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Original Article

Microbiology Section

Mycological Profile in Otomycosis Patients and their Drug Sensitivity: A Cross-sectional Study at Union Territory of Puducherry, India

ANUSHEELA HOWLADER[1], PRITHIVIRAJ NAGARAJAN[2], LATHA RAGUNATHAN[3]

ABSTRACT

Introduction: In clinical Otorhinolaryngology practice, otomycosis is a common problem to overcome caused by *Aspergillus* and *Candida* species. Although, we come across many difficulties to diagnose and treat otomycosis infections, many patients show drug resistance to antifungal agents with a high prevalence rate.

Aim: To elucidate the pattern of mycological agents and susceptibility testing of fungal species in samples collected from patients with an ear infection at a tertiary hospital.

Materials and Methods: The present hospital-based cross-sectional study conducted in the Department of Microbiology and Department of Ear, Nose, and Throat (ENT) at Aarupadai Veedu Medical College and Hospital, Puducherry, India, from August 2018 to January 2020. A total of 126 samples were collected, transported and analysed for bacteriological and mycological analysis using direct inspection and culture techniques. By using the disc diffusion technique, the antifungal susceptibility testing was carried out to determine the sensitivity and resistance against ketoconazole, itraconazole, fluconazole, and amphotericin B. Data were analysed in terms of frequency using the Statistical Package for the Social Sciences (SPSS) software 25.0 version.

Results: Out of 126 samples, fungal isolates were 92, out of which the most common fungal isolate was *Aspergillus niger* followed by *A. flavus*, *Candida albicans (C. albicans)*, *A. terreus*, *and A. fumigatus*. Also, the major common bacterial isolates were *Staphylococcus aureus* followed by *Pseudomonas*, *Proteus*, *Escherichia coli (E. coli)*, and *Klebsiella* spp. Prevalence in males (58.73%) was more as compared to the females (41.27%), and itching was the most common presenting symptom. Otomycosis in Chronic Suppurative Otitis Media (CSOM) mainly affected males (40%) and is common in middle-aged groups (20-35 years) of outdoor and indoor workers, housewives and farmers, particularly during the monsoon season. Antifungal susceptibility testing of *A. flavus* and *A. niger* isolates revealed high sensitivity to amphotericin B, itraconazole and ketoconazole drugs but low efficacy to fluconazole drugs.

Conclusion: Otomycosis should be suspected clinically to prevent the unnecessary use of antibiotics. A high incidence of otomycosis has been encountered in many tropical countries. In present study, *Aspergillus* spp. and *Candida* spp. were the commonest fungal isolates in otomycosis. In our community, currently two drugs, amphotericin B and ketoconazole are the most frequently used for fungal infection. Properly identifying fungal agents and host factors involved in otomycosis can improve such patients' outcomes.

Keywords: Antifungal drugs, Antifungal susceptibility testing, Fungal infection, Predisposing factors

INTRODUCTION

In otorhinolaryngology outpatients, otomycosis is a common external ear canal fungal infection caused by a perforated tympanic membrane [1]. Some of the symptoms are inflammation, itching, scaling, a hole in the tympanic membrane, hearing loss and discharge from the ear [2]. The otomycosis frequency depends on the tropical and subtropical climatic conditions with hot, humid regions (as high as 54%) and dusty areas [3,4]. Otomycosis is a common medical problem and a health hazard in India [2,4]. There are numerous predisposing factors for otomycosis, which may be systemic or local; several predisposing factors, such as increased and indiscriminate use of topical antibiotics, instillation of hot oil/water in the ear, unhygienic mopping of the ear, use of hearing aids, swimming in contaminated water, cytotoxic chemotherapies and immunosuppressive diseases, etc., have increased the incidence of otomycosis [1,4].

Fungi can either be the primary pathogen or be superimposed on bacterial infections [5]. A wide range of fungi causes otomycosis, but *Aspergillus* and *Candida* species are the most common types of fungi that cause it [4]. *Aspergillus* alone accounts for 75% of cases, with *A. niger* being the most common, followed by *A. flavus* and *A. fumigatus* [6]. As a secondary contaminant in instances of external otitis, the fungus may be associated with bacterial infections [7]. In most cases, the infection is unilateral and is characterised by inflammatory pruritis, scaling, and otalgia [8]. Local debridement, local and systemic antifungal medications and stopping of topical antibiotics have been recommended as treatments. Otomycosis may sometimes be difficult to manage in terms of long-term care and follow-up, despite its recurrence rate being high [9].

