

Clinico-Mycolological Profile Of Pityriasis Versicolor Cases Attending At A Tertiary Care Hospital In Eastern India --- A Cross-Sectional And Observational Study.

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Abstract: *Malassezia* spp. are lipophilic unipolar yeasts recognized as commensals of skin that may be pathogenic under certain conditions. Different *Malassezia* species can induce superficial skin infections, and most frequent is pityriasis versicolor (PV), a chronic and recurrent skin disease occurring primarily in hot and humid climates. The purpose of this study was to determine the species prevalence of *Malassezia* yeasts among clinical cases of Pityriasis versicolor. 96 clinically suspected PV patients were subjected to detailed history, clinical examination and investigations. Skin scrapings were processed by direct microscopy and culture. Isolates were processed by colony morphology, gram staining, biochemical characteristics & tween assimilation test. Association of *Malassezia* species with clinical & epidemiological characteristics was studied. *M.furfur* was the most common isolate (44.79%) followed by *M. globosa* (13.54%) and *M. sympodialis* (10.42%). Females were slightly more affected (56.25%) than the males (43.75%). The most common age group in which *Malassezia* encountered was 11-20 years in both sexes. Majority of the cases were students (32.29%). Majority of cases belongs to lower middle socio-economic group (43.75%). PV showed seasonal occurrence mostly in summer and rainy seasons. Maximum patients (81.25%) were associated with excessive sweating. Most common type of lesion was macular, scaly hypopigmented, bilaterally asymmetrically distributed and having well defined margin. Neck was the most affected site (28.13%) followed by back (20.83%). Overall this study has given the clear insight into the Clinico-mycological profile of PV infection observed in a tertiary care setting in eastern India.

Keywords: *Malassezia*, pityriasis versicolor, lipophilic, tween assimilation

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I. Introduction

Pityriasis versicolor is a chronic, benign skin disease that is generally asymptomatic. It occurs worldwide and is very common in tropical and temperate regions.^[1,2] It predominately affects young adults of both genders. The etiological agent is lipophilic yeast belonging to genus *Malassezia*. *Malassezia* species are lipophilic yeasts that are members of the normal microbiota of the skin and mucosal sites of a variety of homeothermic animals, utilising triglycerides and esters of sebum to produce diglycerides, monoglycerides and free fatty acids^[3]. The hair infundibulum is the ecological niche of *Malassezia* yeasts in our body^[4]. Genus *Malassezia* consists of fastidious, opportunistic, saprophytic "yeast like fungi"^[5] characterised morphologically by small cells unilateral, enteroblastic and repetitive percurrent budding^[6]. There are 14 described species, namely, *M. furfur*, *M. pachydermatis*, *M. sympodialis*, *M. globosa*, *M. obtusa*, *M. restricta*, *M. slooffiae*, *M. equina*, *M. dermatis*, *M. japonica*, *M. nana*, *M. capre*, *M. yamatoensis*, & *M. cuniculi*. Recently, the 15th member, *M. arunalokii* has been proposed from India^[7]. Hence this study was conceived to look for the most common species prevalent in this region and its correlation with clinical features.

II. Materials and Methods

This study was conducted in collaboration with the Departments of Microbiology and Dermatology of R.G. Kar Medical College & Hospital, Kolkata over a period of one year from April 2016 to March 2017. The study was approved by Institutional Ethics Committee. Written consent was collected from all the patient before sample collection. On behalf of child participant informed written consent was taken from guardian.

Clinical assessment

Relevant detailed history was taken from suspected PV cases attending Dermatology OPD and important clinical examination findings were noted. Wood's lamp examination was done, and findings were noted.

