Original Article

Species of Malassezia associated with psoriatic patients

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Summary:

Background: Malassezia spp. are lipophilic unipolar yeasts recognized as commensals of skin that may be pathogenic under certain conditions. Yeasts of the genus Malassezia are known to be members of the skin micro flora of human and other warm-blooded vertebrates. Psoriasis is a common cutaneous disease of unknown etiology, may be triggered by infections, including those due to fungi.

Objectives: Although the role of Malassezia spp. in the pathogenesis of psoriasis is still not fully understood, it is thought that these lipophilic yeasts might be a trigger factor in the exacerbation of psoriatic lesions. Malassezia is associated with the development of skin lesions in psoriasis because of the response of the scalp lesions in psoriasis to antifungal agents.

Materials and methods: Twenty three patients with psoriasis were included in this study, who attended Al-Kadhumyia teaching hospital / Dermatology department, from the 30th of October 2010 to the 1st of April 2011. Fifteen (15) were males and eight (8) were females, with the mean age of 44.61±14.65 years (ranging between 3months to 70 years old). The diagnosis was established by clinical examination done by consultant Dermatologist. The site of scraping were face, lower and upper limbs. Control included 14 apparently healthy individuals were randomly selected from entities, primary and secondary schools in Al-Aubaidi city (10 males and 4 females) with a mean age of 26.83±15.68 years (ranging between 1-70 years old). Both groups were investigated for Malassezia spp., cultivation and identification of Malassezia spp. included Sabourauds dextrose agar with and without olive oil.

Results: Malassezia globosa had a high percentage overall Malassezia spp. with psoriatic patients (17.04%). According to gender, males had higher infection rate than females among psoriatic patients. psoriatic patients with age group of (41-50) years had a high percentage among others (56.60%). Oily skinned patients revealed psoriasis disease.

Conclusions: From these findings it was suggested that M. globosa reported a high percentage overall Malassezia spp. with psoriatic patients.

Keywords: Malassezia spp, psoriasis.

J Fac Med Baghdad 2012; Vol.54, No. 4
Received Mar .2012
Accepted Oct.2012

Introduction:

Psoriasis is a common cutaneous disease of unknown etiology, may be triggered by infections, including those due to fungi(1). Although of unknown etiology, but has been associated with the development of psoriatic skin lesions, and differences have been observed in the Malassezia spp. distributions in healthy subjects and patients with psoriasis (2). Malassezia spp. are lipophilic unipolar yeasts recognized as commensals of skin that may be pathogenic under certain conditions (3). Yeasts of the genus Malassezia are known to be members of the skin micro flora of human and other warm-blooded vertebrates (4). Being lipid dependent, they are normally found in areas that are rich in sebaceous glands, current evidence indicates a high rates of skin colonization in healthy adults, in contrast with the low rate of colonization in prepubertal children (5).

The yeasts of the genus Malassezia have been associated with a number of diseases affecting the human skin, such as pityriasis versicolor, Malassezia (Pityrosporum) folliculitis, seborrheic dermatitis, dandruff, steroid acne, atopic dermatitis and psoriasis (6). And less commonly with other dermatologic disorders such as confluent and reticulated papillamatosis, onychomycosis, and transient acantholytic dermatoses, although Malassezia yeasts are a part of the normal micro flora, under certain conditions they can cause superficial skin infections (7). Although the role of Malassezia spp. in the pathogenesis of psoriasis is still not fully understood, it is thought that these lipophilic yeasts might be a trigger factor in the exacerbation of psoriatic lesions (8). Malassezia is associated with the development of skin lesions in psoriasis because of the response of the scalp lesions in psoriasis to antifungal agents (9).

Materials and methods:

Twenty three patients suffering psoriasis diseases who attended Al-Kadhumyia Teaching hospital, and (AL-Shahama primary school, AL-Abed and AL- Nabigha secondary schools) in AL-Aubaidi city were included in this study as 23 patients and 14 control individuals (from 30th of October 2010 to the 1st of April 2011), clinical diagnosis were done by dermatologist.

Twenty three samples were collected from patients with

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induced psoriasis. Samples used were skin scrapings, forceps and surgical blade were used for collecting skin samples respectively. Direct and indirect methods were applied for diagnosis (10).

Scales specimens were subjected for direct examination by placing on a clean slide mounted with a drop of 10 % KOH (to dissolved keratinized material), covered with a cover slip. The slides were warmed gently (but not boiled to prevent crystallization of KOH) and examined under microscope (40X) (11).

