taken. Following a detailed history (age of onset of DM, duration, type of DM, treatment taken, and risk factors), a thorough general physical and systemic examination was carried out. Every patient underwent haematological and biochemical investigations, including fasting and post-prandial blood glucose and HbA1c, and findings were recorded on a predesigned proforma.

All nails were cleansed with sterile alcohol and examined for any clinical evidence of OM. Clinical photographs were taken and stored in JPEG format. Onychoscopy was performed using Dinolite AM 7515 MZT dermoscope on all the nails. Based on clinical features (onycholysis, subungual hyperkeratosis, chromonychia, nail plate thickening, and dystrophy), a representative nail with suspected OM was identified for sample collection.

Sample collection for direct microscopic examination (DME) with potassium hydroxide (KOH), culture, and histopathology

Based on the morphological features, nail clippings, nail plate surface scrapings, or subungual debris were collected from the most proximal part of the affected nail in order to minimize contamination. Two consecutive nail samples were sent in order to diagnose NDMs. These samples were sent for DME in 40% KOH preparation to assess for long-branched filamentous hyphae with or without arthroconidia suggesting dermatophytes, pseudo hyphae suggesting candidiasis, or mycelia, arthroconidia, or yeast cells indicating NDM infection. A part of the nail clipping was sent in a sterile envelope for culture on the Sabouraud's dextrose agar medium with and without chloramphenicol (0.05 g/l), gentamycin (20 mg/l), and cycloheximide (0.5 g/l) at 25 degrees Celsius. The inoculation tubes were examined at one week, two weeks, and four weeks. In case of growth, colony characteristics were studied on lactophenol cotton blue mount preparation. The culture was considered negative if no growth was observed after four weeks of inoculation. The involved part of the nail plate was biopsied using a nail splitter or a 3 mm punch and sent for histopathology (hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS) staining) after fixing in 10% buffered formalin solution. The presence of subungual hyperkeratosis in nail bed, hyphae, and/or polymorphs in the nail lamellae and a few fungal hyphae in the stratum corneum were suggestive of OM. Pseudohyphae and ovoid-to-round yeast forms in the hypertrophic stratum corneum and underlying nail plate were indicative of *Candida* species (spp.) [Figures 1 and 2].[13] The criteria described by Tosti *et al*.[14] were used to diagnose NDMs as the etiological fungi for OM.

The criteria used to diagnose molds as pathogen was:

1. Nail abnormalities consistent with diagnosis.
2. Positive direct microscopy visualizing hyphae in the nail keratin.
3. Failure to isolate a dematophyte in the culture
4. Growth of more than five colonies of the same mold in atleast two consecutive nail samplings.

The patient was diagnosed to have OM, if the clinical suspicion was corroborated with at least two positive results of the four tests viz onychoscopy, direct microscopic examination in KOH, culture, and histopathology with PAS staining.

Statistical analysis was done using SPSS (Statistical Package for Social Sciences) version 20.0 (IBM SPSS, Chicago, IL, USA). Distribution of data with respect to sociodemographic factor was expressed in proportions. Comparative analysis was done using Chi-square test of significance/Fischer's exact test and the value of $P < 0.05$ was considered significant.

Results

The demographic and other baseline characteristics of OM positive (102) and OM negative (198) patients have been listed in Table 1. Significantly, more male patients had OM ($P < 0.001$). Significantly, a greater number of patients with OM belonged to the higher age group (>60 years) as compared to the negative group ($P < 0.001$). In patients with OM (102), the median duration of the diabetes was significantly longer than in OM negative group ($P = 0.02$). About two-third of the patients with OM were obese (68/102 = 66.6%) and this

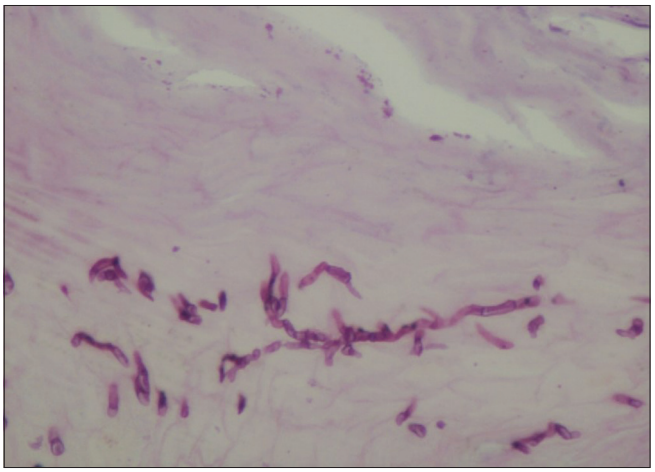

Figure 1: Histopathology with PAS: Thin regular fungal hyphae in the stratum corneum of nail plate (PAS, 400x)

