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Relation of *Malassezia* species with atopic dermatitis patients in Baghdad

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Abstract

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. *Malassezia spp.* are suspected to be involved in the development of skin lesions in atopic dermatitis (AD) when the response of adult AD to anti-inflammatory treatments is poor. Nineteen patients with atopic dermatitis 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. Patient included were twelve (12) males and seven (7) females, with the mean age of 25.72±14.65 years (ranging between 3months to 20 years old). The diagnosis was established by clinical examination done by consultant dermatologist. Control included 24 apparently healthy individuals were randomly selected from entities, primary and secondary schools in Al-Aubaidi city (14 males and 10 females) with a mean age of 26.83±15.68 years (ranging between 1-20 years old). Both groups were investigated for *Malassezia* spp., cultivation and identification of *Malassezia* spp. included sabouraud's dextrose agar with and without olive oil. Results revealed that *Malassezia obtusa* had the high percentage overall *Malassezia* spp. with atopic dermatitis patients (15.80%). According to gender, males had the higher infection rate than females. Atopic dermatitis patients with age group of (<10) years had a high percentage among others (73.30%), dry skinned patients revealed atopic dermatitis. From these findings it was suggested that *M. obtusa* reported a high percentage overall *Malassezia* spp. with atopic dermatitis patients.

Key words: *Malassezia* spp., Identification, Atopic dermatitis, Patient gender, Baghdad.

Introduction

Atopic dermatitis (AD) is one of the most common chronic, recurrent, inflammatory skin disease, its pathogenesis is still not fully understood, the lipophilic yeasts *Malassezia* spp., members of the normal human cutaneous flora, can be the factor that may contribute to AD, specific IgE antibodies to *Malassezia* spp., treatment with proper antifungal agents has been shown to be of great value (Bielenska and Nowicki, 2005).

Initial symptoms of AD or subsequent exacerbations may be triggered by emotional stress, infections, mechanical or chemical irritants, sweating, or allergens, the allergens implicated include food allergens ; aeroallergens, such as pollens and house dust mite (Lindgren *et al*., 1995) and allergens from cutaneous commensals (Nordvall *et al*., 1992) or pathogens (Nissen *et al*., 1992).

Malassezia spp. are lipophilic unipolar yeasts recognized as commensals of skin that may be pathogenic under certain conditions (Kindo *et al.,* 2004*).* Yeasts of the genus *Malassezia* are known to be members of the skin micro flora of human and other warm-blooded vertebrates (Moniri *et al*., 2009). 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 (Juncosa *et al*., 2002*).*

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 (Gupta and Kogan, 2004). 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 (Wei *et al*., 2010).

Malassezia spp. are suspected to be involved in the development of skin lesions in atopic dermatitis when the response of adult AD to anti-inflammatory treatments is poor, however a comparative analysis of *Malassezia* flora between adults and children with AD has not been performed (Takahata *et al*., 2007).

Materials and Methods

Nineteen patients with atopic dermatitis who attended Al-Kadhemyia Teaching hospital, were included in this study as 19 patients and 24 control individuals from (Al-Shahama primary school, Al-Abed and Al- Nabigha secondary schools) in Al-Aubaidi city (from 30th of October 2010 to the 1st of April 2011), clinical diagnosis were done by dermatologist.

Nineteen samples were collected from patients with atopic dermatitis. Samples used were skin scrapings. Forceps and surgical blade were used for collecting skin samples. Direct and indirect methods were applied for diagnosis. (Khosravi *et al*., 2009)

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 (Al-Hamadani, 1997).

For microscopic examination of yeast cells, the suspension of yeast cells were prepared. A loopful of culture were stained with lacto phenol cotton blue on a slide.

Scales were inoculated into sabouraud's dextrose agar containing 0.05 gm/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 (Shokohi *et al*., 2009). 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 (Kim *et al*., 1999).

Catalase test was applied by using a drop of 3% hydrogen peroxide, and production of gas bubbles was considered as a positive reaction (Guillot *et al*., 1996).

According to the method reported by Guillot *et al.*, (1996). Yeast cells of (2x10 to 3x10 cfu/ml) was suspended in 1 ml sterile distilled water and poured into plate containing SDA with 0.05 gm/L chloramphenicol, penicillin at a concentration of 0.4 ml/L and streptomycin at concentration of 2 ml/L cooled at about 50^{o}C. 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^{o}C. Utilization of tween was assessed by the degree of growth and/or reaction (precipitation) of the lipophilic yeasts around the wells (Guillot *et al*., 1996).

