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Study of the distribution of *Malassezia* species in patients with pityriasis versicolor and healthy individuals in Tehran, Iran

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Abstract

Background: Pityriasis versicolor is a superficial infection of the stratum corneum which caused by a group of yeasts formerly named pityrosporium. The taxonomy of these lipophilic yeasts has recently been modified and includes seven species referred as *Malassezia*. The aim of this study is to compare the distribution of *Malassezia* species isolated from pityriasis versicolor lesions and those isolated from healthy skins.

Methods: Differentiation of all *malassezia* species performed using morphological features and physiological test including catalase reaction, Tween assimilation test and splitting of esculin.

Results: In pityriasis versicolor lesions, the most frequently isolated species was *M. globosa* (53.3%), followed by *M. furfur* (25.3%), *M. sympodialis*(9.3%), *M. obtusa* (8.1%) and *M. slooffiae* (4.0%). The most frequently isolated species in the skin of healthy individuals were *M. globosa*, *M. sympodialis*, *M. furfur*, *M. sloofiae* and *M. restricta* which respectively made up 41.7%, 25.0%, 23.3%, 6.7% and 3.3% of the isolated species.

Conclusions: According to our data, *M. globosa* was the most prevalent species in the skin of healthy individuals which recovered only in the yeast form. However, the Mycelial form of *M. globosa* was isolated as the dominant species from pityriasis versicolor lesions. Therefore, the role of predisposing factors in the conversion of this yeast to mycelium and its subsequent involvement in pityriasis versicolor pathogenicity should be considered.

Background

Yeasts of the genus *Malassezia* are known to be members of the skin microflora of human and other warm-blooded vertebrates [1,2]. These lipophilic yeasts are associated with various human diseases, especially pityriasis versicolor (PV), a chronic superficial scaling dermatomycosis [3]. This disease is common in late teens and young adults of both sex and characterized by well-demarcated scaling patches with variable pigmentation [4].

Although PV had been described at the beginning of nineteen century [5], until recently classification of its etiologic agent was a matter of debt. This controversy may be caused by various morphological features and fastidious growth requirements of *Malassezia* yeasts *in vitro*.

The genus of *Malassezia* has undergone several taxonomic revisions [1,6]. In the last reclassification by Gueho *et al*, seven distinct species were recognized within this genus, namely *M. furfur*, *M. pachydermatis*, *M. sympodialis*, *M. globosa*, *M. obtusa*, *M. restricta* and *M. slooffiae* [7]. Furthermore, recently three new species were included in this genus namely, *M. dermatis*, *M. equi* and *M. nana* [8,9]. However, the acceptance of these new species is still under investigation. There is only scanty information about the epidemiology and ecology of *Malassezia* species available and the clinical significance of these species is not completely recognized. Therefore, the aim of this study is to establish whether there is any association between the various species of *Malassezia* and PV lesions as well as determining *Malassezia* species microflora of healthy individuals.

Methods

Subjects

Ninety four outpatients at Razi hospital and medical mycology unit in the school of public health were included in this study. 100 age- and sex-matched clinically healthy individuals (without any dermatosis) were also conducted as control. A questionnaire was used to getting informative data about history of each person.

Collection and culture of samples

Mycological examinations were performed to confirm the diagnosis of pityriasis versicolor. Specimens were taken by scraping the lesions with a scalpel. Moreover, in normal subjects and in cases which there were not sufficient scales, samples were taken by means of sellotape.

Direct microscopy with KOH 20% and methylene blue staining were carried out in the PV lesions as well as normal samples. All samples were also inoculated in plates containing modified Dixon medium. The plates were incubated at 31°C for two weeks and examined at frequent intervals for developing colonies.

Identification

Malassezia species were identified according to their morphological features and physiological properties. Isolated colonies on modified Dixon agar were used for identification. Among *Malassezia* species, only *M. pachydermatitis* is able to grow on Sabouraud agar [7]. However, further tests are essential for identification of other *Malassezia* species such as Tween assimilation test, catalase reaction and splitting of esculin [10,11].

Tween assimilation test

According to the method reported by Guillot *et al* [10], ability to utilize different Tween compounds as a unique lipid supplement by *Malassezia* species was evaluated. Briefly, yeast suspension (at least 10^7 cfu/ml) was made in 2 ml sterilized distilled water and poured into plate containing Sabouraud dextrose agar at 45°C. The inoculum was then spread evenly. After solidification of each plate, four wells were made and filled with 30 μl of a Tween compound, i.e. Tween 20, 40, 60 and 80, respectively. These plates were incubated for a week at 31°C and the growth was assessed around the individual wells after 2, 4 and 7 days.

