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Frequency of Different *Malassezia* Species in Scalp Dandruff

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Background: Members of the *Malassezia* genus are often lipophilic, observed as budding yeasts and found as commensals in the skin of humans. This genus opportunistically reside in several areas including scalp where under the influence of particular predisposing factors, their proliferation is increased (e.g., high activation of sebaceous glands), and leads to dandruff and seborrheic dermatitis, which together affects >50% of human beings. The proliferation of yeasts in scalp creates health and hair hygiene problems. In this study we determined the type and frequency of *Malassezia* species in scalp dandruff in order to have epidemiologic and therapeutic understanding.
Materials and Methods: Differentiation tests were done for scalp samples, including: morphology, Tween 20, 40 and 80 assimilation tests, hydrolysis of bile-esculin, catalase and growth on Sabouraud dextrose agar with chloramphenicol and cycloheximide (SCC) and sediment production on mDixon agar medium.
Results: Frequency of various *Malassezia* species from 140 scalp samples from volunteers of both gender were found as: *M. globosa* (46.5%), followed by *M. furfur* (27.0%), *M. restricta* (12.7%), *M. sympodialis* (6.5%) and *M. slooffiae* (0.8%).
Conclusion: In view of high prevalence of *M. globosa*, its invasive characteristics and the role of predisposing factors in the more proliferation of this species in scalp should be considered.

Keywords: Malassezia, Scalp, Dandruff

1. Background

Malassezia is a lipophilic fungus, appearing in yeast form. It exists as a commensal in the skin of more than 90% humans depending upon the influence of particular predisposing factors, and its abnormal proliferation increases opportunistically leading to dandruff and seborrheic dermatitis, which together may affect more than 50% of humans (1-4). The distribution of the yeasts increases in scalp, especially in youth males and leads to scalp-flaking and dandruff that not only causes physical hygienic problem, but may trouble mentally (5-8). Furthermore, treatment of this problem is time consuming and expensive because of consecutive changes in the type of shampoos. Diagnosis, identification and prevalence of fungal species in scalp have a therapeutic and epidemiologic importance. In addition, the yeast causes systemic disease such as fungemia and is involved in other diseases such onychomycosis, psoriasis, sinusitis, peritonitis, fungemia, blepharitis, folliculitis and inflammation of the lacrimal duct (3,9). According to modern classification, *Malassezia* members consist of 14 species as follows: *M. furfur, M. obtusa, M. globosa, M. slooffiae, M. sympodialis, M. pachydermatis, M. restricta, M. dermatis, M. equina, M. japonica, M. nana, M. yamatoensis, M. caprae and M. cunicoli* (10).

The proliferation of yeasts in scalp creates problem for their health and hair hygiene. Thus, it is important to determine kind and frequency of *Malassezia* species in order to have epidemiologic and therapeutic understanding.

2. Objectives

The aim of this study was to determine the frequency of *Malassezia* species in scalp dandruff that were isolated from volunteers with scalp dandruff.

3. Materials and Methods

3.1. Sampling

Samples were obtained from scalps of 140 adult male and female volunteers by scraping by scalpel. Scrapings were then transferred on a slide containing one drop of sterile distilled water. A second slide was placed over it and two smears were prepared by pressing the slides against each other. After drying at room temperature, smears were heat fixed and stained with methylene blue.

3.2. Direct Microscopic Examination (DME)

The direct microscopic examination of stained slides was performed for the diagnosis of different yeast species. Yeast quantification in scalp (dandruff) was done as described previously (8, 11) and recorded in the following manner under 40X magnification: average counting 0-5 yeasts in 5 high power fields (mild); average counting 6-15 yeasts in 5 high power fields (moderate); average counting>15 yeasts in 5 high power fields (intense).

3.3. Culture and biochemical differential tests

Samples were inoculated to modified Dixon agar medium (mDixon) and incubated at 32°C with humidity for 1-2 weeks. The grown colonies of *Malassezia* were subcultured to obtain high amount of yeast colonies for biochemical differential tests as described previously (3, 12, 13). The tests were performed including: i) Tween 20, 40 and 80 assimilation tests through culturing yeast colonies suspensions on Sabouraud dextrose agar with chloramphenicol and cycloheximide (SCC) medium, as follows: suspensions with 10^6-10^8 yeasts were prepared and were added to SCC tubes at 50°C, mixed and distributed in plates. After cooling of agar, three wells (3-4mm diameter) were begat on plates and tweens with certain concentrations were added to wells and the plates were incubated at 32°C and proper humidity for a week. Results of yeast growth around the wells were recorded. ii) Study of bile hydrolysis on bile esculin agar medium was done as follows: some of yeast colonies were transferred to medium tubes with the tip of a lance and positive

results were observed with color transforming of media to black after incubation at 32°C and proper humidity for a week. iii) Use of 3% Hydrogen peroxide for slide catalase test to observe positive reactions as rising of air bubbles from H_2O_2 after mixing of some colonies on the slide. iv) culture of some colonies on SCC and keeping them at 32°C for differentiation of *Malassezia pachydermatis*. v) Study of sediment production on mDixon agar medium at 32°C by culturing primary colonies in this medium to observe production of insolvable fatty acids.

