Relation of *Malassezia* spp. with steroids acne and folliculitis

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Abstract: Background: Yeasts of the genus *Malassezia(M.)* are known to be members of the skin micro flora of human and other warm-blooded vertebrates. Acne is a multi factorial disease involving the pilosebaceous unit, and is most frequent and intense in areas where sebaceous glands are largest and most numerous. Folliculitis is a superficial infection of the hair follicles with purulent material in the epidermis. **Aims:** To identify of *Malassezia* spp. are suspected to be involved in the development of steroids acne and folliculitis. **Materials and Methods:** Ninety three patients with steroids acne and folliculitis 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. Fifty six (56) were males and thirty seven (37) were females, with the mean age of (19.16 ± 2.55 years for steroids acne patients and 28.60 ± 1.63 years for folliculitis patients) (ranging between 3months to 70 years old). The diagnosis was established by clinical examination done by consultant Dermatologist. Control included 64 apparently healthy individuals were randomly selected from entities, primary and secondary schools in Al-Aubaidi city (43 males and 21 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 Sabouraudós dextrose agar with and without olive oil. **Results:** *Malassezia furfur* reported high percentage overall *Malassezia* spp. with steroids acne and folliculitis patients (15.10%) and (20.0%), respectively. According to gender, males had higher infection rate than females among dermatological problems. Steroid acne patients with age group of (11-20) years had a high percentage among others (74.0%). folliculitis patients with age group of (21-30) years had a high percentage among others (50.0%). Oily skinned patients revealed steroids acne and folliculitis. **Conclusions:** from these findings it was suggested that *M. furfur* reported a high percentage overall *Malassezia* spp. with steroids acne and folliculitis patients.

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Key Words: *Malassezia* spp., steroids acne, folliculitis.

1.Introduction:

Acne is a multi factorial disease involving the pilosebaceous unit, and is most frequent and intense in areas where sebaceous glands are largest and most numerous, those who have refractory inflammatory papules and pustules on the face and mandibular have received long-term antibiotic therapy **(1).**

Folliculitis is a superficial infection of the hair follicles with purulent material in the epidermis. A carbuncle is a coalescence of several inflamed follicles into a single inflammatory mass with purulent drainage from multiple follicles, both folliculitis and carbuncles involve the epidermis but not the deeper soft tissue **(2).**

Malassezia species 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).**

Malassezia folliculitis may be misdiagnosed as acne, and vice versa, some patients appear to develop it after beginning a tetracycline for acne, although it may be that the *Malassezia* folliculitis is just more apparent after the acne is removed, the peak age groups for acne are teens and young adults, and for *Malassezia* folliculitis are young and middle-aged adults overlap **(5).**

The role of *Malassezia* yeast in *pityrosporum* folliculitis is further endorsed by the fact that topical antifungal treatment is effective in most cases **(6).**

2. Materials and methods:

Ninety three patients suffering from steroids acne and folliculitis 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 93 patients and 64 control individuals (from 30th of October 2010 to the 1st of April 2011), clinical diagnosis were done by dermatologist.

Ninety three samples were collected from patients with induced steroids acne and folliculitis. Samples used were skin scrapings, forceps and surgical blade

were used for collecting hair and skin samples. Direct and indirect methods were applied for diagnosis **(7)**.

Scales and hair follicles 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) **(8).**

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 and hair follicles were inoculated into Sabouraudós 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 **(9).** 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 **(10).**

Catalase test was applied by using a drop of 3% hydrogen peroxide, and production of gas bubbles was considered as a positive reaction **(11).**

According to the method reported by **Guillot *et al.*, (1996)(11).** 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 50ºC. 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 32ºC. Utilization of Tween was assessed by the degree of growth and \ or reaction (precipitation) of the lipophilic yeasts around the wells **(11).**

Glucosidase activity was assayed by using esculin agar tube. Using a loop, the yeast inoculum was deeply inoculated into the agar and incubated at 32ºC 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 **(12).**

A suspension of yeast cells (105 cell\ml) were cultured on modified 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 32ºC, 37ºC and 41ºC respectively for 4-7days **(13).**

Yeast cells were cultured on modified Dixons medium which was prepared earlier addition of 0.6% of trytophane 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 32ºC for 2 to 4 weeks **(12).**

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.

