

Identification of *Malassezia* species isolated from seborrheic dermatitis patients

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ABSTRACT

Background: Yeasts of the genus *Malassezia* are known to be members of the skin micro flora of human and other warm-blooded vertebrates. Seborrheic dermatitis is a common inflammatory dermatoses that may affect infants, adolescents, and adults of all ethnicities and races, *Malassezia* spp. have been found in both healthy skin and at sites affected with seborrheic dermatitis.

Aims: To identify of *Malassezia* spp. are suspected to be involved in the development of seborrheic dermatitis and normal subject.

Materials and Methods: Thirty one patients with seborrheic 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. Twenty five (25) were males and six (6) were females, with the mean age of 36.29 ± 4.65 years (ranging between 3 months to 70 years old). The diagnosis was established by clinical examination done by consultant Dermatologist. Control included 48 apparently healthy individuals were randomly selected from entities, primary and secondary schools in Al-Mashtale city (25 males and 23 females) with a mean age of 15.68 ± 15.68 years (ranging between 1-70 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: *Malassezia obtusa* and *M. pachydermatis* had a high percentage overall *Malassezia* spp. with seborrheic dermatitis patients (12.90%). According to gender, males had higher infection rate than females. Seborrheic dermatitis patients with age group of (31-40) years had a high percentage among others (25.80%). Oily skinned patients revealed seborrheic dermatitis.

Conclusions: from these findings, it was suggested that *M. obtusa* and *M. pachydermatis* reported a high percentage overall *Malassezia* spp. with seborrheic dermatitis patients.

KEY WORDS: *Malassezia* spp., seborrheic dermatitis.

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Introduction:

Seborrheic dermatitis is a common inflammatory dermatoses that may affect infants, adolescents, and adults of all ethnicities and races, *Malassezia* spp. have been found in both healthy skin and at sites affected with seborrheic dermatitis. It is still controversial whether *Malassezia* spp. organism counts consistently diminish in association with improvement of SD (1). Seborrheic dermatitis presents clinically as scaling and inflammation on the areas of the body rich in sebaceous glands, such as the face, scalp, and upper trunk (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 (5). The distribution of *Malassezia* spp. on healthy adult human skin, vary significantly among studies, and there are two possible explanations for this. First, there are genuine differences in the distribution of species on the skin of individuals in different countries. A second explanation is that the use of swabbing (6). *Malassezia* yeasts play an important role in the etiology of SD., most of the evidence for which comes from demonstrated responsiveness to treatment with antifungal agents. Its aetiology, however, is far from being resolved. Some believe that it is the immune response of the skin to the *Malassezia* that is the cause of the disease. Traditional treatments of SD have

been the use of keratolytic agents or corticosteroids. Since the discovery of ketoconazole, a considerable amount of research has been focused on determining the efficacy of various antifungal agents (7).

Materials and methods:

Thirty one patients suffering from seborrheic dermatitis disease who attended Al-Kadhumyia Teaching hospital, and (AL-Shahama primary school, AL-Tawiyah and AL- Jazeera secondary schools) in AL-Mashtale city were included in this study as 31 patients and 48 control individuals (from 30th of October 2010 to the 1st of April 2011), clinical diagnosis were done by dermatologist.

Thirty one samples were collected from patients with seborrheic dermatitis. Samples used were skin scrapings. Forceps and surgical blade were used for skin sampling. Direct and indirect methods were applied for diagnosis.(8)

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) (9).

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 0.05gm/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. (10). 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 (11). Catalase test was applied by using a drop of 3% hydrogen peroxide, and production of gas bubbles was considered as a positive reaction (12). According to the method reported by Guillot *et al.*, (1996) (12). 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 a concentration of 0.4 ml\l and Streptomycin at concentration of 2 ml\l cooled at about 50°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°C. Utilization of Tween was assessed by the degree of growth and \ or reaction (precipitation) of the lipophilic yeasts around the wells (12).

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°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 (6)

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°C, 37°C and 41°C respectively for 4-7days (14). Yeast cells were cultured on modified Dixons medium which was prepared earlier addition of 0.6% of tryptophane instead of peptone to the original medium. After sterilization and cooling at room temperature, the suspension was smeared on the agar medium using sterile swab. The plates were incubated at 32°C for 2 to 4 weeks (13).

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 (St.D).

