Comparison of KOH, Calcofluor White and Fungal Culture for Diagnosing Fungal Onychomycosis in an Urban Teaching Hospital, Hyderabad

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ABSTRACT

Background and Objective: *Onychomycosis is a fungal infection of the finger or toe nail apparatus caused by dermatophytes, non dermatophytemoulds and yeasts (mainly Candida species). Laboratory diagnosis of superficial fungal infection relies on direct microscopic examination of fungal elements in the clinical sample and mycological culture of the particular fungal species concerned. Quality of the sample and experience of the microbiologist are the major factors that determine the successful rate of microscopic examination and culture. All diagnostic laboratories should be able to discriminate between organisms that are likely pathogens and other contamination of the culture plate determine. A direct microscopic examination is the simplest, cheapest method used for the diagnosis of mycotic infections. In this study we want to provide detailed information on diagnosis of onychomycosis and compare the efficacy of common methods employed in microbiology laboratory. This study is valuable in establishing a reliable method for early information on diagnosis of onychomycosis that may be crucial for determining appropriate therapy for the successful treatment of onychomycosis patient.*

Methodology: *This is a cross sectional study conducted at Apollo General Hospital, Hyderabad. A total of 150 patients with clinical features suggestive of fungal infection were selected. All samples were subjected to KOH mount, calcofluor white and fungal culture. Sabourauds dextrose agar with chloramphenicol and cycloheximide were used for the growth of dermatophytes and incubated at 37 0 C for 3 weeks. Two tubes of SDA with chloramphenicol were used for the identification and incubated at 37 and 25 0 C for 3 weeks.*

Result: *Out of the 150 patients, direct microscopy with KOH mount, calcofluor mount and mycological culture showed positive results in 84(56%), 95(63.33%), 59(39.33%) respectively. Mycological culture was the least sensitive method and CFW was the most sensitive method among the 3 methods. Out of the 59 culture positive 27 were positive for Dermatophyte, 27 were non dermatophytic mould and 5 were Candida. Among non dermatophyticmould, Aspergillus was the most common isolate followed by Scopulariopsis. Culture was taken as the gold standard and when KOH mount microscopy was compared to the cul¬ture, sensitivity of KOH microscopy was 83.02% and specificity was 70.1%. When calcofluor white microscopy was compared to the cul¬ture, sensitivity of calcofluor white microscopy was 89.83 % and specificity was 60.44%.*

Conclusion: *In conclusion Calcofluor white is an excellent method to detect fungal agents from clinically suspected onychomycosis cases with high sensitivity and negative predictive value. It can be done in all clinically suspected onychomycosis in any laboratory having adequate technical aids. In resource poor settings, KOH mount serve as an alternative method with comparable sensitivity and negative predictive value.*

Keywords: *Onychomycosis, KOHmount, Calcofluor white, Trichophtonrubrum, Sabourauds dextrose agar*

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INTRODUCTION

Onychomycosis is a fungal infection of the finger or toe nail apparatus caused by dermatophytes, non dermatophyte moulds and yeasts (mainly Candida species) which results in discoloration, thickening, and separation from the nail bed. Clinical diagnosis based on physical examination alone is not a reliable method for onychomycosis. Accurate diagnosis is the mainstay for treating onychomycosis successfully.

Onychomycosis is classified clinically as distal and lateral subungual onychomycosis (DLSO), superficial white onychomycosis (SWO), proximal subungual onychomycosis (PSO), candidal onychomycosis and total dystrophic onychomycosis.

The dermatophytes are the commonest cause of superficial white onychomycosis and most common isolate belongs to T. mentagrophytes. .The occurrence of superficial white onycho mycosis is less common than DLSO and affects the surface of the nail plate rather than the nail bed. Proximal subungual onychomycosis (PSO) without evidence of paronychia is an uncommon variety of dermatophyte infection often related to Immune suppressed patients.

A large number of non-dermatophyte moulds can cause infection in traumatized nails; the most common fungal agents include *Scopulariopsis brevicaulis*, the *Aspergillus versicolor* group and *Fusarium spp*. However, nail tissue is often contaminated with fungal spores and isolation of a non-dermatophyte mould should only be considered significant if it is positive for direct examination and cultured from the given clinical sample.

