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Original Article

Microbiology Section

Evaluation of Diagnostic Efficacy of Two Different Microscopic Techniques and Fungal Culture in Onychomycosis

N SUBATHRA, N BHARATHI SANTHOSE

ABSTRACT

Introduction: Onychomycosis is a term used to describe any fungal infection of nail apparatus and is caused mainly by dermatophytes, non-dermatophytic moulds and yeast. Clinical examination based on physical findings alone is not a reliable method of diagnosing onychomycosis. Laboratory diagnosis of fungal nail infection relies on microscopic visualization of fungal cells from the material obtained as well as definitive identification of fungus by culture.

Aim: To find out the diagnostic efficacy of two different microscopic techniques and fungal culture in onychomycosis.

Materials and Methods: It is a cross-sectional lab based study conducted at Government Tertiary Hospital in Coimbatore district, India, from 85 clinically suspected cases of having fungal nail infections. All nail samples were subjected to routine KOH mount, modified KOH with 40% DMSO (Dimethyl Sulfoxide) and fungal culture on two tubes of Sabouraud's Dextrose agar with chloramphenicol and cycloheximide/ Sabouraud's Dextrose agar with chloramphenicol at 37ºC and at 25ºC for 3 weeks.

Results: Out of 85 samples, total culture positive cases were 46 (54%) and all the three groups namely dematophytes, non-dermatophytic moulds and yeasts were isolated. Total KOH positive cases were 43 (51%). Conventional microscopy method positivity was 48% and modified microscopy method positivity was 51%. Twenty five cases (29.41%) were negative by both the techniques. The culture and microscopy were equally sensitive (p-value $\geq$ 0.05) in this study. Two microscopic techniques (KOH alone and modified KOH with DMSO) were also compared. The sensitivity of both the techniques was equal, 50.6% (p = 0.588) and 48.2% respectively.

Conclusion: Both microscopy and fungal culture should be considered complementary to each other in diagnosing onychomycosis. The modified KOH with DMSO mount had allowed fastest and better visualization of fungal elements at 10 minutes instead of routine 30 minutes.

Keywords: Dermatophytes, Fungal infections, Onychopathie

INTRODUCTION

Fungal nail infections affects approximately 5% of the population worldwide [1] and represents 20-40% of onychopathies [2] and about 30% of the mycotic cutaneous infections [3]. Dermatophytes, non-dermatophytic moulds and yeasts (*Candida* spp) have been implicated as causative agents for onychomycosis. The clinical presentation of dystrophic nails should alert the clinician to suspect the possibility of fungal nail infection. However, care should be taken to identify the signs of fungal infections in conditions which mimic onychomycosis. These include psoriasis, lichen planus, idiopathic onycolysis, and yellow nail syndrome so on [4]. So the use of appropriate laboratory diagnostic techniques including microscopy and culture is important to ensure accurate diagnosis and initiation of early treatment. The clinical examination alone will not always identify the correct etiological agent. Most of the fungal nail infections require long term use of antifungal agents when compared to skin and hair infections [5]. Even the newer antifungal agents are not completely devoid of side 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 [5,6]. Identification of fungi at least to the genus level is necessary to initiate therapy. This study is aimed to evaluate diagnostic efficacy of laboratory methods microscopy, modified microscopic technique and culture to identify fungal isolates causing nail infections. This study is valuable to establish the accurate diagnosis and to start antifungal treatment.

OBJECTIVES

1. To perform microscopy and fungal culture from nail clippings and scrapings
2. To evaluate and compare the diagnostic efficacy of microscopy and fungal culture from fungal nail samples
3. To evaluate the diagnostic sensitivity and usefulness of two microscopic techniques, KOH without DMSO and KOH with 40% DMSO for nail samples

MATERIALS AND METHODS

This cross-sectional lab based investigational study was conducted at Department of Microbiology at Coimbatore Medical College Hospital, Coimbatore, Tamil Nadu, India, for a period of eight months from January 2011 to August 2011. Institutional ethical committee clearance was obtained before starting the study and informed consent was obtained from patients before collecting nail samples.

Nail clippings, nail scrapings and subungual debris were collected from 85 clinically suspected onychomycosis patients who attended the Dermatology Outpatient Department at All patients with clinically suspected to have fungal 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.

The affected area of the nail was thoroughly cleaned with 70% alcohol to remove surface contaminants.The alcohol was allowed to dry by evaporation. The nail clippings were taken by using sterile metal nail clipper and nail scrapings were taken by using sterile 15 no. scalpel blade, from the advancing edges of diseased parts of the nails depending on the clinical type [7].The scalpel blades and nail clippers were sterilized in autoclave at 121°C in 15 lbs for 20 minutes. The samples were collected and transported in a sterile dry container to the laboratory.

