

Original Research Article

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Comparison of KOH Mount Using Standard Technique and Cellophane Tape Method for Diagnosis of Superficial Fungal Infections

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ABSTRACT

Superficial fungal infections include Tinea versicolor and Dermatophytosis. Tinea versicolor infects stratum corneum which is caused by lipophilic yeast-like fungi i.e. *Malassezia* species. Dermatophytosis infects keratinised structures (skin, hair and nails) caused by a group of keratinophilic fungi called dermatophytes. KOH mount is the preliminary, convenient and inexpensive test for diagnosis of superficial fungal infections. This study compares the detection rate and visualisation of fungal elements by KOH mount using standard technique and cellophane tape method. This study was carried out in the Department of Microbiology, Dr B R Ambedkar Medical College, K G Halli, Bangalore for a period of 6 months from August 2016 to January 2017. A total of 250 skin scrapings were collected from clinically diagnosed patients with superficial fungal infections (Tinea versicolor- 76, Dermatophytosis- 174). Preliminary KOH mount examination was done by standard technique. The same patients were also studied by a modified technique by using cellophane tape method for KOH mount. Out of 250 clinical samples, 71 were positive for *Malassezia* species, 170 were positive for Dermatophytes and 9 were negative for fungal elements. Standard technique and cellophane tape method of KOH mount both were positive for fungal elements in 91.6% of samples. Standard technique of KOH mount was negative but cellophane tape method was positive in 4.8% of samples. Both the methods were negative in 3.6% of the samples. The present study concludes that there is a higher rate of detection and the morphology of fungal elements were better visualised by cellophane tape method of KOH. Also, the standard technique of KOH mount is time consuming compared to cellophane tape method. Cellophane tape method of KOH mount is easier and convenient for collection and transport of specimen as well. Thus, cellophane tape method of KOH mount serves as a better preliminary diagnostic tool than the standard conventional technique.

Keywords

Standard technique,
Cellophane tape
method, KOH
mount, *Malassezia*
species,
Dermatophytes.

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Introduction

The most common cutaneous fungal infections in the world are *Tinea versicolor* and Dermatophytosis. It is more common in tropical countries (63%) like India due to factors like heat and humidity, over population and poor hygienic conditions.

The high humidity and temperature provides a fertile ground for the abundant growth of these fungi^{2,9}. Patients with a propensity for developing Tinea infections may have a genetic component that predisposes them to disease, either immunosuppression or

hyperhidrosis^{1, 4}. *Tinea versicolor* is caused by *Malassezia* species which is a lipophilic yeast, which are part of the normal skin flora. They sometimes assume a pathogenic state within the epidermis.

When this occurs, patients will present with hypopigmented and/or hyperpigmented scaling lesions on the upper trunk independent of the immune status of the patients^{1, 4}. The other contributing factor is colonization of *Malassezia* species in anatomically vulnerable areas of the skin. Exogenous factors such as sunlight, corticosteroids, and oil-based products encourage the growth of *Malassezia* yeast forms.

Dermatophytes form a closely related group of keratinophilic fungi, causing infection of the superficial keratinized horny layer of skin, the hair and the nails and do not cause systemic infection³. Dermatophytosis are widespread both in developed and developing countries particularly due to the advent of immunosuppressive drugs, frequent usage of antibiotics and various conditions like organ transplantations, lymphomas, leukaemia and Human immunodeficiency virus (HIV) infection⁵.

The clinical presentation, though very typical of fungal infection, is very often confused with other skin disorders particularly due to rampant application of broad-spectrum steroid containing skin ointments and creams leading to further misdiagnosis and mismanagement. So, there is a need for correct, rapid and efficient laboratory methods for diagnosis of these fungal infections⁹.

KOH mount is the preliminary, convenient and inexpensive screening test done for the diagnosis of superficial fungal infections. It can be done as an outpatient procedure and the results are available within minutes. Only drawback is that it requires an expert to

identify and appreciate fungal elements and report the same. It is the most useful and reliable test with the result of which the Dermatologist can start an empirical treatment based on the morphological differences of fungal elements seen in *Tinea versicolor* and Dermatophytosis. Further confirmation can be done by culture and biochemical reactions which help in speciation of the isolates.

The main of this study includes, finding the age and sexing distribution of superficial fungal infections in the study population. And to compare the methods of KOH mount using standard technique and cellophane tape method.

The objectives of this study includes, to compare the detection rate and visualisation of fungal elements from samples of superficial fungal infection patients by KOH mount using standard technique and cellophane tape method and to compare the time taken for reporting of the same by these methods.

Inclusion criteria

Clinically diagnosed cases of *Tinea versicolor* and Dermatophytosis.

Cases of all age groups and both sexes.

Exclusion criteria

Clinical diagnosis of *Tinea nigra*.