Catalase reaction

Presence of catalse was determined by using a drop of hydrogen peroxide (3% solution) and production of gas bubbles was considered as a positive reaction. Lack of catalase activity is a characteristic feature of *M. restricta* [10].

Splitting of esculin

The β-glucosidase activity of different *Malassezia* species was assayed using method described by Mayser *et al* [11]. 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. This test was used to distinguish *M. furfur*, *M. slooffiae* and *M. sympodialis* from other *Malassezia* species.

Statistical analysis

Quantitative data were analyzed by the group *t*-test. The data of the patient and healthy controls were analyzed using chi-square test. Correlation of predisposing factors with PV as well as the difference between isolates from patients and normal individuals was evaluated by Fisher exact test. Specificity and sensitivity of direct exam versus culture were computed. Besides, the effects of predisposing factors on culture results in both groups were eliminated by Mantel-Haenszel tests. A *P*-values of <0.05 were considered significant.

Results

From 94 patients with PV, 52.1% of the cases were female. The average and median ages of patients were 29.19 ± 11.14 and 27 years, respectively. The highest prevalence of

Table 1: Frequency of culture results and hyperhydrosis in patients with pityriasis versicolor compared with normal subjects.

Group	Patient						Normal						
Culture results	Positive		Negative		Total		Positive		Negative		Total		statistical result
Hyperhydrosis	No	%	No	%	No	%	No	%	No	%	No	%	
Positive	37	74.0	13	26.0	50	100.0	9	60.0	6	40.0	15	100.0	f.p = 0.23
Negative	67	72.8	25	27.2	92	100.0	51	60.0	34	40.0	85	100.0	$X^2 = 3.27$ P = 0.07
Total	104	73.2	38	26.8	142	100.0	60	60.0	40	40.0	100	100.0	$X^2_{MH} = 3.74$ P = 0.053

f.p: fisher exact test p-value; X^2: Chi square; P: P value; X^2_{MH}: Mantel-Haenszel Chi square No: Number; %: Percent.

Table 2: Frequency of culture results and allergy in patients with pityriasis versicolor compared with normal subjects.

Group	Patient						Normal						
Culture results	Positive		Negative		Total		Positive		Negative		Total		statistical result
Allergy	No	%	No	%	No	%	No	%	No	%	No	%	
Positive	19	63.3	11	36.7	30	100.0	6	45.5	5	45.5	11	100.0	f.p = 0.44
Negative	85	75.9	27	24.1	112	100.0	54	60.7	35	39.3	89	100.0	$X^2 = 5.38$ P = 0.02
Total	104	73.2	38	26.8	142	100.0	60	60.0	40	40.0	100	100.0	$X^2_{MH} = 4.77$ P = 0.03

f.p: fisher exact test p-value; X^2: Chi square; P: P value; X^2_{MH}: Mantel-Haenszel Chi square No: Number; %: Percent.

tinea versicolor was seen in patients with 20–30 years of age. Significant differences in the distribution of predisposing factors (i.e. allergy, hyperhydrosis, diabetes) were observed between patient groups (58.5%) and healthy control (30 %)($P < 0.001$).

Direct examination of specimens was positive in 98.9% of PV lesions, in which hyphae were seen in 89.4% of positive cases together with budding yeasts. However, only 79.8% of the specimens yielded *Malassezia* in culture. Besides, culture positive cases were higher in patients than healthy controls and these differences was statistically significant ($P < 0.05$). Specificity and sensitivity of direct exam in comparison with culture were determined 62.8% and 99.4%, respectively.

Actual data related on predisposing factors and culture results were presented in tables 1, 2 and 3. Statistical analysis revealed that in both groups with and without predisposing factors (allergy, hyperhydrosis and diabetes), the rate of positive culture cases was higher in patients than controls. Regarding elimination the effects of predisposing factors by Mantel-Haenszel test, only hyperhydrosis could cause difference in the culture of samples in patients compared with those in healthy individuals.

In pityriasis versicolor lesions, the most commonly isolated species was *M. globosa* (53.3%), followed in frequency by *M. furfur* (25.3%), *M. sympodialis*(9.3%), *M. obtusa* (8.1%) and *M. slooffiae*(4.0%). However, the most frequently detected species in healthy individuals were *M. globosa*, *M. sympodialis*, *M. furfur*, *M. slooffiae* and *M. restricta* which respectively made up 41.7%, 25.0%, 23.3%, 6.7% and 3.3% of the isolated *Malassezia* flora. Table 4 and 5, show the distribution of Malassezia species, based on the sites of sample collection. Overall, no differences in distribution of *Malassezia* were noted between patients group and healthy controls ($P = 0.1$).