Processing of Samples

1. **Direct Microscopic Examination:** For microscopic examination of fungal elements two methods were applied:-

   (i) Microscopy (40% KOH mount without DMSO)

   Part of nail samples were crushed and immersed in 40% KOH on a clean grease free glass slide for 30 minutes. Gentle heat was applied to facilitate keratin clearance. A cover slip was then applied and the slide was examined under low and high power objectives for the presence of fungal elements.

   (ii) Modified microscopy (40% KOH with 36% DMSO)

   Another part of nail samples were crushed and immersed in drop of solution of 40% KOH with 36% DMSO on slide. A cover slip was applied and examined after 10 minutes without applying heat.

2. **Culture:** The nail fragments were inoculated aseptically onto two sets of fungal culture media at different sites, one containing Sabouraud's Dextrose agar with 0.05% chloramphenicol, other containing Sabouraud's dextrose agar with 0.05% chloramphenicol and 0.5% cycloheximide.

One set of culture media was incubated at 37°C and another set at 25 -30°C for a period of three weeks. Culture slopes were examined for fungal growth daily in the first week and twice weekly from 2nd week onwards. Fungi grown on both type of culture media was considered as a dermatophyte. Fungi grown on Sabouraud's Dextrose agar with chloramphenicol without cycloheximide was considered as Non Dermatophytic Moulds (NDM). Definitive diagnosis of NDM was done by "Walse and English" criteria [7,8]. That is microscopy positive for fungal elements and culture also positive for non dermatophytic moulds with the absence of concomitant dermatophytic growth with at least four to five inocula sites give rise to same growth from same specimen inoculated at different places of culture media. Yeasts were identified by their typical creamy white smooth moist colonies on Sabouraud's Dextrose agar. Fungal isolates were identified based on colony morphology, pigmentation, Lacto phenol cotton blue mount, slide culture, urease test where ever applicable.

STATISTICAL ANALYSIS

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

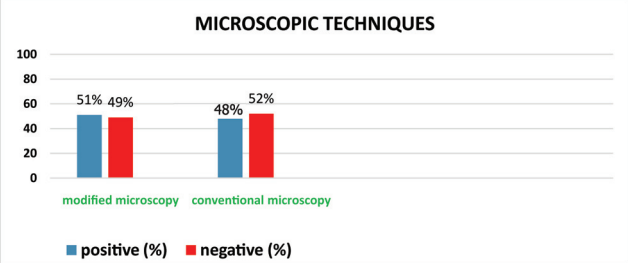
RESULTS

Eighty five nail samples were processed and the comparative results of microscopy and culture in [Table/Fig-1]. It is evident that from 85 nail samples processed, total microscopy alone positive were 43 (51%) and total culture alone positive were 46 (54%) [Table/Fig-2]. The combined sensitivity is 70% i.e., 60 out of 85 samples showed positive result either by microscopy or culture or by both. Total 25 samples (30%) were negative by both the methods.

The two microscopic techniques (KOH alone and KOH with DMSO) were also compared and the results were shown in [Table/Fig-3-6]. The sensitivity of both the techniques was 50.6% and 48.2% respectively.

Culture	Microscopy (KOH)		Total (%) n = 85
	Positive (%)	Negative (%)	
Positive (%)	29 (34.12)	17 (20)	46 (54.12)
Negative (%)	14 (16.47)	25 (29.41)	39 (45.88)
Total (%)	43 (50.59)	42(49.41)	85 (100)

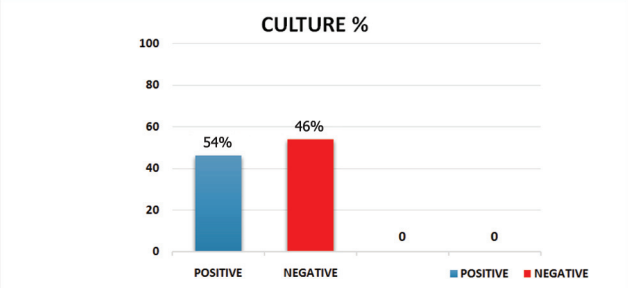
[Table/Fig-1]: Correlation between microscopy and culture results of 85 cases of onychomycosis.



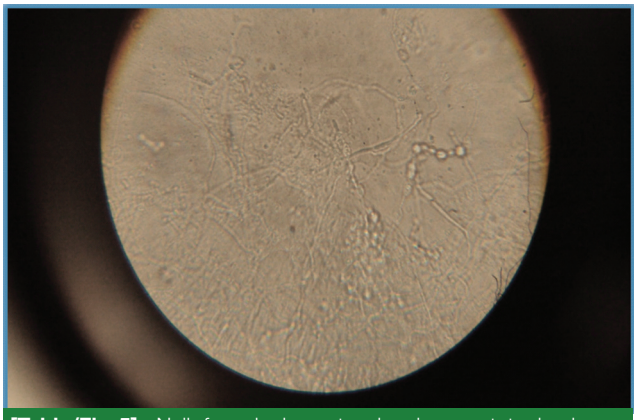
[Table/Fig-2]: Sensitivity of microscopy - modified versus conventional method.