Sample collection

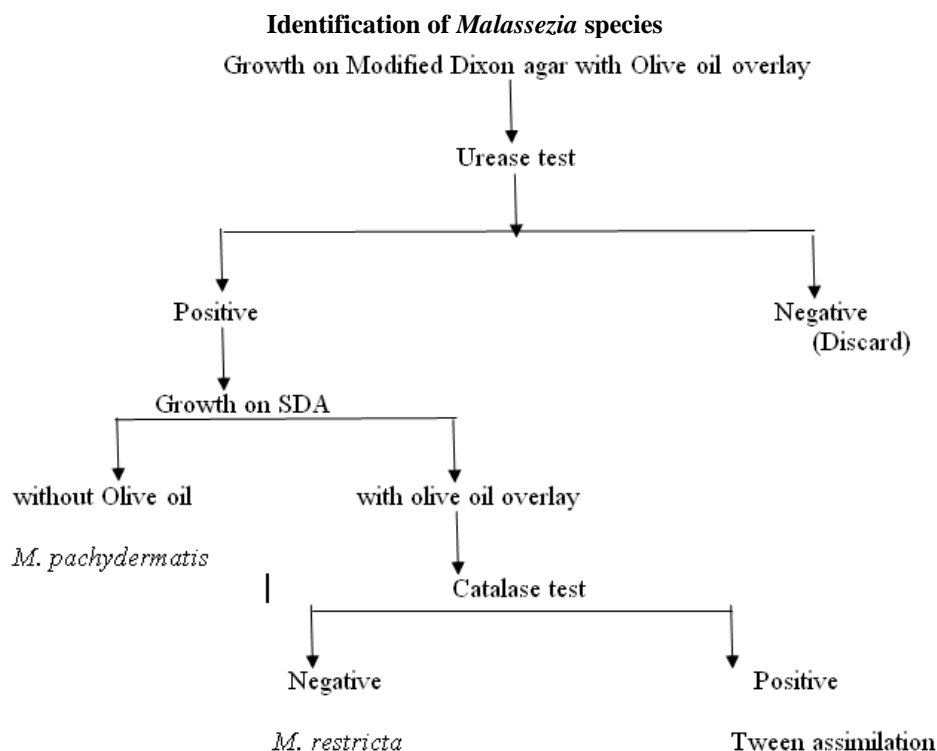
Samples (skin scrapings) were collected aseptically with the help of cello tape and flame-sterilised no.15 scalpel from the active lesions or from the junction of active lesion & normal skin in sterilised petridishes for onward transfer to Mycology laboratory.

Direct Microscopy and culture

A portion of the sample was used for direct microscopic examination with 10% KOH and the remaining portion was used for culture in modified Dixon's agar (HIMEDIA) and SDA (HIMEDIA) with olive oil overlay and then incubated at 32°C for 14 days. Culture plates were examined on days 3 and 7 then weekly intervals up to two weeks. Identification of growth on culture was based on its gross colony morphology on culture media and on its microscopic morphology as described by Crespo Erchiga *et al*^[8] and Gueho *et al*^[9].

Biochemical reactions and Tween assimilation

Then confirm diagnosis of *Malassezia* species were done by their gross colony morphology, microscopic characteristics by Gram stain and catalase test, urease test, esculin hydrolysis, temperature sensitivity and Tween assimilation test.



Tween assimilation test:

Yeast suspension (10^7 CFU/ml) were made in 2 ml distilled water and spread evenly by pour plate method into SDA at 45°C . After solidification, 4 wells were made and filled with 15 μl of each of Tween 20 (10%), Tween 40 (0.5%), Tween 60 (0.5%) and Tween 80 (0.1%) clockwise with proper marking. The plates were incubated for 7–10 days at 32°C in a moist environment and turned upside down on the second day to delay their dehydration.

Utilization of Tween was assessed by the degree of growth and /or reaction (precipitation) of the lipophilic yeasts around individual wells Gueho *et al* ^[9], Guillot *et al* ^[10] and Chander J ^[11].

Table:1 Important physiological and biochemical characteristics of clinically significant *Malassezia species*

<i>Malassezia species</i>	Lipid dependence	Growth at 37°C	Growth at 40°C	Catalase	Esculin splitting	Tween 20 (10%)	Tween 40 (0.5%)	Tween 60 (0.5%)	Tween 80 (0.1%)
<i>M. furfur</i>	+	+	+	+	-	+	+	+	+
<i>M. globosa</i>	+	-	-	+	-	-	-	-	-
<i>M. sympodialis</i>	+	+	+	+	+	-	+	+	+
<i>M. restricta</i>	+	v	-	-	-	-	-	-	-
<i>M. pachydermatis</i>	-	+	+	v	v	+	+	+	+
<i>M. obtusa</i>	+	-	-	+	+	-	-	-	-
<i>M. slooffiae</i>	+	+	+	+	-	+	+	+	-

Statistical analysis was done with the help of Fisher's exact test & Chi-square test. Software used for this study, were SPSS version 22 and Graph pad Prism 7.

III. Results and Analysis

96 clinically suspected pityriasis versicolor cases have been included in the study. Females were slightly more affected (56.25%) than the males (43.75%), with female to male ratio was 1.29:1 The mean age of affected cases was 26.25 years. The most common age group affected was 11-20 years. Majority of the patients were students (32.29%) followed by homemakers (20.83%). Most of them belongs to lower middle socio-economic group (43.75%), followed by lower socio-economic group (28.12%), according to B.G. Prasad scale.

