

## Comparative study for the reliability of cellophane tape and standard KOH mount in diagnosis of pityriasis versicolor

Hussein M. M. Hassab-El-Naby, MD, Ahmad Sadek Mohamed Salem, MD  
Hamed Mohammed Abdo, MD, Hassan Mohammed Hassan, M.B.B.Ch

*Department of Dermatology, Venereology & Andrology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt*

### ABSTRACT

Pityriasis versicolor (PV), a mycotic infection, is characterized by discrete or conrescent, scaly, discolored or hypopigmented areas. The clinical presentation, though very typical, may be confused with other skin disorders. This study was aimed to evaluate the reliability of cellophane tape as a method for diagnosis of PV versus the standard KOH method.

Fifty patients suffering from PV were included. Every patient was subjected to scraping of lesions with a blunt scalpel, and then a drop of 10% KOH solution was added. Another sample was obtained using adhesive cellophane tape applied over the affected site, then stucked on the surface of a glass slide after placing a drop of 10% KOH. Both samples were then examined for fungus under microscope.

The cellophane tape method was positive in 49 cases out of 50 (98%) while the KOH mount was positive in 48 cases (96%). Fungal elements were detected by both methods and were better visualized by the tape method. All negative cases were of hypopigmented type. Similar reliability was observed with the two procedures as there was no significant difference between results of both cellophane tape and standard KOH methods.

KEYWORDS: Pityriasis versicolor, KOH mount, cellophane tape.

### INTRODUCTION

Lipophilic yeasts of the genus *Malassezia* (formerly called *Pityrosporum*) are part of the normal cutaneous microflora of humans and other warm-blooded animals.<sup>1</sup> However, under the influence of predisposing factors, these yeasts can become pathogenic and associated with several skin diseases and even systemic infections.<sup>2</sup>

Pityriasis versicolor is a superficial fungal infection where *Malassezia* species play a definite causative role. The diagnosis is generally simple and lies on the clinical appearance and can be confirmed by microscopic examinations of the lesions.<sup>3-5</sup>

In experienced hands, KOH mount is one of the most useful procedures in medical mycology. It

has been adjudged more reliable than culture for demonstration of dermatophytes.<sup>6</sup>

Stripping keratin layer's fragments from cutaneous or mucocutaneous surfaces by vinyl adhesive tape has largely been used over time for diagnostic purposes, especially in dermatology.<sup>7</sup>

This study was aimed to evaluate the reliability of cellophane tape as a method for diagnosis of PV versus the standard KOH method.

### PATIENTS AND METHODS

Fifty patients with typical clinical lesions of PV were included in this study. They were 31 males and 19 females, aged between 12 and 50 years. Patients that have received systemic antifungal treatment for the last four weeks and/or topical

*Correspondence:* Dr. Hussein M. M. Hassab-El-Naby MD, Professor of Dermatology and Dermatopathology, Al-Azhar university, Cairo, Egypt  
Tel: 002 012 3104605, Office: 002 02 224199089, E-mail: [helnaby@yahoo.com](mailto:helnaby@yahoo.com)

antifungal medications for the last two weeks were excluded.

Every patient was subjected to: 1) full history taking included occupation, special habits, residence and any previous treatment, 2) skin examination for PV for: its site, size, shape, color and clinical type, 3) scraping of lesions with a blunt scalpel, then 1-2 drops of 10% KOH solution were added to the collected material, covered by a cover slip and examined for fungus under microscope using low power field  $\times 10$  then high power field  $\times 40$  and 4) another sample was collected using adhesive cellophane tape as follows; a 5cm long and 2 cm wide scotch tape (transparent cellophane tape) was applied over the affected site, pressed firmly (to ensure adequate recovery of scales) and removed. The tape was then stucked on the surface of a glass slide after placing 1-2 drops of 10% KOH solution on its centre then examined under microscope using low power field  $\times 10$  then high power field  $\times 40$ .

Cases showing hyphae and nests of spores “spaghetti and meatballs” were considered positive. Query cases were repeated for confirmation.