However, information on otomycosis is still limited in the rural and tertiary populations [4]. Yet, no proper systematic studies have been documented to identify otomycosis in the union territory of Puducherry's geographical area. Therefore, the present study aimed to elucidate the pattern of mycological agents and their susceptibility testing from patients with an ear infection at a tertiary care hospital.

MATERIALS AND METHODS

The present study was a hospital-based cross-sectional study conducted from August 2018 to January 2020 in the Department of Microbiology and the Department of ENT at Aarupadai Veedu Medical College and Hospital, Puducherry, India. A total of 126 samples were collected (74 males and 52 females) aged between 10-60 years of the suspected fungus-infected patients during the study period. The patients from Aarupadai Veedu Medical College and Hospital were properly informed about the study and got individual consent from each patient before taking an ear swab. The study was duly approved by Institutional Ethics Committee (IEC) (IEC approval no:AV/IEC/2018/109).

Inclusion criteria: Otomycosis patients with symptoms like inflammation, itching, scaling, a hole in the tympanic membrane,

hearing loss, ear discharge., dry matted masses of hyphae or white cheesy material, and those who gave their written consent, were included from the study.

Exclusion criteria: Patients with other ear problems, such as those with perforated tympanic membrane and chronic otitis media, who had recently been treated for otomycosis, and the patients who refused to give their consent forms were excluded from the study.

Study Procedure

Sample collection: Ear examination was done using an otoscope, and samples were collected by sterilising with 70% alcohol from the outside of the ear canal with two sterile cotton swabs under aseptic conditions. The collected specimens were immediately transferred to the Department of Microbiology. To diagnose otomycosis, a detailed history of patients were recorded on predetermined proforma and their clinical examination was done, otoscopic findings, and laboratory identification of fungus were also recorded [10].

Culture processing and identification: After cleaning the ear with spirit two ear swabs were collected aseptically, without touching the surroundings and directly inserting the swab into the ear canal; one ear swab was used for direct microscopy {(Gram's staining, 10% Potassium hydroxide (KOH) wet mount)} to identify yeast-like fungi, and 10% KOH mount, Lacto Phenol Cotton Blue (LPCB) were used to identify filamentous fungi. The second swab was used for the purpose of the mycological and bacteriological cultures. For fungus culture, Sabouraud's Dextrose Agar (SDA) with antibiotic gentamicin 50 ug/mL (HiMedia, India) was used as it is a common medium, which was incubated at 37°C and 25°C for two to three weeks [11]. Candida isolates were morphologically recognised by gram stain, cultural characteristics, germ tube and chlamydospore development and inoculated on HiChrome agar (HiMedia, India) for species identification [12].

Antifungal susceptibility testing: The disc diffusion assay was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines (M44-A2-method for antifungal disk diffusion susceptibility testing of yeast) to determine the susceptibility of *Candida* isolates [13]. Mueller-Hinton Agar (MHA) was supplemented with 2% glucose and 0.5 mg/L methylene blue. The inoculum was taken with a sterile swab from SDA tubes and was standardised to 0.5 McFarland, lawn culture was done on MHA plates with 2% glucose and 0.5% methylene blue and antifungal drug discs itraconazole, fluconazole, ketoconazole and amphotericin B were applied and incubated at 37°C for 24 hours of incubation for proper growth [7]. For the filamentous fungi CLSI guideline (M51-A-Method for antifungal disk diffusion susceptibility testing of non dermatophyte filamentous fungi) was followed [13].