Table 2: Distribution of cases according to age & sex (n=96)

Age	Male No (%)	Female No (%)	Total No (%)
<10 years	1 (1.04)	2 (2.08)	3 (3.12)
11-20 years	15 (15.62)	20 (20.84)	35 (36.46)
21-30 years	15 (15.63)	18 (18.75)	33 (34.38)
31-40 years	5 (5.21)	7 (7.29)	12 (12.5)
41-50 years	4 (4.17)	5 (5.21)	9 (9.38)
>50 years	2 (2.08)	2 (2.08)	4 (4.16)
Total	42 (43.75)	54 (56.25)	96 (100)

Fig. 1: Distribution of cases in relation to occupation (n =96)

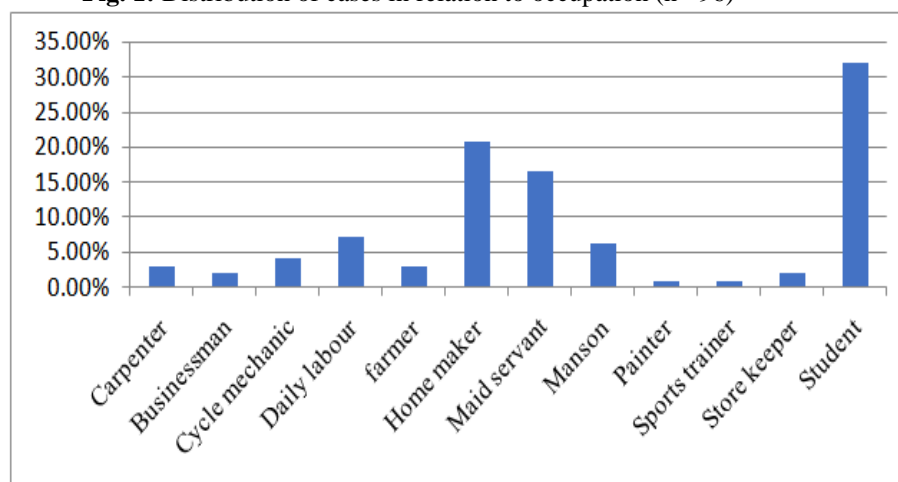
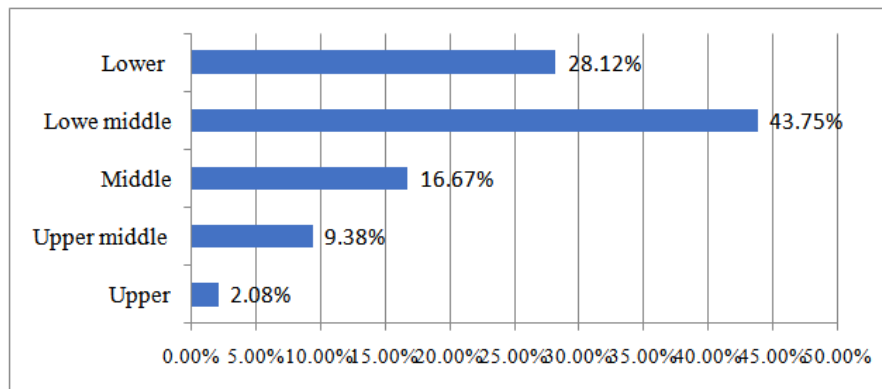


Fig. 2: Distribution of cases according to Socio-economic status* (n = 96)
* According to modified B. G. Prasad scale 2017



PV showed seasonal occurrence mostly (11.46%) in summer and rainy season (in July & August month which was closely followed by May & June month) when atmospheric temperature and relative humidity were very high, favouring its growth. Maximum patients (81.25%) were associated with excessive sweating.

Fig. 3: Seasonal occurrence of cases (n=96)