For many fungal infections, a clinical examination of the affected person and microscopic examination of the sample may be sufficient to determine that a fungal infection is present.[1] Laboratory diagnosis of superficial fungal infection relies on direct microscopic examination of fungal elements in the clinical sample and mycological culture of the particular fungal species concerned. Quality of the sample and experience of the microbiologist are the major factors that determine the successful rate of microscopic examination and culture. All diagnostic laboratories should be able to discriminate between organisms that are likely pathogens and other contamination of the culture plate determine.

Direct microscopic examinations is the simplest, cheapest method used for the diagnosis of mycotic infections.[3] An inexperienced observer may very well misdiagnose the onychomycosis by observing certain artifacts such as cell walls as hyphae. The microscopic examination is usually done by KOH and calcofluor mount.[2-3] Direct mycological examination of specimens in suspected cases of onychomycosis provides early detection when compared to culture, which can take days or weeks. The CFW facilitates the identification of fungal structures, even in relatively small quantities,[4,5] and requires less experience of the observer.[1-4] Calcofluorwhite isaspecial fluorescent stainthatbindss tronglyto structures containing cellulose and chitin.[5]

In this study we want to provide detailed information on diagnosis of onychomycosis and compare the efficacy of common methods employed in microbiology laboratory.

This study is valuable in establishing a reliable method for early information on diagnosis of onychomycosis that may be crucial for determining appropriate therapy for the successful treatment of onychomycosis patient.[6]

1. Outcome of the study may enlighten the importance of rapid microscopic method in early diagnosis of fungal infections.

OBJECTIVES

1. To compare the sensitivity, specificity of rapid direct microscopic examination using culture as gold standard test.

MATERIALS AND METHOD

Methodology

Study design: cross sectional study
Sample population: Patients with clinical features suggestive of fungal infection attending the OPD of Apollo Institute of medical science and Research (AIMSR, General Hospital) will be enrolled in this study.
Sample size: A total of 150 patients with clinical features suggestive of fungal infection were selected. All samples were subjected to KOH mount, calcofluor white and fungal culture. Out of these 150 patients 120 were males and 30 were females. Their age ranged from 15 – 80 years.
Methodology: Nail clippings and nail scrapings were collected in black paper and divided into two parts; one part was used for direct microscopy and the other for culture. Rapid screening of the specimen was done by calcofluor white (*Sigma Aldrich*) fluorescent stain method, and by KOH microscopy using compound microscope.
Sample collection: Subungual debris should be taken from the most proximal part of the infection for onychomycosis caused by Dermatophytes. Dermatophyte infects nail bed rather than nail plate. In DLSO material can be obtained from beneath the nail. Onycholytic nail part can be cut back and material can be scraped off from the underside of the nail as well as from the nail bed.

Because of the paucity of fungal element in the clinical specimen, maximum amount of clinical material should be collected and submitted the mycology laboratory.
KOH mount: wet mount preparation in 10 – 20 % KOH was done to detect the presence of fungal elements.[7-10]
Calcofluor white:[5-10] one drop of calcofluor white stain (comprising 1g/L calcofluor white M2R and Evans blue Sigma Aldrich) would be added specimen on the slide. The slide was then left to stand for 10 minutes and was examined under fluorescence microscopy using blue light excitation (300-400 nm for the emission wavelength with excitation at around 355 nm).
Fungal culture: Sabourauds dextrose agar (SDA) with chloramphenicol and cycloheximide were used for the primary isolation of dermatophytes and incubated at 37^0C for 3 weeks.

Two tubes of SDA with chloramphenicol were used for the identification of other fungal species and the material would be directly inoculated onto the surface of Sabouraud's dextrose agar media and incubated aerobically at 25°C and 37^0C for 3 weeks. The plates would be examined daily during the first week and twice weekly during the next two

weeks. The isolates will be identified by standard laboratory procedures.

