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Role of *Malassezia furfur* and *M. globosa* in Dandruff and Seborrheic Dermatitis

Sibi D[1]*, Silvanose CD[2] and Jibin VG[3]

[1]*Sri Sidhartha Medical College, Tumkuru, Karnataka, India*
[2]*Dubai Falcon Hospital, Dubai, UAE*
[3]*District Hospital, Bundi, Rajasthan, India*

***Address for Correspondence**
Sibi D, Sri Sidhartha Medical College, Tumkuru, Karnataka, India
Email: sdsilvanose@gmail.com

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Abstract

Dandruff is a common problem in both teens and adults. This study is to evaluate the role of bacteria and fungi associated with dandruff and seborrheic dermatitis. *Malassezia furfur* (70%) was the predominant isolate, followed by *Malassezia globosa* (30%) which included mixed infection (15%) of both *M. furfur* and *M. globosa* together adding as the significant causative agents ($p < 0.00001$) as compared to healthy teens. A qualitative in-vitro susceptibility study was performed with Ketoconazole which showed good in-vitro anti-Malassezia activity with a greater inhibitory zone, and similar anti-Malassezia activity was shown by tea tree oil and 1% selenium sulfide. A follow-up study was performed after treatment with 1% selenium sulfide shampoo and showed 92.5% efficiency which suggests a possible solution for dandruff and seborrheic dermatitis.

Introduction

Seborrheic dermatitis (SD) and dandruff are widespread, reportedly affecting 45-50 percent of the global population [1]. Dandruff is a mild form of seborrheic dermatitis. Its hallmark is discarded stratum corneum cells clumped into oily white flakes that are all too obvious on dark clothes, hair, and scalp. There are also changes to the skin that are not visible to the naked eye. While flakes make dandruff apparent, a patient may suffer itching and scalp tightness without visible evidence of cell hyperproliferation [1]. However, many people think it is a dry scalp and are not aware of the condition which can be relieved by proper treatment. Dry skin flakes are generally smaller than dandruff and shed transparent flakes that are barely visible, rather than oily white or yellow flakes. Seborrheic dermatitis has the same etiology as dandruff, but it is an extremely severe form that requires medical treatment [2].

The suspicion of fungi that cause disease is only about a few decades old, but the precise diagnosis is possible now as there are new resources in the scientific toolkit and genetic research which finally provides the means to accurately identify the precise microflora component of *Malassezia* species [3]. Recent genetic research has turned up new evidence that the fungus is causing an agent formerly identified as *Pityrosporum ovale*, an antiquated term that is no longer used, and later identified as *Malassezia furfur*, the fungus producing seborrheic dermatitis and dandruff, but later added another species *M.globosa* and *M. restricta* [4].

Accurate identification of causative agents is essential in treating and preventing seborrheic dermatitis and the less severe form in which it often appears is dandruff. The inflamed skin may also be intensely itchy, and the flakes may be white or yellow. *M. furfur* is known to cause disease, but later *M. globosa* was added, but some specialists were still baffled by an inconsistency. *M. furfur* infections occurring on other body parts look very different from the scalp desquamation associated with seborrheic dermatitis and dandruff [5]. As there are some inconsistencies that exist, this study is to rule out the role of *Malassezia* species which cause dandruff and seborrheic dermatitis in teens, and possible recommendations for clinical solutions. It was a comprehensive study on children which included Bacteriology, Mycology, Cytology, *Malassezia* culture, and anti-Malassezia studies to investigate the role of microbes and associated cellular findings.

Material and Methods

Samples were collected from 40 children between the ages of 12 to 15, using saline-moistened sterile swabs rotating on their scalps. Four samples were collected from each person and processed immediately at Dubai Falcon Hospital (Dubai, United Arab Emirates) for cytology, bacteriology culture, fungi, and *Malassezia* culture. These 40 selected children were included in group 1 and a control group of 20 healthy children who did not have visible dandruff or seborrheic dermatitis was included in group 2. In group 1, 38 children had dandruff and two of them had seborrheic dermatitis, which is characterized by large scaly patches, a flaky and itchy scalp with mild hair falls, and pimples on the scalp. Thus, they were grouped together as they did not have any other health problems. Bacterial and fungal cultures were done to rule out any bacterial and fungal involvement other than the *Malassezia* species.

Cytology

Cellular findings associated with microbial colonization is a rapid diagnostic method and thus cytology smears were prepared; air dried, fixed in ethanol, and stained using commercial preparation of eosin-methylene blue stain (Neat stain, Astral scientific, USA). All stained smears were examined under oil immersion (1000x) and microscopic photography was done using an Olympus microscope camera system (Olympus BX 51 with DP70, Japan).