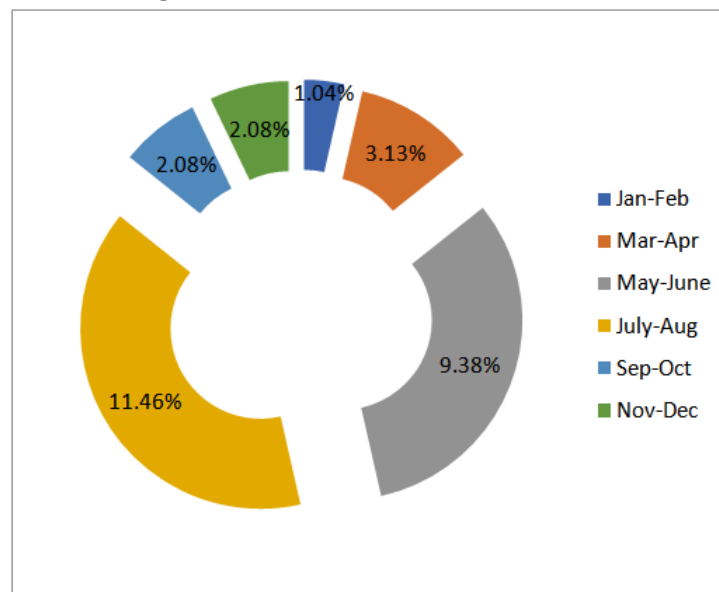
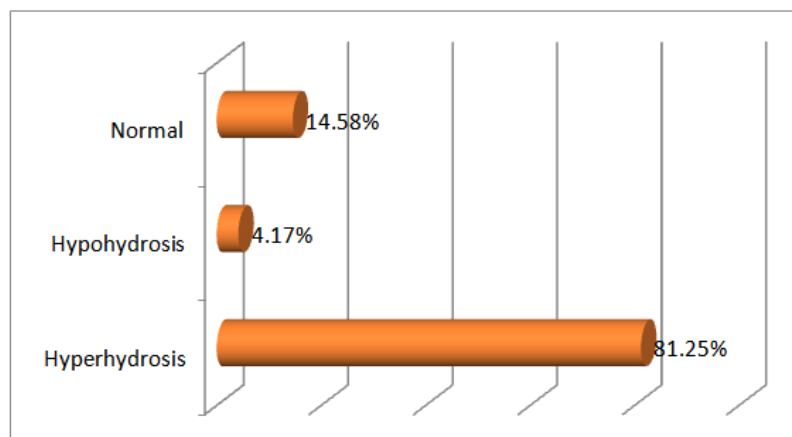


Fig. 4: Distribution of cases according to sweating pattern (n=96)



Most common type of lesion was macular, scaly hypopigmented lesion. They were mainly with well-defined margin and bilaterally asymmetrically distributed. White scale was more common than brown/tan scale. Most affected site of lesion was Neck (28.13%) followed closely by Back (20.83%) and Chest (18.75%).

Fig. 5: Distribution of cases according to type of lesion of PV (n=96)

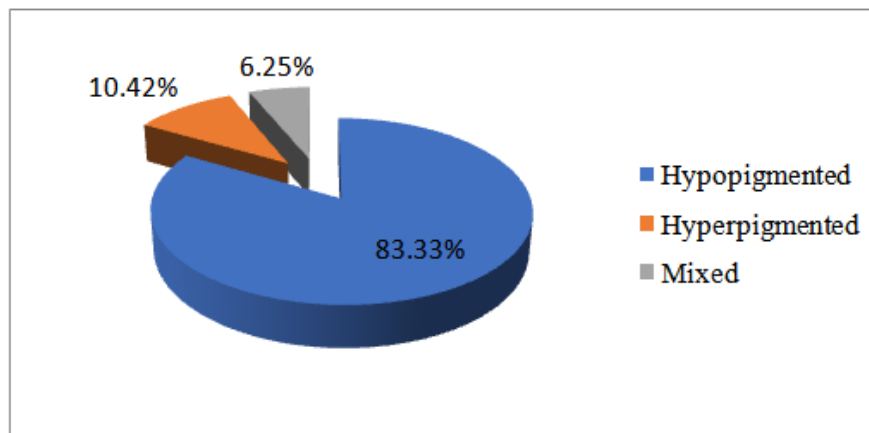


Fig. 6: Distribution of cases according to character of lesion of PV (n=96)

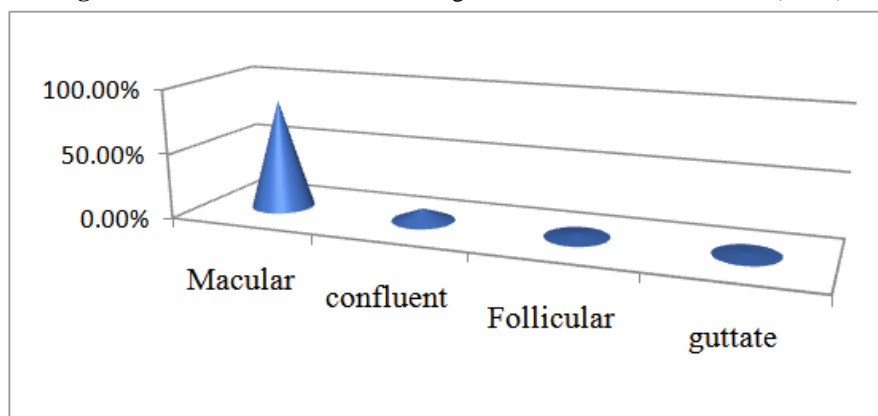


Fig. 7: Distribution of cases according to colour of scale of lesion (n=96)