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^{o}C 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 (Midgley, 2000).

A suspension of yeast cells (105 cell/ml) were cultured on m dixon's agar containing 0.05 gm/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^{o}C respectively for 4-7days (Gouda, 2008).

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^{o}C for 2 to 4 weeks (Gouda, 2008).

Statistical analysis was performed with the statistical Package for Social Sciences (SPSS, 2007) 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 and Discussion

A total of nineteen patients had been included in the present study with age ranging from 3 months to 70 years, with a mean age of 7.47±1.22 years, consisting of 12 males and 7 females (63.2% and 36.8%, respectively) with the most frequent age group (15-29) years.

Control group includes skin scraps collected from 24 apparently healthy individuals, with ages ranging from 1 to 70 years with a mean of (26.83±1.70 years). Males were 14 (58.3%) and females were 10 (42.70%) respectively (Table 1).

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

Study groups	HC	AD
No.	24	19
Mean	26.83	7.47
Std. Deviation	15.68	5.30
Std. Error of Mean	1.70	1.22
P value		

P value=0.001** highly statistical significant difference.
HC: Healthy control
AD: Atopic dermatitis

Identification of *Malassezia* spp. with Biochemical tests:

Isolated colonies on sabouraud́s dextrose agar have been cultured. *Malassezia* spp. were identified according to their morphological features and physiological properties.

The morphology of the yeast cells was studied by lacto phenol cotton blue staining smears of the isolates from SDA after one week incubation at 37℃.

Gross morphology of the colonies on culture media, the colonies were smooth, dry and wrinkled while the color was white to creamy (Ahmed, 2004).

Among *Malassezia* spp. there was no growth on sabouraud́s dextrose agar without overlying oil, ruling out *Malassezia pachydermatis* the only lipid independent species. All *Malassezia spp.* studied exhibited, catalase activity except *Malassezia restricta.* Tween assimilation tests were used to differentiate most of *Malassezia* spp. by appearance of a ring around tiny colonies.

Isolation and identification of *Malassezia* spp.:

Macroscopic appearance:

Skin scrapings collected from different patients and different sites, with different characteristics features (Figure 1). different colonies which were obtained as white to creamy colored with different texture.

Upon stratification of the isolated species of *Malassezia* yeasts according to age groups in atopic dermatitis patients. *M. obtusa* (21.4%) was identified as the major species in the age group ranging between (<10) years old. Whereas *M. dermatis* and *M. sympodialis* (4.2%) were the major one among control group (Table 2). No statistical significant analysis was detected in the age group (<10) years old, but was significant in the age group ranging between (11-20) years old.

Upon stratification of the isolated species of *Malassezia* yeasts according to the gender in atopic dermatitis patients. *M. globosa, M. obtusa and M. pachydermatis* were the most frequently isolated species in males, with a percentage of 16.7%, *while M. restricta* was the most frequently isolated species in females, with a percentage of 28.6%. In contrast, among control groups, *M. dermatis* and *M. sympodialis* were the most predominant species in females, with a percentage of 10.0% (Table 3). No statistically significant difference was observed between atopic dermatitis patients and control groups in females (P≤0.05), while statistically significant difference was observed between atopic dermatitis patients and control groups in males concerning *Malassezia* spp. (P≤0.05).

Upon stratification of the isolated species of *Malassezia* yeasts according to the type of skin in atopic dermatitis patients, *M. globosa* was the most frequently isolated species in oily skinned, with a percentage of 25.0%, while *M. obtusa, M. pachydermatis and M. restricta* were the most frequently isolated species in dry skinned, with a percentage of 18.2% (Table 4).