3. Results:

A total of ninety three patients had been included in the present study with ages ranging from 3 months to 70 years, with a mean age of (19.16 ± 2.55 years for steroids acne patients and 28.60 ± 1.63 years for steroids acne and folliculitis patients), consisting of 56 males and 37 females (60.21% and 39.78% respectively).

Control group includes skin scraps and hair were collected from 64 apparently healthy individuals, with ages ranging from 1 to 70 years with a mean of (26.83 ± 1.70 years). Males were 43 and females were 21 (67.18% and 32.81% respectively) (Table 1).

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

Study groups	HC	St. acne	Fo.
No.	24	73	20
Mean	26.83	19.16	28.60
Std. Deviation	15.68	4.73	7.29
Std. Error of Mean	1.70	2.55	1.63
P value	** 0.001		

**** highly statistical significant difference.**

Identification of *Malassezia* spp. with Biochemical tests:

Isolated colonies on Sabouraudós 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 Sabouraudós dextrose agar after one week incubation at 37ºC.

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 **(13)**.

Among *Malassezia* spp. there was no growth on Sabouraudós 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.*

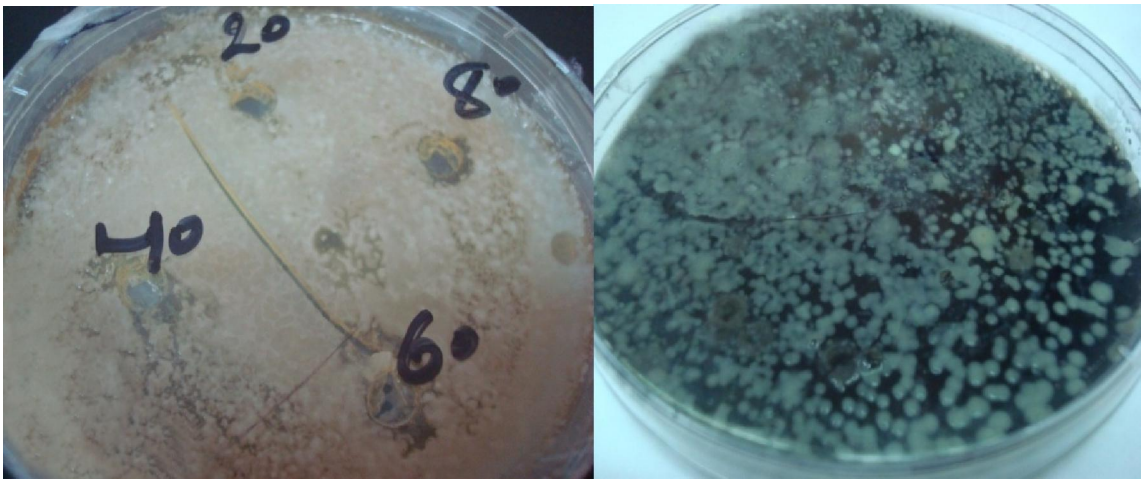
Tween assimilation tests allowed the differentiation of most *Malassezia* spp. in this study population. This phenomenon resulted in characteristic ring of tiny colonies around the corresponding well.

Isolation and identification of *Malassezia* spp.:

Macroscopic appearance:

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

Figure (1): gross appearance of steroids acne in back



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).

In terms of age, among steroids acne patients, the highest rate was in the age group ranging between (11-20) years old, which was 74.0% (54 out of 73), while the lowest rate was in the age group ranging between (31-40) years old, which was 1.4% (1 out of 73). Among control groups, the highest rate was in the age group ranging between (11-20) years old, which was 34.8% (16 out of 46), while the lowest rate was in the age group ranging between (21-30 and 41-50) years old, which was 15.2% (7 out of 46) (Fig. 3). A highly statistical significant difference was observed between steroids acne patients and control groups concerning age ($p \leq 0.001$).

