

A Study on Demographic Profile and Risk Factor Association in Pityriasis Versicolor Cases Attending at a Tertiary Care Hospital in Kolkata.

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ABSTRACT

Background: Pityriasis versicolor (PV), also known as tinea versicolor, is a chronic, mild, superficial and recurrent infection of the stratum corneum, caused by different *Malassezia* spp and seen predominantly in young age group and primarily in hot and humid climates. The aim of this study was to analyze epidemiological parameters and risk factors association in clinically diagnosed PV cases and also the mycological evaluation of those PV cases. **Methods:** A total of 116 patients attending the OPD of Dermatology were included and analysed for detailed history, clinical examination, epidemiological parameters, risk factors and investigations. Skin scrapings collected were processed by direct microscopy with 10% KOH and culture in modified Dixon agar (mDA). Isolates were identified by colony morphology, gram staining, biochemical characteristics & tween assimilation test. **Results:** Females were more affected (56.03%) than the males (43.97%) with F: M ratio 1.27:1. PV affected most commonly (36.21%) in 11-20 years of age group. Students (32.29%) were affected in maximum. Majority of affected patients (65.52%) used oily body creams, whereas 34.48% cases shared their body towels with others. 10.34% cases were associated with seborrheic dermatitis. Seasonal occurrence mostly seen in May - August. Patients with type III (Medium) complexion (56.03%) with normal skin texture (49.14%) were mostly affected. Maximum patients (74.14%) were associated with excessive sweating. 18.96% patients were associated with Type II DM. Most of the cases presented with macular, scaly hypopigmented, bilaterally asymmetrically distributed and having well defined margin. Neck was the most affected site (28.45%) followed by back (20.69%). **Conclusion:** *M.furfur* was the most common isolate (47.06%) followed by *M. globosa* (24.71%) and *M. sympodialis* (15.29%).

Keywords: Pityriasis versicolor (PV), *Malassezia*, modified Dixon agar

INTRODUCTION

Pityriasis versicolor, also known as tinea versicolor is a superficial chronically recurring fungal infection of the stratum corneum, characterized by scaly, dyspigmented irregular macules most often occurring on the trunk and extremities.^[1] It occurs worldwide and is very common in tropical and temperate regions and predominately affects young adults of both genders.^[2] This infection presents most commonly as pigmentation changes in the skin but also can be accompanied with sebure or pruritis.^[3] Genus *Malassezia* has a definitive causal role in the pathogenesis of PV.^[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] Many predisposing factors have been proposed for this disease such as late teen and young adulthood age due to increase sebum secretion after puberty, tropical and subtropical climate, immunosuppression, use of oral contraceptives, hyperhidrosis, malnutrition, poor hygiene, Cushing's disease, pregnancy and a few other conditions.^[8,9] The organism can readily be identified by treating skin scraping with 10% KOH. Microscopical visualization of the fungi appears as short, thick hyphae with variously sized budding yeasts (spaghetti and meat-ball appearance).^[10] They are primarily differentiated by their ability to assimilate various polyoxyethylene sorbitan esters

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(Tween) following the methodology of Guillot et al.^[11]

The objective of our study was to find out different clinical patterns, analyze epidemiological parameters and risk factors association in clinically suspected pityriasis versicolor cases and also the mycological evaluation of those cases at a Tertiary care setting in Kolkata.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology in collaboration with Department of Dermatology of R.G. Kar Medical College & Hospital, Kolkata over a period of one year from April 2016 to March 2017 after approval from Institutional Ethical Committee. Written consent was collected from all the patient before sample collection. 116 patients with clinically suspected pityriasis versicolor attending Dermatology OPD were consecutively included.

Clinical examination

A detailed history including patient's age, sex, occupation, socioeconomic status (according to the modified B.G.Prasad scale), symptoms, duration, history of recurrence, climatic influence, family history, use of cosmetics, talcum powder, shampoo, oil, synthetic clothing and condition of the personal hygiene were recorded. A thorough clinical examination was done to determine the characteristics and distribution of lesions, colour and texture of the skin of the patient and any other associated dermatological or systemic diseases. The cutaneous lesions of suspected pityriasis versicolor were also confirmed by Wood's lamp examination.