Discussion

Although pityriasis versicolor has worldwide occurrence, its frequency is variable and depends on different climatic, occupational and socio-economic conditions [12,13]. This disease is prevalent in Iran, in which almost 6% of all dermatosis and approximately 30% of dermatomycoses are due to these lipophilic yeasts [14,15].

Similar to other investigations [1,3,16,17], the highest prevalence of PV in present study was observed in 20–30 year-old group, suggesting that the peak of the infection is coincided with ages when the sebum production is in the highest level. Although 60% of patients in age range of 10–20 years were female, this proportion was reversed in

Table 3: Frequency of culture results and diabetes in patients with pityriasis versicolor compared with normal subjects.

Group	Patient						Normal						
Culture results	Positive		Negative		Total		Positive		Negative		Total		statistical result
Diabetes	No	%	No	%	No	%	No	%	No	%	No	%	
Positive	2	66.7	1	33.3	3	100.0	2	50.0	2	50.0	4	100.0	f.p = 0.6
Negative	102	73.4	37	26.6	139	100.0	58	60.4	38	39.6	96	100.0	$X^2 = 4.39$ P = 0.04
Total	104	73.2	38	26.8	142	100.0	60	60.0	40	40.0	100	100.0	$X^2_{MH} = 3.97$ P = 0.046

f.p: fisher exact test p-value; X^2: Chi square; P: P value; X^2_{MH}: Mantel-Haenszel Chi square No: Number; %: Percent.

Table 4: Distribution of Malassezia species based on the body sites in Pityriasis Versicolor

	Anatomic site (%)						
Species	Neck	Chest	Abdomen	Back	Limbs	Groin	Total
M. globosa	13 (61.9)	7 (41.2)	2 (25.0)	14 (63.6)	2 (66.7)	2 (50.0)	40 (53.3)
M. furfur	3 (14.3)	7 (41.2)	2 (25.0)	6 (27.4)	1 (33.3)	0 (0.0)	19 (25.3)
M. sympodialis	1 (4.8)	1 (5.9)	2 (25.0)	1 (4.5)	0 (0.0)	2 (50.0)	7 (9.3)
M. obtusa	4 (19.0)	1 (5.9)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	6 (8.1)
M. slooffiae	0 (0.0)	1 (5.8)	1 (12.5)	1 (4.5)	0 (0.0)	0 (0.0)	3 (4.0)
Total	21 (100.0)	17 (100.0)	8 (100.0)	22 (100.0)	3 (100.0)	4 (100.0)	75 (100.0)

Table 5: Distribution of Malassezia species based on the body sites in healthy individuals

	Anatomic site (%)			
Species	Scalp	Chest	Back	Total
M. globosa	8 (28.6)	6 (42.9)	11 (61.1)	25 (41.7)
M. furfur	9 (32.1)	3 (21.4)	2 (11.1)	14 (23.3)
M. sympodialis	8 (28.6)	3 (21.4)	4 (22.2)	15 (25.0)
M. slooffiae	1 (3.6)	2 (14.3)	1 (5.6)	4 (6.7)
M. restricta	2 (7.1)	0 (0.0)	0 (0.0)	2 (3.3)
Total	28(100.0)	14 (100.0)	18 (100.0)	60(100.0)

the age group 20–30. Lower maturity age in female compared with male can be considered as the possible reason of this dissimilarity.

Pityriasis versicolor is uncommon in children [3]. We just found one case of PV in a child with the age less than 10 years. Moreover it is only rarely found in the elderly [1], as we have only two cases of PV over 50s.

The role of sex in propensity to development of PV is still unclear. Some studies found that PV is more common in men than women [18,19]. While others indicated that the incidence of this infection is higher in women [20-22], which may be due to extra attention of women to beauty and skin hygiene. However, similar to many reports [3,4,17], we found no differences in development of PV among both sexes.

Although *Malassezia* species are considered as normal microflora of the human skin, these lipophilic yeasts are associated with many skin disorders in particular PV, in some circumstances [1,23]. It is widely believed that endogenous factors such as administration of corticosteroids, malnutrition and increased plasma cortisol level mediating the development of PV [3,12,16,24]. Besides, role of high temperature and humidity in this condition is

well established [1,25]. In this regard, no significant differences were observed in culture results of patients in comparison with controls after elimination the effects of endogenous factors such as hyperhydrosis by statistical tests. Similar to other investigations [3,26], our results strongly support that hyperhydrosis can be considered as the endogenous factors in mediating the development of this infection.