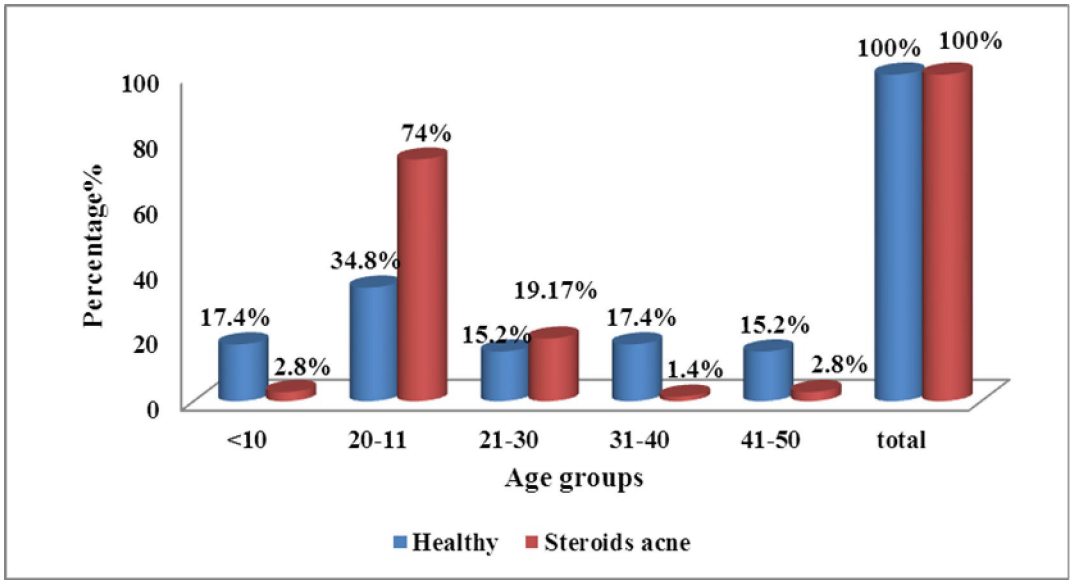


Figure (3):Relation between control groups and steroids acne patients according to their age.

Among folliculitis patients, the highest rate was in the age group ranging between (21-30) years old, which was 50.0% (10 out of 20), while the lowest rate was in the age group ranging between(41-50) years old, which was 5.0% (1 out of 20). Among control groups, the highest rate was in age group ranging between (11-20) years old, which was 55.6% (10out of 18),while the lowest rate was in the age group ranging between(41-50) years old, which was 5.6%(1 out of 18) (Fig. 4). A statistically significant difference was observed between folliculitis patients and control groups concerning age ($p \leq 0.05$).

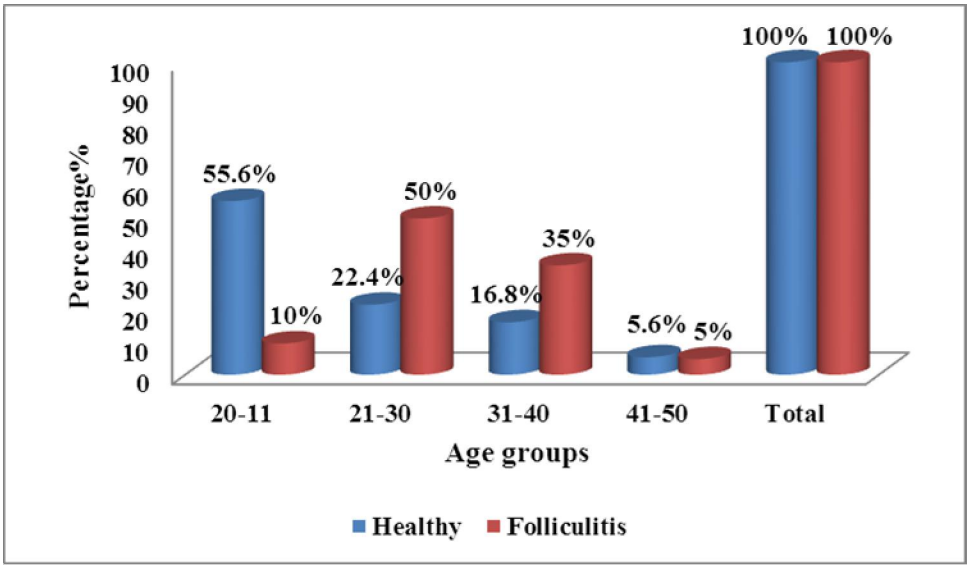


Figure (4):Relation between control groups and folliculitis patients according to their age.