To microscopic observation of yeast cells, the suspension of yeast cells were prepared, loopful of culture were stained with lacto phenol cotton blue on sterile glass slide. Scales were inoculated into Sabourauds dextrose agar containing 0.05gm\L chloramphenicol, Penicillin at a concentration of 0.4 ml\L and Streptomycin at a concentration of 2 ml\L with olive oil or without olive oil . The vials were incubated at 37°C for 1-2 weeks. (12). The suspension was obtained by inoculating 5 ml of sterile distilled water with a loopful of actively growing yeast and the concentration was adjusted to about 105 cell/ml (13).Catalase test was applied by using a drop of 3% hydrogen peroxide, and production of gas bubbles was considered as a positive reaction (14).According to the method reported by Guillot et al., (1996) (14). Yeast cells of (2x10 to 3x10 cfu\ ml) was suspended in 1ml sterile distilled water and poured into plate containing SDA with 0.05 gm\L chloramphenicol, Penicillin at concentration of 0.4 ml\L and Streptomycin at concentration of 2 ml\L cooled at about 50℃. The inoculum was then spread evenly . After solidification, four holes were made by means of a 2 mm diameter punch and filled with 5µl of Tween 20, 40, 60 and 80, respectively. The plates were incubated for 1 week at 32℃. Utilization of Tween was assessed by the degree of growth and \ or reaction (precipitation) of the lipophilic yeasts around the wells (14).

Glucosidase activity was assayed by using esculin agar tube. Using a loop, the yeast inoculum was deeply inoculated into the agar and incubated at 32℃ for 5 days. The splitting of esculin into esculetin and glucose is revealed by darkening of the medium with liberation of soluble ferric salt incorporated in the medium (15).

A suspension of yeast cells (105 cell\ml) were cultured on m Dixon's agar containing 0.05gm\L chloramphenicol, Penicillin at a concentration of 0.4 ml\L and Streptomycin at a concentration of 2 ml\L. Plates incubated at 32℃ , 37℃ and 41℃ respectively for 4-7days (16).Yeast cells were cultured on m Dixons medium which was prepared earlier addition of 0.6% of trytophane instead of peptone to the original medium. After sterilization and cooling at RT, the suspension was smeared on the agar medium using sterile swab. The plates were incubated at 32℃ for 2 to 4 weeks (16).

Statistical analysis:

Statistical analysis was performed with the statistical Package for Social Sciences (SPSS) 16.01 and Excell 2007. Descriptive statistics for categorical data were formulated as frequency and percentage. While numerical data were formulated as mean, standard errors (SE) and standard deviation (SD).

Data analysis was done using Chi-square for comparison of categorical data, while independent sample t-test for comparison of numerical data. P-value of ≤ 0.05 was used as the level of significant.

Results:

A total of twenty three patients had been included in the present study with ages ranging from 3 months to 70 years, with a mean age ± SE (44.61 ± 2.48 years for psoriatic patients), consisting of 15 males and 8 females (65.20% and 34.80% respectively) with the most frequent age group (15-29) years. The control group includes skin scraps collected from 14 apparently healthy individuals, with ages ranging from 1 to 70 years with a mean age ± SE (26.83 ± 1.70 years). Males were 10 and females were 4 (71.42% and 28.58% respectively) (Table 1) .

Table (1): Age of persons involved in the study

Study groups	HC	Ps.
No.	14	23
Mean	26.83	44.61
Std. Deviation	15.68	11.90
Std. Error of Mean	1.70	2.48
P value		

** highly statistical significant difference.

Isolated colonies on Sabourauds dextrose agar were used for culturing. Malassezia spp. were identified according to their morphological features and physiological properties. The morphology of the yeast cells was studied by making lacto phenol cotton blue stained smears of the isolates from SDA after one week incubation at 37℃.Based on the gross morphology of the colonies on culture media the colonies were raised and smooth initially and get dry and wrinkled in time the color of Malassezia colonies was white to creamy. (17).

Among Malassezia spp. there was no growth on Sabourauds dextrose agar without overlying oil, ruling out the presence of Malassezia pachydermatis the only lipid independent species. All Malassezia spp. studied exhibited, catalase activity except Malassezia restricta. Tween assimilation tests allowed the differentiation of most Malassezia spp. in this study population. This phenomenon resulted in characteristic ring of tiny colonies around the corresponding well. (16).Isolation and identification of Malassezia spp.:

Macroscopic appearance: Skin scrapings collected from different patients and different sites, with different characteristics features, (Fig. 1) and (Fig. 2). different colonies which were obtained as whiteto creamy colored with different texture.