4. Results

One hundred and forty volunteers from adult persons of both genders and various age groups were enrolled in the study, of which; 59.5% were males and 40.5% were females. The lowest and highest age of patients was 11 and 80 years, respectively. The average age was 27.2 years old (SD=12.3) and most of the volunteers were in 21-30 age group. The direct microscopic examination (DME) of stained slides revealed different shapes (globular, oval and cylindrical) of budding yeast cells with broadband or narrowband connections. Furthermore, mycelium existence was also surveyed (Fig.1.C and D). This test was positive in 93.5% of samples; however, 6.5% volunteers were negative in DME. All positive cases in DME were also positive in culturing on mDixon agar medium. The result of growth on SCC medium is shown in Figure 1.A. The bile hydrolysis test was performed by culturing on bile esculin agar medium and result of yeast growth around the wells is shown in Figure 1.B.

The results of species frequency were: *M. globosa* (46.5%), followed by *M. furfur* (27.0%), *M. restricta* (12.7%), *M. sympodialis* (6.5%), *M. slooffiae* (0.8%). The results of species frequency based on age groups of males, females, all volunteers, gender and yeast quantity are shown in Table 1 and Table 2. According to the results, the noticeable statistical relationship was found between females age groups (p=0.03) (Table 1) and yeast quantity (p=0.03) (Fig.2).

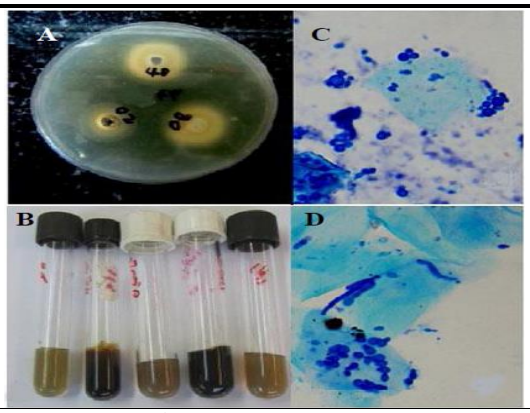

Figure 1. Detection of various *Malassezia* spp. by biochemical tests and direct microscopy. A) Positive Tween assimilation test by *M. furfur* (SCC). B) Positive results of bile esculine hydrolysis (shown in black). C) *M. globosa* in scalp dandruff (DME). D) *M. furfur* associated with mycelium in scalp dandruff (DME).

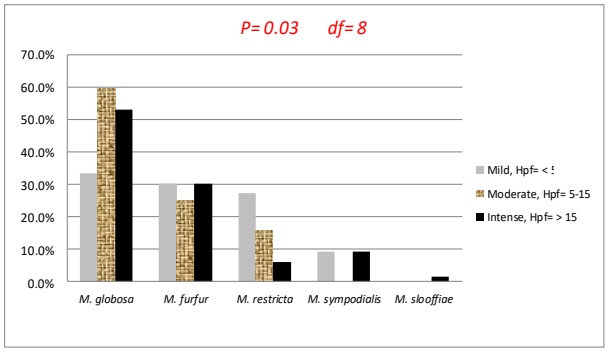

Figure 2. Frequency of different *Malassezia* spp. in dandruff.
*Hpf: High power field, P: Probability Value, df: degree of freedom.

Table1. Frequency of *Malassezia* spp. in scalp dandruff based on age groups in males and females.

	***Malassezia* spp.**							***Malassezia* spp.**						
Age groups of males (years old)	***M. globosa***	***M. furfur***	***M. restricta***	***M. sympodials***	***M. slooffiae***	**Total**		**Age groups of females (years old)**	***M. globosa***	***M. furfur***	***M. restricta***	***M. sympodials***	***M. slooffiae***	**Total**
	N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**			**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
10-20	16 (66.7)	4 (16.7)	3 (12.5)	1 (6.2)	0	24 (100)		10-20	12(80)	3 (20)	0	0	0	15 (100)
21-30	15 (38.5)	13 (33.3)	6 (15.4)	5 (12.8)	0	39 (100)		21-30	8 (36/4)	10 (45.5)	4 (18.2)	0	0	22 (100)
31-40	4 (50)	1 (12.5)	2 (25)	0	1 (12.5)	8 (100)		31-40	4 (36.4)	3 (27.3)	1 (9.1)	3 (27.3)	0	11 (100)
41-50	0	1 (50)	1 (50)	0	0	2 (100)		41-50	2 (100)	0	0	0	0	2 (100)
51-60	2 (100)	0	0	0	0	2 (100)		51-60	0	1 (100)	0	0	0	1(100)
>60	1 (33.3)	2 (66.7)	0	0	0	3 (100)		>60	1 (50)	0	1 (50)	0	0	2 (100)
Total	38 (48.7)	21 (26.9)	12 (15.4)	6 (7.7)	1 (1.3)	78 (100)		Total	27 (50.9)	17 (32.1)	6 (11.3)	3 (5.7)	0	53(100)
Probability Value=0.2 degree of freedom=20								Probability Value=0.03 degree of freedom=15						

Table 2. Frequency of *Malassezia* spp. in scalp dandruff based on age groups in all volunteers and in different genders.