Upon stratification of the isolated species of *Malassezia* yeasts according to the gender in steroids acne patients. ***M. furfur*** was the most frequently isolated species in males, with a percentage of 17.4%, while ***M. Slooffaie*** was the most frequently isolated species in females, when the percentage was 14.8%. In contrast, among control groups, ***M. sympodialis*** was the most predominant species in male, with a percentage of 10.0%, while *M. furfur* was the predominant species in females, with a percentage of 12.5% (Table 2). No statistically significant difference was observed between the patient and control groups concerning gender and presence of *Malassezia* spp. ($p>0.05$).

Table (2): Relation of identified *Malassezia* spp. From Steroids acne patients, with gender.

Malassezia spp.		Gender types					
		Female		Male		Total	
		HC	St. acne	HC	St. acne	HC	St. acne
M. dermatis	Co.	1	1	1	2	2	3
	%	6.3%	3.7%	3.3%	4.3%	4.3%	4.1%
M. furfur	Co.	2	3	0	8	2	11
	%	12.5%	11.1%	0.0%	17.4%	4.3%	15.1%
M. globosa	Co.	0	3	0	4	0	7
	%	0.0%	11.1%	0.0%	8.7%	0.0%	9.6%
M. japonica	Co.	0	3	1	1	1	4
	%	0.0%	11.1%	3.3%	2.2%	2.2%	5.5%
M. nana	Co.	0	1	0	1	0	2
	%	0.0%	3.7%	0.0%	2.2%	0.0%	2.7%
M. obtusa	Co.	0	0	0	1	0	1
	%	0.0%	0.0%	0.0%	2.2%	0.0%	1.4%
M. pachydermatis	Co.	0	2	1	4	1	6
	%	0.0%	7.4%	3.3%	8.7%	2.2%	8.2%
M. restricta	Co.	0	0	0	2	0	2
	%	0.0%	0.0%	0.0%	4.3%	0.0%	2.7%
M. slooffaie	Co.	0	4	0	1	0	5
	%	0.0%	14.8%	0.0%	2.2%	0.0%	6.8%
M. sympodialis	Co.	1	0	3	3	4	3
	%	6.3%	0.0%	10.0%	6.5%	8.7%	4.1%
No growth	Co.	12	10	24	19	36	29
	%	75.0%	37.0%	80.0%	41.3%	78.3%	39.7%
Total	Co.	16	27	30	46	46	73
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	p value	0.135		0.072		0.042	

Co.= Count St. acne= steroids acne ، HC= Healthy Control

Upon stratification of the isolated species of *Malassezia* yeasts according to the gender in the folliculitis patients. *M. furfur* was the most frequently isolated species in females, with a percentage of 30.0%. Among control groups, *M.* pachy*dermatis* and *M. sympodialis* were the most predominant species in females and males, with a percentage of 5.6% (Table 3). No statistically significant difference was observed between the folliculitis patient and control groups according to their gender concerning *Malassezia* spp. ($p>0.05$).

Upon stratification of the isolated species of *Malassezia* yeasts according to types of skin in steroids acne patients. *M. furfur* was the most frequently isolated species in oily and dry skinned, with a percentage of 12.3% and 37.3% respectively. In contrast, among control groups, *M. sympodialis* was the most predominant species in oily skinned, with a percentage of 8.7%, subgroups (Table 4). A statistically significant difference was observed between steroids acne patients and control groups with oily and dry skinned concerning *Malassezia* spp.($p\leq0.05$).

Upon stratification of the isolated species of *Malassezia* yeasts according to types of skin in the folliculitis patients, *M. furfur* was the most frequently isolated species in oily skinned, with a percentage of 23.5%, while *M. globosa* was the most frequently isolated species in dry skinned, with a percentage of 66.7% (Table 5). No statistically significant difference was observed between folliculitis patients and control groups with oily and dry skinned concerning *Malassezia* spp.($p>0.05$).

