Isolation of different fungi from the skin of patients with seborrheic dermatitis

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Background and Purpose: Seborrheic dermatitis (SD) is characterized by erythematous inflammatory patches that mostly appear in the sebaceous gland-rich skin areas. In addition to the key role of Malassezia species in SD, its contribution to other fungal microbiota has been recently addressed in the literature. Regarding this, the present study was conducted to identify and determine the fungal species associated with the incidence of SD.

Materials and Methods: For the purpose of the study, fungal microbiome in scaling samples were collected from SD lesions and then analyzed based on the DNA sequencing of ITS regions.

Results: In addition to Malassezia, several fungal species were detected in the samples collected from the SD lesions. According to the results, 15.5%, 13.3%, and 6.7% of the isolates were identified as Candida parapsilosis, Cryptococcus albidus var. albidus/Rhodotorula mucilaginosa, and Penicillium polonicum, respectively.

Conclusion: Based on the obtained results, C. parapsilosis was the most prevalent non-Malassezia species isolated from SD lesions. Our results provided basic information about a specific fungal population accounting for the incidence of SD.

Keywords: Malassezia, Non-Malassezia, Seborrheic dermatitis

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Introduction

The human skin mycobiome is comprised of a variety of fungal species [1-4]. Fungal microbiota reportedly undergoes a change with the incidence or severity of certain skin diseases [5-8]. For example, the number of Malassezia yeasts is increased 1.5-2 times, compared to the normal levels, on the seborrheic dermatitis (SD)-afflicted crust [7, 8]. The SD is characterized by erythematous patches that mostly appear in sebaceous gland-rich areas in the scalp, forehead, eyelids, nasolabial folds, and upper trunk [9].

Malassezia lipophilic yeasts play a key role in the development of SD; however, its contribution to other fungal microbiota for the recurrence and severity of the disease has been addressed in the literature [10-11]. Simultaneous presence of Candida species and M. pachydermatis in canine SD lesions is associated with the exacerbation of the clinical symptoms of SD. The co-colonization of these two yeasts is related to the formation of a much higher amount of extracellular polymeric substance matrix (biofilm). This matrix protects microbial cells against adverse conditions and causes chronic infections by tightly attaching them to the surface material [12]. Determination of the associations among Malassezia species or between these species and other fungi can facilitate the establishment of therapeutic procedures for SD. Regarding this, the present study was conducted to comprehensively analyze the skin fungal microbiota in SD lesions.

Materials and Methods

Patients and specimens

The Ethics Committee of Alborz University of Medical Sciences, Karaj, Iran, approved all experiments conducted in the study with the code of Abzums.Rec.1395.51. This study was conducted on 19 patients referring to the Skin Department of Bahonar Hospital, Karaj, Iran. The lesions with yellowish scale, pityriasisiform scaling, and greasy seborrhea and erythematous plaques were clinically diagnosed as SD [13]. The patients who had used antifungal drugs or other medications, such as anti-inflammatory and antimicrobial drugs, during the previous 6 weeks [14], were excluded from the study.

Isolating and identification of the fungus inducing seborrheic dermatitis

Microscopic identification was performed on the collected samples using the direct KOH test on a wet
mount. Skin samples collected from the SD lesions were inoculated on modified Dixon’s agar (Sigma, Germany), supplemented with cyclohexamide (0.5%), chloramphenicol (0.05%), and Sabarbo dextrose agar (Sigma, Germany). Subsequently, the samples were incubated for 10 days at 32°C for the isolation of Malassezia and for 3-5 days at 37°C and 24°C for the isolation of other fungal species. The obtained colonies were identified based on the morphological characteristics of the colonies, attendance of catalase, germ tube examination [15], and Tween assimilation test [8].

**Molecular identification**

Polymerase chain reaction (PCR) was performed by isolating the fungal genomic DNA using the phenol-chloroform protocol described by Yamada et al. [16]. The PCR amplification of genomic DNA was carried out to amplify the ITS-5.8S rDNA region using the universal primers ITS4 and ITS5 [17]. The PCR was performed in 25 µl of a 2-µl Taq 2 x PCR Master Mix (SinaClon BioScience Co., Karaj, Iran), 0.5 µl of each primer, and 2 µl DNA templates using a PCR thermal cycler (Peqlab, Belgium). The thermal cycle consisted of denaturation for 5 min at 94°C, 35 cycles of a second denaturation at 94°C for 45 sec, annealing at 56°C for 40 sec, and elongation at 72°C for 2 min. The PCR was completed through a final elongation at 72°C for 10 min.

