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Original Research Article

Identification of Malassezia Species from Clinical Cases of Pityriasis Versicolor in a Tertiary Care Hospital, Ahmedabad

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Abstract

Introduction: Opportunistic yeast of the genus Malassezia have been recognized as members of the microbiological flora of human skin and many other warm-blooded vertebrates. These lipophilic fungi are associated with different types of diseases especially Pityriasis versicolor, a chronic, benign and recurrent superficial infection which is generally occur in tropical and temperate regions This disease occurs worldwide with prevalence as high as 30–40% in populations in tropical area. Warm and humid environment is considered to be among the important precipitating factors.

Present study was undertaken to determine the incidence of Malassezia in patients, clinically suspected of having Pityriasis versicolor and speciate the identified isolates.

Material and Methods: This study was conducted during June 2012 to October 2013 on 55 patients from the OPD and IPD of the hospital, with clinically suspected Pityriasis versicolor, either hypopigmented or hyperpigmented type.

Direct KOH examination and culture was done on Modified Dixon's Agar and Sabourauds dextrose agar and colony was identified by morphology, direct microscopy after Gram stain and other biochemical tests like esculin hydrolysis, urease, tween assimilation and catalase.

Results: Total 55 cases of Pityriasis versicolor, of 1-65 years of age group and both sexes attending outpatient and inpatient department of dermatology were taken Out of 55 patients, 37 (67.3%) were males and 18(32.8%) were females, M: F ratio being 2.05:1. The highest prevalence of Malassezia was seen in young adults ie, patients with 21 -30 years of age. Lowest was seen in the age group 61-70 years. Majority of patients presented with hypopigmented lesion ie, 51 (92.7%) in which the maximum isolated specie was of M. furfur 27(52.9%). Hyperpigmented lesion was seen in 4 patients i.e., (7.3%) and *M. globosa* was isolated i.e., 1(25%). Malassezia species isolated were as follows: *M. furfur, M. globosa, M. obtusa, M. pachydermatis, M. sympodialis.* The predominant isolate was *M. furfur*, 27(67.5%) followed by *M. globosa* 7(17.5%).

Conclusion: The procedure of culture and antifungal testing required to be performed as different species of Malassezia are involved in Pityriasis versicolor and susceptibility is different among different species. It would help in preventing recurrences, systemic complications and any cosmetological problems especially in younger age group.

Keywords: Versicolor, Hypopigmented, Gram Stain, Cosmetologically Problems.

Introduction

Medical mycology is fast catching pace. The catapulting incidence of fungal infections due to increased life span, HIV, malignancies, and immunosuppressive therapies; is inviting attention of many an intellectual.

Opportunistic yeast of the genus Malassezia have been recognized as members of the microbiological flora of human skin and many other warm-blooded vertebrates [1,2]. These lipophilic fungi are associated with different types of diseases especially Pityriasis Versicolor, a chronic, benign and recurrent superficial infection which generally occurs in tropical and temperate regions [3-6]. This disease occurs worldwide with a prevalence as high as 30–40% in populations in tropical areas. A warm and humid environment is considered to be among the important precipitating factors [7,8].

Malassezia species had been recently reclassified on the basis of morphology, genomic composition, and physiological characteristics of the yeast [9] Currently, eleven species were identified which include ten lipid dependent species: *Malassezia dermatis, Malassezia furfur, Malassezia globosa, Malassezia japonica, Malassezia nana, Malassezia obtuse, Malassezia restricta, Malassezia slooffiae, Malassezia sympodialis, Malassezia pachydermatis* was the only non- lipid- dependent species [10] *Malassezia yamatoensis* was the new eleventh species identified.

Our study was undertaken to determine the incidence of Malassezia in patients, clinically suspected of having Pityriasis versicolor and speciate the identified isolates.

Material and Methods

This study was conducted during June 2012 to October 2013 on 55 patients from the OPD and IPD of the hospital, with clinically suspected pityriasis versicolor, either hypopigmented or hyperpigmented type.

Direct examination

Scales were collected in sterile autoclaved paper and mounted in 10% KOH for half an hour to 1 hour to be examined microscopically for detection of septate hyphae or collection of yeast cells and spaghetti and meatball appearance.