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 thirty one patients had been included in the present study with age group ranging from 3 months up to 70 years, with a mean age of 36.29 ± 4.65 years, consisting of 25 males and 6 females (80.6% and

19.4%, respectively) with the most frequent age group (31-40) years.

Control group includes skin scraps collected from 48 apparently healthy individuals, with ages ranging from 1 to 70 years with a mean of (15.68 ± 15.68) years. Males were 25 and females were 23 (52.1% and 47.9%, respectively) (Table 1).

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

Study groups	HC	SD.
No.	48	31
Mean	26.83	36.29
Std. Deviation	15.68	15.97
Std. Error of Mean	1.70	2.87
P value		

**** highly statistical significant difference.**

Identification of *Malassezia* spp. with Biochemical tests:

Isolated colonies on Sabouraud's 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 Sabouraud's dextrose agar after one week incubation at 37°C.

Gross morphology of the colonies on culture media were smooth, dry and wrinkled while the color was white to creamy. (14).

Among *Malassezia* spp. there was no growth on Sabouraud's dextrose agar without 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.:

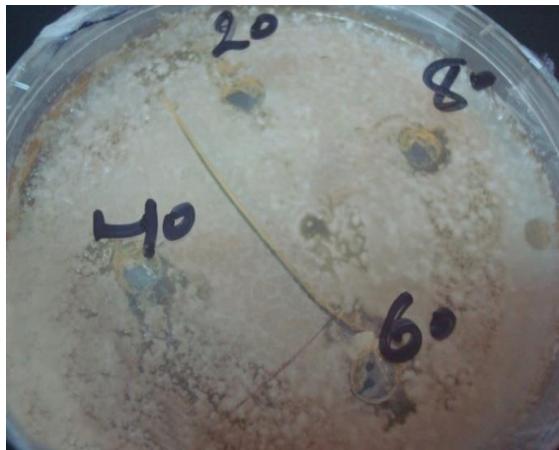
Macroscopic appearance:

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



Figure (1) : Gross appearance of seborrheic dermatitis in face.

A



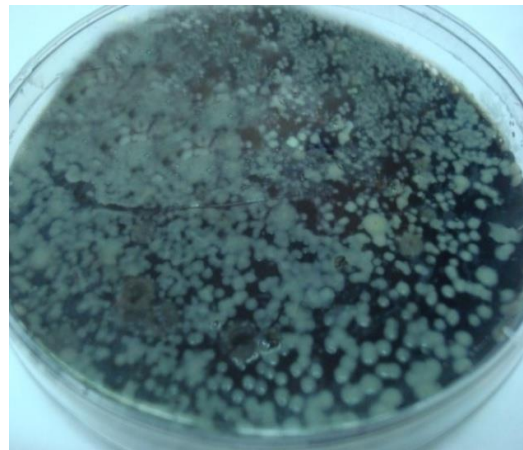
B

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 seborrheic dermatitis. *M. sympodialis* (25.0%) was identified as the major species in the age group ranging between (31-40) years old. seborrheic dermatitis patients and control groups in males concerning *Malassezia* spp. ($p \leq 0.05$).

Whereas among control groups, *M. furfur* and *M. sympodialis* (4.2%) were identified as the major species in the age groups. No statistically significant analysis was detected concerning age groups, but significant in the age group (11-20) years old.

Upon stratification of the isolated species of *Malassezia* yeasts according to the gender in seborrheic dermatitis. *M. pachydermatis* was the most frequently isolated species in males, with a percentage of 16.0%, while *M. sympodialis* was the most frequently



isolated species in females, with a percentage of 33.3%. In contrast, among control groups, *M. sympodialis* was the most predominant species in females, with a percentage of 4.0% (Table 2). No statistically significant difference was observed between seborrheic dermatitis patients and control groups in females ($p > 0.05$), while statistically significant difference was observed between

