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COMPRATIVE STUDY OF TWO DIFFERENT MICROSCOPIC TECHNIQES AND FUNGAL CULTURE FOR ISOLATION OF DERMATOPHYTES.

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Abstract

AIM-
To evaluate the usefulness of two different microscopic techniques for the identification and isolation of dermatophytes from clinical sample.

INTRODUCTION - Fungal infections are very common in man. They are assuming greater significance both in developed & developing countries due to advent of immunosuppressive drugs & disease[(1)] hot & humid climate in tropical & subtropical countries like India makes dermatophytosis or ringworm a very common superficial fungal skin infection. Dermatophytosis is caused by dermatophytes, a group of keratinophilic fungi that require long incubation period to grow. The clinical presentation, though very typical of ringworm infection, is very often confused with other skin disorder particularly due to rampant application of broad – spectrum steroid containing skin ointments and cream leading to further misdiagnosis and mismanagement.

Even the newer antifungal agents are not completely devoid of slide effects which may affect the patient health on long term use. The long duration of treatment which seeks patient compliance and co-operation, every effort should be made to confirm the etiology by the use of appropriate laboratory tests before starting the treatment.

Microscopy is the simplest and cheapest investigation to screen superficial fungal infections. It needs experienced observer to avoid false positive and false negative reports which affect the diagnosis. However, fungal culture still remains the gold standard investigation to confirm the causative agent [(2,3)]. Identification of fungi at least to the genus level is necessary to initiate therapy.

This study is aimed to evaluate diagnosis efficacy of laboratory methods microscopy and modified microscopy technique to identify fungal isolates causing fungal infections. This study is valuable to establish the accurate diagnosis and to start antifungal treatment.

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OBJECTIVES ---

To evaluate the diagnosis sensitivity and usefulness of two microscopic techniques, KOH without DMSO and KOH with 40% DMSO for all skin, hair and nail sample.

MATERIAL AND METHOD --

Cross sectional lab based investigational study was conducted and skin, hair, nail sample from 110 clinically suspected cases of dermatophytosis were taken from OPD of district hospital of central India and were screened by direct microscopic examination using 10% potassium hydroxide (KOH) with and without 40% dimethyl sulphoxide (DMSO) mount.
10% potassium hydroxide (KOH) with 40% Dimethyl sulphoxide mount (DMSO) mixed in equal proportion.

10% KOH MOUNT.

A drop of 10% KOH was kept on a clean, grease free glass slide. The sample (hair, skin and nail clipping) was placed in the KOH drop and slide passed through a burner flame to hasten keratolysis. When keratolysis softened the sample, a clean glass cover slip was kept on the sample and pressed, preventing the formation of air bubbles.
The sample was kept in KOH for a variable duration ranging from 5 minutes to 30 minutes, depending upon the thickness of the scales and examined every 5 minutes. Each slide was thoroughly examined for the presence of filamentous septa and other morphology of fungi.

10% KOH WITH 40% DMSO

The sample was processed in the fashion similar to KOH mount except that sample was kept on a slide with 10% KOH with 40% DMSO. The slide was not passed through flame and was screened for presence of fungus within 5 minutes.
Institutional ethical committee clearance was obtained before starting the study and informed consent was obtained from patients before collecting nail sample.
All patients with clinically suspected to have fungal skin hair and nail infections, irrespective of age and sex were included and patients who were under treatment with antifungal drugs for previous two weeks period were excluded from the study.

Culture

For primary isolation of dermatophytes following media were used
A} Sabouraud Dextrose Agar (SDA) with antibiotics (Himedia)
B} Dermatophytes Test Medium (DTM) with supplements (Himedia)

The SDA and DTM were inoculated in duplicate; one inoculated at 30^0c and other at 37^0c SDA was taken as standard media for primary isolation and other one was compared with it. Isolation of dermatophytes was confirmed by gross morphology of growth ,typical microscopic characteristics , supplements with hair perforation and slide culture as and when needed. To compare the efficiency of the two media of dermatophytes, Chi square test and standard error of difference between two proportion was applied.

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STATISTICAL ANALYSIS ---

Statistical analysis was done to compare the sensitivity of two different microscopic techniques, (conventional and modified microscopy) and culture media by using Chi-square test. The calculated P- value were 0.294 and 0.588(>0.05) respectively.