Image 1: Showing characteristic depigmented, scaly & macular lesions over neck, sometimes coalesce to produce big patch (Confluent).



Image 2: Showing golden yellow fluorescence under wood's lamp examination

Mycological examination

Sample collection

Samples (skin scrapings) were collected aseptically with the help of cellotape 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,^[12] and Gueho et al.^[13]

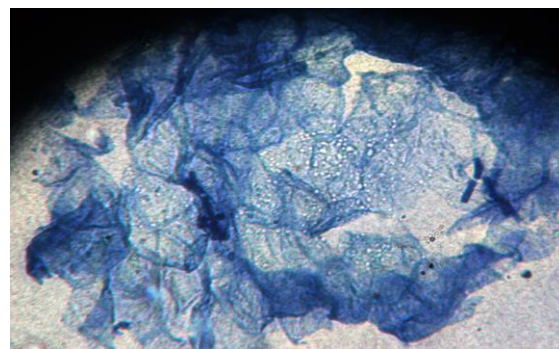


Image 3: Showing budding yeast cells with small hyphae (Spaghetti and meat ball appearance) with 10% KOH and 1 drop methylene blue under 40X



Image 4: Showing colony morphology of Malassezia over modified Dixon agar with olive oil overlay

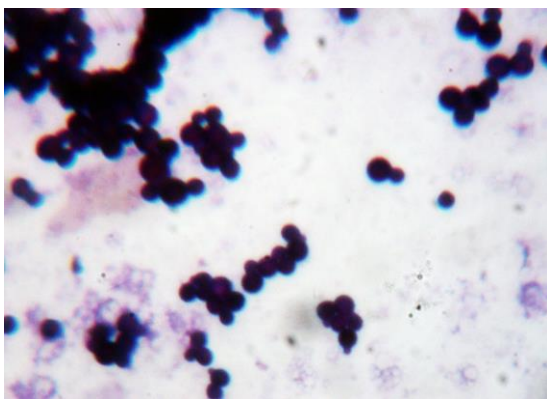


Image 5: Gram stain showing gram positive budding yeast cells

Biochemical reactions and Tween assimilation

Identification of Malassezia species were done following standard laboratory protocol which were based on colony morphology, microscopic morphology and also catalase test, urease test, esculin hydrolysis, temperature sensitivity and Tween assimilation test.

Statistical analysis

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.

RESULTS

Out of the 116 patients of pityriasis versicolor, 65 (56.03%) patients were female and 51 (43.97%) patients were male, with a slight female preponderance (F:M= 1.27:1). Most of the patients were young adults. Majority 42 (36.21%) were in the age group of 11 to 20 years.

Majority of the patients in this study were students (31.90 %). 43.97% patients were from lower middle socioeconomic status followed by lower (28.45 %). 51.72% cases used medicated soaps while bathing. Oil based body creams were more (65.52%) frequently used than petroleum jelly (31.90%).

58.62% cases used talcum powder whereas 34.48% cases shared their body towels with others. 61.21% patients used to apply oil in their hair or body while bathing. 10.34% cases had history of seborrheic dermatitis. Family history was found to be positive in 36.21% cases. Comorbid diseases were noted in 42/116 (36.21%) of the patients in this study. Among them, 18.96% cases were diabetics and having renal disorders in 06.90% and polycystic ovarian disease in 10.34%. 44.83% cases had history of using steroid whereas 29.31% cases had history of using topical/ systemic antifungal agents. Most of the patients (56.03%) had medium (type III) skin complexion (according to Fitz-Patrick Scale) with normal (49.14%) skin texture followed by oily (29.31%). Seasonal occurrence had been noted in 29.31% cases with a peak incidence during summer and rainy seasons (May – August) (41.18%). Majority of cases (74.14%) had history of hyperhidrosis.