Table 1: Demographic profile of diabetic patients with and without onychomycosis (OM)

Variable	OM+ve patients (*n*=102) *n* (%)	OM–ve patients (*n*=198) *n* (%)	*P*
Prevalence of OM			
• Male	63 (61.80)	64 (32.32)	<0.001*
• Female	39 (38.20)	134 (67.68)	
Age (years)			
• <40	8 (7.84)	47 (23.74)	<0.001*
• 41–59	40 (39.22)	79 (39.90)	
• >60	54 (52.94)	72 (36.36)	
Duration of diabetes (years) Median (IQR)	5 (1-10)	3 (1–8)	0.02*
Body mass index			
• Underweight	2 (1.96)	1 (0.50)	0.05*
• Normal	18 (17.64)	17 (8.59)	
• Overweight	14 (13.73)	26 (13.13)	
• Obese	68 (66.67)	154 (77.78)	
HbA1c status			
• Controlled (HbA1c ≤7)	15 (14.71)	31 (15.66)	0.829 (NS)
• Uncontrolled (HbA1c >7)	87 (85.29)	167 (84.34)	
Peripheral neuropathy			
• Yes	41 (40.20)	78 (39.40)	0.893 (NS)
• No	61 (59.80)	120 (60.60)	

*P<0.05- significant; NS- not significant

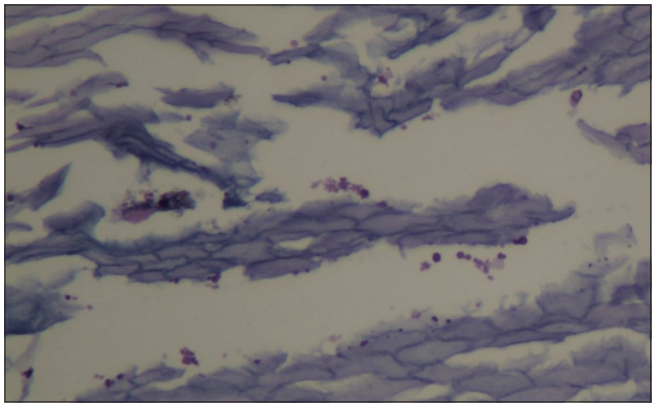

Figure 2: Histopathology with PAS: Pseudohyphae and ovoid to round yeast forms of *Candida* species in the hypertrophic stratum corneum (PAS, 400x)

was statistically significant as compared to OM negative group (P = 0.05). Both OM positive and negative groups had uncontrolled diabetes (HbA1c > 7) in 87 (85.29%) and 167 (84.34%) patients, respectively, and this difference was not statistically significant (P = 0.829). Although peripheral neuropathy is an important risk factor for OM, it was not found to be statistically significant (P = 0.893) in our study. Only one-third (32/102 = 31.37%) of OM-positive cases were aware of the presence of nail abnormalities.

Out of 102 positive cases, 561/2040 nails (toe nails + fingernails) were involved. Distal lateral subungual onychomycosis was the most common clinical variant observed in 80 (78.43%) patients, followed by mixed variant (DLSO+TDO) in 10 (9.80%). Endonyx OM was present in 5 (4.90%) while TDO, PSO, and SO were present in 4 (3.92%), 1 (0.98%), and 2 (1.97%) cases, respectively.

Within four weeks of inoculation, mycological culture was positive in 57/102 (55.88%) patients [Figures 3 and 4]. A total of 63 species were cultured as few of the nails showed growth of more than one organism. Maximum isolates belonged to non-dermatophyte molds (NDMs) (30/63 = 47.6%) followed by *Candida* species in 19 (30.15%) cultures and dermatophytes in 14 (22.22%). Among the NDMs, *Aspergillus* spp. was most common and grew in 15 (50%) cultures followed by *Trichosporon beigelli* in 4 (13.33%). Of the dermatophytes, *Trichophyton mentagrophytes/interdigitale complex* was isolated in 11/14 (78.57%) cultures, followed by *Trichophyton rubrum* in 2/14 and *Trichophyton verrucosum* in 1/14 cultures. Lactophenol cotton blue was used to identify most of the species on microscopy**.** Varying species that were isolated have been tabulated in Table 2.

