International Journal of Research in Dermatology
Thimmappa V et al. Int J Res Dermatol. 2019 Feb;5(1):88-93
http://www.ijord.com

DOI: http://dx.doi.org/10.18203/issn.2455-4529.IntJResDermatol20185515

Original Research Article

Clinico-mycological study and comparison of efficacy of three different techniques of sample collection from skin lesions for potassium hydroxide mount preparation in dermatophytoses

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Received: 11 Deceember 2018
Revised: 27 December 2018
Accepted: 28 December 2018

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ABSTRACT

Background: Dermatophytoses are superficial fungal infections which invade and multiply within keratinized tissue. The KOH mount is one of the useful procedures and believed to be more reliable than culture for demonstrating dermatophytes. A few studies in the past have demonstrated the usefulness of alternative methods of sample collection for KOH preparation, but data on sensitivity and specificity of these methods is lacking. The aim of the study was to study the clinic-mycological aspects of dermatophytoses and to compare the efficacy of three different sampling techniques from skin lesions and correlating KOH mount with culture results.
Methods: 210 clinically diagnosed patients with dermatophytic infection attending outpatient department of Dermatology of a tertiary care hospital for duration of 2 years (September 2015 to October 2017) were included. The samples were collected from skin, hair and nail. These samples were used for direct microscopy by KOH mount and fungal cultures by Sabouraud dextrose agar media.
Results: Of the total of 210 patients, maximum were in age group of <10 years (74 cases), male: female - 1.2:1. Tinea corporis was commonest presentation (40.5%). Overall direct microscopy positivity was 81% while three different techniques from the glabrous skin and groins lesions was scraping method (97%), cellophane tape method (96%), skin surface biopsy (SSB) (98%) and culture in (89%). *T. rubrum* was commonest species isolate (37.7%).
Conclusions: Tinea corporis was the commonest clinical type followed tinea capitis. *T. rubrum* were commonest dermatophytes isolated. All three methods of sampling were suitable for routine sample collection. The KOH mount helped rapid confirmation of clinical diagnosis.

Keywords: Standard scraping method, Cellophane tape method, Skin surface biopsy

INTRODUCTION

Dermatophytoses refers to superficial fungal infection of keratinized tissues caused by dermatophytes.[1] The dermatophytes belong to 3 genera i.e. *Trichophyton*, *Microsporum* and *Epidermophyton*.[2]

The prevalence of superficial mycotic infections varies from 20-25%, making these one of the most frequent forms of infection worldwide. It is common in tropics and may present as epidemic proportions in areas with high rate of humidity, over population and poor hygienic conditions.[3] Worldwide the most common species causing dermatophytoses is *Trichophyton rubrum*.[3-7]

Direct microscopic examination in KOH solution is a reliable rapid test for demonstrating dermatophytes for Out Patient Department (OPD) procedure. Various studies are done on alternative methods for collecting sample for KOH preparation and fungal culture, but sensitivity and specificity of these methods have been lacking.[8-12]

Thus this study was taken up to define current clinical pattern and the ethological prevalence in our locality, to compare the efficacy of three different sampling techniques from skin lesions and to correlate KOH mount results with the culture results.

METHODS

Clinically suspected cases for dermatophytoses attending Dermatology Out Patient Department of a hospital in Mysore over a period of two year (September 2015 to October 2017) were included.

Patient who are on topical and/or systemic antifungal agents in the past 6 weeks or who were on topical steroids and / or systemic steroid in the past 8 weeks or patient on immunosuppressive agents, other than steroids in the past 6 months were excluded

After taking consent, detailed clinical history and through clinical examination was conducted, specimens were collected by following methods,

3 methods of sample collection from skin lesions:

- Standard scraping method: The active border was scraped with a glass slide.
- The cellophane tape method: cellophane tape was stuck on the active border & it was peeled off, which was then stuck on a glass slide.
- Surface skin biopsy (SSB): was done by taking a drop of instant adhesive (fevikwik) on a slide and then the slide is placed on lesion, after few seconds the slide is removed.

Infected hairs from the lesion were plucked and were put on slide. Nails were thoroughly cleaned with 70% alcohol or spirit, and then clipped using scissors or scraping of the nail was obtained from the nail bed and underside (ventral side) of the nail plate from the advancing edge, most proximal to the cuticle.

The above collected samples were subjected to direct microscope examination by KOH mount and culture study. On microscopic examination, long branching septate hyphae or arthrospores were looked in skin and nail scraping. Fungi in infected hair specimens were seen as hyphae or chains or arthrospores and the nature of invasion of hair and spore formation was studied.

Culture examination: For primary isolations, Sabouraud's dextrose agar (SDA) slope was used as selective media. One set was incubated at room temperature for one month. Growth was usually seen by 2 weeks. Growth was identified based on macroscopic appearance and microscopic features in lactophenol cotton blue mount. If no growth was observed at the end of 4th week, the culture was labelled as negative.