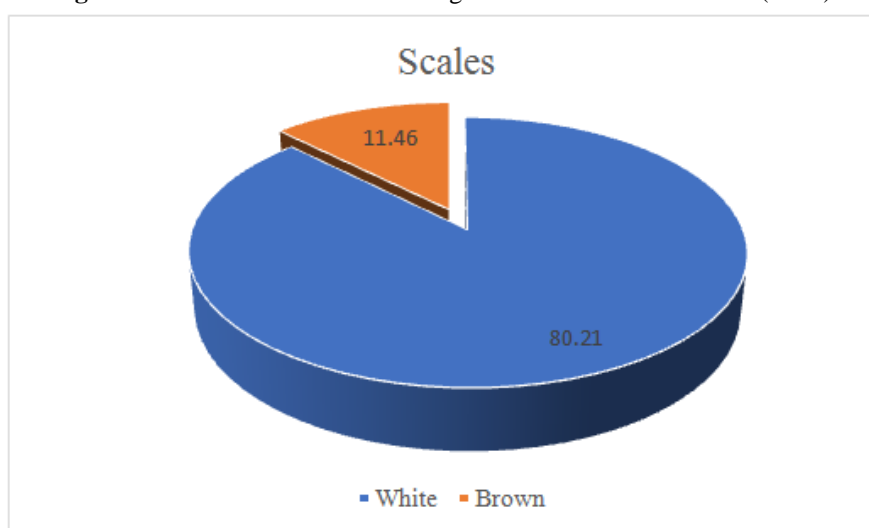


Fig. 8: Distribution of cases according to site of lesion of PV (n=96)

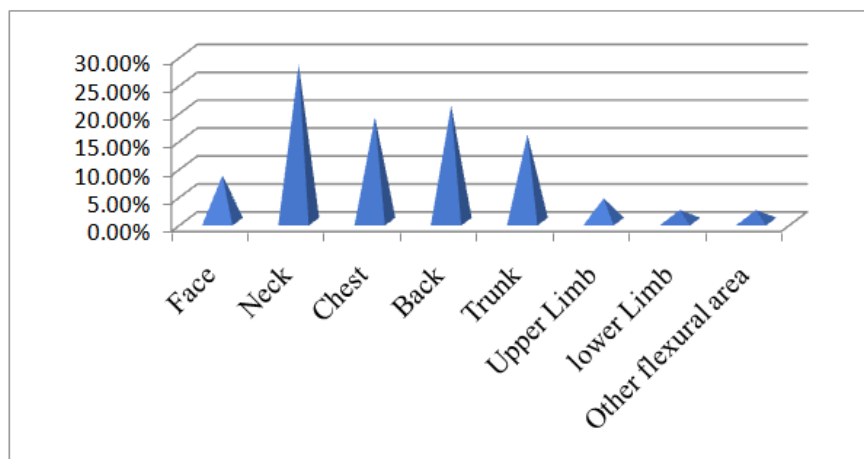
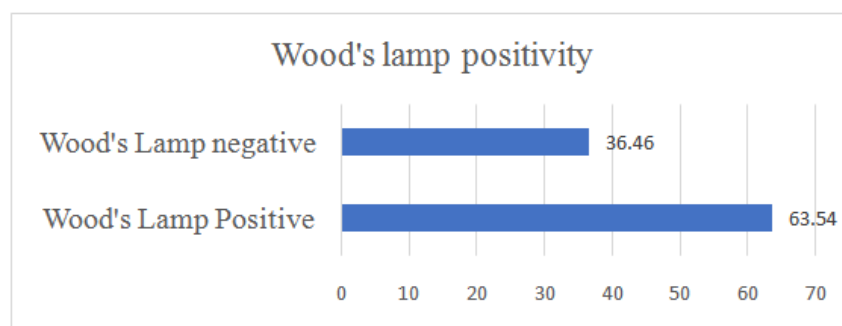


Table 3: Distribution of cases according to margin of lesion and pattern of distribution of lesions (n=96)

Margin of lesion	No (%)
Well defined	66 (68.75)
Ill defined	30 (31.25)
Total	96 (100)
Pattern of distribution of lesion	No (%)
Unilateral	6 (6.25)
Bilaterally symmetrical	18 (18.75)
Bilaterally asymmetrical	72 (75)
Total	96 (100)