Potassium hydroxide (KOH) wet mount

1. A drop of 10% KOH was taken on a clean microscopic slide with the help of sterile dropper.
2. The scales were emulsified with the drop of KOH and covered with a cover slip. The slide was examined under low (10X) and high power (40X) magnification after 10-15 minutes for demonstration of fungal elements.

In the absence of scales celophane tape method was used.

The sticky side of cellophane was pressed firmly against the lesion and then placed over a sterile glass slide containing 10 % KOH and viewed under 10x and 40x for detection of septate hyphae or collection of yeast cells and spaghetti and meat ball appearance and the other tape to be impregnated in plate of Dixon's medium.

The samples were subjected for culture

Culture

Samples were inoculated in plates containing

a. Modified Dixon's agar (prepared by 3.6% malt extract, 0.6% peptone, 2% desiccated oxbile, 1% tween 40, 0.2% glycerol and 1.2% agar) containing chloramphenicol and cycloheximide
b. Sabouraud dextrose agar +olive oil.
c. Potato dextrose agar+olive oil.

The plates will be incubated at 32°C for 10 days and examined every day for the suspected growth of Malassezia before negative results were noted.

Identification

Suspected Malassezia species were identified according to their morphological features and physiological properties by:

a- Morphology of the colonies.
b- Direct microscopy after Gram stain
c- Other Biochemical Tests: Esculin hydrolysis, urease, tween assimilation and catalase.

Results

Total 55 cases of Pityriasis versicolor, of 1-65 years of age group and both sexes attending outpatient and inpatient department of dermatology were taken.

All the 55-skin scrapping showed positive morphology and the characteristic Spaghetti and meatball appearance in KOH preparation, i.e.100%.

Growth of Malassezia was obtained on Modified Dixon agar which represents 40 (72.7%) of the 55-skin scrapping.

Sex-wise distribution:

Out of 55 patients, 37(67.3%) were males and 18(32.8%) were female, M: F ratio being 2.05:1

Age wise distribution:

The highest prevalence of Malassezia is seen in young adults ie, patients with 21 -30 years of age

The lowest was seen in the age group 41-50 years and 61-70 years

Table 1: Age wise distribution

Age group distribution	Numbers(n=55)
0-10	3(5.5%)
11-20	8(14.5%)
21-30	24(43.6%)
31-40	15(27.3%)
41-50	1(1.8%)
51-60	3(5.5%)
61-70	1(1.8%)

Type of lesions:

Majority of patients presented with hypopigmented lesion i.e., 51(92.7%) in which the maximum isolated specie was of *M. furfur* 27(52.9%)

The hyperpigmented lesion was seen in 4 patients i.e., (7.3%) and *M. globosa* was isolated i.e., 1(25%)

Table 2: Type of lesion and species isolated

Type of lesion Species isolated	Hypopigmented lesion 51(92.70%)	Hyperpigmented lesion 4(7.30)
M.furfur	27(52.94%)	-
M.globosa	6(11.76%)	1(25%)
M.obtusa	3(5.88%)	-
M.pachydermatis	2(3.92%)	-
M.sympodialis	1(1.96%)	-

Isolated species:

Malassezia species isolated were as follows: *M. furfur, M. globosa, M. obtusa, M. pachydermatis, M. sympodialis*

The predominant isolate was *M. furfur*, 27(67.5%) followed by *M. globose* (17.5%).

Discussion

Pityriasis versicolor is a major cosmetic health problem all over world; the adverse cosmetic effects of the lesion may lead to significant emotional distress, particularly in adolescent.

Even though *Malassezia* is a component of the normal flora, it can also be an opportunistic pathogen. The organism is considered to be a factor in the other cutaneous diseases, including Pityrosporum folliculitis, confluent and reticulate papillomatosis, seborrheic dermatitis, and some forms of atopic dermatitis. *Malassezia* species have also been shown to be a pulmonary pathogen in patients with immunosuppression due to stem cell transplantation.

Comparison of KOH positivity and culture positivity rates with other studies

Out of 55 cases studied, all the 55(100%) cases were KOH positive and 40(72.7%) were culture positive.

Our study is comparable with study conducted by Karhoot *et al.* [15] in KOH positivity rates, ie 100% whereas culture positivity rates in all the other studies were higher than our study.

