The Prevalence and Species Composition of Malassezia yeasts in Patients with Clinically Suspected Onychomycosis

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ABSTRACT

Introduction: There are limited numbers of studies which focused on the identification of Malassezia yeasts to a species level in onychomycosis. Therefore, the aim of our study was to determine the prevalence and species composition of Malassezia yeasts in patients with clinically suspected onychomycosis and to examine if the range of species varies with patient gender, age, site of involvement and clinical pattern of onychomycosis.

Methods: Specimens were taken from 785 patients presenting signs of onychomycosis and then incubated on Sabouraud dextrose agar and modified Dixon agar. The yeasts isolated were identified according to their macroscopic and microscopic features and physiological characteristics.

Results: Malassezia species were diagnosed both by microscopy and culture in fourteen (1.8%) patients. M. globosa was the predominant, if not only, species identified from nail samples. Mixed cultures were observed in five cases: in 4 cases Malassezia was co-isolated with Candida albicans and in one case with dermatophyte. Fingernails were affected more frequently than toenails (85.7%) and distolateral subungual onychomycosis was the most common clinical type (78.6%).

Conclusion: No significant differences were found in the distribution of Malassezia species isolated according to demographic parameters.

Key words: Malassezia species, isolation, onychomycosis.

1. INTRODUCTION

Malassezia are lipophilic yeasts colonizing the skin of humans and in many warm-blooded animals. In contrast to their commensal roles, these organisms have been implicated in the pathogenesis of various dermatological disorders, including pityriasis versicolor (PV), Malassezia folliculitis, seborrheic dermatitis (SD), atopic dermatitis and psoriasis (1, 2). Additionally, different reports in the literature have linked Malassezia yeasts with cases of nail infection (3-10).

At present, fourteen Malassezia species have been identified from human (M. dermatis, M. japonica and M. yamotensis) and animal skin (M. nana, M. caprae, M. equina and M. cuniculi) (11). A large number of studies have been carried out world-wide on the distribution of Malassezia species on healthy and diseased human skin in which variable results have been reported from different geographical regions (3, 12-19). These variations are likely to have occurred due to different isolation techniques, different media and the various growth characteristics of each species (12).

The etiologic role of Malassezia species in onychomycosis is uncertain because these yeasts have not been shown to degrade keratin, an ability that is necessary to invade the nail.

Moreover, less is known about which of the currently recognized Malassezia species is linked to onychomycosis. The present study was designed to determine the prevalence and species composition of Malassezia yeasts in patients with clinically suspected onychomycosis and to examine if the range of species varies according to demographic parameters.

2. METHODS

Study Population

From 2000 to 2013 clinical specimens from 785 patients with clinically suspected onychomycosis referred to the Department of Dermatovenerology Sarajevo University Clinical Center were examined. Only those subjects who had not used any topical and oral treatment or ultraviolet photo therapy in previous
two months were included in the study. No concomitant diseases were registered.

The Hospital Ethics Committee approved the study and all participants signed a written informed consent.

Samples

Samples of nail clippings and scrapings, including some debris from a nail bed, were collected with sterile surgical blades after cleaning the site with 70% ethanol.

A part of this material was examined microscopically after the treatment with 30% solution of potassium hydroxide and Parker blue ink; the rest was cultured into Sabouraud dextrose agar and into modified Dixon agar consisting of 3.6% malt extract, 0.6% mycological peptone, 2.0% desiccated ox bile (Sigma Chemical Co. Ltd, Dorset, UK), 1% Tween 40, 0.2% glycerol, 0.2% oleic acid, 0.05% chloramphenicol, 0.05% cycloheximide, and 1.2% agar pH 6.0. The medium was always used within one week of preparation and the cultures were inoculated at 32°C for seven days.

Identification of Malassezia yeasts

Malassezia species were identified according to their macroscopic and microscopic features and physiological characteristics.

The macroscopic features of the predominant colonies included their shape, size, color, consistency, and the characteristics of medium around them.

Microscopic features of the yeast cells in culture were described after lactophenol staining and included the predominant morphology, size and budding base of the yeasts.

To assess the physiological properties of the yeasts catalase reaction was determined by using a drop of hydrogen peroxide (30%) onto a culture smear on a glass slide. The production of gas bubbles, indicative of release of oxygen, was considered a positive reaction. Utilization of Tween compounds was done according to the test originally described by Guillot et al. (20) and later modified by Gupta et al. (21). Yeast suspension, obtained by inoculating 5 mL of sterile water with a loopful of actively growing yeasts, was inoculated on Sabouraud glucose agar. The inoculum was then spread evenly. Each plate was divided into four sections and 5 mL of Tween 20, 40, 60 and 80 were added into a hole made in center of each section and incubated for a week at 32°C. Utilization of Tweenes was assessed by the degree of growth and/or reaction of the lipophilic yeasts around individual holes.