Statistical method

All the statistical calculations were done through SPSS for windows (v 16.0) - The Chi-Square Test procedure tabulates was done.

RESULTS

In this study of 210 patients, dermatophytoses was more common in males (111) than in females (99), (M: F= 1.2:1). Maximum number of cases were encountered in the age group of <10 years of age (74 cases) (Table 1). The most common clinical pattern of dermatophytic infection observed was tinea corporis in 83 cases (40.5%) followed by tinea capitis 74 cases (35.2%), (Table 1).

Overall positivity by culture was in 188 patients and direct microscopy was in 170 cases (Table 2). Fungal element from the skin samples was detected by three methods (standard-97%, SSB-98%, Cellophane tape method-96.2%), Scraping method, cellophane tape method, SSB method of sample collection was found to be almost equally effective (Table 3).

Table 1: Cross tabulations between clinical types and age distribution.

Clinical types	Age groups (years)						Total
	<10	11-20	21-30	31-40	41-50	50+	
Tinea capitis	62	10	2	0	0	0	74
Tinea faciei	1	1	0	0	0	1	3
Tinea corporis	6	5	15	24	17	18	85
Tinea cruris	0	1	1	2	0	1	5
Tinea pedis	1	0	0	0	1	2	4
Tinea mannum	0	0	1	1	1	0	3
Tinea unguium	4	2	7	5	8	5	31
Tinea corporis + t cruris	0	1	1	0	1	1	4
Tinea pedis + tinea mannum	0	0	0	0	1	0	1
Total	74	20	27	32	29	28	210

Table 2: Cross tabulation between direct microscopy and fungal culture.

KOH		Culture		Total
		+ve	-ve	
	+ve	160	10	170
	-ve	28	12	40
Total		188	22	210

Table 3: Comparison three different methods sample collection for KOH from skin.

Type of KOH from skin samples	Positive	Negative	Total
Scraping	102	3	105
SSB	103	2	105
Cellophane tape	101	4	105

Table 4: Cross tabulation between fungal isolates and clinical types.

Fungal Isolates	Clinical types								Total
	T.capitis	*T.faciei*	*T.corporis*	*T.cruris*	*T.pedis*	*T.mannum*	*T.unguium*	More than one clinical forms	
No growth	4	0	1	0	0	0	17	0	22
Trichophyton rubrum	13	1	33	4	4	1	8	7	71
Trichophyton mentagrophytes	8	2	20	2	1	0	4	0	37
Trichophyton schoenllenii	0	0	1	0	0	0	0	0	1
Trichophyton violaceum	41	0	17	3	1	2	1	0	65
Microsprorum cani	8	0	1	0	0	1	1	0	11
Epidermophyton floccoum	0	0	3	0	0	0	0	0	3
Total	74	3	76	9	6	4	31	7	210

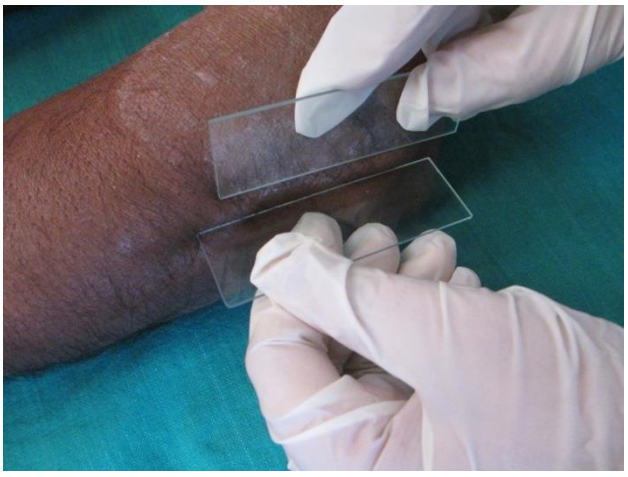

Figure 1: Scraping method.

Overall positivity by culture was in 188 patients and direct microscopy was in 170 cases (Table 2). Fungal element from the skin samples was detected by three methods (standard-97%, SSB-98%, Cellophane tape method-96.2%), Scraping method, cellophane tape method, SSB method of sample collection was found to be almost equally effective (Table 3).