The correlation of different clinical variants of OM to the etiological fungus was evaluated. Eighty patients with DLSO grew 49/63 (77.77%) species on culture, majority being NDMs in 25 (51.02%), followed by *Candida* spp. in 14 (28.57%), and dermatophytes in 10 (20.40%). Of the 10 cases with mixed type OM, 9/63 (14.29%) growths were observed on the culture. TDO, PSO, and SO grew 3/63 (4.76%), 1/63 (1.58%), and 1/63 (1.58%) species, respectively. No growth in culture was observed in five cases

of endonyx. The clinico-etiological correlation of different types of OM and culture growth has been summarized in Table 3.

Discussion

Owing to the presence of immune suppression, vascular compromise, and peripheral neuropathy, diabetic patients are highly predisposed to develop OM. However, there is a paucity of published literature regarding its prevalence, etiological fungus, and clinico-etiological correlation in this special population. Therefore, we attempted to assess these parameters in this study.

Table 2: Fungal species cultured (*n*=63) among the OM positive cases (*n*=102)

Species cultured	Number of cultures *n* (%)
Dermatophytes	14 (22.22)
• *Trichophyton (T.) mentagrophytes/ interdigitale complex*	• 11
• *T. rubrum*	• 2
• *T. verrucosum*	• 1
Candida spp.	19 (30.15)
NDMs	30 (47.61)
• *Aspergillus (A.) flavus*	• 9
• *A. niger*	• 2
• *A. versicolor*	• 4
• *Trichosporon beigelli*	• 4
• *Chetomium*	• 1
• *Cladosporium*	• 1
• *Geotrichum*	• 2
• *Fonsecaea*	• 2
• *Fusarium* solani complex	• 1
• *Scopulariopsis brevacaulis*	• 1
• Others	• 3
Total	63

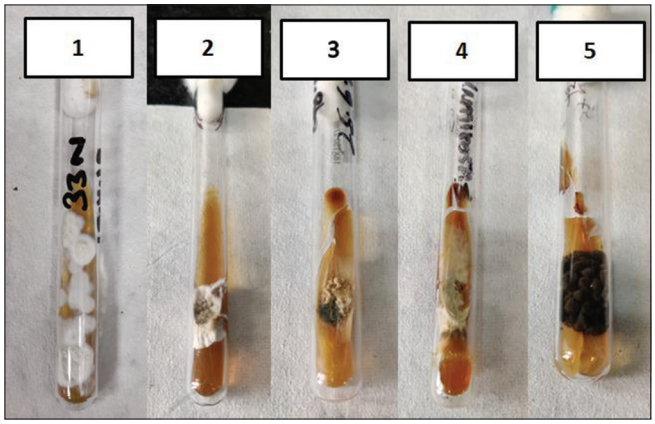

Figure 3: Inoculation tubes showing fungal growths in the following order (Obverse) 1. *Trichophyton mentagrophytes/interdigitale complex* 2. *Aspergillus versicolor* 3. *Trichosporon beigelli* 4. *Fusarium solani complex* 5. Fonsecaea

The prevalence of OM was found to be 34% on screening 300 diabetic patients. There is only one similar study from North India[9] conducted two decades ago that reported a prevalence of 17% among 400 diabetic subjects. Few studies have been conducted across the globe and India [Table 4][15–17] to assess the prevalence and causative fungus for OM in patients with DM, and the results are variable, possibly due to the difference in the time period, geographic locations, methodology, and diagnostic criteria for OM.