## RESULT

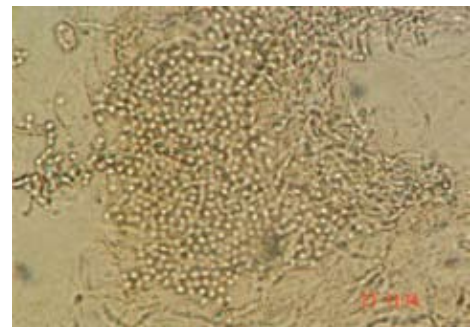
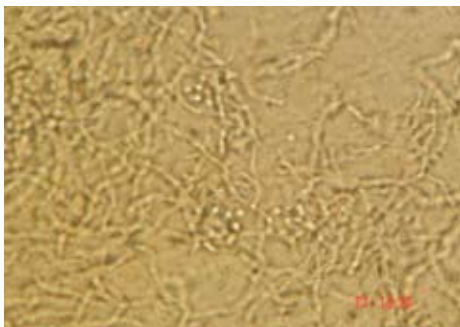
The collected data was organized, tabulated and statistically analyzed using SPSS software statis-

tical computer package. For qualitative data, number and percent of the cases were calculated. For quantitative data, the mean and standard deviation were calculated

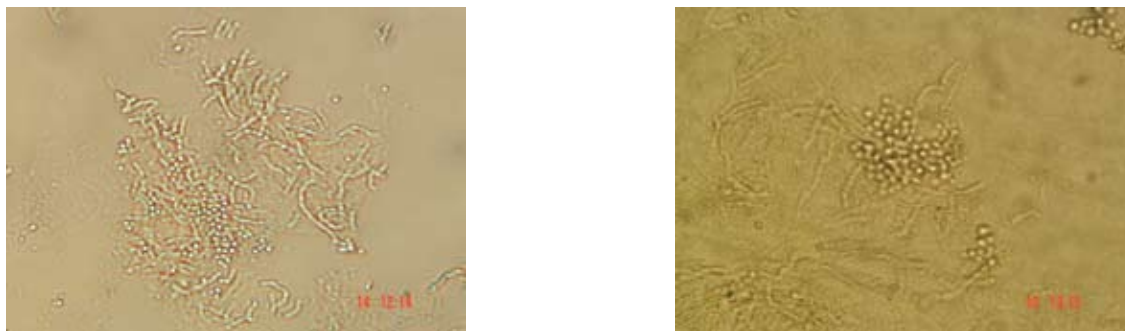
From 50 patients with PV, 31 were men (62%) and 19 were women (38%) with male to female ratio of 1: 0.61. Their ages ranged from 12 to 50 years (mean  $\pm$  SD;  $22.88 \pm 7.95$ ). Hyperpigmented PV was found in 44 cases out of 50 (88%), while hypopigmented PV was found in 6 cases (12%) with hyperpigmented to hypopigmented ratio of 1: 0.14.

This study showed that both cellophane and standard KOH methods have nearly equal results. The cellophane tape method was positive in 49 cases out of 50 (98%) while the standard KOH mount was positive in 48 cases (96%). Fungal elements were detected by both methods, revealed scattered hyphae and nests of spores “spaghetti and meatballs” and were better visualized by the tape method (Fig. 1 A, B & 2 A, B). All negative cases were of hypopigmented type.

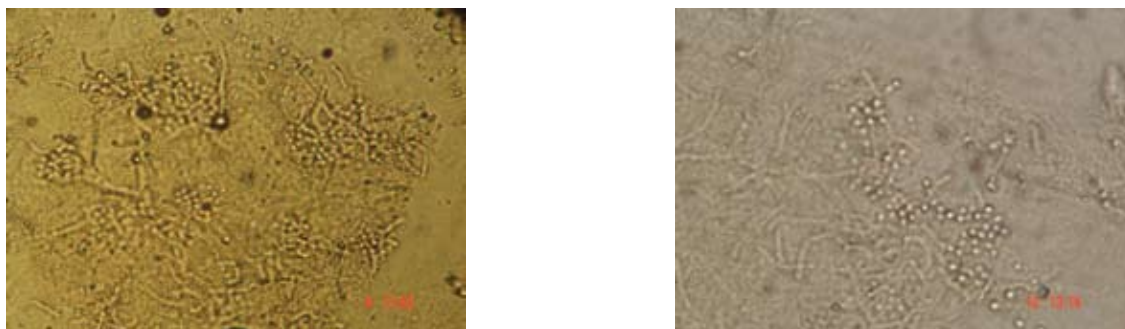
The density of hyphae and spores using the cellophane tape method was marked in 25 cases (50%), moderate in 15 (30%) and mild in 9 (18%). While in the KOH mount, marked density was observed in 17 cases (34%), moderate in 27 (54%) and mild in 4 (8%) (Table 1).