Bacteriology

Samples collected from both groups were performed for bacteriology culture in 5% sheep blood agar and MacConkey's agar (Liofilchem, Italy) to evaluate any involvement of bacteria associated with dandruff conditions or seborrheic dermatitis, whether as a

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causative or triggering agent. Culture plates were incubated at 37°C for 48 hours in an aerobic incubator (Shel lab, USA). Bacteria isolated were identified using cultural characteristics, gram stain, and biochemical characteristics using a Vitek 2 analyzer (Biomeriux, France).

Mycology

Swabs collected from both groups were inoculated for mycology culture in *CHROMagar Malassezia* with Tween 40 and Glycerol (CHROMagar, France) and Sabouraud Chloramphenicol agar (Liofilchem, Italy). *Malassezia* species were identified by morphological characteristics in *CHROMagar Malassezia* and a microscopic appearance in a stained smear. Identification of *M. furfur* and *M. globosa* was correctly set by *CHROMagar Malassezia* colony characteristics including color and morphology, which was established based on molecular analysis, and thus both correlate to 100% sensitivity and specificity [6]. Culture plates were incubated at 37°C for 72 hours in an aerobic incubator (Shel lab, USA).

Qualitative Antifungal Studies by Disc Diffusion Technique

Antifungals and pure essential oils were used to test against *Malassezia* species to determine the qualitative growth inhibition. The essential oils used in this study included lemon grass oil (*Cymbopogon citratus*), Clove oil (*Syzygiumaromaticum)*, Tea tree oil (*Melaleuca alternifolia*), Basil Oil (*OcimumBasilicum*), eucalyptus oil (*Eucalyptus globulus*) and Neem seed oil (*Azadirachta indica*). 1% Ketoconazole, 1% selenium sulfide, and Glycerol (propane- 1, 2, 3-triol) also aka Glycerin included the anti-Malassezia activity.

All microbiology work was done inside a microbiology safety cabinet (Faster, Italy). Anti-Malassezia studies were performed on *CHROMagar Malassezia* with standard disc diffusion techniques. All culture plates were incubated at 37°C for 72 hours in an aerobic incubator and the inhibitory zones were noted for qualitative assessment of anti-Malassezia activity.

Follow-up Studies

A follow-up study was undertaken for group 1 after treatment with Selsun anti-dandruff shampoo (Abbott Healthcare Ltd, India) which contains 1% selenium sulfide as an active ingredient. Anti-dandruff shampoo treatment was performed four times with an interval of 7 days of each treatment and samples were collected for cytology and fungal studies after 30 days of initial sample collection. A follow-up bacteriology culture was done in one case of SD after treatment with Fucidin which was earlier isolated with *Staphylococcus aureus.*

Results

Cytology findings from the smears, bacteriology, and mycology culture results were documented separately for comparison between groups.

Cytology Results

Excess Superficial squamous cellular exfoliation was seen in association with moderate and severe colonization of *Malassezia* species (100%) in group 1 with dandruff and SD. The superficial exfoliation was minimal in healthy teens of group 2, which includes <5 squamous cells per field of high-power magnification of microscope with scanty *Malassezia* species (40%) while in group1, aggregates of superficial squamous cells (90%) were seen per field with heavy colonization of *Malassezia* species. *M. furfur* is oval '8' shaped yeast-like budding cells, like *Candida* species (Figure 1) and *M. globosa* are spherical budding cells (Figure 2). Smear findings also include cocci in groups resembling *Staphylococci* species (20%) and isolated large cocci resembling Micrococcus species (22%) and mixed types of cocci (35%) in group 1 with dandruff and SD. Similar findings were also seen in the healthy control group and it is including cocci in groups resembling *Staphylococcus* species (15%) and isolated large cocci resembling *Micrococcus* species (15%) and mixed cocci (30%).

The cytology findings suggest that the colonization of *Malassezia* species with excess exfoliation of superficial squamous cells is the only obvious association with dandruff and SD cases.

Bacteriology Results

The bacteria isolated from both groups include normal skin flora except S*taphylococcus aureus* isolated from an SD case. Non-pathogenic *Staphylococcus* species (50%) and *Micrococcus* species (45%) are the major isolates in group 1 with a mixed growth of 35% isolates. Similar skin flora bacteria were isolated in group 2, which includes non-pathogenic *Staphylococcus* species (65%) and *Micrococcus* species (40%) with a mixed growth of 30% isolates. *Micrococcus* species and *Staphylococcus* species isolated from both groups were compared and the results are statistically not significant at $p<0.01$. Table 1 shows the summarized bacteriology culture results of both groups, which shows the normal skin flora organism except *S. aureus* isolated in a single case with SD.