Technique	Positive (%)	Negative	Chi-square value	p-value (>0.05)
40% KOH & DMSO	43 (50.6)	42 (49.4)	0.953	0.294
40% KOH	41 (48.2)	44 (51.8)	0.329	0.588

[Table/Fig-3]: Comparision of results of two microscopic methods.



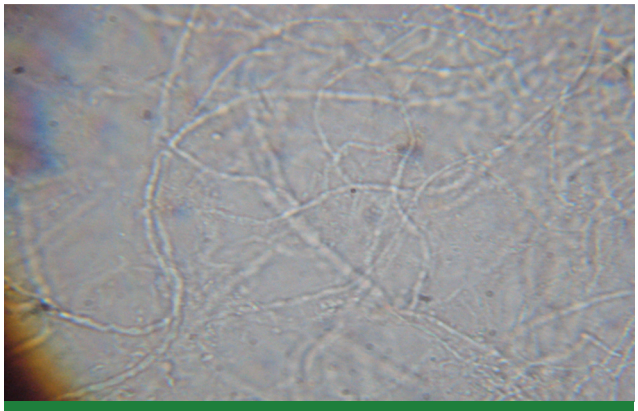
[Table/Fig-4]: Sensitivity of fungal culture.



[Table/Fig-5]: Nail fungal elements showing septate hyphae - conventional microscopy (40% KOH) (40X 10 magnification).

DISCUSSION

The incidence of onychomycosis varies 0.5-5% from various studies in India [9-11]. There are two currently available microbiological methods to diagnose fungal nail infections are KOH microscopy and culture. In our study the sensitivity of KOH microscopy is about 51% and the sensitivity of fungal culture is slightly high, 54%. It is in concurrent with studies conducted by Grover S et al., and Kaur R et al., [10-12].



[Table/Fig-6]: Nail fungal elements with septate hyphae-modified microscopy (36% DMSO with 40% KOH).

Whereas, it is in contrast with Singh et al., study and Das et al., study [6,11].

The results of two methods (microscopy vs culture) were compared and showed that there is no significant differences in the sensitivity results of microscopy and culture, that is when statistically taken both the methods are equally sensitive and the difference is negligible.While analyzing results of eighty five samples, if microscopy alone was considered we would have missed 50% of infections; if culture alone was taken we would have missed 40% of infections.

When both the methods considered together, there has been 30% false negative results in this study and similar observations are noted in other studies also [10,13,14]. These false negative results could be attributed to various factors like methods followed in nail 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.

Direct microscopy using KOH mount is simple, rapid and easy method to visualize fungal elements. Many modifications have been evolved to increase the specificity and sensitivity of KOH microscopy results like use of 5% glycerol, addition of 36% DMSO, and addition of Parker's blue [15]. In this study we compared the results of conventional KOH mount and a modified technique (40% KOH with DMSO) [16]. As shown in [Table/Fig-3] the results were compared statistically and p-value was calculated. For both the tests there was no significant difference (p>0.05) in the sensitivity. So it is inferred that the use of keratolytic agents (DMSO or 5% glycerol) with KOH, do not alter the sensitivity of microscopic technique and it lead to clear visualization of fungal elements than conventional KOH mount.

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 of mount is also not required while using DMSO.

As literatures say, Isolation and identification of fungus to species level requires the use of fungal culture which is considered as the gold standard technique. In this study also culture is equally sensitive and specific to microscopic method similar to other studies [11,13].

This study shows the future need for developing newer rapid screening methods to visualize fungal elements from nail samples and to adopt modified microscopic techniques rather than conventional microscopy for detecting fungal elements.

CONCLUSION

Microscopy and fungal culture both should be considered complementary to each other and coupled together in the diagnosis of fungal nail infections. The study also reveals that there is definite advantage in adding DMSO with KOH mount as a rapid keratolytic agent for faster and better visualization of fungal elements in nail samples without altering the results outcome.

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AUTHOR(S):

1. Dr. N Subathra
2. Dr. N Bharathi Santhose

PARTICULARS OF CONTRIBUTORS:

1. Senior Assistant Professor, Department of Microbiology, Government Mohan Kumaramangalam Medical College, Salem, Tamil Nadu, India.
2. Senior Assistant Professor, Department of Microbiology, Coimbatore Medical College, Coimbatore, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. N Subathra,
A2, Jayamangala Apartment, Sakthivinayagar Nagar,
Narasothipatti, Salem, Tamil Nadu-636004, India.
E-mail: drsubi98@yahoo.in

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