Table (3): Relation of identified *Malassezia* spp. From Folliculitis patients, with gender.

Malassezia spp.		Gender types					
		Female		Male		Total	
		HC	Fo.	HC	Fo.	HC	Fo.
M. dermatis	Co.	0	0	0	1	0	1
	%	0.0%	0.0%	0.0%	10.0%	0.0%	5.0%
M. furfur	Co.	0	3	0	1	0	4
	%	0.0%	30.0%	0.0%	10.0%	0.0%	20.0%
M. globosa	Co.	0	1	0	1	0	2
	%	0.0%	10.0%	0.0%	10.0%	0.0%	10.0%
M. nana	Co.	0	0	0	1	0	1
	%	0.0%	0.0%	0.0%	10.0%	0.0%	5.0%
M. obtusa	Co.	0	0	0	1	0	1
	%	0.0%	0.0%	0.0%	10.0%	0.0%	5.0%
M. pachydermatis	Co.	0	1	1	1	1	2
	%	0.0%	10.0%	7.7%	10.0%	5.6%	10.0%
M. slooffaie	Co.	0	1	0	0	0	1
	%	0.0%	10.0%	0.0%	0.0%	0.0%	5.0%
M. sympodialis	Co.	0	0	1	0	1	0
	%	0.0%	0.0%	7.7%	0.0%	5.6%	0.0%
No growth	Co.	5	4	11	0	16	8
	%	100.0%	40.0%	84.6%	41.3%	88.9%	40.0%
Total	Co.	5	10	13	10	18	20
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	p value	0.287		0.251		0.084	

Fo.= Folliculitis

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

Malassezia spp.		Gender types					
		Oily		Dry		Total	
		HC	St. acne	HC	St. acne	HC	St. acne
M. dermatis	Co.	1	3	1	0	2	3
	%	2.9%	4.6%	8.3%	0.0%	4.3%	4.1%
M. furfur	Co.	1	8	1	3	2	11
	%	2.9%	12.3%	8.3%	37.5%	4.3%	15.1%
M. globosa	Co.	0	6	0	1	0	7
	%	0.0%	9.2%	0.0%	12.5%	0.0%	9.6%
M. japonica	Co.	1	4	0	0	1	4
	%	2.9%	6.2%	0.0%	0.0%	2.2%	5.5%
M. nana	Co.	0	2	0	0	0	2
	%	0.0%	3.1%	0.0%	0.0%	0.0%	2.7%
M. obtusa	Co.	0	1	0	0	0	1
	%	0.0%	1.5%	0.0%	0.0%	0.0%	1.4%
M. pachydermatis	Co.	1	4	0	2	1	6
	%	2.9%	6.2%	0.0%	25.0%	2.2%	8.2%
M. restricta	Co.	0	2	0	0	0	2
	%	0.0%	3.1%	0.0%	0.0%	0.0%	2.7%
M. slooffaie	Co.	0	5	0	0	0	5
	%	0.0%	7.7%	0.0%	0.0%	0.0%	6.8%
M. sympodialis	Co.	4	3	0	0	4	3
	%	11.8%	4.6%	0.0%	0.0%	8.7%	4.1%
No growth	Co.	26	27	10	2	36	29
	%	76.5%	41.5%	83.3%	25.0%	78.3%	39.7%
Total	Co.	34	65	12	8	46	73
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	p value	0.05		0.042		0.049	

Table (5): Relation of identified *Malassezia* spp. From folliculitis patients, with skin types.