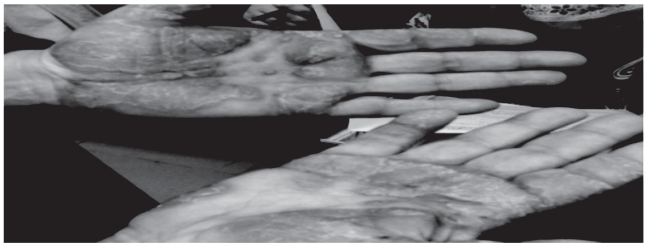
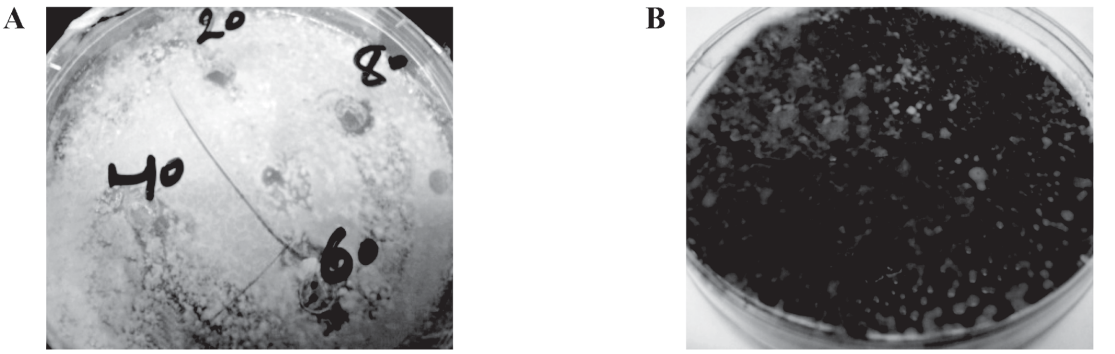


Figure (1) : Gross appearance of psoriasis in upper limbs.



Figure(2): Malassezia spp. colonies on Sabouraud dextrose agar. (A) Tween assimilations (incubated at 32C for 1 week) and (B) Malassezia spp. colonies on Tween 60-Esculin agar (incubated at 32C, for 5 days).

Upon stratification of the isolated species of Malassezia yeasts according to age groups in psoriatic patients, M. globosa, M. restricta and M. slooffaie (15.4%) were identified as the major species in the age group ranging between (41-50) years old. No statistically significant analysis was detected concerning age groups, but significant in the age group (<10) years old.
Upon stratification of the isolated species of Malassezia yeasts according to the gender in the psoriatic patients. M. restricta was the most frequently isolated species in males, with a percentage of 20.0%. whereas M. globosa was the most frequently isolated species in females, with a percentage of 25.0% (Table 2). No statistical significant difference was observed between the psoriatic patients and control groups in females ($p \leq 0.05$), while statistical significant difference was observed between psoriatic patient and control groups in males concerning Malassezia spp. ($p \leq 0.05$).

Table (2): Relation of identified Malassezia spp. from psoriatic patients, with gender.

Malassezia spp.		Gender types					
		Female		Male		Total	
		HC	Ps	HC	Ps	HC	Ps
M. furfur	Co.	0	1	0	2	0	3
	%	0.0%	12.5%	0.0%	13.3%	0.0%	13.0%
M. globosa	Co.	0	2	0	2	0	4
	%	0.0%	25.0%	0.0%	13.3%	0.0%	17.4%
M. japonica	Co.	0	0	0	1	0	1
	%	0.0%	0.0%	0.0%	6.7%	0.0%	4.3%
M. restricta	Co.	0	0	0	3	0	3
	%	0.0%	0.0%	0.0%	20.0%	0.0%	13.0%
M. slooffaie	Co.	0	1	0	1	0	2
	%	0.0%	12.5%	0.0%	6.7%	0.0%	8.7%
M. sympodialis	Co.	0	0	0	2	0	2
	%	0.0%	0.0%	0.0%	13.3%	0.0%	8.7%
No growth	Co.	4	4	10	4	14	8
	%	100.0%	50.0%	100.0%	26.7%	100.0%	34.8%
Total	Co.	4	8	10	15	14	23
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	p value	0.392		0.042		0.018	

Upon stratification of the isolated species of Malassezia yeasts according to types of skin in the psoriatic patients, M. globosa was the most frequently isolated species in oily skinned, with a percentage of 22.2%, while M. furfur was the most frequently isolated species in dry skinned, with a percentage of 40.0% (Table 3). A statistically significant difference was observed between the patient and control groups in oily skinned concerning Malassezia spp. ($p > 0.05$), but not with dry skinned ($p \leq 0.05$).