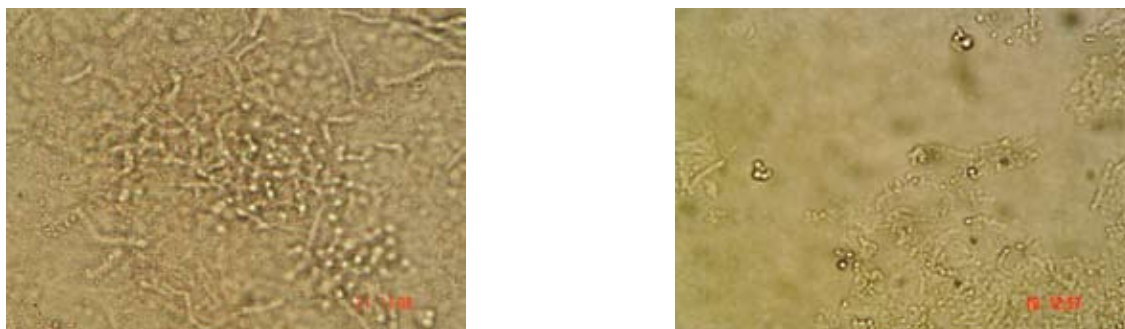
**Fig. 1 A.** Hyphae and spores observed on cellophane tape method in hyperpigmented PV.



**Fig. 1 B.** Hyphae and spores observed on cellophane tape method in hypopigmented PV.



**Fig. 2 A.** Hyphae and spores observed on KOH preparation in hyperpigmented PV.



**Fig. 2 B.** Hyphae and spores observed on KOH preparation in hypopigmented PV.

The density of hyphae and spores in hyperpigmented PV (n. 44), was markedly observed by standard KOH in 16, moderate in 25 and mild in 3 cases versus 24, 14 and 6 cases observed by cellophane tape respectively. In hypopigmented type (n. 6), marked density was observed by standard KOH in 1, moderate in 2 and mild in 1 cases versus 1, 1 and 3 cases observed by cellophane tape respectively (Table 2).

Although there was no actual difference in the

morphology of the fungus seen in the two methods, the fungal elements were generally better demonstrated and minimally distorted by the tape technique.

#### DISCUSSION

It is relatively easy to diagnose PV. However, the varied presentation of the lesions, the differential diagnosis including vitiligo, melasma, tinea corporis, seborrhoeic dermatitis, pityriasis rosea,

**Table 1** Degree of density of hyphae and spores in the two methods

	Standard KOH		Cellophane tape	
	N.	Percent	N.	Percent
No (-ve)	2	4.0%	1	2.0%
Mild	4	8.0%	9	18.0%
Moderate	27	54.0%	15	30.0%
Marked	17	34.0%	25	50.0%

**Table 2** Distribution of density of hyphae and spores between hyperpigmented and hypopigmented types (n. 50)

Density of hyphae and spores	Clinical type			
	Hyperpigmented (n. 44)		Hypopigmented (n. 6)	
	Standard KOH	Cellophane tape	Standard KOH	Cellophane tape
Absent (-ve )	----	----	2	1
Mild	3	6	1	3
Moderate	25	14	2	1
Marked	16	24	1	1

pityriasis alba, erythrasma, confluent and reticulated papillomatosis of Gougerot and Carteaud, pityriasis rotunda, secondary syphilis and pinta may confuse the inexperienced clinician.<sup>8</sup>

Wood's light examination may help in the diagnosis of PV however; Wood's light provides a positive response in only one-third of cases.<sup>9</sup>

Skin biopsy is generally not necessary in the diagnosis of PV. As the causative agent requires a lipid-rich medium for growth, culture in standard media will not allow the yeast to grow. In addition, as *Malassezia* yeasts are found on 90–100% of the normal population, growth of the organism in culture does not necessarily indicate PV.<sup>10,11</sup>

Considering all of the above, mycological micro-

scopic examination can be used to confirm the diagnosis of PV. The present study was planned to evaluate the reliability of cellophane tape as a method for diagnosis of PV versus the standard KOH method in a random sample of PV Egyptian patients.