Materials and Methods

A study was undertaken with 250 clinical samples collected from clinically diagnosed patients of superficial fungal infections (*Tinea versicolor*-76, Dermatophytosis-174) attending the Dermatology and Venereology outpatient department of Dr B R Ambedkar Medical College and Hospital, Bangalore during a period of 6 months from August

2016 to January 2017. The diagnosis of superficial fungal infection was based on the clinical history and physical examination. This study was carried out in the Department of Microbiology, Dr B R Ambedkar Medical College and Hospital, Bangalore.

Sample collection

The affected area was cleaned aseptically with 70% ethyl alcohol and allowed to dry. Skin sample was collected directly on to a sterile black paper envelope using a pre-flamed blunt scalpel from the edge of the lesion for standard technique⁶. The same patients were also studied by a modified method by applying a transparent cellophane tape over similar lesion, pressed firmly and removed. The tape was stuck on a sterile glass slide over which 3-4 drops of 10% KOH solution was placed.

Microscopic examination

Preliminary examination was done by direct microscopy by KOH mount. It was done by adding 2-3 drops of 10% KOH solution to the collected sample on a sterile glass slide and a cover slip was placed. The slide was observed for fungal elements after allowing 15-20 minutes for digestion and clearing of keratin making the fungal elements evident.

The same patients were also studied by a modified method using cellophane tape for collection of sample. This slide was observed immediately on receiving in the laboratory considering the fact the time required for digestion of keratin is covered in the time taken for transport of the slide.

For samples of which KOH mount were positive by cellophane tape method but negative by standard technique were confirmed further by culturing the same sample on to antibiotic incorporated Sabouraud dextrose agar (SDA) media with

chloramphenicol 50 mg/L and cycloheximide 500 mg/L for dermatophytes and with a sterile olive oil overlay on to SDA with antibiotics for *Malassezia* species.

Results and Discussion

KOH was positive with short hyphae and round yeast cells ("spaghetti and meatball" appearance) in case of *Tinea versicolor* and with long septate hyphae in case of Dermatophytes. Out of 250 clinical samples, 71 were positive for *Malassezia* species and 170 were positive for Dermatophytes and 9 were negative for fungal elements.

The majority of patients were of the age group 20-40 years constituting 60.4% of which males were 36.4% and females were 24%.

Followed by the age group <20 years constituting 30% of patients and 9.6% of patients were of age group >40 years. On the whole, males (62.4%) were more affected than females (37.6%) by superficial fungal infections in this study (Table 2).

Standard technique and cellophane tape method of KOH mount for detecting fungal elements both were positive for 91.6% of samples. Standard technique of KOH mount was negative but cellophane tape method positive for 4.8% of samples. Both the methods were negative for 3.6% of the samples (Table 1).

Also, the morphology of fungal elements was better visualised by cellophane tape method compared to standard technique. All of the 12 samples which were KOH mount negative by standard technique but positive by cellophane tape method showed positive culture growth on SDA with antibiotics thus confirming the validity of the cellophane tape method. In the present study with 250 clinical samples, 229 (91.6%) were positive for fungal elements by

both the methods under study whereas 12 (4.8%) of the samples were positive for fungal elements only by cellophane tape method but negative by standard technique and 9 (3.6%) of samples were negative for fungal elements by both methods (Figs 1–5).

Similarly, a study conducted by Thirumurthy M *et al.*,¹⁰ showed that out of 15 clinical samples tested by both methods all were positive for fungal elements by both methods but fungal morphology was better appreciated by cellophane tape method.

Table.1 Comparison of standard technique of KOH mount with cellophane tape method

Standard technique of KOH Mount	Cellophane tape method of KOH Mount	Results Total no. (%)
Positive	Positive	229 (91.6%)
Negative	Positive	12 (4.8%)
Negative	Negative	9 (3.6%)

Table.2 Age and gender distribution of superficial fungal infections

Age (years) / Infection	<i>Tinea versicolor</i>		Dermatophytosis		Total
	Male	Female	Male	Female	
<20	13	8	32	22	75 (30%)
20-40	21	23	70	37	151 (60.4%)
>40	8	3	12	1	24 (9.6%)
Total	42	34	114	60	250 (100%)
	76 (30.4%)		174 (69.6%)		