STATISTICAL ANALYSIS

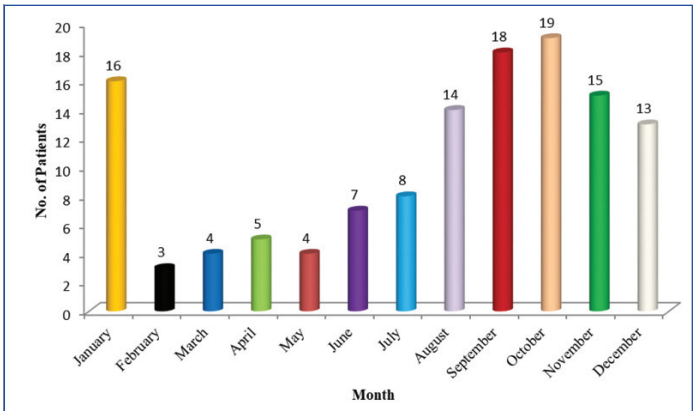
Statistical analysis was done using the one-way Analysis of Variance (ANOVA) method and a p-value of <0.05 was taken as significant. For the antibiotic sensitivity test, data were extracted and converted to an excel sheet, and data were analysed in terms of frequency using the SPSS 25.0 version.

RESULTS

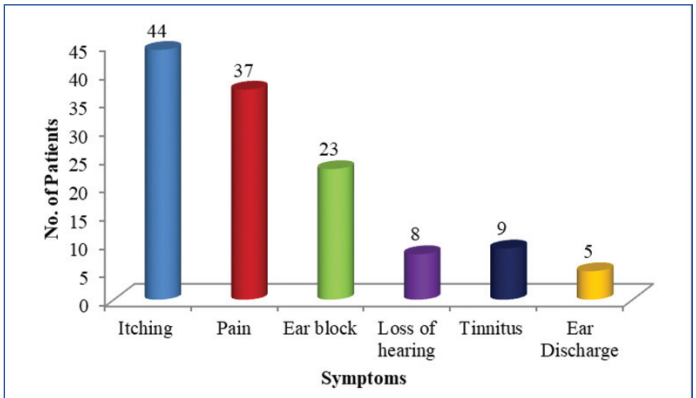
Demographic profile of patients: A total of 126 cases that fulfilled the inclusion criteria were included in this study. The majority of them were 52 (41.27%) females and 74 (58.73%) were males. In present study, otomycosis was frequently observed in all age groups. The most prevalent age group was 16-30 years while few numbers of the patients were reported in the paediatric age group of 0-15 years and above 60 years [Table/Fig-1]. In the seasonal variation, the monsoon showed the highest incidence of otomycosis in the month of October 19 (15.08%) and September 18 (14.29%) [Table/Fig-2]. Occupationally maximum number of patients were seen in indoor workers 46 (36.51%) followed by housewives and housemaids 37 (29.37%), while minimum cases were seen in other groups of outdoor (mechanics, drivers, shopkeepers, welding, handcraft workers and teachers) workers and agricultural workers [Table/Fig-1]. The common presenting symptoms solely or in combination encountered in the study group have been summarised in [Table/Fig-3].

Age group (years)	Male	Female	Total (%)
0-15	3	2	5 (3.97)
16-30	29	19	48 (38.1)
31-45	23	16	39 (30.95)
46-60	16	13	29 (23.01)
Above 60	3	2	5 (3.97)
Total	74 (58.73%)	52 (41.27%)	126
Occupation-wise distribution			
Occupation			**Total (%)**
Miscellaneous indoor worker			46 (36.51)
Housewife and housemaid			37 (29.37)
Labour (Mechanic, drivers, shopkeepers, welding, handcraft workers and teachers).			26 (20.63)
Agriculturists			17 (13.49)

[Table/Fig-1]: Age, sex and occupation distribution of the otomycosis patients (N=126).



[Table/Fig-2]: Month-wise prevalence of otomycosis (N=126).



[Table/Fig-3]: Symptoms of otomycosis patients (N=126).

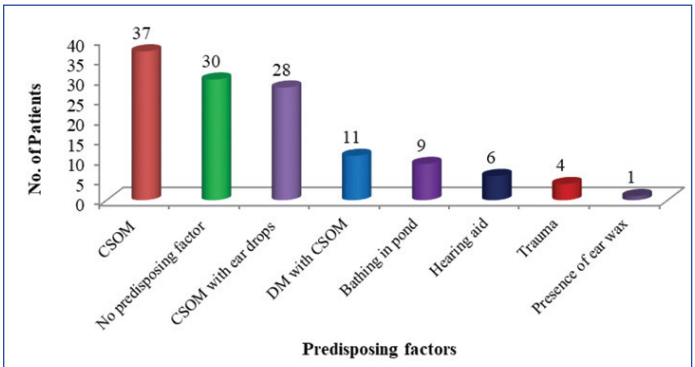
Laterality distribution of otomycosis patients: In the present study, total 8 (6.35%) cases represented as bilateral infection among that three cases were immunocompromised individuals. Among unilateral cases, the maximum number of otomycosis cases recorded in the right side ear showed predominantly 62 (49.2%) compared to the left ear at 56 (44.4%) [Table/Fig-4].