Fig. 9: Distribution of cases according to wood's lamp positivity (n=96)



Wood's lamp examination with positive golden yellow fluorescence was found in maximum cases (44.79%) with significant association with *M. furfur* ($p < 0.0001$). KOH preparation of specimens showed short hyphae with budding yeast cells (spaghetti and meatball appearance) in maximum cases (76.04%). Growth on modified Dixon's agar with olive oil overlay was noticed in only 72.92% cases, *M. furfur* was the most common isolate (44.79%) followed by *M. globosa* (13.54%) and *M. sympodialis* (10.42%).

Fig. 10: Distribution of cases according to culture positivity of *Malassezia* spp. (n=96)

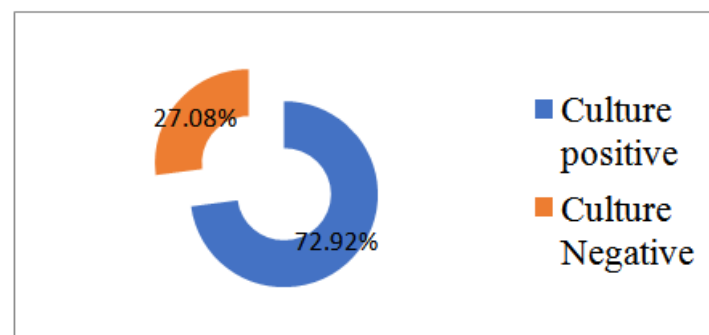


Fig. 11: Distribution of different *Malassezia* isolates (n=96)

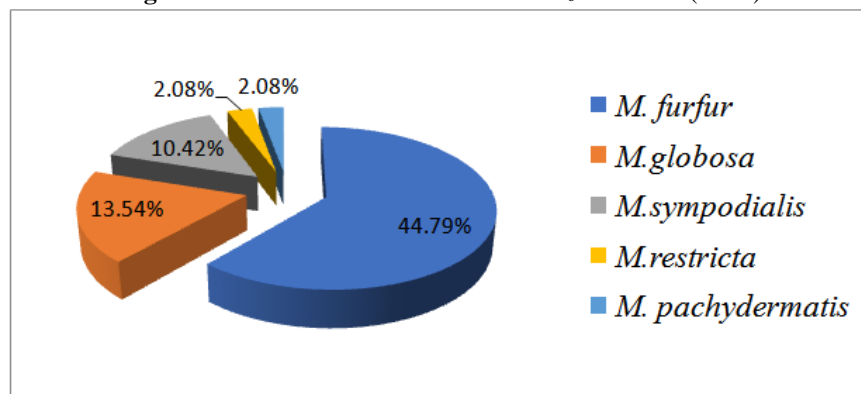


Image 1: Classical hypopigmented patch



Image 2: Hyperpigmented patch

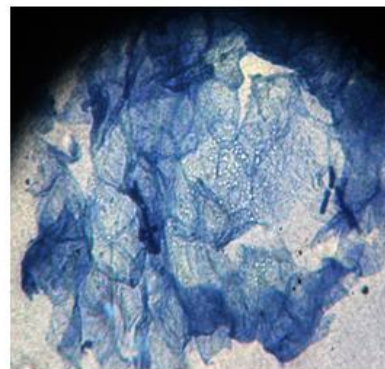
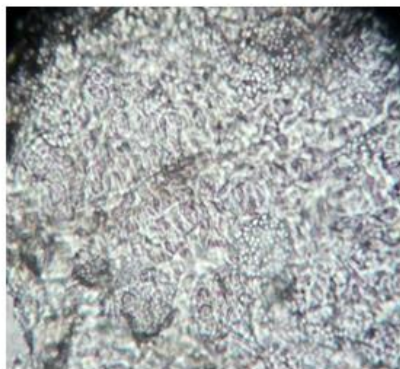


Image 3/ 4 : 10% KOH preparation (w/ methylene blue) showing budding yeast cells with short hyphae (Sphaghetti and meat ball appearance)