Malassezia spp.		Gender types					
		Oily		Dry		Total	
		HC	Fo.	HC	Fo.	HC	Fo.
M. dermatis	**Co.**	0	1	0	0	0	1
	%	0.0%	5.9%	0.0%	0.0%	0.0%	5.0%
M. furfur	**Co.**	0	4	0	0	0	4
	%	0.0%	23.5%	0.0%	0.0%	0.0%	20.0%
M. globosa	**Co.**	0	0	0	2	0	2
	%	0.0%	0.0%	0.0%	66.7%	0.0%	10.0%
M. nana	**Co.**	0	1	0	0	0	1
	%	0.0%	5.9%	0.0%	0.0%	0.0%	5.0%
M. obtusa	**Co.**	0	1	0	0	0	1
	%	0.0%	5.9%	0.0%	0.0%	0.0%	5.0%
M. pachydermatis	**Co.**	1	2	0	0	1	2
	%	6.3%	11.8%	0.0%	0.0%	5.6%	10.0%
M. slooffaie	**Co.**	0	1	0	0	0	1
	%	0.0%	5.9%	0.0%	0.0%	0.0%	5.0%
M. sympodialis	**Co.**	1	0	0	0	1	0
	%	6.3%	0.0%	0.0%	0.0%	5.6%	0.0%
No growth	**Co.**	14	7	2	1	16	8
	%	87.5%	41.2%	100.0%	33.3%	88.9%	40.0%
Total	**Co.**	16	17	2	3	18	20
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
	p value	0.113		0.136		0.084	

4. Discussion:

The percentage of *Malassezia* spp. according to the age group of steroids acne patients were more frequent among (11-20) years old which represent (74.0%) (Fig.3). *M. furfur* had a high percentage (14.80%), followed by *M. pachydermatis* (11.10%) and then *M. globosa* and *M. slooffaie* (9.30%), among other spp. This result disagreed with **Kang and Kim (1999)(14); Song *et al.*(2011)(15)** who revealed that *M. restricta* was the predominant with age group of (11-20) years old.

The percentage of *Malassezia* spp. according to the gender of steroids acne patients were in males (63.0%), while in females (37.0%). *M. furfur* was the predominant among other spp. (17.40%), followed by *M. globosa* and *M. pachydermatis* (8.70%) and *M. sympodialis* (6.50%) in males, while, (14.8%) in females when *M. slooffaie* was the predominant, followed by *M. furfur*, *M. globosa* then *M. japonica* were recovered in equal proportions (11.1%) (Table 2), these results were the first in our country and outside, there were no such publishment to compare our study with.

The percentage of *Malassezia* spp. according to the types of skin of steroids acne patients were more frequent among oily skinned patients which represent (89.0%). *M. furfur* was more frequent with oily skinned patients (12.30%), followed by *M. globosa* (9.2%) and then *M. slooffaie* (7.70%), while *M. furfur* was more frequent with dry skinned patients (37.50%), followed by *M. pachydermatis* (25.0%) (Table 4), there were no published results to compare our results with.

These results due to wrong treatment, excessive cleaning, application of corticosteroid, cleansing for acne skin is to gently remove surface debris, sweat, and excessive skin lipids without irritating or drying the skin. many acne patients often mistakenly believe that aggressive scrubbing of their skin with soap and water several times a day will alleviate the oiliness and "dirty feeling" on their skin **(16).**

The percentage of *Malassezia* spp. according to the age group were more frequent among (21-30) years old which represent (50.0%) (Fig.4). *M. furfur*, *M. pachydermatis* and *M. nana* had a high presence percentage (10.00%) among other spp. This result disagreed with **Jong *et al.*, (2011)(17)** who revealed that age group of (11-20) years old had the highest percentage (63.30%), followed by age group of (31-40) years old with a percentage of (31,70%) and then age group of (21-30) years old with a percentage of (5.00%), *M. restricta* was the most frequently isolates among other spp. (23.30%).

In terms of gender, for the folliculitis patients group, *Malassezia* spp. reported equal proportions appeared in males and females, with 50.0%. *M. furfur*, was the predominant (30.0%) in females, (Table 3). This result disagreed with **Jang *et al.*,**

(2003)(18), who revealed that *M. restricta* and *M. globosa* had a high percentage of infection, in females using cosmetics and different types of soaps may play a role in this result.

Malassezia spp. were more frequent among oily skinned patients which represent (85.0%). *M. furfur* had a high percentage among oily skinned (23.50%), followed by *M. pachydermatis* (11.80%), while *M. globosa* had a high percentage among dry skinned (66.7%) (Table 5), there were no published results to compare our results with. Long-term of cosmetics and antibiotics commonly used to treat acne may suppress normal bacterial flora and allow over growth of *Malassezia* **(19).**

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 furfur reported high percentage overall *Malassezia* spp. with steroids acne and folliculitis patients (15.10%) and (20.0%), respectively.

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