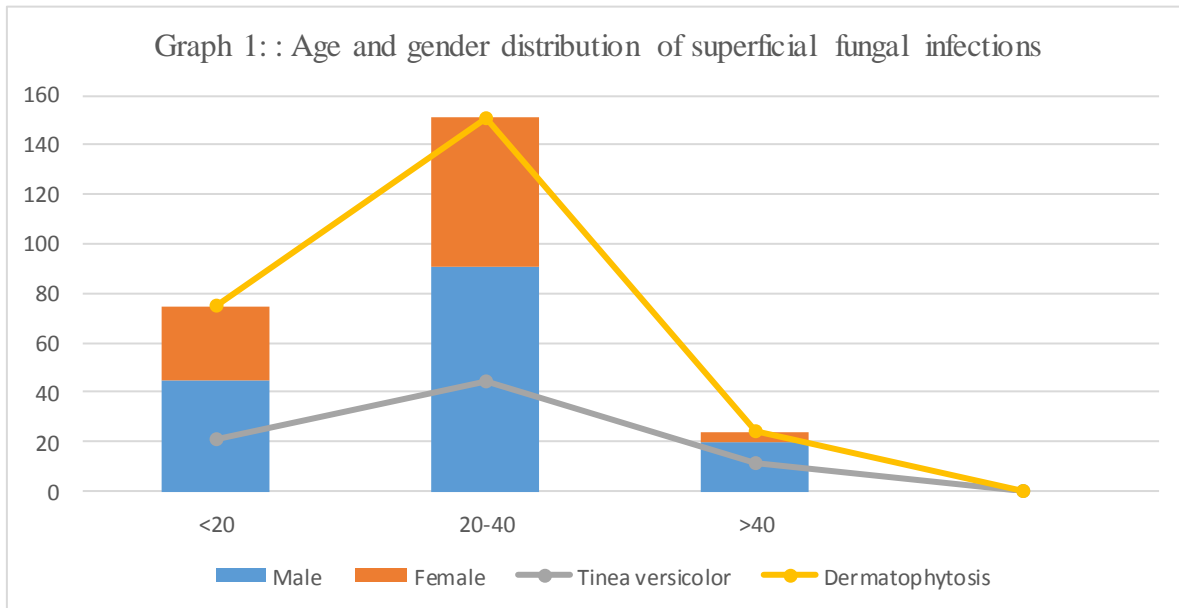


Fig.1 Slides of KOH mount by standard technique and cellophane tape method



Fig.2 KOH mount of *Tinea versicolor* by standard technique

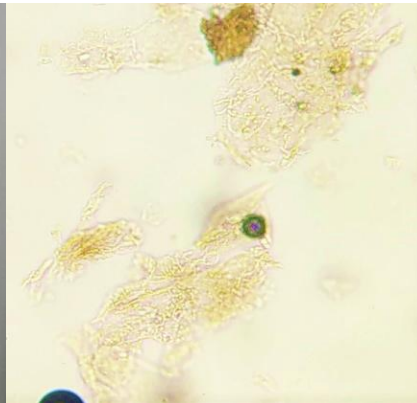


Fig.3 KOH mount of *Tinea versicolor* by cellophane tape method

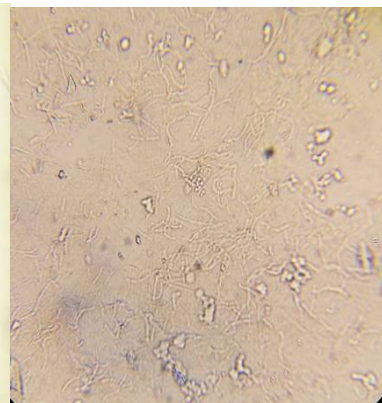


Fig.4 KOH mount of Dermatophytosis by standard technique

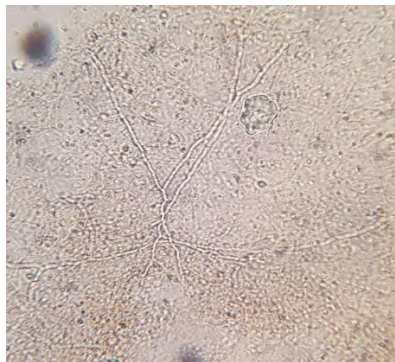
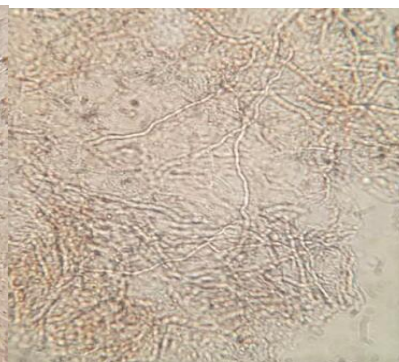


Fig.5 KOH mount of Dermatophytosis by cellophane tape method



As per the study by Kurade *et al.*,⁷ transportation of sample by cellophane tape method is easier compared to the standard technique. Milne *et al.*,⁸ studied the use of cellophane tape method for detection of deep seated cutaneous mycoses after the usefulness of this method for *Malassezia* species, *Candida* species and dermatophytes was proved.

The present study concludes that there is a higher rate of detection by cellophane tape method compared to standard technique of KOH mount. Also, the morphology of fungal elements was better visualised by cellophane tape method of KOH mount reason being the

hyphae remain undisturbed in the sample. The standard technique of KOH mount is time consuming compared to cellophane tape method as it takes 15-20 minutes for KOH solution to digest the keratin material and reveal the fungal elements. Whereas by the cellophane tape method fungal elements are detected within few minutes of slide reaching the laboratory as KOH is added at the time of collection of specimen itself.

Cellophane tape method of KOH mount is easier and convenient for collection and transport of specimen as well. Cellophane tape method also has an advantage of collecting good amount of sample especially

when only scanty amount of sample is available for collection by scraping the edge of the lesion for standard technique of KOH mount. Thus, cellophane tape method of KOH mount serves as a better preliminary diagnostic tool than the standard conventional technique.

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