Comparative Study of two Different Microscopic Techniques and Fungal Culture for Isolation of Dermatophytes

Abstract: 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. 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. Materials and Methods: 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. Results: 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 in this study we compared the results of conventional KOH mount and a modified technique (40% KOH with DMSO). When doing direct microscopic. Conclusion: The modified KOH with DMSO mount had allowed fastest and better visualization of fungal elements at 10 minutes instead of routine 30 minutes.

Keywords: Jaundice, etiology, mode of termination, maternal mortality, Pregnancy, Feto-maternal outcome.

INTRODUCTION:
Among all fungus, dermatophyte is one of the cutaneous fungi. They have both keratinophilic and keratinolytic properties. They are capable of invading human and animal keratinized tissue causing dermatophytosis. Dermatophytosis has several distinct cutaneous manifestations. The severity of the disease depends on various factors including- strain or species of infecting dermatophyte, the sensitivity of the host and the site of infection. Dermatophyte belongs to three group named as Trichophyton, Epidermophyton and Microsporum. Further they are divided into anthropophilic, zoophilic and geophilic according to their natural habitat.

Dermatophytosis lesion is called annular lesion. It takes single or multiple ring shape lesions with inflammatory edges. Itching, redness, and scaling edges with blister is also notifiable. Another term used as tinea infection according to their anatomical location like tinea capitis, tinea barbae, tinea corporis, tinea cruris, tinea manuum, tinea pedis and tinea unguium.

Both healthy and immune compromised patients are affected with this infection. The estimated lifetime risk of acquiring dermatophytic infection is 10-20%. Their geographical distribution is widely variable. Climate, lifestyle, involvement of outdoor activities, pre-existing co-morbidities (diabetes mellitus, hypothyroidism, malnutrition etc) are responsible for the heterogeneous prevalence. Prevalence of dermatophytosis varies between 13% to 49% depending on the geographical distribution of the countries. Although dermatophytes are not life-threatening fungus, it turns into major public health problem due to high morbidity as well as cosmetic damage.
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.

MATERIALS AND METHODS
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°C and other at 37°C 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.

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

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<th>Culture</th>
<th>Microscopy (KOH)</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>Positive (%)</td>
<td>Negative (%)</td>
</tr>
<tr>
<td>Positive</td>
<td>46(91.37)</td>
<td>4(8%)</td>
</tr>
<tr>
<td>Negative</td>
<td>21(35%)</td>
<td>39(65%)</td>
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<tr>
<td>Total</td>
<td>67(60.90%)</td>
<td>43(39.09%)</td>
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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 in this study we compared the results of conventional KOH mount and a modified technique (40% KOH with DMSO). 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 compared 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.(6,8,10). 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.

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(10). 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.

<table>
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<tr>
<th>Technique</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
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<tbody>
<tr>
<td>40% KOH &amp; DMSO</td>
<td>110(100%)</td>
<td>00</td>
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<tr>
<td>40% KOH</td>
<td>106(96.40%)</td>
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SDA - Sabouraud dextrose agar
DTM – Dermatophytes test medium

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.

REFERENCE