A B

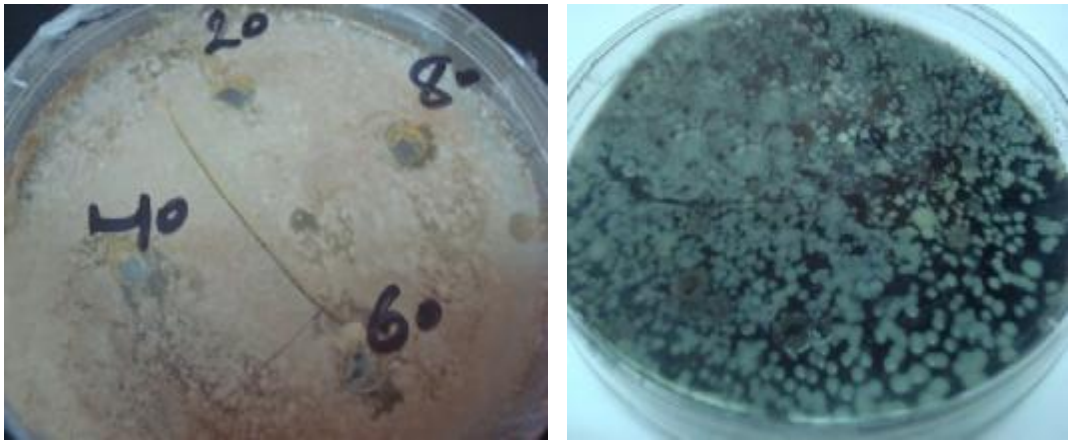

Figure (1): *Malassezia* spp. colonies on sabouraud dextrose agar. (A) Tween assimilations (incubated at 32C for 1 week) and (B) *Malassezia* spp. colonies on tween 60-esculin agar (incubated at 32°C, for 5 days).

Table (2): Relation of identified *Malassezia* spp. from atopic dermatitis patients, with age group.

Malassezia spp.	Count	Age groups					
		<10 years		11-20 years		Total	
		HC	AD	HC	AD	HC	AD
M. dermatis	Count	0	0	1	0	1	0
	%	0.0%	0.0%	5.6%	0.0%	4.2%	0.0%
M. globosa	Count	0	2	0	0	0	2
	%	0.0%	14.3%	0.0%	0.0%	0.0%	10.5%
M. japonica	Count	0	1	0	0	0	1
	%	0.0%	7.1%	0.0%	0.0%	0.0%	5.3%
M. obtuse	Count	0	3	0	0	0	3
	%	0.0%	21.4%	0.0%	0.0%	0.0%	15.8%
M. pachydermatis	Count	0	2	0	1	0	3
	%	0.0%	14.3%	0.0%	20.0%	0.0%	15.8%
M. restricta	Count	0	1	0	1	0	2
	%	0.0%	7.1%	0.0%	20.0%	0.0%	10.5%
M. sympodialis	Count	0	0	1	1	1	1
	%	0.0%	0.0%	5.6%	20.0%	4.2%	5.3%
No growth	Count	6	5	16	2	22	7
	%	100.0%	35.7%	88.8%	40.0%	91.7%	36.8%
Total	Count	6	14	18	5	24	19
	%	100.0%	100.0%	100.0%	100.0%	100.0 %	100.0%
	P value	0.220		0.048		0.003	

Table (3): Relation of identified *Malassezia* spp. from atopic dermatitis patients, with gender.

Malassezia spp.		Gender types					
		Female		Male		Total	
		HC	AD	HC	AD	HC	AD
M. dermatis	Count	1	0	0	0	1	0
	%	10.0%	0.0%	0.0%	0.0%	4.2%	0.0%
M. globosa	Count	0	0	0	2	0	2
	%	0.0%	0.0%	0.0%	16.7%	0.0%	10.5%
M. japonica	Count	0	0	0	1	0	1
	%	0.0%	0.0%	0.0%	8.3%	0.0%	5.3%
M. obtusa	Count	0	1	0	2	0	3
	%	0.0%	14.3%	0.0%	16.7%	0.0%	15.8%
M. pachydermatis	Count	0	1	0	2	0	3
	%	0.0%	14.3%	0.0%	16.7%	0.0%	15.8%
M. restricta	Count	0	2	0	0	0	2
	%	0.0%	28.6%	0.0%	0.0%	0.0%	10.5%
M. sympodialis	Count	1	1	0	0	1	1
	%	10.0%	14.3%	0.0%	0.0%	4.2%	5.3%
No growth	Count	8	2	14	5	22	7
	%	80.0%	28.6%	100.0%	41.7%	91.7%	36.8%
Total	Count	10	7	14	12	24	19
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	p value	0.139		0.025		0.003	

A statistically significant difference was observed between atopic dermatitis patients and control groups with oily skinned (P≤0.05), but not in dry skinned (P≤0.05).