Image 5: Colony morphology over modified Dixon agar with olive oil overlay

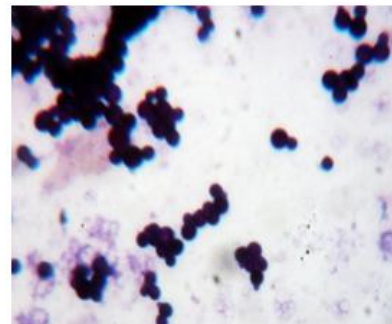


Image 6: Gram stain showing budding yeast cells

IV. Discussion

In the present study, 96 clinically diagnosed cases of PV were studied. Females were slightly more affected (56.25%) with Female to male ratio was 1.29:1. A similar trend was observed in studies by Imwidththaya *et al.* ^[12] (1.13:1), Santana *et al.* ^[13], Uneke *et al.* ^[14]. As females are more conscious about skin related problem, they reported to doctor early. This may be a major factor that could have contributed to the higher prevalence of PV in females in this study as PV is a major cosmetic problem. It was also seen that maximum patients had the habit of applying oil to their hair while bathing. This habit made hair allow contacting with neck and /or back, probably contributing to development of the disease. The most common age group affected was 11 to 20 yrs. of age (36.46%) followed by 21 to 30 years of age (34.38%). It was similar with Dutta *et al.*, (age group: 11 to 30 years) ^[15], Krishnan *et al.*, (age group: 15 to 29 years) ^[16] and Rao *et al.*, (2002) (age group 21 to 30 years) ^[17]. According to Dutta *et al.*, In India, the disease prevalence has been recorded for somewhat younger individuals, between 10 and 30 years old. ^[15] It was probably due to the period of highest sebum secretion which is linked with increased release of hormones especially androgens in this age group. The presence of long chain fatty acids >C12 is required for the growth of *Malassezia* species, as they are unable to synthesis their own. The ability of *Malassezia* to split lipids present in sebum into cholesterol and utilize them for growth helps in their maintenance both in the skin and in the scalp.

Students were found to be the most commonly affected ones in our study, hence are more conscious of their lesions. It may also be since the period of highest sebum secretion which is linked with increased release of hormones especially androgens in this age group. Apart from these factors, as students are not involved in earning of daily wages in most of the cases, they can easily access to hospital. A similar finding was noted in Ghosh *et al.* ^[18], Sharma *et al.* ^[19], and Pramanik *et al.* ^[20]. Majority of the cases belongs to lower middle (43.75%) and Lower socio-economic group (28.12%), which was also similar with the findings by Ghosh *et al.* ^[18] and Jena *et al.* ^[21].

In summer and rainy season, the environmental temperature and relative humidity rises in our state (Above 37 °C temperature and >80% humidity). This leads to profuse sweating predisposing to development of PV. In the present study, 29.17% showed seasonal occurrence. Majority of patients 11.46% developed lesions in July & August month which was closely followed by May & June month by 9.38% cases. In the study of Dutta *et al.* ^[15], maximum number of the cases presented during the period July to September and Rao *et al.* ^[17] also reported clustering of cases (35%) during the summer months. This seasonal trend was also consistent with the study of Ghosh *et al.* ^[18], Sharma *et al.* ^[19], and Pramanik *et al.* ^[20]. 81.25% cases showed hyperhidrosis which is a strong predisposing environmental factor to PV development.

In this study, the number of hypo pigmented achromic PVs (83.33%) outnumbered the hyperpigmented chromic (10.42%) type. It was similar with the study of Thayikkannu *et al.* ^[22]. Almost similar observation was noted in study of Krishnan *et al.* ^[16], Rao *et al.* ^[17], and Ghosh *et al.* ^[18]. Azelaic acid and several tryptophan metabolites produced by *Malassezia*, which can interfere with melanisation, are considered important in the skin pigmentation changes seen in PV ^[23]. 85.42% cases presented with macular type of skin lesion. Macules were most common type of lesion found in some other studies also i.e. Krishnan *et al.* ^[16], Rao *et al.* ^[17] (86.60% Macules) & Ghosh *et al.* ^[18]. In our study, maximum patients (91.67%) presented with scaly lesions, out of which, 80.21% cases presented with white scale and 11.46% cases presenting with brown/tan scale. A similar finding was noted in study of Rao *et al.* ^[17], Ghosh *et al.* ^[18] (89.09%), and Sharma *et al.* ^[19].

The most affected site of lesion in our study was Neck (28.13%) followed closely by Back (20.83%) which were similar with the study of Rao *et al.* ^[17] (The disease was seen commonly on the neck, back and chest), Shah *et al.* ^[24] (The most common sites affected in patients were neck followed by back and chest). Similar findings were also noted by Dutta *et al.* ^[15], Krishnan *et al.* ^[18], Sharma *et al.* ^[19], and Kaur *et al.* ^[25] revealing neck, back, upper trunk and face to be the commonly involved sites. Distribution of lesions on various sites depends on the density of the sebaceous gland. This type of finding may also be seen due to that most of the patients had the habit of applying oil to their hair and taking daily baths. When women plait their hair with oil with our traditional costume, saree and blouse / or any other dress, it will allow its contact with the skin of the neck and back mostly, and exposure to sunlight could have contributed to development of pityriasis versicolor.