Table 1: Distribution of cases according to age & sex (n=116)

Age	Male No (%)	Female No (%)	Total No (%)
<10 years	1 (0.86)	2 (1.73)	3 (2.59)
11-20 years	17(14.66)	25 (21.55)	42 (36.21)
21-30 years	19(16.38)	21 (18.10)	40 (34.48)
31-40 years	7(6.03)	8 (6.90)	15 (12.93)
41-50 years	4(3.45)	7 (6.03)	11 (9.48)
>50 years	3(2.59)	2 (1.72)	5 (4.31)
Total	51 (43.97)	65 (56.03)	116 (100)

Table 2: Distribution of cases according to personal hygiene

	Male (%)	Female (%)	Total (%)	p value
Medicated soap	22 (18.96)	38 (32.76)	60 (51.72)	0.1011
Body creams	24 (20.69)	52(44.83)	76 (65.52)	0.0002
Petroleum jelly	16 (13.79)	21 (18.10)	37 (31.90)	0.9225
Bleaching and toning creams	14 (12.07)	25(21.55)	39 (33.62)	0.1270
Talcum powder	28 (24.14)	40 (34.48)	68 (58.62)	0.4713
Share body towel	28 (24.14)	12 (10.34)	40 (34.48)	0.0000
Synthetic clothing	11 (9.48)	25 (21.55)	36 (31.03)	0.0509
Oil	42 (36.21)	29 (25%)	71 (61.21)	0.0000

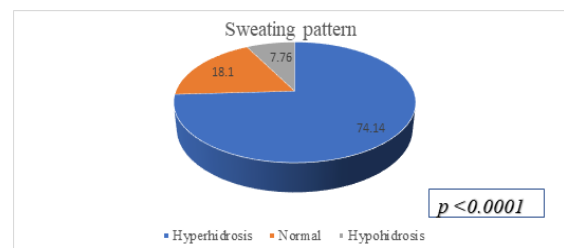


Figure 1: Distribution of cases according to sweating pattern (n=116)

Table 3: Distribution of cases according to characteristics of lesion (n=116)

Type	Cases (%)
Hypopigmented	97 (83.62)
Hyperpigmented	12 (10.34)
Mixed	7 (6.03)
Scaly	106 (91.38)
White scale	85 (73.28)
Brown scale	21 (18.10)
Pruritus	56 (48.28)
Lesion	
Macular	99 (85.34)
Confluent	11 (9.48)
Follicular	4 (3.45)
guttate	2 (1.73)
Margin of lesion	
Well defined	80 (68.97)
Ill defined	36 (31.03)
Pattern of distribution	
Unilateral	7 (6.03)
B/L Symmetrical	22 (18.97)
B/L asymmetrical	87 (75)

Most common type of lesion was macular (85.34%), scaly (91.38%) hypopigmented (83.62%) lesion. They were mainly with well-defined margin (68.97%) and bilaterally asymmetrically distributed (75%). White scale was more common (73.28%) than brown/tan scale. Itching was associated with 48.28% cases. Most affected site of lesion was Neck (28.45%) followed closely by Back (20.69%) and Chest (18.97%).

Wood's lamp positivity was noted in 47.41% cases with significant association with *M. furfur* (p < 0.0001). 10% KOH preparation of specimens showed short hyphae with budding yeast cells (spaghetti and meatball appearance) in maximum cases (75.86%).

Table 3: Distribution of cases according to KOH positivity & Culture positivity (n=116)

	Culture positivity (%)	Culture negativity (%)	Total (%)
KOH positivity	75 (64.66)	13 (11.20)	88 (75.86)
KOH negativity	10 (8.62)	18 (15.52)	28 (24.14)
Total	85 (73.28)	31 (26.72)	116 (100)