Common predisposing factors: In the present study, common predisposing factors noted were CSOM 37(29.37%) followed by CSOM with ear drops 28 (22.22%), Diabetes Mellitus (DM) with CSOM 11 (8.73%), Bathing in a pond 9 (7.14%), 30 (23.81%) of

S. No.	Side	Male	Female	Total
1	Right	36	26	62 (49.21%)
2	Left	33	23	56 (44.44%)
3	Bilateral	5	3	8 (6.35%)

[Table/Fig-4]: Laterality distribution of otomycosis patients (N=126).

cases had no predisposing factors. The predisposing factors of various data for otomycosis are represented in [Table/Fig-5]. Out of 126 samples only 92 (73.02%) were positive for the presence of fungal elements, 34 (26.98%) were negative both by microscopy and culture process.



[Table/Fig-5]: Distribution of predisposing factors (N=126).

Microbiological findings: Out of 126 specimens collected, 92 (73.02%) were positive for fungal growth by culture. The most common fungal isolates belonged to the species of *Aspergillus*. The [Table/Fig-6] shows *Aspergillus, Candida* and gram positive and gram negative isolates.

Antifungal susceptibility testing (AST): The efficacy of antifungals against different species of cultures were tested such as amphotericin B, fluconazole, ketoconazole, and itraconazole [Table/Fig-7]. The maximum (100%) resistance was seen in *A. terreus* when treated with amphotericin B, followed by *A. niger* (19.6%) and *A. flavus* (17.39%). The highest percentage of sensitivity among mold was observed with amphotericin B. *A. fumigatus, C. parapsilosis, C. albicans, C. glabrata, Pencillium* were sensitive to fluconazole and amphotericin B and itraconazole showed the maximum resistance against *A. fumigatus* (20%) followed by *A. niger* (6.5%) and *A. flavus* (4.3%). The other antifungal ketoconazole showed a relatively good effect against *Candida* species, as it was resistant at 16.7%.

Fungal isolates	Number of patients, n (%)
Aspergillus niger	46 (50%)
Aspergillus flavus	23 (25%)
Aspergillus terreus	7 (7.61)
Aspergillus fumigatus	5 (5.44%)
Candida albicans	6 (6.52%)
Candida parapsilosis	2 (2.17%)
Candida glabrata	1 (1.09%)
Pencillium	2 (2.17%)
Total fungal isolates	n=92
Bacterial Isolates	
Coagulase negative *Staphylococcus*	10 (8.93%)
Micrococcus	7 (6.25%)
Staphylococcus aureus	32 (28.57%)
Pseudomonas aeruginosa	25 (22.32%)
Proteus mirabilis	15 (13.39%)
Escherichia coli	10 (8.93%)
Klebsiella species	8 (7.14%)
Proteus vulgaris	5 (4.47%)
Total bacterial isolates	n=112

[Table/Fig-6]: Spectrum of fungal and bacterial isolates among patients.

DISCUSSION

One of the most common external auditory canal fungal infection is otomycosis, often known as fungal otitis externa [1]. Tropical and subtropical regions of the world often encounter it. Otomycosis is more prevalent in hot, humid and dusty areas and depends on many climatic conditions [2]. This is congruent with present study data, which showed that august to december had the highest prevalence of fungal infections. The present study was carried out in Puducherry Union Territory, India. The density of dust particles in the air has increased in recent years due to several new projects in and around Puducherry city. During the rainy season, there is a high relative humidity of around 70 percent. Compared to other researchers, present study found a lot of fungal otitis externa in tropical and subtropical countries, like India, in the last few decades during the rainy season [3-5].