The ability of the various Malassezia species to growth on mDA at 38°C was studied. A sample of actively growing cultures was transferred to mDA and incubated on 38°C for seven days after which the ability to grow was investigated.

The β-glucosidase activity (spliting of esculin) of different Malassezia species was assayed using method described by Mayser et al. (22). Briefly, a loop of fresh yeast was inoculated deeply in the esculin agar tube and incubated for 5 days at 32°C. The splitting of esculin is revealed by darkening of the medium.

Statistics

Chi-squared test with Yates’ correction for a small sample size was carried out to determine the statistical significance of differences in proportions. We used a statistical package Minitab 13.0. Significance level was set at P < 0.05.

3. RESULTS

Malassezia species were diagnosed both by microscopy and culture in fourteen (1.8%) cases. Only one Malassezia species was recovered from each of the samples.

The microscopic examination of samples showed the presence of yeast cells in 14 (1.8%) patients. Typical large and spherical yeasts producing buds on narrow base, which are identical in appearance to those of M. globosa, dominated; they were found in 8 (57.1%) cases. Smaller yeast cells, oval or cylindrical, with buds forming on narrow base, were seen in remaining 6 slides (42.9%), suggesting different Malassezia species. Short filaments were not observed in any sample.

Growth of Malassezia cultures was observed from all microscopically positive samples. Four species were isolated: the most common species was M. globosa (8; 57.1%), followed in frequency by M. sympodialis (3; 28.6%), M. restricta (2; 14.3%) and M. furfur (1; 7.1%).

Malassezia was isolated by culture as the only fungus in nine (64.3%) cases; in remaining five cases Malassezia was co-isolated with Candida albicans in four cases (28.6%) and in one case with dermatophyte (7.1%).

Distribution of Malassezia species isolated according to demographic parameters

Gender

Men and women were equally affected. No statistically significant differences were found between the genders in the species isolated (p>0.05).

| species       | Gender | Age groups | Site of involvement | Clinical pattern |
|---------------|--------|------------|---------------------|-----------------|
|               | F      | M 0-15     | 16-30               | 31-45 | 46-60 | > 60 | FN | TN | DLSO | PSO | TDO |
| M. globosa    | 5      | 3          | 0                   | 1     | 1     | 5    | 1  | 7  | 1    | 6   | 1   |
| M. sympodialis| 1      | 2          | 0                   | 0     | 0     | 2    | 1  | 2  | 0    | 2   | 0   |
| M. restricta  | 1      | 1          | 0                   | 0     | 1     | 1    | 0  | 2  | 0    | 2   | 0   |
| M. furfur     | 0      | 1          | 0                   | 0     | 0     | 0    | 0  | 1  | 0    | 1   | 0   |
| TOTAL         | 7      | 7          | 0                   | 1     | 2     | 8    | 3  | 12 | 2    | 11  | 2   |

Table 1. Malassezia species distribution from patients with clinically suspected onychomycosis according to age, gender, site of involvement and clinical pattern. FN; Fingernails, TN; Toenails, DLSO, Disto–Lateral Subungal Onychomycosis; PSO, Proximal Subungal Onychomycosis; TDO, Total Dystrophic Onychomycosis
Age

Patients were aged between 22 and 79 years (average 52.8 years). According to their age, they were divided into five groups as follows: less than 15 years (n=0; 0%), 16-30 years (1; 7.1%), 31-45 years (n=2; 14.3%), 46-60 years (n=8; 57.1%) and more than 60 years (n=3; 21.4%). No statistically significant differences were found in the species isolated in these five groups (p>0.05).

Site of involvement

Fingernails were affected in twelve of fourteen patients (85.7%) and alterations on both the fingernails and toenails in a single patient were not seen.

We found no statistically significant difference in the distribution of isolated Malassezia yeasts according to the site of involvement (p>0.05).

Clinical pattern of onychomycosis

The infection presented in eleven patients as disto-lateral subungual onychomycosis (DLSO) (78.6%), in two patients as proximal subungual onychomycosis (PSO) (14.3%), while total dystrophic onychomycosis (TDO) was noted in one patient (7.1%).

There was no statistically significant difference in the distribution of isolated species according to clinical type of onychomycosis (p>0.05).

Distribution of Malassezia species isolated from patients with clinically suspected onychomycosis according to demographic parameters is demonstrated in Table 1.

4. DISCUSSION

Malassezia yeasts, the recognized etiological agent of PV, has occasionally been reported as an agent of onychomycosis, although it may exist as a colonizer of subungual debris of patients with nail infections (23). Risk factors that could affect nail plate, such as nail trauma and psoriasis, are considered to be important in the development of Malassezia onychomycosis (3-5).

With the exception of M. pachydermatis, all of the Malassezia species are obligatory lipid-dependent and show an absolute requirement for lipids in culture media. This property influences their distribution in sebum rich areas of the skin such as scalp, face and the trunk (1, 23). Since a source of lipids is essential for their pathogenicity, the etiologic role of Malassezia in onychomycosis is speculative because these yeasts do not possess keratolytic properties and have no ability to invade nails, as they are not good source of lipids.

