

Original article:

The role of mycological investigations in the diagnosis of Pityriasis Versicolor and Seborrheic Dermatitis

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Date of submission:18 September 2014 ; Date of Publication: 15 December 2014

Abstract:

Introduction: Pityriasis Versicolor is a mild, chronic infection of the skin, caused by *Malassezia* yeast. It involves stratum corneum characterized by discrete or confluent, scaly hypo or hyperpigmented areas mainly on the upper trunk. Present study evaluates the role of mycological investigations in the diagnosis of Seborrheic dermatitis and Pityriasis versicolor infection.

Materials and methods: The consecutive patients were selected from the patients attending Nair OPD on the basis of various signs and symptoms they show. The symptoms were confirmed by two separate dermatologists. Patients were shown patient information sheet and a written informed consent was taken from each.

Observations and results: The KOH positivity was found to be 78.38% as against KOH negativity which was 21.62% of all 40 cases.

The patients of Pityriasis versicolor mostly present themselves between 1-6 months of duration of the illness. One striking finding seen is the number of patients presenting after the duration of 3 yrs was 7.

Conclusion: The diagnosis of Pityriasis versicolor and Seborrheic dermatitis can be done on the basis of clinical findings and KOH examination. Culture and biochemical reactions are only required for the confirmation of *Malassezia* species.

Introduction

Pityriasis Versicolor is a mild, chronic infection of the skin, caused by *Malassezia* yeast. It involves stratum corneum characterized by discrete or confluent, scaly hypo or hyperpigmented areas mainly on the upper trunk²². It is sometimes called 'tinea versicolor', although the term 'tinea' should strictly refer to infection with dermatophyte fungus.³ Internationally, the prevalence has been reported to be as high as 50% in the humid, hot environment of

Western Samoa and as low as 1.1% in the colder temperatures of Sweden¹⁶. Some regional studies are carried out in parts of India. One such study carried out in Imphal shown that the prevalence of Pityriasis versicolor in all the skin infections was 3% of 451560 cases assessed²⁸.

Dandruff appears to be a precursor of Seborrheic dermatitis, which gradually progresses to Seborrheic dermatitis⁵. The affected skin is pink, edematous and covered with yellow-brown scales and crusts. The

disease has wide range from mild to severe⁴.The synonym *eczema flannellaire* stems from an idea that retention of skin surface lipids by clothing promotes or aggravates Seborrheic dermatitis⁴. Dandruff, the mildest form of this dermatitis, is probably far more common and is present in an estimated 15-20% of the population¹⁵.The prevalence of Seborrheic dermatitis is much higher in patients with HIV infection⁵. It is 40-80% in AIDS patients⁴. Seborrheic dermatitis occurs slightly more often in males and has two age peaks one in infancy within first three years of life and second around fourth decade to seventh decade of life⁴.Yeasts of the genus *Malassezia* are known to be the members of the skin microflora of the warm-blooded vertebrates. These lipophilic yeasts are yeasts are associated with various infections especially Pityriasis versicolor, a chronic superficial scaling dermatomycosis². Seborrheic dermatitis being a more inflammatory condition is rare in occurrence. Present study evaluates the role of mycological investigations in the diagnosis of Seborrheic dermatitis and Pityriasis versicolor infection. The commonest reason for the patients with Pityriasis versicolor to not seek medical attention was its asymptomatic nature. They come to a doctor mainly for cosmetic reasons. This has caused the disease to increase in the community even after availability of easy treatment. Though disease not a public threat, people should be made aware of the disease due to its resemblance, sometimes, with lepromatous patches in Hansen's disease⁵.

Materials and methods

The consecutive patients were selected from the patients attending Nair OPD on the basis of various signs and symptoms they show. The symptoms were confirmed by two separate dermatologists. Patients were shown patient information sheet and a written

informed consent was taken from each.The disease was diagnosed either Pityriasis versicolor or Seborrheic dermatitis based on following criteria.