In the present study, the age group analysis revealed that otomycosis could affect any age group from 1-60 years old. However, the incidence was highest at 48 (38.1%) in the age group

Fungus	No of isolates	Drug sensitivity	Amphotericin B, n (%)	Itraconazole, n (%)	Fluconazole, n (%)	Ketoconazole, n (%)
A. niger	46	Sensitive	37 (80.4%)	43 (93.5%)	42 (91.3%)	41 (89.1%)
		Resistant	9 (19.6%)	3 (6.5%)	4 (8.7%)	5 (10.9%)
A. flavus	23	Sensitive	19 (82.61%)	22 (95.7%)	21 (91.3%)	21 (91.3%)
		Resistant	4 (17.39%)	1 (4.3%)	2 (8.7%)	2 (8.7%)
A. terreus	7	Sensitive	0	7 (100%)	6 (85.7%)	6 (85.7%)
		Resistant	7 (100%)	0	1 (14.3%)	1 (14.3%)
A. fumigatus	5	Sensitive	4 (80%)	4 (80%)	5 (100%)	4 (80%)
		Resistant	1 (20%)	1 (20%)	0	1 (20%)
C. albicans	6	Sensitive	6 (100%)	6 (100%)	6 (100%)	5 (83.3%)
		Resistant	0	0	0	1 (16.7%)
C. parapsilosis	2	Sensitive	2 (100%)	2 (100%)	2 (100%)	2 (100%)
		Resistant	0	0	0	0
C. glabrata	1	Sensitive	1 (100%)	1 (100%)	1 (100%)	1 (100%)
		Resistant	0	0	0	0
Pencillium	2	Sensitive	2 (100%)	2 (100%)	2 (100%)	2 (100%)
		Resistant	0	0	0	0

[Table/Fig-7]: Antifungal susceptibility testing.

of 16-30 years, while a small number of patients were reported in the paediatric age group of 0-10 years. Similar to the findings mentioned by Fasunla J et al., and Ologe FE and Nwabuisi C [4,6]. Due to work exposure, travel and other factors, these age groups are more exposed to mycelia than older and younger age groups, which results in a higher incidence in these age groups [7]. The present study found that males were more likely to be infected than females, which was consistent with other studies, as males spend more time outdoors, leading to more exposure to fungal spores [5]. Present findings were consistent with those of Kaur R et al., and Ho T et al., who reported a 60% and 56% male incidence, respectively [7,8]. Present study found unilateral involvement in 118 (93.65%) patients with predisposing characteristics, which was consistent with the study of Barati B et al., in which unilateral involvement was 97% [9]. Ho T et al., reported that 7% of the patients were affected on both sides, which was the same as what present study found: 6.35% of the patients had bilateral otomycosis [8].

According to the literature, itching, ear discharge, ear ache, blocking sensations, decreased hearing and tinnitus were the most common symptoms in all patients with otomycosis [3,10]. In present study, the most common symptoms were 44 (34.92%) itching, followed by 37 (29.37%) pain, 23 (18.25%) ear block, 8 (6.35%) loss of hearing, 9 (7.14%) tinnitus and 5 (3.97%) ear discharge. Similarly, Gupta S and Mahajan B, and Sangavi AKB et al., found itching to be a prevalent presenting symptom in their respective investigations [11,12]. CSOM is one of the most important risk factors for otomycosis. In the present study, CSOM was noted 37 (29.37) followed by CSOM with ear drops 28 (22.22%), DM with CSOM 11 (8.73), bathing in a pond 9 (7.14%), 30 (23.81)% of cases had no predisposing factors, in which present results also correlated with Punia RS et al., showed that 49% of patients had CSOM as a predisposing factor [14], and the usage of antibiotics and steroid ear drops was 21.4%, which also correlated with Prasanna V et al., whose findings showed 56.25% usage of ear drops [15]. Interestingly, in present study group, 30 (23.81%) of cases had no predisposing factors in isolates clinically diagnosed with otomycosis. The Agarwal P and Devi LS, study also supports present study that, 32% had no predisposing factors and showed that 42% of fungal isolates were diagnosed with otomycosis [1].