In our study Malassezia was isolated in 1.8% of the patients diagnosed with onychomycosis, alone in 64.3% of cases, but was associated with Candida albicans in 28.6% of cases which is in concordance with study of Escobar et al. (5) who found an association between two genera in 30%. Similarly, in a study conducted in Brasil, Malassezia yeasts were isolated in 3.8% of patients with clinically suspected onychomycosis, but in 3 cases co-isolated with Candida albicans or Trichophyton rubrum (4). Furthermore, Crespo-Erchiga et al. claimed that they co-isolated Malassezia yeasts with Candida as subungual flora members. Thus, because of its low incidence in the subungual debris, the authors could not substantiate that Malassezia is a pathogenic agent in onychomycosis (24).

We assume from these observations that initially the etiologic agent might have been a dermatophyte or yeast and that the nail damage caused by the supposed fungus might have promote the consecutive invasion by Malassezia yeasts. How long they can survive as a colonizer of subungual debris without lipids necessary for their growth, and whether their presence affects the clinical picture of the nail, remains to be established.

To date, the most common species cultured from healthy and diseased human skin are M. globosa, M. sympodialis and M. restricta. M. sympodialis emerges as the predominant species on healthy skin, especially on the trunk, where it can be recovered in great numbers in more than 40% of individuals (2, 12-14), while M. globosa is reported to be a causative agent of PV, found in filamentous form in the scales from this skin disorder (2, 14-16). In our study, M. globosa was the major isolate found in more than a half of all isolate (57.1%) and M. sympodialis was the second most common agent (28.6%). The remaining two species, M. restricta and M. furfur, were recovered in lower frequencies, 14.4% and 7.1%, respectively. M. restricta is isolated regularly from the scalp and face of healthy subjects and of patients with SD (2, 17-19), while M. furfur, besides colonizing the skin, also may cause systemic fungal infection, usually in neonatal and adult patients who are receiving parenteral nutrition (25, 26). Contrary to our findings, studies that have attempted to identify Malassezia to a species level, reported isolation of M. furfur from fingernails (6, 8-10) and/or from toenails (6, 7). However, all these studies reported single cases, in contrast to our study, which included fourteen cases of Malassezia-associated onychomycosis over a longer period.

The factors permitting the transition of the yeast to the mycelial phase are still little known but the production of various enzymes such as lipase, phospholipase and lipooxygenase could contribute to the pathogenic activity of these yeasts (27). The hyphal stage is considered to play a role in the pathogenesis of PV, as hyphae are abundantly demonstrable in scales from lesions (2, 14, 15). No evidence of mycelial phase in our study further supports the proposal that Malassezia yeasts are rather colonizer than etiological agents of onychomycosis.

Chowdhary et al. reported a case of onychomycosis in which the nail invasion of Malassezia was identified by conventional and molecular techniques and additionally was proven by histopathology. In addition, other concomitant diseases that could alter the nail plate were excluded (6). Furthermore, no comorbidities were registered in our study, contrary to the study of Zawar and Chuh (8) which reported a patients having PV with clinical and mycological evidence of fingernail infection related to Malassezia. The results of the present study showed that Malassezia onychomycosis was more frequent in fingernails, what is in concordance with some aforementioned studies (6, 8-10). Scratching, regardless of whether the skin is clinically healthy or in the presence of certain skin disorders, can lead to the transmission of Malassezia fungi from the skin where they reside as commensals or pathogens, to the nails, colonizing subungual debris. We thus postulate that these species were isolated from the nail samples as a part of the normal cutaneous micro flora. Moreover, the
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The presence of Malassezia yeast in subungal debris of fingernails would be relevant as a source of systemic infections particularly in the context of colonization of patients in hospital care units (25, 26).

Clinically, onychomycosis is classified into various types, of which Disto–Lateral Subungal Onychomycosis (DLSO) was found to be the commonest clinical pattern (28). The present study showed that DLSO was the commonest clinical pattern which was seen in 78.6% patients. Proximal Subungal Onychomycosis (PSO), noted in two cases, is considered extremely rare, being described in the literature in a single case (10).

Overall, onychomycosis is more common in adults than children and its prevalence increases with age (28, 29). In the present study, the majority of patients (57.1%) were above 46 years of age (78.6%) and the age group 46–60 contained the highest prevalence of Malassezia onychomycosis (57.1%). This finding is somehow in accordance with studies examining onychomycosis caused by other fungi (dermatophytes, yeasts and molds), as no relevant studies regarding Malassezia onychomycosis were found in the literature.

To conclude, no evidence of mycelial phase supports the proposal that Malassezia yeasts are rather colonizer than etiological agents of onychomycosis. Whether these yeasts are capable of inducing clinical symptoms of onychomycosis remains to be established. However, their presence in nail material is important as a possible source of systemic infections.

CONFLICT OF INTEREST: NON DECLARED.

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