Further gel extraction of the PCR products and sequencing were performed using the Biosystems 3730 XL Bioneer DNA analyzers (Korea). The molecular database recorded at NCBI Medical Library, Bethesda, MD, USA (http://www.ncbi.nlm.nih.gov/BLAST/) was also employed for the identification of the isolated fungal species.

**Statistical analysis**

The data were analyzed using SPSS software, version 15 (SPSS Inc., Chicago, IL, USA). The Chi-square test was used to determine the potential relationship between SD and two variables of age and gender. A p-value less than 0.05 was considered statistically significant.

**Results and discussion**

In the present study, the universal primers ITS4 and ITS5 were used to amplify the ITS5.8S rDNA region. A total of 23 isolates of Malassezia strains and 22 isolates of other fungal species were taken from 19 patients with SD (Table 1). Analysis of the relationship between SD and demographic characteristics did not show a significant association between SD and age and gender (P>0.07). According to the data, the mean age of the participants was 30.4 years (Table 1).

The patients had no predisposing factor contributing to their infection. The fungal isolates detected in the samples included Cryptococcus albidus var. albidus (n=6, 13.3%), Candida parapsilosis (n=7, 15.5%), Rhodotorula mucilaginosa (n=6, 13.3%), and Penicillium polonicum (n=3, 6.7%; Table 1, Figure 1). These microorganisms rarely cause opportunistic fungal infections in immunocompromised hosts despite their common presence on the skin surface of the healthy subjects [3]. Nevertheless, in this study, Candida parapsilosis (15.5%; P<0.001) was found to be the most common non-Malassezia species isolated from SD lesions.

Candida parapsilosis, as an opportunistic pathogen in human, can cause different infections, such as cerebritis, pneumonia, endocarditis, peritonitis, osteomyelitis, arthritis, and onychomycosis [18, 19]. Recent evidence is suggestive of the associations between the increased resistance level of SD lesions in dogs and the co-colonization of two yeasts, namely C. parapsilosis and M. pachydermatis [12]. This can be explained by the growth of predominant strains by the first colonizing organism, which crowds out others in the same niche. Moreover, C. parapsilosis can produce more biofilms associated with Malassezia species. The amount of biofilm produced from the co-colonization of these yeasts was higher than that produced from single strains in vitro. This biofilm could exacerbate clinical symptoms by increasing the obstruction risk of sebaceous glands, leading to skin inflammation. It also

| Table 1. Isolation of non-Malassezia and Malassezia clinical strains from patients with seborrheic dermatitis |
|-------------------------------------------------|----------------|------------------------------|----------------|
| Fungal species | Number (%) | Gen bank accession numbers | Gender | Age (Year) |
| Cryptococcus albidus var. albidus | 6 (13.3) | MN044934, MN044938 | Male=8 | Range=17-48 |
| Candida parapsilosis | 7 (15.5) | MN044946, MN044933, MN044944, MN080442, MN044945 | Female=11 | Mean=30.4 |
| Penicillium polonicum | 3 (6.7) | MN044940, MN044941, MN044942, MN044943 | | |
| Rhodotorula mucilaginosa | 6 (13.3) | MN044927, MN044931, MN044932, MN044928, MN044930, MN044929 | | |
| M. furfur | 9 (20.01) | | | |
| M. globosa | 7 (15.5) | | | |
| M. slooffie | 3 (6.7) | | | |
| M. sympodialis | 3 (6.7) | | | |
| M. restricta | 1 (2.2) | | | |
Figure 1. Gel electrophoresis of polymerase chain reaction products of isolated non-Malassezia species from SD scales on 1% agarose gel: lanes 1-4) Cryptococcus albidas var. albidas, Candida parapsilosis, Rhodotorula macluginosa, and Penicillium polonicum, lane M) 1-kb DNA ladder

serves as a drug sponge with antifungal sequestration abilities that can reduce azole activities against fungi [12, 20]. The results of the present study regarding the fungal communities on the lesions of SD provided new evidence and confirmed previous findings. The current study is the first attempt targeting fungal microbiota in SD lesions in Iranian samples.

In conclusion, the results of the present study may provide basic information about the presence of a specific fungal population in SD lesions. However, our samples are too small to prove this claim. Therefore, further studies are recommended to investigate a larger sample size in order to evaluate the relationship between fungal species and SD.

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Author’s contribution
All the authors provided their comments and ideas during the different stages of the study.

Conflicts of interest
The authors declared no conflicts of interest regarding the publication of the present paper.

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There are no financial conflicts of interest to disclose.