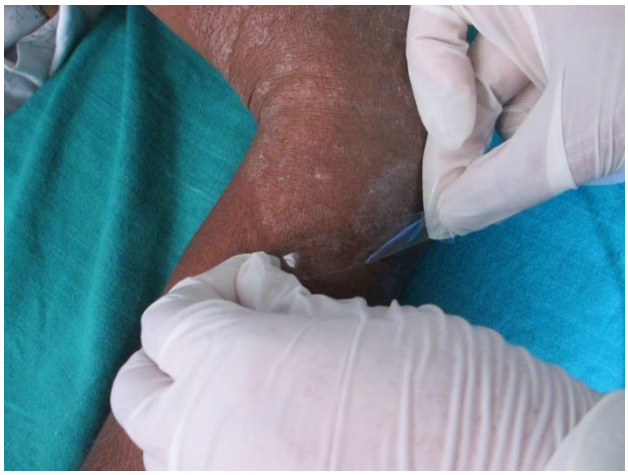

Figure 2: Cellophane tape method.

Out of 74 hair clipping samples, 53 were positive for fungal element by direct microscopy and 71 were positive

by culture. Out of 31 samples of nail clipping 16 (51.6%) were positive for fungal element by direct microscopy and 14 (45%) were positive by culture.

Figure 3: KOH mount.

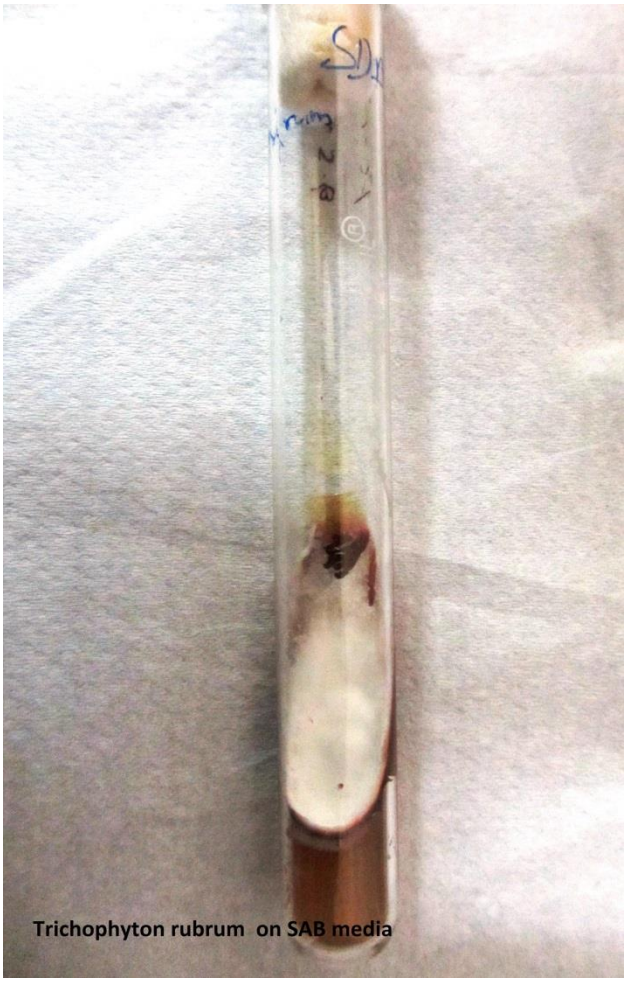

Figure 4: *Trichophyton rubrum* in SDA media.

All the 3 genera of dermatophytes i.e. *Trichophyton*, *Epidermophyton* and *Microsporum* have been isolated as the causative agent in this study (Table 4, Figure 4 to 6). Over all, the *Trichophyton* genera dominated in the fungal isolates (92.35%, 174/188) followed by *Microsporum* 11cases (5.8%) and *Epidermophyton* 3cases (1.5%). *Trichophyton rubrum* (71) was the most common species isolated followed by *Trichophyton violaceum* in 65 cases.

Figure 5: *E. floccosum* in SDA media.

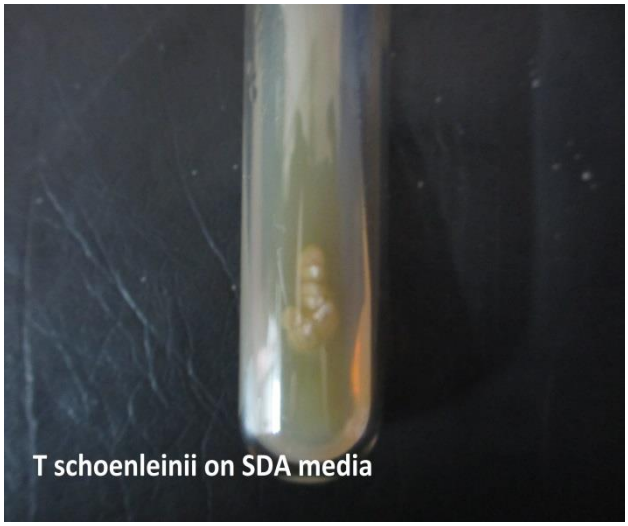

Figure 6: *T. schoenleinii* in SDA media.