Age groups of all volunteers (years old)	*Malassezia* spp.						Gender	*Malassezia* spp.					
	M. globosa	*M. furfur*	*M. restricta*	*M. sympodials*	*M. slooffiae*	Total		*M. globosa*	*M. furfur*	*M. restricta*	*M. sympodials*	*M. slooffiae*	Total
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
10-20	28 (71.8)	7 (17.9)	3 (7.7)	1 (2.6)	0	39 (100)	Male	38 (48.7)	21 (26.9)	12 (15.4)	6 (7.7)	1 (1.3)	78 (100)
21-30	23 (37.7)	23 (37.7)	10 (16.4)	5 (8.2)	0	61 (100)							
31-40	8 (42.1)	4 (21.1)	3 (15.8)	3 (15.8)	1 (5.2)	19 (100)	Female	27 (50.9)	17 (32.1)	6 (11.3)	3 (5.7)	0	53 (100)
41-50	2 (50)	1 (25)	1 (25)	0	0	4 (100)							
51-60	2 (66.7)	1 (33.3)	0	0	0	3 (100)	Total	65(49.6)	38 (29)	18 (13.7)	9 (6.9)	1 (0.8)	131 (100)
> 60	2 (40)	2 (40)	1 (20)	0	0	5 (100)							
Total	65 (49.6)	38 (29)	18 (13.7)	9 (6.9)	1 (0.8)	131 (100)							
Probability Value=0.2 degree of freedom=20							Probability Value=0.8 degree of freedom=4						

5. Discussion

According of the Leeming's and other studies, the use of good culture media and sampling can increase the chance of *Malassezia* isolation from the skin up to 98% (14). Various species have been isolated from different parts of the body like the scalp of healthy people (1, 3, 14, 15). For sampling, different methods have been used such as: scraping, scotch gold tape, soap, plate contact and hair brushing (16-19). In this study, scraping method was used, which is a proper method for obtaining a large number of samples at any time. All positive samples on DME were inoculated on mDixon agar media were found positive, although the growth rate at 30% of cases after 20 days was so weak and limited to one colony but we could gain mass of the colonies by repeated passage on a new mDixon agar medium. Similar reports are available from Korea, where 90% positivity has been reported on culture by Jung (18). Scalp culture of healthy people in Iran was found positive in 100% of cases (20). Gupta and colleagues (2004) showed culture positivity in ages of over 14 years old to be 90% (16). For culture, it is important that the culture medium should be fresh with optimum temperature of 32-35°C and humidity must be maintained (21). Another noteworthy point is that, despite its high quantity in the scalp, yeasts may be missed after the one month period of time.

In the present study, the highest prevalence of *M. globosa* in comparison to other species is compatible to other studies (20, 22) and those carried out on patients with seborrheic dermatitis in Iran (17, 20, 23, 24). The direct role of the *Malassezia* species in etiology of seborrheic dermatitis is not so clear, whether yeasts are etiologic agents of disease or abnormal proliferation of yeasts results from disease (3). Findings from a healthy scalp indicate that the species exist as normal flora, and appearance of suitable condition makes the yeasts to proliferate abnormally.

To find a significant effect of age groups, gender and yeast quantity on species frequency, statistical computation was done and the results showed no significant difference in frequency of various species with gender, age groups of all volunteers and age groups of males (Table 1 and 2) ($p>0.05$), however; significant differences were noticed with yeast quantity ($p=0.03$) (Fig. 2) and age groups of females ($p=0.03$) (Table 1). It is probable that early puberty in females and increased activity of sebaceous glands may create the appropriate environment for colonization and proliferation of *M. globosa* (20). In our study, only adult volunteers were enrolled and if immature were enrolled too, probably it would have given the differences as observed in other studies (18, 19, 25-28). For example, in study of Gupta and colleagues, *M. sympodialis* was the prevalent species in 15-25 age group, while, in other age groups *M. globosa* was more prevalent (16). Also, according to study by Lee and colleagues, *M. restricta* has been reported as a prevalent species in adolescents and adults less than 50 years old, while, in above of 50 years old, *M. globosa* has been found to be the prevalent species (10, 25). The main reasons for these differences in such studies have been reported to be because of differences in composition and volume of secreted sebum in different ages associated with difference in the prevalence of different species of *Malassezia* in varied geographic regions. Noticeable statistical relationships were observed between species and yeast quantity ($p= 0.03$) (Fig. 2) which could be due to invasive characteristics of *M. globosa* (20).

6. Conclusion

M. globosa was seen as predominant species in dandruff of persons which emphasizes on its invasive characteristics and the role of predisposing factors in proliferation of this species in scalp which should be considered in therapeutic purposes.

Conflict of Interests

The authors declare they have no conflict of interests.

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Authors' Contributions

All of authors contribute to this study.

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