

Original Research Article

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Comparison of KOH, Calcofluor White and Fungal Culture in the Diagnosis of Onychomycosis

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ABSTRACT

Onychomycosis is a fungal infection of the finger or toe nail caused by various fungal agents like dermatophytes, non dermatophytes moulds and yeasts. It is necessary to diagnose the infection with proper laboratory evidences before treating them with antifungal drugs, which require prolonged duration of treatment and may have adverse side effects. This study involves comparison of standard laboratory tests in the diagnosis of onychomycosis, namely, potassium hydroxide mount (KOH mount), Calcofluor white staining and fungal culture of the nail clippings. A total of 100 patients with clinically suspected onychomycosis were selected. Nail scrapings and clippings were subjected to KOH mount, Calcofluor staining for direct microscopic examination and culture using Sabouraud's dextrose agar. Out of the 100 patients, direct microscopy with KOH mount, calcofluor mount and fungal culture showed positive results in 69(69%), 80(80%), 54(54%) respectively. Out of the 54 culture positive 29 were Dermatophyte, 20 were non dermatophytes mould (*Aspergillus*) and 5 were *Candida* species. Culture was taken as the gold standard and was compared with KOH mount and calcofluor white microscopy. The sensitivity and specificity of KOH microscopy was 83.05% and 56.09% respectively, whereas calcofluor white microscopy showed 92.3% and 54.2% respectively. Calcofluor white is the best method to detect fungal agents from clinically suspected onychomycosis cases with high sensitivity. It is easy to perform, rapid, and give significantly higher rates of detection of onychomycosis compared to the KOH mount and fungal culture.

Keywords

Onychomycosis,
KOH mount,
Calcofluor white,
fungal culture

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Introduction

Onychomycosis is a fungal infection of the finger or toe nail caused by dermatophytes, non dermatophytes moulds and yeasts, mainly by *Candida* species which leads to the discoloration, thickening, and separation of nail from the nail bed. The dermatophytes are the commonest cause of onychomycosis and most common isolate belongs to *T. mentagrophytes*. The non-dermatophytes

moulds can cause infection in traumatized nails; the most common agents include *Aspergillus* species, *Scopulariopsis* species and *Fusarium* species.

But, nail tissue is generally contaminated with fungal spores and isolation of a non dermatophytes mould should only be taken as significant if it is positive for both direct microscopic examination and culture from the given clinical samples (Bolognia *et al.*, 2003).

Onychomycosis can be diagnosed based on physical examination and microscopic examination of the specimen. Laboratory diagnosis of onychomycosis depends on direct microscopic examination of fungal elements and mycological culture of the particular fungal species in the clinical sample.

Every diagnostic laboratory must be able to differentiate between pathogens and contaminants in the culture plate.

The microscopic examination is generally done by KOH and calcofluor white fluorescent stain (Rippon, 1988; Miller *et al.*, 1993). Direct microscopic examination of specimens in suspected cases of onychomycosis provides early detection when compared to culture, which may take days or weeks. KOH examination is very simple, fast and most cost effective technique used for the diagnosis of fungal infections but, an inexperienced observer may misdiagnose certain artifacts as hyphae and it does not allow species identification (Lilly *et al.*, 2006; Kaur *et al.*, 2008). The calcofluor white stain is very sensitive technique which helps in the identification of fungal elements, even in small quantities and less experience of the observer, but it is an expensive technique which requires purchasing a fluorescence microscope (Rippon, 1988; Evans *et al.*, 1989; Thomson *et al.*, 1989; Miller *et al.*, 1993; Bologna *et al.*, 2003; Weinerg *et al.*, 2003; Guzman, 2004; Gupta *et al.*, 2008). Cultures are more specific than KOH but have a higher percentage of false negatives (Weinerg *et al.*, 2003; Shenoy *et al.*, 2008).

The confirmation of the diagnosis of onychomycosis is difficult and there are various diagnostic techniques available, this study was undertaken to determine the reliable method in early diagnosis of onychomycosis for initiating appropriate therapy for the successful treatment of onychomycosis case.

Objectives

To compare the sensitivity, specificity of direct microscopic examination by KOH and CFW stain using fungal culture as gold standard test.