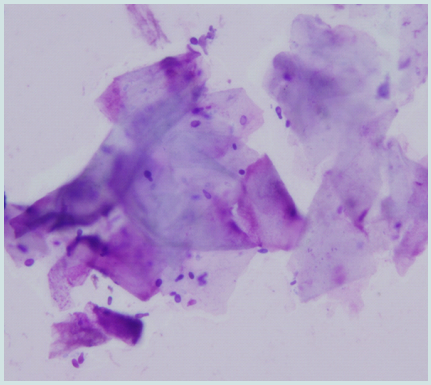

Figure 1: Cytology from Group 1 shows exfoliated superficial squamous cells and *M. furfur*.

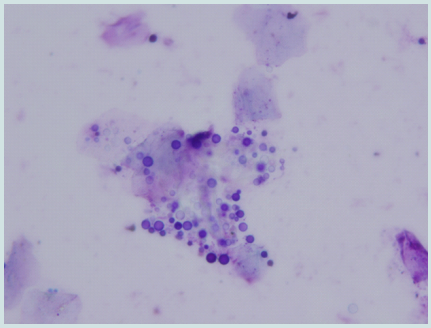

Figure 2: Cytology from Group 1 shows exfoliated superficial squamous cells and *M. globosa*.

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Mycology Results

Malassezia species were isolated in *CHROMagar Malassezia*, while the culture was negative for other fungi or *Candida* species. *M. furfur* colonies were easily distinguishable from those of other *Malassezia* species in *CHROMoagar Malassezia* due to their characteristically large pale pink colonies and *M. globosa* were small, purple, and smooth colonies (Figure 3). Table 2 shows the *Malassezia* culture results from both groups and it revealed significant findings in association with dandruff and SD.

Heavy colonization of *Malassezia* species (100%) was isolated from all dandruff cases in group 1 but mild colonization of *Malassezia* species (20%) was isolated in healthy cases belonging to group 2. Among the dandruff cases, *M.furfur* (70%) was more predominant than *M. globosa* (30%), including mixed isolates of *M.furfur* and *M.globosa* (15%). Isolation of *Malassezia* species was significantly higher ($p < 0.00001$) in dandruff cases as compared to healthy teens.

Follow-up Study Results

There is no visible dandruff noticed in group 1 after treatment with 1% selenium sulfide shampoo and the exfoliation of squamous cells was minimal in cytology smears, like the healthy control group. Among the treated cases, 37 cases (92.5%) were negative for *Malassezia* species in culture and cytology smears, which was a significant finding and thus it suggests a solution to the problem. *S. aureus* is a pathogenic bacteria isolated in one case with SD, and it was treated by applying Fucidin on the infected area and became negative on treatment.

Table 1: Shows the Bacteriology findings of Group 1 (n=40) with dandruff or seborrheic dermatitis and Group 2 (n=20), a healthy control group.

Bacteria isolated	Group1 (n)*	Group 2 (n)*
Staphylococcus aureus	1	0
Staphylococcus epidermidis	9	6
Staphylococcus citrus	10	7
Micrococcus species**	18	8

*Number of isolates, **Mixed growth of *Micrococcus* species and *Staphylococcus* species isolated (14 mixed isolates from Group 1 and 6 mixed isolates from Group 2)

Table 2: Shows the Mycology findings of Group 1 with dandruff or seborrheic dermatitis and Group 2, the healthy control group.

Category (n)*	*M.furfur*	*M.globosa*	Negative
Group 1 (n=40)	28	12**	0
Group 2 (n=20)	4	4	12

*Number of isolates. ** Six cases with mixed isolates of *M. globosa* and *M. furfur*

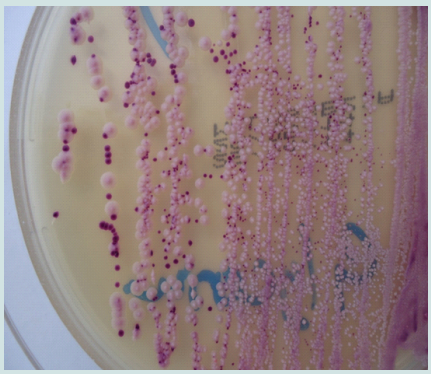

Figure 3: *Malassezia furfur* colonies were easily distinguishable on CHRO Magar Malassezia due to their characteristically large pale pink colonies and *M. globosa* was small purple colonies.

Table 3: Shows the zone of inhibition of herbal oils, azole, and other chemical agents against *M. furfur* and *M.globosa* which were isolated from the scalp of teens.