$\chi^2 = 26.592$, $df = 1$, $\chi^2/df = 26.59$, $p(\chi^2 > 26.592) = 0.0000$

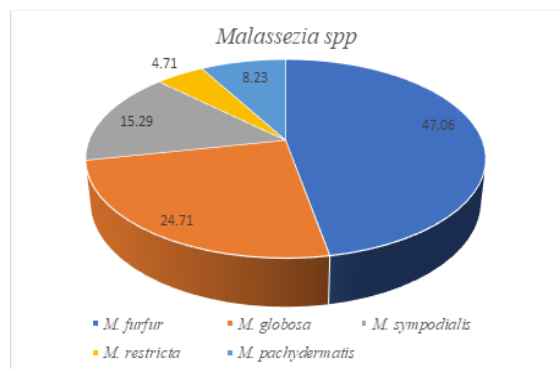


Figure 2: Distribution of cases different Malassezia isolates

Only 18/116 (15.52%) cases were KOH negative and Culture negative, thus ruling out Pityriasis versicolor. Out of 98/116 (84.48%) PV positive cases, growth on modified Dixon's agar with olive oil overlay was noticed in only 85/116 (73.28%) cases. Out of them, *M. furfur* was the most common isolate (47.06%) followed by *M. globosa* (24.71%) and *M. sympodialis* (15.29%).

Table 4: Distribution of cases in relation with various risk factors

Risk factors	PV positive (%)	PV negative (%)	p value
Medicated soap	48 (41.38)	12 (10.34)	0.1675
Body creams	70 (60.34)	6 (5.17)	0.0018
Petroleum jelly	26 (22.41)	11 (9.48)	0.0060
Bleaching and toning creams	28 (24.14)	11 (9.48)	0.0072
Talcum powder	56 (48.28)	12 (10.34)	0.4508
Share body towel	34 (29.31)	6 (5.17)	0.9181
Synthetic clothing	31 (26.72)	5 (4.31)	0.7453
Oil	66 (56.90)	5 (4.31)	0.0015
Family h/o	32 (27.59)	10 (8.62)	0.0631
Seborrheic dermatitis	8 (6.90)	4 (3.45)	0.0904
Seasonal occurrence	28 (24.14)	6 (5.17)	0.6833
Type II DM	12 (10.34)	10 (8.62)	0.0001
Use of Steroid	40 (34.48)	12 (10.34)	0.0427
Use of Antifungals	28 (24.14)	6 (5.17)	0.6833
Hyperhidrosis	80 (68.97)	6 (5.17)	0.0000

Significant association was noted with Type II DM, hyperhidrosis along with the use of oil, body creams, petroleum jelly, bleaching and toning creams and steroid.

DISCUSSION

In the present study, out of the 116 patients of pityriasis versicolor, 56.03% patients were female, and 43.97% patients were male, with a slight female preponderance (F:M= 1.27:1). Similar study was noted in studies by Imwidthaya et al.^[14] (1.13:1), Santana et al.^[15] Uneke et al.^[16] This may be since females are more conscious about skin related problem, they reported to doctor early 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. Most of the patients were young adults. Majority of patients (36.21%) were in the age group of 11 to 20 years followed by the age group of 21-30 years (34.48%). It was similar with Dutta et al., (age group: 11 to 30 years),^[17] Krishnan et al. (age group: 15 to 29 years) [18] and Rao et al., (2002) (age group 21 to 30 years).^[19] According to Dutta et al.,^[17] In India, the disease prevalence has been recorded for somewhat younger individuals, between 10 and 30 years old.^[17] It was probably since in this age group increased

release of androgens is associated with the period of highest sebum secretion. The presence of long chain fatty acids >C12 is required for the growth of *Malassezia* species, as they are unable to synthesize 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.