Distal lateral subungual onychomycosis was the most common variant, observed in 78.43% cases of OM followed by mixed type (9.80%), endonyx (4.90%), TDO (3.92%), SO (1.97%), and PSO (0.98%). Similar results were observed in previous studies.[6,7,9]

The percentage positivity of all four diagnostic tests has been tabulated [Table 5]. We obtained positive growth on culture in 55.88% cases, with a total of 63 species as few nails showed growth of more than one organism. Non-dermatophytic molds constituted 47.61% of these, followed by *Candida* species (30.15%) and dermatophytes (22.22%). Among the 14 dermatophytes, the isolates were *Trichophyton mentagrophytes/ interdigitale complex* (11), *Trichophyton rubrum* (2) and *Trichophyton verrucosum* (1). Another study in diabetic population in 2002 showed yeasts to be the most common (48%) isolates followed by dermatophytes and NDMs.[9] In the studies done by Gupta *et al*. (1998)[6] and Eba *et al*. (2016),[7] dermatophytes were the predominant group (*Trichophyton rubrum* > *Trichophyton mentagrophytes/interdigitale complex*). Another study from North India involving non-diabetic patients reported that *Trichophyton mentagrophytes/interdigitale complex* was the most common species among dermatophytes followed by *Trichophyton rubrum* and *Trichophyton violaceum*.[18]

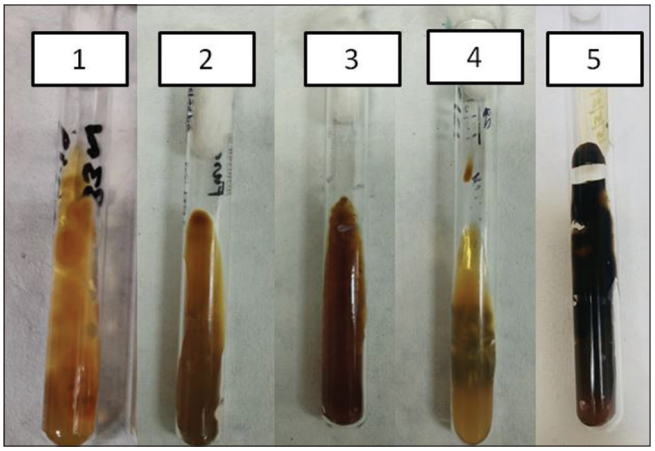

Figure 4: Inoculation tubes showing fungal growths in the following order (Reverse) 1. *Trichophyton mentagrophytes/interdigitale complex* 2. *Aspergillus versicolor* 3. *Trichosporon beigelli* 4. *Fusarium solani complex* 5. Fonsecaea

Table 3: Clinico-etiological correlation of the morphological variant of OM and etiological fungi

Species	Clinical pattern (*n*=102)						
	DLSO (80)	PSO (1)	SO (2)	EO (5)	TDO (4)	Mixed (10)	Total (*n*)
Dermatophytes	10						
- *T.M/T.I complex*	8	-	-	-	-	3	11
- T.R	1	1	-	-	-	-	2
- T.V	1	-	-	-	-	-	1
Non-dermatophyte molds	25	-	-	-			
- *Aspergillus*	12	-	-	-	1	2	15
- *Trichosporon beigelli*	3	-	1	-	-	-	4
- *Geotrichum*	1	-	-	-	-	1	2
- *Fonsecaea*	2	-	-	-	-	-	2
- *Fusarium solani*	1	-	-	-	-	-	1
complex	1	-	-	-	-	-	1
- *Chetomium*	1	-	-	-	-	-	1
- *Scopulariopsis brevicaulis*	4				-	-	3
- Others							
Candida spp.	14	-	-	-	2	3	19
Total	49	1	1	0	3	9	63

*T.M/T.I complex- *Trichophyton mengtragophytes/interdigitale complex*, T.R- *Trichophyton rubrum*, T.V- *Trichophyton verrucosum*, DLSO- Distal and lateral subungual Onychomycosis, PSO- Proximal Subungual Onychomycosis, SO- Superficial Onychomycosis, TDO- Total Dystrophic Onychomycosis

Table 4: Prevalence of OM and the etiological fungus in diabetic patients across the globe