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

<i>Malassezia</i> spp.		Gender types					
		Female		Male		Total	
		HC	SD	HC	SD	HC	SD
<i>M. dermatis</i>	Co.	1	0	0	0	1	0
	%	4.3%	0.0%	0.0%	0.0%	2.1%	0.0%
<i>M. furfur</i>	Co.	2	0	0	2	2	2
	%	8.7%	0.0%	0.0%	8.0%	4.2%	6.5%
<i>M. globosa</i>	Co.	1	0	0	0	1	0
	%	4.3%	0.0%	0.0%	0.0%	2.1%	0.0%
<i>M. japonica</i>	Co.	0	0	0	1	0	1
	%	0.0%	0.0%	0.0%	4.0%	0.0%	3.2%
<i>M. nana</i>	Co.	0	0	0	2	0	2
	%	0.0%	0.0%	0.0%	8.0%	0.0%	6.5%
<i>M. obtuse</i>	Co.	1	1	0	3	1	4
	%	4.3%	16.7%	0.0%	12.0%	2.1%	12.9%
<i>M. pachydermatis</i>	Co.	0	0	0	4	0	4
	%	0.0%	0.0%	0.0%	16.0%	0.0%	12.9%
<i>M. restricta</i>	Co.	1	1	0	2	1	3
	%	4.3%	16.7%	0.0%	8.0%	2.1%	9.7%
<i>M. slooffaie</i>	Co.	0	1	0	0	0	1
	%	0.0%	16.7%	0.0%	0.0%	0.0%	3.2%
<i>M. sympodialis</i>	Co.	1	2	1	0	2	2
	%	4.3%	33.3%	4.0%	0.0%	4.2%	6.5%
No growth	Co.	16	1	24	11	40	12
	%	69.6%	16.7%	96.0%	44.0%	83.3%	38.7%
Total	Co.	23	6	25	25	48	31
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	p value	0.070		0.006		0.005	

Upon stratification of the isolated species of *Malassezia* yeasts according to the types of skin in seborrheic dermatitis. *M. pachydermatis* was the most frequently isolated species in oily skinned patients, with a percentage of 12.0%, while *M. obtusa* was the most frequently isolated species in dry skinned, with a percentage of 33.3%

(Table 3). A statistically significant difference was observed between seborrheic dermatitis patients and control groups with oily and dry skinned concerning *Malassezia* species ($p \leq 0.05$).

Table (3): Relation of identified *Malassezia* spp. from seborrheic

dermatitis patients, with skin types

<i>Malassezia</i> spp.		skin types					
		Oily		Dry		Total	
		HC	SD	HC	SD	HC	SD
<i>M. dermatis</i>	Co.	1	0	0	0	1	0
	%	2.6%	0.0%	0.0%	0.0%	2.1%	0.0%
<i>M. furfur</i>	Co.	1	2	1	0	2	2
	%	2.6%	8.0%	11.1%	0.0%	4.2%	6.5%
<i>M. globosa</i>	Co.	1	0	0	0	1	0
	%	2.6%	0.0%	0.0%	0.0%	2.1%	0.0%
<i>M. japonica</i>	Co.	0	1	0	0	0	1
	%	0.0%	4.0%	0.0%	0.0%	0.0%	3.2%
<i>M. nana</i>	Co.	0	2	0	0	0	2
	%	0.0%	8.0%	0.0%	0.0%	0.0%	6.5%
<i>M. obtusa</i>	Co.	1	2	0	2	1	4
	%	2.6%	8.0%	0.0%	33.3%	2.1%	12.9%
<i>M. pachydermatidis</i>	Co.	0	3	0	1	0	4
	%	0.0%	12.0%	0.0%	16.7%	0.0%	12.9%
<i>M. restricta</i>	Co.	1	2	0	1	1	3
	%	2.6%	8.0%	0.0%	16.7%	2.1%	9.7%
<i>M. slooffaie</i>	Co.	0	1	0	0	0	1
	%	0.0%	4.0%	0.0%	0.0%	0.0%	3.2%
<i>M. sympodialis</i>	Co.	2	2	0	0	2	2
	%	5.1%	8.0%	0.0%	0.0%	4.2%	6.5%
No growth	Co.	32	10	8	2	40	12
	%	82.1%	40.0%	88.9%	33.3%	83.3%	38.7%
Total	Co.	39	25	9	6	48	31
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	p value	0.036		0.080		0.005	

Discussion:

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 (15).