Majority of the patients of our study were students (31.90 %), hence are more conscious of their lesions. It may also be since the period of highest sebum secretion was noted in this age group. This finding was consistent with the study of Ghosh et al [10], Sharma et al,^[20] and Pramanik et al.^[21] 43.97% patients were from lower middle socioeconomic status followed by lower (28.45 %) in our study, which was also similar with the findings by Ghosh et al,^[10] and Jena et al.^[22]

The study showed that personal hygiene is important factor for the development of PV. It was also noted that more female used talcum powder, body creams and bleaching/toning creams, whereas male used to share body towel with others and apply oil to hair or body while bathing and the differences were found to be significant. Similar finding was noted in the study of Ibekwe et al.^[24] Significant association was noted with the use of oil, body creams, petroleum jelly, bleaching and toning creams and steroid in our study. A major factor may be the higher use of occlusive cosmetics and skin lightening creams (some of which may also contain corticosteroid) known to impair the integrity of the skin immune surveillance against superficial infections. Daily use of cosmetics has been observed to increase concentration of carbon dioxide in the epidermis thus inducing a change in the microflora, producing low skin pH and encourage growth of the *Malassezia* yeast.^[15]

In the present study, 10.34% cases had history of seborrheic dermatitis. A similar finding was noticed in study of Ghosh et al,^[10] (10%). Similar coexistence (11.60%) was also noted in study of Rao et al,^[19] Meanwhile, there were no significant coincidence observed between seborrheic dermatitis and PV; thus, the fact that an individual with dandruff is not susceptible to PV. Probably this could be because the etiological agent of the seborrheic dermatitis was not necessarily the source of the PV. Further studies are needed to clarify this. Ibekwe et al.^[24] Family history was found to be positive in 36.21% cases, in our study, which is consistent with the study of Ibekwe et al,^[24] (36.5%), Rao et al,^[19] (38.30%). It was a bit lower in study of Ghosh et al,^[10] (25.55%). PV is not a contagious disease; it may be due to increased sweat and sebum secretion mainly with genetic makeup. There was also some evidence of genetically determined susceptibility. In a study it was observed that more than one sibling in a family could get the infection. While despite long intimate contact between the

parents, infection found to be restricted to one of them. Workers found no higher frequency of the disease in married couples than in the general population. Our study also didn't find any familial association in the study population.

In the present study, seasonal occurrence had been noted in 29.31% cases with a peak incidence during summer and rainy seasons (May – August) (41.18%). In summer and rainy season, the environmental temperature and relative humidity rises in our state (Above 37 0 c temperature and >80% humidity). This leads to profuse sweating predisposing to development of PV. In the study of Dutta et al,^[17] maximum number of the cases presented during the period July to September and Rao et al.^[19] also reported clustering of cases (35%) during the summer months. This seasonal trend was also consistent with the study of Ghosh et al,^[10] Sharma et al,^[20] and Pramanik et al.^[21] In this study, majority of patients (56.03%) belongs to type III (Medium) complexion according to Fitz-Patrick scale. 49.14% patients were of Normal skin texture followed by oily (29.31%). Similar findings were noted in the study of Ghosh et al,^[10] (55.45% patients had medium skin complexion with normal 75.45% skin texture followed by oily and dry skin), Thayikkannu et al,^[23] and Morais et al,^[25] (Normal skin most frequent in the study, found in 39.7% of the patients followed by dry skin 36.2% and oily skin 24%).

36.21% of the patients in this study presented with various comorbidities. Among them, 18.96% cases were diabetics and having renal disorders in 06.90% and polycystic ovarian disease in 10.34%. 44.83% cases had history of using steroid whereas 29.31% cases had history of using topical/ systemic antifungal agents. A similar finding was noted in the study of Ghosh et al,^[10] Rao et al,^[19] Reed et al,^[26] Faergemann et al,^[27] and Hashim et al.^[28] In the present study, 74.14% cases showed hyperhidrosis, which was on a little higher side in comparison to the study of Ibekwe et al,^[24] Sharma et al,^[20] (48.09%) and Thayikkannu et al.^[23] This may be since the environmental temperature and relative humidity rises in our state (Above 37 0 c temperature and >80% humidity) in the summer and rainy season and maximum number of patients of our study population (belonging to lower and middle lower socio economic status) was involved more in outdoor activities, this leads to profuse sweating, which is a significant predisposing environmental factor to PV development.