Inclusion criteria:

- a. For Pityriasis versicolor –
 - 1) Patches on skin: Hyperpigmented or Hypopigmented
- b. For Seborrheic dermatitis –
 - 1) Papules
 - 2) Itching
 - 3) Erythema
 - 4) Scaling

Exclusion criteria:

- a. Patients with a history of antifungal taken within previous 4 weeks
- b. Patients with a history of topical steroids taken within previous 7 days
- c. Patients with a history of systemic steroids taken within previous 15 days

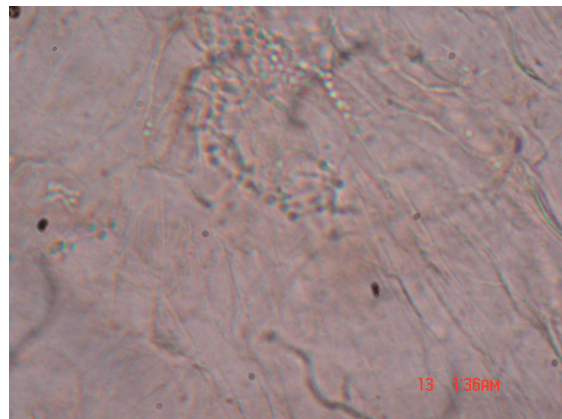
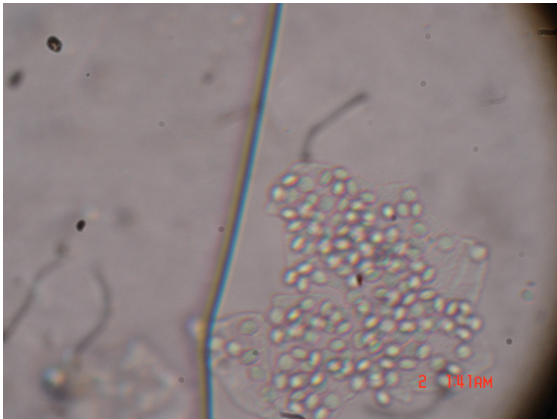
The study included total 50 patients (40 Pityriasis versicolor and 10 Seborrheic Dermatitis). After clinical confirmation, Wood's lamp examination was carried out. This was particularly useful to demarcate the lesions in which the pigmentation was not much different from the normal skin. Skin scrapings were collected from the patients after cleaning of that part with spirit and proper drying. The lesions were scraped with sterile blade from the centre to the periphery so that normal flora of the skin is avoided. Scrapings were collected from the lesions all over the body. Samples taken on glass slide and KOH examination was done with 20% KOH, both under low and high magnification.

The samples were taken to microbiology laboratory in a sterile test tube or autoclaved filter papers. They were stored for a day in total asepsis. Culture was done on the following day on Sabouraud's Dextrose

agar slant with two to three drops of olive oil. The samples were inoculated at room temperature and 37°C; followed up for a month for checking any growth. The slants on which there was no growth in this 1-month period were reported negative and then were discarded. Cultures showing growth were refrigerated. Care was taken not to let the culture

Microscopy

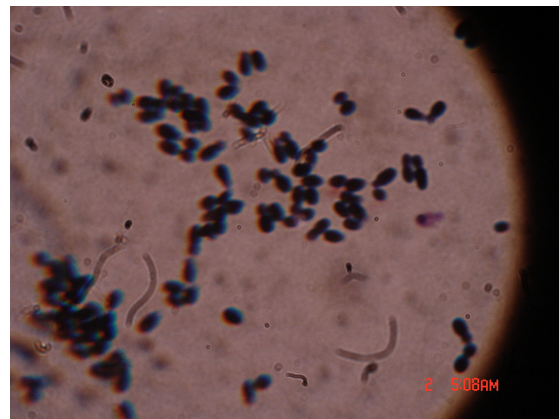
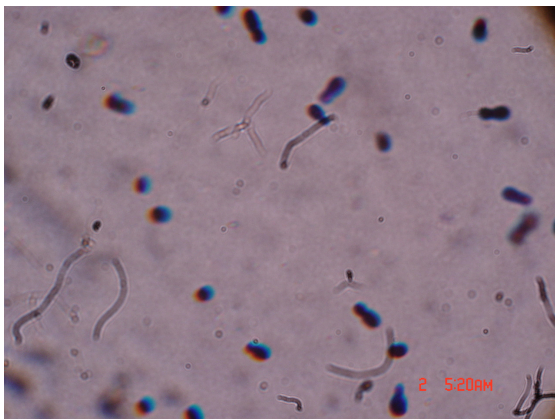
Potassium Hydroxide Mount (KOH)