Table (3): Relation of identified Malassezia spp. from psoriatic patients, with skin types.

		Types of skin					
		Oily		Dry		Total	
		HC	Ps	HC	Ps	HC	Ps
M. furfur	Co.	0	1	0	2	0	3
	%	0.0%	5.6%	0.0%	40.0%	0.0%	13.0%
M. globosa	Co.	0	4	0	0	0	4
	%	0.0%	22.2%	0.0%	0.0%	0.0%	17.4%
M. japonica	Co.	0	0	0	1	0	1
	%	0.0%	0.0%	0.0%	20.0%	0.0%	4.3%
M. restricta	Co.	0	3	0	0	0	3
	%	0.0%	16.7%	0.0%	0.0%	0.0%	13.0%
M. slooffaie	Co.	0	2	0	0	0	2
	%	0.0%	11.1%	0.0%	0.0%	0.0%	8.7%
M. sympodialis	Co.	0	2	0	0	0	2
	%	0.0%	11.1%	0.0%	0.0%	0.0%	8.7%
No growth	Co.	13	6	1	2	14	8
	%	100.0%	33.3%	100.0%	40.0%	100.0%	34.8%
Total	Co.	13	18	1	5	14	23
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	p value	0.015		0.549		0.018	

Discussion:

Accurate identifications of the species are needed to obtain a better understanding of the role of each individual species in the etiology of disease, and to facilitate adequate treatment, this can be determined based on species-specific susceptibilities to antifungal agents (18).

Many factors play role in Malassezia pathogenicity such as increased oil (sebum) production (oily hair),hormonal fluctuations, stress, illness, infrequent shampooing, food allergies, vitamin B deficiency, hair curlers and blow dryers, cold weather (winter), use of hair sprays, gels and hair coloring chemicals (19).

The role of Malassezia in psoriasis comes from successful use of ketoconazole in patients (14). Although ketoconazole may act by a direct antifungal mode of action, it has also been shown to suppress Malassezia-induced proliferation of lymphocytes from psoriatic patients (19).

The percentage of Malassezia spp. according to the gender were (65.20%), in males, while (34.80%) in females. M. restricta was the predominant among other spp. (20.0%) in males, while, in females the percentage of M. globosa was the predominant (25.0%) (Table 2). This result agrees with Gupta et al., (2001)(20) ; Hernandez et al., (2003)(21); Prohic (2003)(2) and Rendic et al., (2003)(22) who revealed that M. globosa was the most prevalent among other spp. in psoriatic skin lesions, and disagreed with Baroni et al., (2004) (23) who revealed that M. furfur was the most predominant among psoriatic patients, and disagreed with Nakabayashi et al., (2000)(24); Gupta et al., (2001)(20); Crespo and Delgado (2002)(25) and Sugita et al., (2002)(26) who revealed that M. sympodialis was the most predominant among psoriatic patients. These results may be due to long-term use of oral corticosteroids, chemotherapy and the use of broad-spectrum antibiotics (Rhie et al., 2000(2)). The age group of patients (41-50) years old had a high percentage of infection with psoriasis which represent (56.50%) than other age groups. M. globosa, M. slooffaie and M. restricta revealed high percentage (15.04%) among other spp., this result agrees with Prohic (2003)(2) who revealed that age group (40-55) years old had a high percentage of infection with psoriasis (50.09%) and M. globosa revealed high percentage (55%%) followed by M. slooffaie and M. restricta (15.3% and 10%), respectively. Hereditary and immune suppressant factors may play a role in Malassezia pathogenicity.

Malassezia spp. had a high percentage with oily skinned patients (78.30%). M. globosa appeared in a high percentage among oily skinned (22.20%), followed by M. restricta (16.7%), while M. furfur appeared in a high percentage among dry skinned patients (40.0%) (Table 3), there were no published results to compare our results with.

Conclusions:

From this study we concluded the followings:

New Malassezia species were isolated in this study (M.

pachydermatis, M. slooffaie, M. dermatis, M. japonica and M. nana). Malassezia globosa reported high percentage overall Malassezia spp. with psoriatic patients (17.04%).

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