In the present study, 68.75% cases were presented with bilaterally asymmetrically distributed well-defined margin of the lesions. A similar finding was noted in Sharma *et al.* ^[19] (67.9% of which were having well-defined border), Pallai *et al.* ^[26].

In our study, 61 (63.54%) cases were found to show fluorescence under wood's lamp with all 43 (44.79%) cases with *M. furfur* isolates, which was similar with the study of Rao *et al.* ^[19] (78.30%), Shah *et al.* ^[24] (88.48%). Under wood's light examination pityriasis versicolor lesions may fluoresce a characteristic bright yellow or gold colour. The colour of the fluorescence may also aid in differential diagnosis, as it is unique to the mycelial form of *Malassezia*. Recent evidence ^[27,28] suggests that only *M. furfur* produces the indole compounds

that fluoresce under Wood's light, indicating that this species is implicated in at least some cases of pityriasis versicolor^[29].

In this study, KOH preparation of specimens showed short hyphae with budding yeast cells in 76.04% cases. Similar study was noted in study of Thayikkannu *et al*^[21], Pallai *et al*^[25], Rao *et al*^[17] & Ghosh *et al*^[18]. Growth on modified Dixon's agar with olive oil overlay was noticed in only 72.92% cases. Ibekwe *et al*^[30] showed culture positivity in their study was 57.6%. Agarwal *et al*^[31] showed culture positivity was 62%. Culture positivity was 62.78% in the study of Remya *et al*^[32], 54.34% in the study of Ahmed *et al*^[33], 89.79% in the study of Pramanik *et al*^[20] and 68.57% in the study of Kindo *et al*^[34]. This difference in culture positivity may be due to adequacy of sampling and the different culture media^[8].

M.furfur (44.79%) was the predominant isolate in our study followed by *M. globosa* (13.54%) and *M. sympodialis* (10.42%). This finding was similar with the study of Sharma *et al*^[19] (77.3%), Pramanik *et al*^[20] (60.23%). Krisanty RI *et al*^[35] conducted a study in Indonesia and found *M. furfur* to be most common (42.9%) like De Quinzada MM *et al*^[36] and Miranda KC *et al*^[37].

Several studies reported *M. furfur* to be the commonest species associated with PV in the tropical and subtropical regions (Razanakolona *et al*^[38]; Eidi *et al*^[39]; Shoeib *et al*^[40]; and Ibekwe *et al*^[30]). Canteros *et al*^[41] and Giusiano *et al*^[43] also found that *M. furfur* was the predominant species. *M. furfur* was reported to be the commonest species in West Bengal^[44]. This can be explained by the fact that *M. furfur* produces an indole alkaloid pityriacitrin which has the ability to protect this fungus against ultraviolet exposure and renders *M. furfur* more resistant to sun exposure^[44].

In contrast to our study, Aspiroz *et al*^[45], Nakabayashi *et al*^[46], Crespo Erchiga *et al*^[8], Tarazooie *et al*^[47] and Shah *et al*^[25] all isolated *M. globosa* as the most common species, at the frequencies of 58.2%, 55%, 55%, 53.3% and 48.57% respectively. Dutta *et al*^[15] also revealed that 54% of isolates belonged to *M. globosa* and the next common species being *M. furfur* (30%). In most of these studies, *M. globosa* was the predominant species in the PV lesions^[43]. Its pathogenicity might be explained by high lipolytic activity^[8].

Whereas Kindo *et al*^[34] in South India revealed that *M. sympodialis* to be the commonest agent. Gupta *et al*^[2] also reported *M. sympodialis* (71%) to be the predominant isolate.

This differences between various studies could be explained not only by ethnic and geographical factors, but also adequacy of sampling and the different culture media (Leeming and Notman agar or modified Dixon agar)^[48].

V. Conclusion

Thus, this study has given as the clear insight into the clinical and mycological aspects of pityriasis versicolor and throws a light on the predominant association of *Malassezia furfur* and *Malassezia globosa* with the disease. The results of this study supported the proposed hypothesis that geographical, climatic and racial variations can contribute towards the differences in the distribution of *Malassezia* species. This study differs significantly with many other studies in the distribution of *Malassezia* species suggesting the role of geographical, ethnic, or racial variation in causing PV.

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