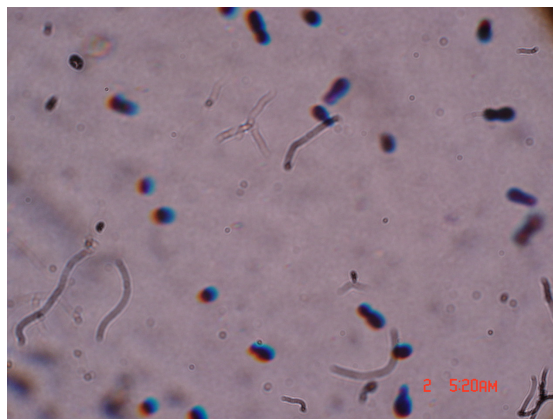
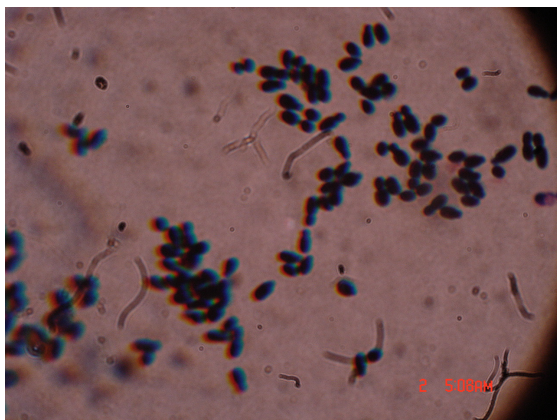


become dry. To prevent this repeated sub cultures were done from time to time.

Subcultures were done from the original cultures on mDixon agar (Mycological peptone – 3g, Malt extract – 30g, Ox-bile – 20g, Tween 40 – 10ml, Glycerol – 2ml, Agar –15g, Distilled Water – 1L) ¹⁷. The cultures were followed up for 7 days.

Gram's Staining





Observations and Results

Table No. 1: Evaluating the relation between type of pigmentation and culture positivity of Malassezia

| Isolation | | Hypo pigmentation | Hyper pigmentation | Total |
|------------------|-----|-------------------|--------------------|-------|
| Culture Positive | No. | 22 | 4 | 26 |
| | % | 84.62 | 15.38 | 100 |
| Culture Negative | No. | 9 | 5 | 14 |
| | % | 64.29 | 35.71 | 100 |
| Total | No. | 31 | 9 | 40 |
| | % | 77.50 | 22.50 | 100 |

By Fischer's Exact test, p (one tail) = 0.1423 (NS).

As the table shows, cases with hypo pigmented lesions (77.50%) were more compared to hyperpigmented lesions (22.50%).

Table No. 2: Evaluating the relation between type of pigmentation and KOH positivity of Malassezia

| KOH | | Hypo pigmentation | Hyper pigmentation | Total |
|--------------|-----|-------------------|--------------------|-------|
| KOH Positive | No. | 29 | 8 | 37 |
| | % | 78.38 | 21.62 | 100 |
| KOH Negative | No. | 2 | 1 | 3 |
| | % | 66.67 | 33.33 | 100 |
| Total | No. | 31 | 9 | 40 |
| | % | 77.50 | 22.50 | 100 |

By Fischer's Exact test, p (one tail) = 0.5450 (NS)

The KOH positivity was found to be 78.38% as against KOH negativity which was 21.62% of all 40 cases.