References
1. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. Nat Rev Microbiol. 2018; 16(3):143-55.
2. Underhill DM, Biever ID. The mycobiota: interactions between commensal fungi and the host immune system. Nat Rev Immun. 2014; 14(6):405-16.
3. Zhang E, Tanaka T, Tajima M, Tsuboi R, Nishikawa A, Sugita T. Characterization of the skin fungal microbiota in patients with atopic dermatitis and in healthy subjects. Microbiol Immunol. 2011; 55(9):625-32.
4. Niemeyer van der Kolk T, van der Wall HE, Balmforth C, Van Doorn MB, Rissmann R. A systematic literature review of the human skin microbiome as biomarker for dermatological drug development. Br J Clin Pharmacol. 2018; 84(10):2178-93.
5. Rodrigues Hoffmann A. The cutaneous ecosystem: the roles of the skin microbiome in health and its association with inflammatory skin conditions in humans and animals. Vet Dermatol. 2017; 28(1):60-e15.
6. Findley K, Grice EA. The skin microbiome: a focus on pathogens and their association with skin disease. PLoS Pathog. 2014; 10(10):e1004436.
7. Theelen B, Cafarchia C, Gaitanis G, Bassukas ID, Boekhout T, Dawson TL Jr. Malassezia ecology, pathophysiology, and treatment. Med Mycol. 2018; 56(Suppl 1):S10-25.
8. Hedayati M, Hajheydari Z, Hajjar F, Elsani A, Shokohi T, Mohammadpour R. Identification of Malassezia species isolated from Iranian seborrhoeic dermatitis patients. Eur Rev Med Pharmacol Sci. 2010; 14(1):63-8.
9. Prohic A, Jovovic Sadikovic T, Krupalija J, Prohic M, Radijic M. Malassezia species in healthy skin and in dermatological conditions. Int J Dermatol. 2016; 55(5):494-504.
10. Zoulia EN, Widaty S, Ksienty IA, Wahid MH. Identification of Malassezia species and the severity of seborrhoeic dermatitis on scalp in Dr. Cipto Mangunkusumo Hospital Jakarta. Adv Sci Let. 2018; 24(9):66-49.
11. Faeregannan J. Management of seborrhoeic dermatitis and pityriasis versicolor. Am J Clin Dermato. 2000; 1(2):75-80.
12. Bumroongthai K, Chetanachan P, Niyomtham W, Yurayat C, Prapasarakul N. Biofilm production and antifungal susceptibility of co-cultured Malassezia pachydermatis and Candida parapsilosis isolated from canine seborrhoeic dermatitis. Med Mycol. 2016; 54(5):544-9.
13. Chowdhry S, Gupta S, D’souza P. Topical antifungals used for treatment of seborrhoeic dermatitis. J Bacteriol Mycol Open Access. 2017; 4(1):76.
14. Mahmoudi E, Saedi M, Marashi MA, Moafi A, Mahmoudi V, Zeinolabedini Zanami M. In vitro activity of kambucha tea ethyl acetate fraction against Malassezia species isolated from seborrhoeic dermatitis. Curr Med Mycol. 2016; 2(4):30-6.
15. Kalantar E, Marashi SM, Pormazaheri H, Mahmoudi E, Hatami S, Barati MA, et al. First experience of Candida non-albicans isolates with high antibiotic resistance pattern caused oropharyngeal candidiasis among cancer patients. J Can Res Ther. 2015; 11(2):388-90.
16. Yamada Y, Makimura K, Merhendi H, Ueda K, Nishiyama Y, Yanaguchi H, et al. Comparison of different methods for extraction of mitochondrial DNA from human pathogenic yeasts. Jpn J Infect Dis. 2002; 55(4):122-5.
17. Gupta AK, Boekhout T, Theelen B, Summerbell R, Batra R. Identification and typing of Malassezia species by amplified fragment length polymorphism and sequence analyses of the internal transcribed spacer and large-subunit regions of ribosomal DNA. J Clin Microbiol. 2004; 42(9):4253-60.
18. Trofa D, Gaeber A, Nosanchuk JD. Candida parapsilosis, an emerging fungal pathogen. Clin Microbiol Rev. 2008; 21(4):606-25.
19. Kohler JR, Casadevall A, Perfect J. The spectrum of fungi that infects humans. Cold Spring Harb Perspect Med. 2015; 5(1):a019273.
20. Yin W, Wang Y, Liu L, He J. Biofilms: the microbial “protective clothing” in extreme environments. Int J Mol Sci. 2019; 20(14):E3423.