To study the microbiological profile causing Onychomycosis.

Materials and Methods

A total of 100 patients with clinically suspected onychomycosis attending the OPD of AIMS, B.G. Nagara were enrolled for this study.

Nail clippings and nail scrapings from subungual debris were collected in black paper and divided into two parts; one part was used for direct microscopy (KOH and CFW) and the other for culture. Ethical committee clearance was taken from the institute

KOH mount

Specimen was placed on a slide, and a drop of 20% KOH was added. Incubation was done for 2 hours or more (up to 48 hours) until softening or digestion of the specimen occurred (Kurade *et al.*, 2006).

Slides were evaluated for the presence of branching thread-like structures (hyphae). Samples with the presence of hyphae was considered to be a positive.

Calcofluor white staining (CFW)

Add 1 drop of Avistain calcofluor white stain (comprising 1g/L calcofluor white M2R and Evans blue) over KOH digested specimen on the slide. The slide was examined under fluorescence microscopy using Ultraviolet light with K-530 excitation filter and a BG12 barrier filter.

Fungal culture

Culture was done using Sabouraud's dextrose agar (SDA) with and without antibiotics (chloramphenicol and cycloheximide). The plates were examined daily during the first week and twice weekly during the next two weeks. The isolate was identified by culture characteristics and microscopy.

Results and Discussion

In this study, 62 (62%) of the patients were female and majority of patients were from 35-45 years of age group.

Out of the 100 patients, direct microscopy with KOH mount, calcofluor mount and fungal culture showed positive results in 69 (69%), 80 (80%), 54 (54%) respectively (Table 1). CFW was the most sensitive method among the three methods.

Out of the 54 culture positive isolates, 29 were Dermatophyte, 20 were non dermatophytic mould and 5 were Candida species (Figure 1).

Among dermatophytes majority of isolates were *Trichophyton rubrum* followed by *Trichophyton mentagrophytes*, whereas among non dermatophytic mould, *Aspergillus* species

was the most common isolate (Table 2).

Culture was taken as the gold standard, when KOH mount microscopy was compared to the culture, sensitivity was 83.05% and specificity was 56.09%. When calcofluor white microscopy was compared to the culture, sensitivity and specificity was 92.3% and 54.2% respectively (Table 3, 4 and 5).

Onychomycosis is a growing global health problem which is difficult to treat using antifungal agents because of the inherent slow growth of the nail (Elewski, 1998). The poor efficacy and longer duration of treatment using older antifungal agents, make them unsuitable for treating onychomycosis, whereas newer antifungal agents like itraconazole and terbinafin renders higher cure rate. Newer drugs also have some potential adverse effect on patients. It is very important to confirm the clinical diagnosis with proper laboratory evidence before initiating treatment.

Out of the 100 patients, direct microscopy with KOH mount, calcofluor mount and mycological culture showed positive results in 69 (69%), 80 (80%), 54 (54%) respectively (Fig. 2 and 3).