DISCUSSION

There has been a changing pattern of the dermatophytes isolates in India in the past 5–7 years, with an alarming rise in overall number of case and increasing proportion of atypical clinical types and unusual manifestations, due to a complex interplay of host, agent, environmental or pharmacologic factors.[13]

In the present study of 210 cases, dermatophytoses was more common in male; similar findings have been

reported by other workers.[2-4] The higher incidence in male could be due to greater physical activity and increased sweating.

While maximum number of cases were encountered in the age group of <10 yrs (35.2%) followed by 31-40 years (15.2%). Although majority of studies done observed higher incidence in the 3rd decade, a study done at Calicut observed higher incidence in the 2nd decade.[1] These differences may be due to regional variation in the prevalence of various dermatophyte species and affinity of certain species for particular anatomical sites. The higher frequency in males may be because they frequently visit saloons for their hair dressing compared with females, where they use contaminated razors and hence spreading infection from one child to another. The lower frequency in females could be due to the custom of regular application of vegetable oil over the scalp, which has fungistatic properties.[14]

Mixed clinical types were seen among the enrolled patients. Tinea corporis (85) was the highest (40.5%), followed by 74 cases of tinea capitis (35.2%). This is in accordance with the findings of Bindu et al and other workers.[1] In a study done at Kolkata tinea capitis was the commonest dermatophytic infection in children.[5] In a study by Sardari et al it has been reported that tinea cruris was the commonest clinical type.[6] In another study in North East India, Tinea pedis (29.2%) was the most common dermatophytoses followed by tinea cruris (26.2%).[7]

The laboratory diagnosis of dermatophytoses relies first on the KOH mount on direct microscopic observation of the pathogen in samples from the affected area and identification of the fungal agents can be done based on the colony morphology by the macroscopic characteristics: upper and bottom side of colonies as well as pigmentation and slide culture growth forms by lactophenol cotton blue (LPCB) tease mounts for microscopic characteristics: formations of macro and microconidia, respectively.

For the laboratory to provide the optimum performance, the quantity and the quality of the material examined are critical. Because for most samples, both culture and microscopic examination will be performed, it is essential that as much clinical material as possible is submitted to the laboratory to allow both diagnostic methods to be carried.[15]

In the present study out of 210 clinical cases it was possible to demonstrate fungi on direct microscopy with potassium hydroxide in 81%. Fungal element from skin samples was detected by three methods (standard-97%, SSB-98%, Cellophane tape method-96.2%), (Figure 1-3). However, cellophane method and SSB method were noted to be much better in both collection and transport of samples, over the standard method, especially in lesion with minimal scales.

A few studies in the past have demonstrated the usefulness of alternative methods of sample collection for KOH preparation and fungal culture, but data on sensitivity and specificity of these methods is lacking.

Overall positivity by culture was 89%. In comparison, a study done at Thane showed culture positivity of 71%, but a study done at Aurangabad showed very low rate of culture positivity (22.8%).[4,11]

Correlating clinical and mycological data it shows that in *T. rubrum* (Figure 4) was isolated in most cases followed by *T. violaceum. T. rubrum* was the common isolate in cases of tinea cruris. Out of 14 isolates from tinea unguium, 8 were *T. rubrum.* And out 74 cases of tinea capitis most common isolate was *T. violaceum* (55.4%) followed by *T. rubrum* (17.6%). It was therefore concluded that *T. rubrum* was the main organism isolated from the infections of glabrous skin and groins followed by tinea capitis, which was again supported by other studies from India and other countries.[8,9,12] Overall, the *Trichophyton* species dominated with 90% of the isolates followed by *Epidermophyton* and *Microsporum*. The reports from other parts India showed *T. rubrum* as the commonest species, 87.12% in Bombay, 80% in Punjab, 73.27% in Baroda, 66.2% in Calicut.[1,3,11] This variance is possibly due to the different geographical regions in these studies which have different prevailing dermatophyte flora.

CONCLUSION

The present study has given us a clear insight regarding the mycological pattern of dermatophytoses in this region, tinea corporis as the commonest clinical pattern followed by tinea capitis. *Trichophyton rubrum* was commonest fungal isolate.

The fact that laboratory diagnosis of dermatophytoses has been generally a neglected area in clinical practice. The present study shows KOH mount is a useful rapid diagnostic method. Cellophane tape and SSB are equally effective alternate methods to standard scraping method of specimen collection for KOH mount with added advantages of lesions with minimal scaling can be easily collected and transported, scales which can lost and lead to air born infection is prevented.

Culture is not essential for diagnosis but it is useful in identifying the species. The collaborative effort involving dermatologists, microbiologists, and public health professionals is required to address this emerging public health problem.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

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Cite this article as: Thimmappa V, Shastry V, Betkerur J. Clinico-mycological study and comparison of efficacy of three different techniques of sample collection from skin lesions for potassium hydroxide mount preparation in dermatophytoses. Int J Res Dermatol 2019;5:88-93.