In an overview of the literature, *Aspergillus niger* and *Candida* found to be the most common fungal isolates causing otomycosis worldwide, similar to the studies conducted by Agarwal P and Devi LS, Aneja KR et al., and Hagiwara S et al., [1,16,17]. Among the *Aspergillus* species, *A. niger* was the most common isolate, followed by *A. flavus*, *A. terreus*, and *A. fumigatus*. *C. albicans* 6 (6.52%) was the most common *Candida* species isolated from otomycosis cases, followed by *C. glabrata*, and *Pencillum*. Similar results have been reported previously by Agarwal P and Devi LS, where the authors found *Aspergillus* spp. 302 (87.3%) to be the predominant fungi isolated from the cases followed by *Candida spp*. 35 (10%) and *Penicillium* 2 (0.6%) [1]. Gupta S and Mahajan B, in their study, they found *A. niger* 20 (51.3%), *A. flavus* 7 (17.9%), *A. fumigatus*, and *Candida* spp. 6 (15.4%), to be the most common fungal isolates recovered from clinically suspected cases of otomycosis respectively [11]. Likewise, Sangavi AKB et al., have reported *A. niger* 15 (46.9%) followed by *A. flavus* 6 (18.8%) and *Candida* spp. 10 (31.3%) to be the most common fungal isolates were recovered from 32 samples [12]. In a study done by Ashopa V et al, *Aspergillus* species and *Candida* species were highly isolated fungal pathogens in otomycosis 76 (70.37%) and 17 (15.74%), respectively [18].

In the index study, the susceptibility test disc diffusion method was performed against itraconazole, ketoconazole, fluconazole and amphotericin B. Present results showed *Aspergillus* were more sensitive to amphotericin B, followed by itraconazole, ketoconazole, and fluconazole and two *Penicillium* isolates were 100% sensitive to amphotericin B and itraconazole these findings were correlated with Kazemi A et al., the study showed, *Penicillium* is more sensitive to amphotericin B [19]. Among 46 isolates, 37 (80.43%) *A. niger* were sensitive and 9 (19.57%) were resistant to amphotericin B and *A. flavus* 19 (82.6%) were sensitive to amphotericin B and 4 (17.39%) were resistant, these findings match with Misra R et al., showed amphotericin B are resistant to isolates [20]. Similarly, over 23 isolates, 22 (95.7%) of *A. flavus* were sensitive to itraconazole and 1 (4.3%) was a resistant isolate. Present findings were correlated with Misra R et al., and Karaarslan A et al., studies showed *A. flavus* were 100% sensitive to amphotericin B and showed no resistance to itraconazole [20,21]. In present study observations among nine *Candida* species, six were *C. albicans*, two were *C. parapsilosis* and one was *C. glabrata* as identified. Interestingly all nine species of *Candida* were 100% sensitive to amphotericin B whereas itraconazole, fluconazole showed high resistance to other fungal isolates. In among that *C. albicans* were more sensitive to ketoconazole (16.7%) compared to other azoles [22]. Overall present study finding indicates, a majority of fungi that led to otomycosis were sensitive to amphotericin B, though some were resistant to fluconazole and itraconazole.

Limitation(s)

A limitation of the current study was the small number of cases (n=126). Also, ear swabs were used to get samples, which is not a good way to figure out what kind of otomycosis a person has. There is also a need to study the increase in Minimum Inhibitory Concentration (MIC) values over a period developed by different bacterial isolates in particular geographic locations.

CONCLUSION(S)

Early microbiological diagnosis and microscopic examination of fungal culture are needed for prompt and effective treatment to avoid severe complications in otomycosis. The present study highlights the increased prevalence of otomycosis in males as compared to females, with the majority of the cases occurring in the rainy season. It was commonly found in people working in dusty environments, particularly agricultural and indoor workers, housewives, and labourers. Further educating and providing proper awareness to the Puducherry rural population and agriculturists is another important concern to be addressed, and the proper identification of fungal agents and host factors involved in otomycosis will improve the outcome for such patients.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Microbiology, Aarupadai Veedu Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry, India.
2. Assistant Professor (Research), Multi-Disciplinary Centre for Biomedical Research, Aarupadai Veedu Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry, India.
3. Professor and Head, Department of Microbiology, Aarupadai Veedu Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Prithiviraj Nagarajan,
Assistant Professor (Research), Multi-Disciplinary Centre for Biomedical Research, Aarupadai Veedu Medical College and Hospital, Vinayaka Mission's Research Foundation, (Deemed to be University), Kirumampakkam, Puducherry-607403, India.
E-mail: prithivinaga@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: May 13, 2022
- Manual Googling: Jul 30, 2022
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- Was informed consent obtained from the subjects involved in the study? Yes
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