In this survey, the most affected areas were the trunk and neck, which is concordant with the majority of studies worldwide [3,21,27]. The distribution of *Malassezia* species on back and chest is parallel with the density and activity of pilocebaceous glands in these areas. However, there are few reports indicated that PV lesions can occur in unusual location such as the nipple, genital areas and groin [28-30]. Similar to previous studies [27], we found no statistical difference in the distribution of *Malassezia* species on various body sites.

Diagnosis of PV is generally simple and lies on the clinical manifestations and microscopic examinations of the lesions [13,23]. In the direct examination 98% of PV samples yielded positive results which is the same as the results reported by Erchiga *et al* [31]. Two patients with negative results in this study had also been received topical antimycotic treatment.

In 89.4% of positive cases of PV, classical feature so-called "spaghetti and meatball" were seen. Our results are consistent with those previously published and confirm the significance of the yeast-mycelium conversion in pathogenesis of this infection [23,31]. Regarding high sensitivity and acceptable specificity of direct exam, diagnosis of PV is based on observation of short hyphae and yeast in the scales. However, in cases that only hyphae were presented in the scales, direct examination of samples with KOH especially by unskillful technicians, may fail to reveal the infection. Hence, we suggest staining the scales prior to performing light microscopic examination to avoid false-negative results.

Culture is necessary to distinguish the *Malassezia* species by morphological and physiological methods. In the present study, the recovery rate of *Malassezia* species from the PV lesions was 87%, which was most comparable to recent study by Nakabayashi *et al* [19]. But, our result is higher than some previous studies [27,32-34]. The difference may be due to this fact that margin of the PV lesions might be used to collect specimens. However as it shown by Erchiga *et al* [23], unlike other dermatomycosis, center of the PV lesions yields more viable materials for culture. Hence we scraped center of the lesions instead of the borders to increase recovery rate and avoid isolation of surrounding commensal species. Besides, more recovery rate which was reported by Gupta *et al* [21] may reflect the difference in culture media and sampling method. Although Leeming & Notman agar which used by them enhances recovery of *Malassezia* spp., modified Dixon agar provides features of colonies [1,33].

Based on many studies, more than one species can be recovered from each sample [21]. On the other hand, providing a pure culture and discriminating a species from mixed sample is too difficult. This might be due to this point that fast growing species usually cover other species in the culture. Besides, because of hydrophobic characteristic of *Malassezia* yeast, preparing homogenous suspension is very difficult to separate them by culture [1]. Moreover, some *Malassezia* species may loss their viability after several subcultures [7]. That's why we selected single separated colony in each species for analyzing.

As we mentioned above, *Malassezia* species are members of the normal skin flora and can be recovered from different sites of the body especially the sebaceous-rich areas. In healthy skin, we found *Malassezia* species by direct examination and culture with the frequency of 62% and 60%, respectively. These rates of positive results in our study are lower than those from recent study of Gupta *et al* [21] and may suggest the difference in sampling method and culture medium. Similar to the majority of other investigations [19,34,35], we found *M. globosa* as the most frequent species in healthy skin. By contrast, *M. sympodialis* was the main isolated species in some other investigations [17,22,36,37].

In this survey, the most common isolated species in PV lesions was *M. globosa*, which is concordant with the majority of studies worldwide [19,31,32,38]. This was contrary to observation of Makimura *et al*, which isolated *M. furfur* and *M. sympodialis* as the predominant species in PV lesions [37]. Although Gupta *et al*, were also found *M. sympodialis* as the predominant agent of PV in temperate climate, they reported *M. globosa* as the main agent in tropical regions [27]. Moreover, *M. furfur* was the second most frequent species isolated from PV lesions in the present study which was similar to the report of Dutta [32]. However they failed to isolate *M. slooffiae* from PV lesions.

Conclusions

Collectively, *M. globosa* was also the most prevalent species in healthy individuals and recovered from healthy skin only in yeast form. Furthermore, our results suggested that *M. globosa* especially in the mycelial form, is the main agent of PV and *M. furfur* is the second agent in importance. This hypothesis gained strength because of the fact that *M. globosa* is a species with high levels of esterase and lipase enzymes with probable importance in

pathogenicity [38]. It remains an open question, if there are any differences in enzyme components of its mycelial phase with yeast form.

Competing interests
None declared.

Authors' contributions
All authors contributed equally in the study design, literature search, data analysis and manuscript preparation. All authors read and approved the final manuscript.

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