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 (Anonymous, 2010).

The percentage of *Malassezia* spp. according to the gender was (63.20%) in male while (36.80%) in females. *M. pachydermatis, M. globosa* and *M. obtusa* were the predominant among other spp. (16.70%) in males, while, in females *M. restricta* was the predominant among other spp. (28.60%) (Table 3). This result agrees with Tay *et al.* (2002), who showed that there were slightly more boys with atopic dermatitis among the younger children, but more girls were affected among the 16 year olds, and disagreed with Saghazadeh *et al.* (2010) who reported that *M. globosa* as a major spp. in females. These results were due to immune suppressant and hormones exercise, heat and sweating, grass intolerance, thick clothing and stress (Tay *et al.*, 2002).

The percentage of *Malassezia* spp. according to the age group were more frequent among (<10) years old which represent (73.70%). *M. obtusa* shows high percentage (21.40%), followed by *M. pachydermatis* and *M. globosa* (14.30%) among other spp. (Table 2). This result disagreed with Yim *et al.* (2010) who improved the highest percentage for the age group of (11-20) years old (59.00%) and disagreed with Nakabayashi *et al.* (2000), Nakabayashi and Guillot, (2000) who improved that *M. furfur* was the predominant among other spp. (21%) and disagreed with Rincon *et al.* (2005) who revealed that *M. furfur* followed by *M. restricta* were the predominant among other spp. (27% and 21%), respectively. The dryness and cracking of AD skin, as a result of trans epidermal water loss caused by altered lipid content, may facilitate *Malassezia* colonization (Baker, 2005), also long-term use of cosmetics.

Table (4): Relation of identified *Malassezia* spp. from atopic dermatitis patients, with skin types.

Malassezia spp.		Types of skin					
		Oily		Dry		Total	
		HC	AD	HC	AD	HC	AD
M. dermatis	Count	1	0	0	0	1	0
	%	4.8%	0.0%	0.0%	0.0%	4.2%	0.0%
M. globosa	Count	0	2	0	0	0	2
	%	0.0%	25.0%	0.0%	0.0%	0.0%	10.5%
M. japonica	Count	0	0	0	1	0	1
	%	0.0%	0.0%	0.0%	9.1%	0.0%	5.3%
M. obtusa	Count	0	1	0	2	0	3
	%	0.0%	12.5%	0.0%	18.2%	0.0%	15.8%
M. pachydermatis	Count	0	1	0	2	0	3
	%	0.0%	12.5%	0.0%	18.2%	0.0%	15.8%
M. restricta	Count	0	0	0	2	0	2
	%	0.0%	0.0%	0.0%	18.2%	0.0%	10.5%
M. sympodialis	Count	1	0	0	1	1	1
	%	4.8%	0.0%	0.0%	9.1%	4.2%	5.3%
No growth	Count	19	4	3	3	22	7
	%	90.5%	50.0%	100.0%	27.3%	91.7%	36.8%
Total	Count	21	8	3	11	24	19
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	p value	0.029		0.405		0.003	

Faergemann (1997) showed that *M. furfur* extracts increased IL-4, IL-10 and IgE synthesis in patients with atopic dermatitis. The role of *Malassezia* yeasts as a trigger factor in AD is probably due to an allergic reaction (Yim *et al.*, 2010). According to the types of skin and *Malassezia* spp. were more frequent among dry skinned patients which represent (57.90%). *M. globosa* had a high percentage among oily skinned (25.0%), while *M. obtusa, M. pachydermatis* and *M. restricta* had a high percentage among dry skinned (18.20%) (Table 4). This result disagreed with Sugita *et al.* (2001) who improved that oily skin was the predominant among atopic dermatitis patients, and *M. restricta* had a high percentage among oily skinned patients. Long-term use of cosmetics, immune suppressant and hormonal factors had explained the results.

Conclusions

We concluded that new *Malassezia* species were isolated in this study (*M. pachydermatis, M. dermatis* and *M. japonica*). *Malassezia obtusa* reported high percentage overall *Malassezia* spp. with atopic dermatitis patients (15.80%).

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