Table No. 5: Cutaneous areas affected in Pityriasis versicolor patients

| | Head & Neck | Chest | Back | Extremities | Other |
|-----------------------|-------------|-------|-------|-------------|-------|
| Pityriasis Versicolor | 80% | 65% | 72.5% | 52.5% | 20% |

From the table it can be stated that the sites affected mainly are Head and neck region (including shoulders-80%) and Back (72.5%) closely followed by Chest (65%) and Extremities (52.5%). Among others, the sites affected are Axilla (5%), Abdomen (7.5%), Face (2.5%), Knee (2.5%) and Buttocks (2.5%).

Table No. 3: The duration of illness in the patients of Pityriasis versicolor

| | | <1m | 1-6m | 6m-1yr | 1-3yr | >3yrs | Total |
|-----------------------|-----|------|------|--------|-------|-------|-------|
| Pityriasis Versicolor | No. | 5 | 17 | 8 | 3 | 7 | 40 |
| | % | 12.5 | 42.5 | 20 | 7.5 | 17.5 | 100 |

The patients of Pityriasis versicolor mostly present themselves between 1-6 months of duration of the illness. One striking finding seen is the number of patients presenting after the duration of 3 yrs was 7.

Table No. 4: Correlation of Clinical finding (itching) with the KOH positivity of Malassezia

| | | Itching present | Itching absent | Total |
|--------------|-----|-----------------|----------------|-------|
| KOH Positive | No. | 19 | 18 | 37 |
| | % | 51.35 | 48.65 | 100 |
| KOH Negative | No. | 2 | 1 | 3 |
| | % | 66.67 | 33.33 | 100 |
| Total | No. | 21 | 19 | 40 |
| | % | 52.50 | 47.50 | 100 |

By Fischer's Exact Test, p (one tail) = 0.5384 (NS)

The cases with positive history of itching (52.50%) were approximately equal to those without any such history (47.50%).

Table No. 5: Results of various Biochemical reactions used for identification and speciation of *Malassezia* Genus

| | Catalase | | | Esculin | | | Urease | | |
|-----------------------|----------|-----|-------|---------|-------|-------|--------|-------|-------|
| | +ve | -ve | Total | +ve | -ve | Total | +ve | -ve | Total |
| Pityriasis Versicolor | 13 | 13 | 26 | 6 | 20 | 26 | 22 | 4 | 26 |
| | 50 | 50 | 100 | 23.08 | 76.92 | 100 | 84.62 | 15.38 | 100 |

Various biochemical tests used show that Urease is positive in 84.62% of culture positive cases as opposed to 50% Catalase positive and 23.08% Esculin positive.

Discussion

This study was carried out in the patients attending Nair Hospital OPD in order to study the diseases- Pityriasis versicolor and Seborrheic dermatitis, with respect to their clinical, microscopic, cultural and biochemical characteristics. The patients of Seborrheic dermatitis were very less (20%) as compared to Pityriasis versicolor (80%). So the comparison of the two diseases is not statistically possible.

The mean age in the present study was found to be 27yrs for Pityriasis versicolor. Age distribution shows peak occurrence of the disease is in the 16 to 30 yrs group (72.5%). Only 2 cases (5%) of age below 15 yrs were found [Table No.1]. In a study done by Kanta et al.²¹ showed 75.0 per cent cases were in the age group of 11.30 years. Rathi SK et al.²³ found in his study that the mean age was 18 -32 yrs which is almost similar to the age range in our study. Most probable reason for this can be given by the fact that disease occurs more commonly after puberty as lipid secretion in the sebum favors the growth of the fungus¹⁹. In the Seborrheic dermatitis the mean age was found to be 36yrs in 10 patients. And peak incidence was found to be in 31 to 45 yrs age group.

The sex ratio in Pityriasis versicolor patients was found to be 37:3 i.e. 93% of the patients were males [Table No.2]. In the study of Kanta et al.²¹ the ratio was 2:1 for males:females. Patients presenting with hypopigmented lesions were more (77.50%) as compared to those presenting with hyperpigmented lesions (22.50%). The association of KOH positivity and culture positivity with pigmentation was not significant as evaluated with the Fischer's Exact Test. [Table No.3 and 4] In the study of Rathi SK et al.²³, all the patients had hypopigmented lesions and scaling.