Both cellophane and standard KOH methods have nearly equal results. The cellophane tape method was positive in 49 cases out of 50 (98%) while the standard KOH mount was positive in 48 cases (96%). Several studies reported almost the same results.<sup>5,12,13</sup>

Fungal elements were more prominent, minimally distorted and better visualized by the tape method. Positive cases revealed scattered hyphae and nests

of spores “spaghetti and meatballs” observed with varying degree of density. The hyphae appeared more organized and numerous than those seen in KOH preparations. These observations are comparable with those of El-Hefnawi et al.<sup>14</sup> All negative cases were of hypopigmented type.

Porto in 1953,<sup>15</sup> who was the first to describe the cellophane method in patients with PV, revealed hyphae and spores by both standard KOH and cellophane tape methods in all the nine cases (100%). This was also similar to the results of Thirumurthy et al,<sup>16</sup> who reported 100% positivity in their cases by both methods. Also a diagnosis of PV was confirmed by Miranda and Silva, 2003<sup>17</sup> in ten PV patients out of sixty patients with superficial mycosis. All cases yielded positive results on vinyl tape preparations (100%).

The presence of negative results in the current study (two negative cases in standard KOH and one case in cellophane method) and the absence of negative results in the previous researches may be explained by the relatively large sample of patients and the presence of random selection (hyperpigmented and hypopigmented types), which might not have been present in other studies.

This difference could also be attributed to using a staining method by previous researchers, such as methylene blue, to enhance both detection level and imaging quality of fungi which has not been used by us or could be explained by prior antifungal medications unnoticed or denied by our patients. Tarazooie et al, 2004<sup>13</sup> claimed that two patients with negative results in their study had also been received topical antimycotic treatment. On the other hand, negative cases in this work could be related to the category of the so called PV alba or achromia parasitica (depigmentation after healing of lesions). In these cases, hypopigmentation

develops following the hyperpigmented stage of PV, either spontaneously or under the influence of UV light;<sup>18,19</sup> so, the examination of these negative cases may be done during the healing process.

While demonstration of yeast cells and hyphae in hyperpigmented areas is thought to be certain,<sup>20</sup> controversial data exist concerning demonstration of the infectious agent in depigmented areas: both positive and negative results have been reported.<sup>19</sup> In a comparative study, Galadari and El Komy, 1992<sup>21</sup> found lower number of pathogens in depigmented areas than in hyperpigmented lesions. In the older literature, there are observations reporting high numbers of yeast cells and hyphae in early lesions of PV, followed by reduction (only demonstration of hyphae) and finally even pathogen-free depigmented areas.<sup>22</sup> Such a time course could explain the recent controversial findings and shows that depigmentation may outlast the presence of *Malassezia* yeasts. This would also explain the non-response of PV alba to antimycotics.<sup>19</sup>

In all of positive cases of PV, classical feature, the so-called “spaghetti and meatballs”, were seen. Our results are consistent with those previously published (4, 13) and confirm the significance of the yeast-mycelium conversion in pathogenesis of this infection.<sup>12,23</sup>

Regarding the accuracy of direct exam, diagnosis of PV is based on observation of both short hyphae and yeast cells in the scales. In cases when only hyphae or spores were present in the scales, direct examination of samples may fail to reveal the infection and/or differentiate it from dermatophytosis. Therefore, we suggest staining the scales prior to performing light microscopic examination to avoid false negative results.

## CONCLUSION

Similar reliability was observed with the two procedures as there was no significant difference between them. However, the cellophane tape technique offers some advantages: 1) harmless lesion sampling especially in anxious children; 2) more suitable in large studied groups; 3) avoidance of possible skin injury and dissemination of infection to nearby areas during the test; 4) can be kept for longer period for future observation in contrast to KOH preparation which dries up within short time ; and 5) Moreover; the fungal hyphae were generally better demonstrated and minimally distorted by the tape technique than KOH mount.

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