RESULTS AND DISCUSSION

Table 1

Culture	Microscopy (KOH)		Total
	Positive (%)	Negative (%)	
Positive	46(91.37)	4(8%)	50 (45.45)
Negative	21(35%)	39(65%)	60(54.54)
Total	67(60.90%)	43 (39.09%)	110

Direct microscopy using KOH mount is simple, rapid and easy method to visualize fungal elements. Many modification have been evolved to increase the specificity & sensitivity of KOH microscopy results like use of 5% glycerol, addition of 36% DMSO, and addition of parker's blue[(4)] in this study we compered the results of conventional KOH mount and a modified technique (40%KOH with DMSO)[(5)]. When doing direct microscopic examination it was observed that DMSO produced rapid clearing of keratin and faster visualization of fungal hyphae as all the sample could we examine within 5 minutes compered to plain KOH required 10-15 minutes for complete clearing of keratin.

Two microscopic techniques (KOH alone and modified KOH with DMSO) were also compared. The sensitivity of both the techniques was equal, 50.6% (P= .588) and 48.2% respectively.

There are two currently available microbiological methods to diagnose fungal nail infections are KOH microscopy & culture.

In our study the sensitivity of KOH microscopy is about 60% and sensitivity of fungal culture is slightly 50 %. It is in concurrent with studies conducted by Grover S et al., and Kaur R et al; [(6-8)]. Where as, it is in contrast with Singh et al., study and Das et al., study [(3,7).]

The result of two methods (microscopy and culture) were compared and show that there is no significance differences in the sensitivity results of microscopy and culture that is when statically taken both the methods are equally sensitive and the difference is negligible. While analyzing results of 110 samples, if microscopy alone was considered we would have missed 50% infection; if culture alone was taken we would have missed 40% of infection.

When both the methods considered together, there has been 30% false negative results in this study and similar observation are noted in other studies also [(6,9,10).] These false negative results could be attributed to various factors like methods followed in, sample collection, site of sample collection, observer expertise in microscopy, adequacy and processing of nail material for culture etc. To avoid such false negative results proper and adequate sample collection, expertise well trained in microscopic observation are necessary.

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Table 2

The comparative evaluation of the isolation of dermatophytes on SDA and DTM has been reported by Yavuzdemir who found no significant difference in the isolation rate of these media[11]. The effectiveness of SDA was 93.5% and that of DTM was 95.4% in this study of 225 samples. We found in the study of 110 samples, SDA to be 96.31% effective and DTM 98.27% effective in isolation of dermatophytes.

Growth on SDA	Growth on DTM	No of isolates
+	+	106 (96.36%)
-	+	2 (1.8%)
-	-	2(1.8%)
Total		110

Table 3

In Sorbothra et al KOH & DMSO 43% KOH 41% in our study KOH & DMSO 69% and only KOH 60%. Aqueous potassium hydroxide (KOH) has been used as a clearing agent for direct demonstration of fungi in skin, nail, or hair scrapings[1] but addition of dimethyl sulphoxide as described by Rebell et al in 1971[12] was found to be better preparation over plain KOH [12-14]. Addition of DMSO permits rapid clearing of keratin and almost immediate examination of sample without warming of slide[14] It also prevent rapid drying of the fluid and thus is a better option. KOH preparation tends to absorb carbon dioxide from air and form carbonate crystals thus reducing the effective hydroxide [14]. Also hydroxide preparation tends to saponify when gently heated thus forming fat globules in the slide and reducing effective visualization of fungal hyphae. The faster keratolysis by addition of DMSO in probably due to increased transport of chemicals through the stratum corneum[15,16].

The metabolic end products of dermatophytes are such that an increase in the alkalinity of the surrounding medium is noticed in contrast to saprophytic fungi, which make the medium acidic[17,18]. The early release of alkali is supposed to be of important in the attack of keratin by fungi[19].This property has been used to prepare media using indicators for isolation of dermatophytes for rapid presumptive identification, particularly useful for non- mycologist as results can be evaluated simply by color change in the medium without detailed knowledge of morphology.

Addition of DMSO helped in faster clearing of keratin without formation of KOH crystals leading to better visualization of fungal hyphae at 10-15 minutes instead of more than 1 hour in conventional KOH for nail samples. Heating not required while using DMSO.

Technique	**Positive (%)**	**Negative (%)**
40% KOH & DMSO	110(100%)	00
40% KOH	106(96.40%)	04

SDA - Sabouraud dextrose agar

DTM – Dermatophytes test medium

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CONCLUSION

The modified KOH with DMSO mount had allowed fastest and better visualization of fungal elements at 10 minutes instead of routine 30 minutes.

- Clear visualization
- Heating not required
- Sensitivity is almost equal.
- Less time required
- Disadvantage being high cost but can be optimally used in centers S with high sample load.

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