Predominant area involved was Neck and Shoulder region (80% cases). Other areas involved were Back (72.5%), Chest (65%), Extremities (52.5%) and other sites in 20% of the cases. [Table No.5]. In the study done by Tarazooie et al.² the commonest areas of affection were trunk and neck. Kanta et al.²¹ found that the predominant sites involved were chest, back, neck, shoulders and abdomen. The lesions were distributed mainly on upper back, shoulders, neck and chest as studied by Rathi SK et al.²³.

Duration of the illness in 42.5% of cases was in between 1-6 months [Table No.6]. This roughly coincides with the duration 2 month to 1 yr (Average 5 months) as was found in the study done by Rathi SK et al.²³.

The present study also evaluates the correlation of itching and KOH and culture positivity [Table No. 7 and 8]. Itching was found to be associated with 52.5% of the cases of Pityriasis versicolor. The correlation was not found to be statistically significant. Out of thirty patients in the study done by Rathi et al.²³, 17 patients (56.67%) complained of mild itching which worsened after sweating. Family history was found to be associated in only 20% of the cases of Pityriasis versicolor [Table No. 9]. Wood's lamp examination for showing yellow-orange fluorescence was found to be positive in 70% of the cases [Table No. 10].

KOH positivity was found to be in 37 out of 40 cases i.e. 92.5%. The culture positivity was found to be 65% on Sabouraud's Dextrose agar with Olive oil overlayed upon it. In the study done by Rajshekhar et al.²⁶, the direct KOH examination of the skin scrapings revealed fungal elements in all the 100 cases studied, the culture gave positive growths only in 60 cases i.e., 60%. The oil was used to enhance the growth of the organism as was shown by Kanta

et.al.²¹, who found that the rate of isolation was increased to 17% from 6% after addition of Olive oil.

Further culture on Sabouraud's Dextrose agar without any oil was also done to check for the isolation of *M.pachydermatis*. Subcultures done on mDixon agar showed positive results in 16 of the 26 Sabouraud's agar. Out of these, one of the isolates was confirmed to be *Candida parapsilosis*. Out of remaining 15 isolates, commonest was *M.restricta* (46.67%). The other species isolated were *M.slooffiae* (26.67%), *M.globosa* (20%) and single isolate of *M.nana*(6.67%).

Among various biochemical tests done Urease was found to be positive in 84% of the cases. This confirms that the isolated species was not *Candida*. Also, Catalase was positive in 50% of the cases, may be caused due to high isolation of *M.restricta*. Esculin test was positive in only 23% of the cases [Table No. 13]. Tween assimilation was also done with Tween 20,40,60 and 80 concentrations. The test requires expertise to do. It was found conclusive in 15 isolations and helped to a larger extent in confirming the species of the organism.

Conclusions

- 1) The diagnosis of Pityriasis versicolor and Seborrheic dermatitis can be done on the basis of clinical findings and KOH examination. Culture and biochemical reactions are only required for the confirmation of *Malassezia* species.
- 2) Age distribution of Pityriasis versicolor was in concurrent with previous studies confirming increase in incidence of the disease

only after puberty. The incidence is least among 0-15yrs.

- 3) Various precautions are required to be taken while doing KOH mounts and culture. Total asepsis should be maintained at each level right from scrapping of the lesion to handling of the agar.
- 4) For the culture of the organism belonging to genus *Malassezia*, Sabouraud's Dextrose agar with overlay of olive oil can be used as the universal culture medium showing growth of all the species in the genus. Special culture media such as Sabouraud's Dextrose agar without oil and mDixon agar are required for the speciation of the organism.
- 5) Among biochemical tests, the generic test was found to be Urease which can differentiate *Malassezia* species from *Candida* species. Among others, Catalase is given positive by most of the *Malassezia* except *M.restricta*. Esculin is useful for speciation. The most confirmatory test is Tween assimilation test which is very much difficult to carry out and requires expertise.
- 6) The speciation thus found can be used for epidemiological purposes as to find which species is more prevalent in which area. Also, antifungal sensitivity tests can be carried out for specific species.

This can help in giving species
specific treatment of the disease

(Pityriasis versicolor and
Seborrheic dermatitis)

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