Authors, Reference	Year of study	Place	Number of patients	Prevalence of OM	Causative organisms
Alteras *et al*.[15]	1979	Israel	100	73%	*T.rubrum* > *C.albicans* > *T.mentagrophytes*
Buxton *et al*.[16]	1996	Edinburgh	100	12%	NA
Gupta *et al*.[6]	1998	Canada	550	26%	Dermatophytes > NDMs > *Candida* spp.
Dogra *et al*.[9]	2002	India (Chandigarh)	400	17%	*Candida* spp. > Dermatophytes > NDMs
Pierard *et al*.[17]	2005	Belgium	190	65.3%	Dermatophytes > NDMs > *Candida* spp.
Saunte *et al*.[8]	2006	Denmark	271	22%	Dermatophytes > NDMs
Eba *et al*.[7]	2016	Cameroon	152	50.7%	Dermatophytes *T.rubrum* > *T.mentagrophytes* > *T.tonsurans*
Dogiparthi *et al*.[10]	2018	India (Chennai)	205	80.5%	NA
Present study	2020	India (Delhi)	300	34%	NDMs > *Candida* spp. > Dermatophytes

NA- Not available, NDMs- Non-dermatophyte molds

The role of NDMs and *Candida* spp. in causation of OM is increasing.[19,20] The difference in growth pattern in our study could be due to climate variations in different geographical locations. The higher prevalence of NDMs in our study could be attributed to the favorable growth pattern in the tropical and humid climates.

The clinico-mycological correlation was performed to look for the association of various fungi with the clinical type of OM. Among the 80 cases of DLSO, 49 cases were culture positive, and the growth comprised NDMs (25/49) > *Candida* spp. (14/49) > dermatophytes (10/49) {*T.mentagrophytes/interdigitale complex* (8), *T.rubrum* (1), *T.verrucossum* (1)}. One case of PSO was caused by *T. rubrum*. This finding was consistent with the findings by Dogra *et al*.[9] Only one out of the two cases of SO showed growth, which was by *Trichosporon beigelli*. Three out of four cases of TDO showed growth {*Candida* spp. (2) and *Aspergillus* spp. (1)}. Endonyx variant did not show any growth, while mixed variant was caused by *T.mentagrophytes/interdigitale complex, Candida* spp., *Aspergillus* species, and *Geotrichum*. Most of the growths in our study were of varied NDM species, majority being *Aspergillus* spp. Similar findings were observed in a study from Southeast Rajasthan, India, in general population, where NDMs (53/150 = 35.33%) were reportedly the most common isolates followed by dermatophytes (28/150 = 18.66%) and Candida spp. (15/150 = 10%).[19]

Table 5: Percentage positivity of various diagnostic modalities for OM (*n*=102)

Diagnostic modality	Frequency *n* (%)
DME with KOH	87 (85.29)
Histopathology with PAS	70 (68.62)
Mycological culture	57 (55.88)
Onychoscopy	58 (56.86)
DME + Histopathology	99 (97.05)
DME + Mycological culture	93 (91.17)
DME + Onychoscopy	101 (99.01)

DME- Direct microscopic examination, KOH- Potassium hydroxide mount, PAS- Periodic acid-Schiff stain, OM- Onychomycosis

Authors have studied the clinico-etiological correlation of OM in general population in 2015 and 2019. In 2015, *T. interdigitale* (61%) was the predominant etiological agent followed by *T. rubrum* (34%) and *T. verrucosum* (5%).[21] Recent study in 2019, on the other hand, showed dermatophytes (*T.rubrum* 18 (38.3%) > *T. mentagrophytes/interdigitale complex* 10 (21.3%) > *T.violaceum* 1 (2.1%) > *T.tonsurans* 1 (2.1%)) to be the most common etiological agents followed by *Candida* spp. in ten (21.3%) and NDMs in seven (14.8%).[22] Thus, the etiological fungus causing OM in diabetic population is clearly different.

Limitations

The study posed some limitations. The culture positivity rate could have been higher if there were better mycological growth conditions. This could have led to the isolation of more fungi resulting in better culture positivity and clinico-etiological correlation.

Conclusion

The prevalence of OM among the Indian diabetic population is high (34%). Distal and lateral subungual onychomycosis is the most commonly encountered clinical variant caused by NDMs in the majority followed by *Candida* species and dermatophytes. Among the dermatophytes, *Trichophyton mentagrophytes/interdigitale complex* is the most common species. Non-dermatophytic molds are increasingly incriminated as the causative organisms for OM in DM and must be considered as potential pathogens in the present scenario, thus necessitating the change in the treatment options accordingly.

Statement of ethics

Authors state that subjects have given their written informed consent and that the study protocol was approved by the institute's committee on human research.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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