In this study, majority of lesions were hypo pigmented (83.62%). It was similar with the study of Thayikkannu et al.^[23] Almost similar observation was noted in study of Krishnan et al,^[18] Rao et al.^[19] and Ghosh et al.^[10] 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.^[29] Most common type of lesions in our study was macular (85.34%) and scaly (91.38%), out of which 73.28% was white scale. A similar finding was noted in study of Ghosh et al,^[10] (89.09%), Rao et al. (86.60% Macules),^[19] and Sharma et al.^[20] The most affected site of lesion in our study was Neck (28.45%) followed closely by Back (20.69%) and Chest (18.97%). Similar study was also noted in the study of Rao et al.^[19] (The disease was seen commonly on the neck, back and chest), Shah et al.^[30] (The most common sites affected in patients were neck followed by back and chest). Dutta et al,^[17] Sharma et al,^[20] Krishnan et al,^[18] also showed that neck followed by 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, majority of cases were presented with bilaterally asymmetrically distributed (75%) lesions with well-defined margin (68.97%). A similar finding was noted in Sharma et al,^[20] (67.9% of which were having well-defined border) and Pallai et al.^[31]

In our study, 63.79% cases were found to show golden yellow fluorescence under wood's lamp with all 40 (47.06%) cases with *M. furfur* isolates ($p < 0.0001$), which was similar with the study of Rao et al.^[19] and Shah et al.^[30] The colour of the fluorescence may also aid in differential diagnosis, as it is unique to the mycelial form of *Malassezia*. Recent evidence,^[32,33] 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.^[34]

In the present study, itching was associated with 48.28% cases. This finding was almost similar with the study of Ghosh et al,^[10] (47.27%). Symptomatic PV was noted in 72% cases in study of Thayikkannu et al,^[23] whereas Rao et al,^[1] showed that only 30% cases presented with mild itching.

In this study, 75.86% cases showed short hyphae with budding yeast cells in 10% KOH preparation of specimens. Similar study was noted in study of Thayikkannu et al,^[23] Pallai et al,^[31] Ghosh et al,^[10] & Rao et al.^[19] When a good representative sample is used for KOH it gives significant correlation with growth (Thayikkannu et al).^[23] In our study cultural outcome was significantly correlated with KOH positivity. Pallai et al.^[31] showed 100% KOH positivity whereas; Ghosh et al,^[10] showed 83.64% KOH positivity. Growth on modified Dixon's agar with olive oil overlay was noticed in only 73.28%

cases which was a little bit higher with the study of Ibekwe et al.^[24] (57.6%), Agarwal et al,^[36] (62%), Remya et al,^[37] (62.78%), Ahmed et al,^[38] (54.34%), Pramanik et al,^[21] (89.79%) and Kindo et al.^[39] (68.57%). This difference in culture positivity may be due to adequacy of sampling and the different culture media.^[12]

M.furfur (47.06%) was the predominant isolate in our study followed by *M. globosa* (24.71%) and *M. sympodialis* (15.29%). Similar finding was also noted with the study of Sharma et al,^[20] (77.3%), Pramanik et al,^[21] (60.23%), Krisanty RI et al.^[40] (42.9%), De Quinzada MM et al.^[41] and Miranda KC et al.^[42]

Several studies reported *M. furfur* to be the commonest species associated with PV in the tropical and subtropical regions (Razanakolona et al.^[43] Eidi et al,^[44] Shoeib et al.^[45] and Ibekwe et al.^[24] Canteros et al.^[46] and Giusiano et al.^[47] also found that *M. furfur* was the predominant species. *M. furfur* was reported to be the commonest species in West Bengal.^[48] This can be explained by the fact that *M. furfur* produces an indole alkaloid pityriacitrin which can protect this fungus against ultraviolet exposure and renders *M. furfur* more resistant to sun exposure.^[49] Thus, this study has given as the clear insight into the clinical and mycological aspects of pityriasis versicolor and throws a light on the association with various risk factors for development of PV.

CONCLUSION

M.furfur was the most common isolate (47.06%) followed by *M. globosa* (24.71%) and *M. sympodialis* (15.29%).

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