Table 3: Sex wise comparison in different studies

Studies	Male	Female
Shah *et al*(2012) [11]	83(59.71%)	56(40.28 %)
Our study (2013)	37(67.30%)	18(32.80%)
Ahmed *et al*(2015) [13]	82(89.11%)	10(10.85%)
Prasanna *et al*(2020) [14]	37(74%)	13(26%)

Male preponderance is seen in the Our study,ie 37(67.3%) males and 18 (32.8%) females which is comparable with other studies conducted in above table.

Male dominance seen in above studies may be due to the fact that they are more involved with outdoor activities, which place them at high risk of exposure to the factors like high temperature and humidity.

Table 4: Distribution of type of lesions in studies conducted by different authors

Investigator / Culture	Chaudhry *et al* (2010) [12] n=100	Karhoot *et al* [15] (2008) n=100	Ahmed *et al* (2015) [13] n=118	Our study (2013) n=55
KOH positive	90(90%)	100(100%)	114(96.61%)	55(100%)
Culture positive	87(87%)	79(79%)	105(92%)	40(72.7%)

Table 5

Studies	Hypopigmented lesion	Hyperpigmented lesion
Chaudhry *et al* [12](2010)	91(91%)	9(9%)
Shah *et al* [11] (2012)	117(84.10%)	12(8.60%)
Our study (2013)	51(92.70%)	4(7.30%)
Prasanna *et al* [14] (2020)	80%	20%

The lesion of PV can be hypopigmented, hyperpigmented, both or erythematous. Majority of patients in our study had hyperpigmented lesions ie 51(92.7%) and 4(7.3%) had hyperpigmented lesion.

The findings are same in other studies conducted which are shown in table. The hypopigmentation induced by this fungus can be explained on the basis of production of dicarboxylic acids, main component of which is azelaic acid. This acid acts through competitive inhibition of DOPA tyrosinase and perhaps has direct cytotoxic effect on hyperactive melanocytes. The pathogenesis of hyperpigmentation is also not fully understood but it may be due to increased thickness of keratin layer and more pronounced inflammatory cell infiltrate in these individuals act as a stimulus for melanocystes.

Out of 55 patients with clinically suspected of having PV, the maximum number of patients i.e., 24(43.6%) were in age group of 21 to 30 years followed by 15(27.3%) patients who were in the age group 31 to 40 years. This is similar to the finding published by Ahmed *et al*(2015)[13] and Prasanna *et al* (2020)[14]. —

This could be explained by the fact that sebum production is at its peak in this age group.

Table 6: Comparison of *Malassezia* species isolated in different studies

Species isolated / Studies	Most common species isolated	2nd most common species isolated
Krisanty et al [16] (2008)	*M.furfur*(33.7%)	*M.sympodialis*(27.5%)
Shah et al [11] (2012)	*M.globosa*(50.3%)	-
Our study (2013)	*M.furfur*(67.5%)	*M.globosa*(17.5%)
Ahmed et al [13] (2015)	*M.globosa*(76%%)	*M.furfur*(20%)
Prasanna et al [14] (2020).	*M.furfur*(50%)	*M.globosa*(27%)

Total 40(72.7%) patients out of 55 yielded growths of Malassezia spp. We found all the results consistent with *M. furfur* which was the predominanat spp isolated 27(67.5%), this was followed by *M. globosa* 7(17.5%) and *M. obtusa* 3(7.5%). The findings are similar to the study conducted by Krisanty *et al.* (2008)[16] and Prasanna *et al.*(2020)[14] in which predominant islolate was also *M. furfur* and was molecularly identified. The findings of *M. furfur* in the tropics might be explained by the recovery of pityriacitrin, an indole alkaloid produced by *M. furfur*. Pityriacitrin has the ability to protect fungus against ultraviolet exposure, which renders *M. furfur* more resistant to sun exposure.

Conclusion

Malassezia species can now be added to the growing list of normal skin flora organisms of low virulence that may cause mild recurrent skin infections and serious systemic skin infections in susceptible host. Clinicians must be aware of the patient population at risk of infection, and they must communicate the laboratory the need to include special procedures to recover the organism. Thus, the procedure of culture and antifungal testing required to be performed as different species of Malassezia are involved in Pityriasis versiclor and susceptibility is different among different species. It would help in preventing recurrences, systemic complications and any cosmetologically problems especially in younger age group.

Owing to the limited number and ambiguity of the phenotypic tests used for differentiating Malassezia species, it is difficult to accurately identify those using phenotypic methods alone. Molecular

methods are essential for the confirmation of the species.

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