Fig.1 Results of fungal culture

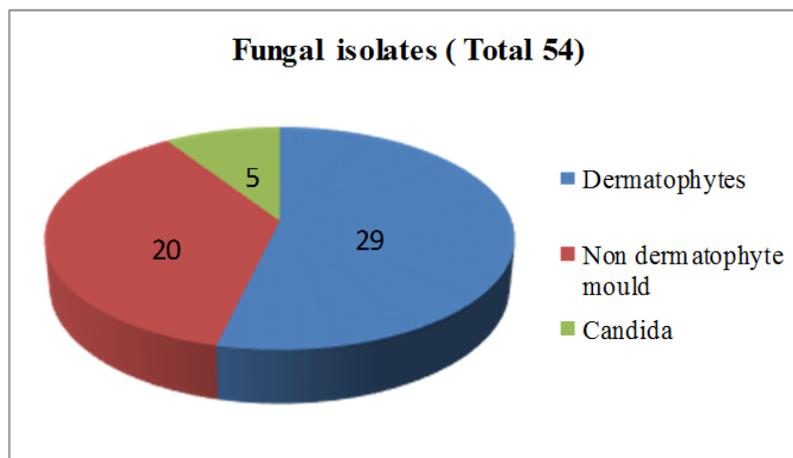


Fig.2 KOH mount microscopy

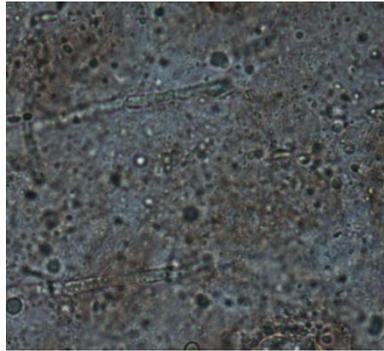


Fig.3 Calcofluor White fluorescent stain showing fungal hyphae



Table.1 Number of positive by different methods

Sl. No	Test	Positive (total no of samples = 100)	Percentage %
1	KOH	69	69
2	Calcofluor white stain	80	80
3	Culture	54	54

Table.2 Fungal agents isolated

Sl. No	Fungal group	Species	Number of isolates
1	Dermatophyte (29)	<i>Trichophyton rubrum</i>	18
		<i>Trichophyton mentagrophytes</i>	10
		<i>Epidermophyton floccosum</i>	1
2	Non dermatophytes mould (20)	<i>Aspergillus</i>	18
		<i>Fusarium</i>	02
3	Yeast (5)	<i>Candida albicans</i>	04
		<i>Non albicans Candida</i>	01

Table.3 KOH mount microscopy and fungal culture

	Gold standard culture	
	Culture positive	Culture Negative
Test positive	True Positive = 49	False Positive =18
Test negative	False Negative = 10	True Negative =23

Table.4 Calcofluor white microscopy and fungal culture

	Gold standard culture	
	Culture positive	Culture Negative
Test positive	True Positive = 60	False Positive =16
Test negative	False Negative = 5	True Negative =19

Table.5 Statistical analysis

Sl no	Test	Sensitivity (%)	Specificity (%)
1	KOH	83.05	56.09
2	Calcofluor white	92.3	54.2

Culture was taken as gold standard because of high specificity and it is the only confirmatory test in routine use for identifying the species causing Onychomycosis. High false negative results in mycological culture may be due inappropriate or insufficient sample collection and inoculation of clinical specimen

Direct KOH mount is a rapid, simple and inexpensive test, which requires minimum technical aids (Baron *et al.*, 1990). When compared to fungal culture, KOH mount was able to detect more fungal agents from clinical specimens. In our study direct KOH mount showed 69% positivity which was higher than fungal culture (54%). When KOH mount was compared with culture, sensitivity and specificity of KOH mount was 83.05 and 56.09% respectively.

Calcofluor white is rapid, reliable, more sensitive but less specific than KOH mount microscopy. It is a non-specific Fluorescent whitener having ability to bind particularly on cellulose and chitin contained in the fungal cell wall. Cotton fibers will fluoresce strongly and must therefore be differentiated from fungal hyphae (Dass *et al.*, 2015). In this study Calcofluor white showed 92.3% sensitivity and

specificity of 54.2% when compared to fungal culture.

Fungal culture was the least sensitive method and Calcofluor white microscopy was the most sensitive method among the three methods used. These results are similar to the study of Dass *et al.*, (2015).

Out of the 54 culture positive isolates, *Trichophyton rubrum* was the most common isolate. *Trichophyton. rubrum* is the commonest cause of human fungal infection of skin and nail. The identification of the mould is necessary in patient management. This result is similar to the study of Elewski *et al.*, (1998).

It is difficult to interpret the role non dermatophyte moulds because they can often be recovered as contaminants from nail tissue, and also occasionally as pathogens. They should only be considered as pathogen, if hyphae are seen on direct microscopy and the same organism is repeatedly isolated (Elewski *et al.*, 1998).

Delay in the diagnosis of onychomycosis leads to total nail dystrophy, which may not regain its normal structure in spite of adequate therapy. It

is necessary to use appropriate laboratory techniques for early diagnosis of onychomycosis and help clinician in choosing the appropriate therapy. Calcofluor white is a most sensitive and excellent method to detect fungal agents from clinically suspected onychomycosis cases. It can be done in any laboratory having adequate infrastructure. In resource poor settings, KOH mount can be used as an alternative method

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