	Growth inhibition zone size of *M.furfur* (38)*		Growth inhibition zone size of *M.globosa* (16)*	
	Median (mm)	Range (mm)	Median (mm)	Range (mm)
Ketoconazole 1%	28	24 -32	28	26 -34
Selenium sulfide 1%	28	22 – 30	28	20 -30
Glycerol (propan- 1, 2, 3-triol)	0	0	0	0
Neem *(Azadirachta indica)* oil	8	0 - 8	8	0-10
Clove (Syzygiumaromaticum) oil	20	18 – 22	20	16 – 24
Lemon grass *(Cymbopogon citratus)*oil	18	16 – 24	18	14 – 24
Tea tree *(Melaleuca alternifolia)*) oil	28	24 – 30	28	24 – 30
Eucalyptus (*Eucalyptus globulus*) oil	10	0 – 12	10	0 – 12
Ocimum Basilicum (basil) oil	8	0 -10	8	0 – 10

*Number of isolates tested with antifungals and oils

Discussion

Members of the genus Micrococcus are found in the environment, and it is a frequent isolate as transient flora on the skin of humans [7]. Similarly, coagulase-negative *Staphylococcus* species such as *S. epidermidis* and *S. citrus* are skin flora organisms while *S. aureus* is a pathogenic organism as it produces several enzymes that may contribute to its virulence and it is one of the common causes of skin infections such as folliculitis, impetigo, furuncles and carbuncles [7] and it was treated successfully by applying Fucidin.

Malassezia sp. is a lipid-dependent yeast that grows well in *CHROMagar Malassezia* with Tween 40 and glycerol. Glycerol is a growth enhancer of *Malassezia* species, and it may be avoided as an ingredient in shampoos, which may have a reverse effect. An earlier study in adults shows that the number of *Malassezia* sp. retrieved was significantly higher ($P<0.001$) in dandruff cases (84%) as compared to healthy individuals (30%). *M. restricta* was the single most predominant (37.8%) isolate from patients of the northern part of India and *M. furfur* (46.4%) from patients of the southern part of India [4]. *Malassezia* species start the cycle of seborrheic dermatitis and dandruff, whether mild or severe, and variants of the condition may share a common etiology. As teens also suffer high rates of dandruff-like adults, this study was important to confirm the microbial isolates associated with dandruff and SD, which could be the causative or triggering agents.

An in-vitro study of *M. furfur* which was formerly known as *P.ovale* recorded good anti-Malassezia activity against zinc pyrithione, ketoconazole, and other azole compounds [8]. Similarly, herbal ingredients like tea tree oil, rosemary oil, coleus oil, clove oil, pepper extract, neem extract, and basil extract also recorded anti-

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Malassezia activity with lower MIC values [9-13]. Selenium sulfide has excellent anti-fungal properties and is generally safe and effective for seborrheic dermatitis/dandruff, but patients need to follow the directions and frequent washing which relieves most of the scalp seborrhea and dandruff.

Ketoconazole is a broad-spectrum anti-fungal agent that controls the scale and itches. It is available as shampoo but not recommended for patients under 12 years of age and should not be used on broken skin. In another in vitro study by agar dilution method, *M. furfur* showed a MIC of 10mg/ml to zinc pyrithione which showed effective inhibition followed by 100mg/ml for tea tree oil [14]. Zinc pyrithione and Ketoconazole were recorded as having good antidandruff activity among synthetic ingredients with an ability to reduce the growth of the test organism by 67% and 44% respectively [14]. Tea tree oil scored good activity among herbal ingredients with a recorded 78% reduction in microbial growth [12]. The fungicidal activity of clove essential oil is documented against *Candida albicans and dermatophytes* [15], but clove oil was found to be cytotoxic at higher concentrations because of eugenol [16]. Tea tree oil shows moderate toxic effects at higher concentrations, such as the effect of terpinen-4-ol, γ-terpinene, 1,8-cineole, α-terpinene, and α-terpineol [12]. Thus, concentrated clove oil and tea tree oil cannot be used directly on the scalp and require further studies to establish the concentration of herbal oil for its safe usage. *M. globosa* and *M. restricta* were added later as the causative agent and the antifungal studies show similar activity with 1% ketoconazole, 1% selenium sulfide, and tea tree oil [17].

Conclusion

M. furfur and *M. globosa* were the isolates associated with dandruff and seborrheic dermatitis in teens and it has a significant role as a causative or triggering agent with $p<0.00001$. Ketoconazole 1% showed good anti-Malassezia activity with a greater inhibitory zone, and similar anti-Malassezia activity was shown by tea tree oil and 1% selenium sulfide. A follow-up study was performed after four shampoo treatments with 1% selenium sulfide shows 92.5% efficiency, which suggests a possible solution for dandruff and seborrheic dermatitis.

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