The percentage of *Malassezia* spp. according to the gender were (80.60%) in males while (19.40) females. *M. pachydermatis* was the predominant among other spp. (16.0%), followed by *M. obtusa* (12.0%) in males, while in females *M. sympodialis* was the predominant among other spp. (33.30%) (Table 2). This result agrees with Stoll *et al.*, (2001)(16); Laschinsky (2010)(17) and Sasseville *et al.*, (2011)(18) who reported that males affected more frequently than females, while disagreed with Hedayati *et al.*, (2010)(19) who revealed that *M. globosa* was the predominant among other spp. in females. Males had larger oil gland which can lead to oily scalp and dandruff, also some research indicated hormones may be a factor, these reasons explained the previous result. According to the age group and *Malassezia* spp. were more frequent among (31-40) years old which represent (25.80%). *M. sympodialis* had a high percentage (25.0%) among other spp., this result agrees with Laschinsky (2010)(17) who revealed

that young adults and middle aged adulthood were the most frequent than other age groups, and agrees with Hedayati *et al.*, (2010)(19) who revealed the highest prevalence of SD, which was seen in age group of (20-29) years old. and agrees with Niambli *et al.*, (1998)(20) who revealed that percentage of *Malassezia* spp. increase with age, and disagreed with Bernier *et al.*, (2002)(21) who reported that seborrheic dermatitis most frequently present among neonates than other age groups. and this disagreed with Byung *et al.*, (2010)(22) who reported that *M. restricta* as a major spp. with age group of (30-40) years old, *M. sympodialis* as the major spp. with age group of (40-50) years old.

Malassezia spp. represent more among oily skinned (80.60%) than dry skinned patients. *M. pachydermatis* had a high percentage among oily skinned patients (12.0%), while *M. obtusa* had a high isolation percentage among dry skinned (33.30%) (Table 3). There were no published review to compare our result with.

Conclusions:

From this study we concluded the followings:

New *Malassezia* species were isolated in this study (*M. pachydermatis*, *M. dermatis*, *M. slooffiae*, *M. nana* and *M. japonica*). *Malassezia pachydermatis* reported high percentage overall *Malassezia* spp. with seborrheic dermatitis patients (12.90%).

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تشخيص أنواع المالايسيزيا المعزولة من مرضى الأكزيما الدهنية

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الخلاصة:

خلفية الموضوع: الخمائر العائدة لجنس المالايسيزيا هي أحد أنواع النبيت الطبيعي للإنسان والفقرات ذوات الدم الدافئ. الأكزيما الدهنية مرض جلدي التهابي شائع يصيب الأطفال والشباب والبالغين وكل الأعمار والأعراق. أنواع المالايسيزيا توجد على سطح جلد الأصحاء والمواقع المتأثرة بالأكزيما الدهنية.

الأهداف: عزل أنواع المالايسيزيا المتسببة في تطور الأكزيما الدهنية .

المواد و طرائق العمل: شملت هذه الدراسة واحد وثلاثون شخص يعانون من الأكزيما الدهنية ممن راجعوا قسم الجلدية في مستشفى الكاظمية التعليمي ((للفترة من الثلاثين من شهر تشرين الأول ولغاية الأول من شهر نيسان)) وكان عدد المرضى المشمولين بالدراسة خمسة وعشرون مريضاً وستة مريضات وبمعدل عمري 36.29 ± 4.65 والمتراوح بين 3 شهور الى 70 سنة . تم التشخيص بوساطة الفحص السريري من قبل أخصائي بأمراض الجلدية. أما عينات السيطرة أخذت من 48 من الأشخاص الأصحاء تم اختيارهم عشوائياً من مدارس مختلفة في منطقة المشتل بنسبة 25 من الرجال و 23 من النساء تتراوح أعمارهم بين 1-70 سنة وبمعدل 15.68 ± 15.68 سنة وللاستقصاء عن فطر المالايسيزيا تم عزل وتشخيص أنواع المالايسيزيا على وسط السبارويد دكستروز-أكار الزرعي مع إضافة زيت الزيتون وبدون إضافة الزيت.

النتائج: سيادة نوع *Malassezia obtusa* و *M. pachydermatis* لدى مرضى الأكزيما الدهنية وبنسبة

(12.90%) الذكور سجلوا أعلى نسبة إصابة بالمرض مقارنة بالأنثى. سجلوا المرضى ممن هم بأعمار تتراوح (31-40) سنة كانوا أعلى إصابة ونسبة (25.80%). الجلد الدهني كان سائداً لدى المصابون بالمرض. الاستنتاجات: من هذه النتائج نقترح بأن تشكل *M. pachydermatis* و *Malassezia obtusa* أعلى نوع مابين أنواع المالايزيا عند المرضى. مفتاح الكلمات: أنواع المالايزيا، الأكزيما الدهنية.

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