IMPLICATIONS

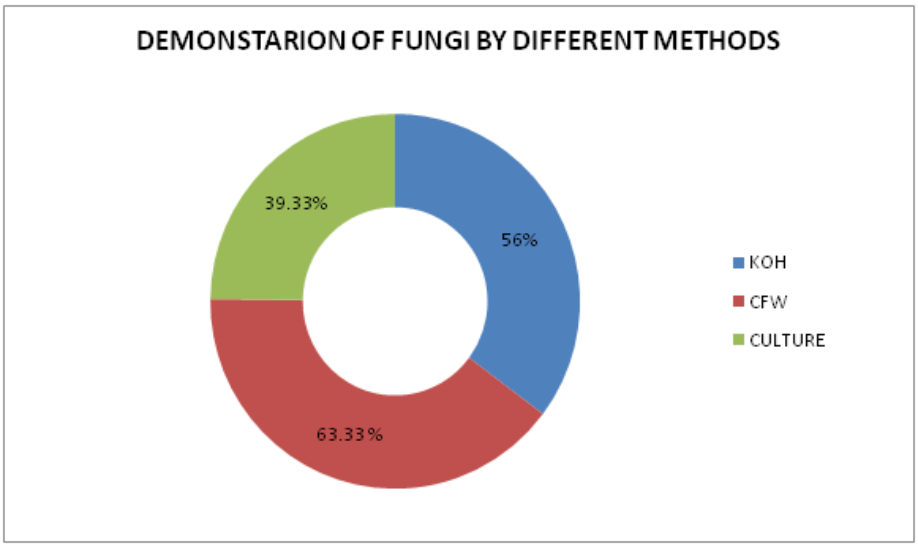
1. Early diagnosis of the fungal infection is important, so that the Physician can give effective and faster treatment.
2. To understand the importance of differentiating the fungal infections from bacterial infections as the symptoms produce by fungal and bacterial infections are almost similar.

RESULT

Out of the 150 patients, direct microscopy with KOH mount, calcofluor mount and mycological culture showed positive results in 84 (56%), 95(63.33%), 59(39.33%) respectively (**Table 1**). CFW was the most sensitive method among the 3 methods.

Table 1: Patient positivity by different methods

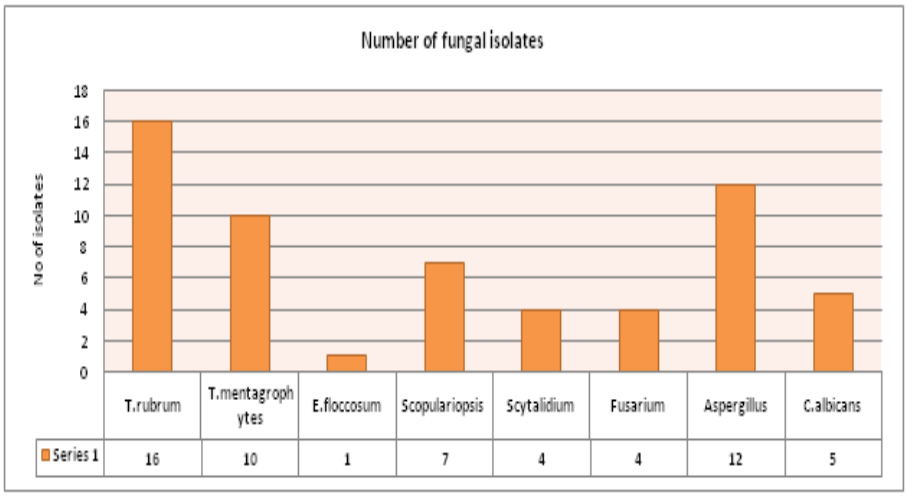
S. No.	Test	Positive (total no of samples = 150)	Percentage %
1	KOH	84	56%
2	CFW	95	63.33 %
3	CULTURE	59	39.33 %



RESULT OF MYCOLOGICAL CULTURE

Table 2

S. No.	Fungal Group	Species	Number of Isolates (Total No : 59)
1	Dermatophyte	Trichophyton rubrum	16
		Trichophyton mentagrophyte	10
		Epidermophyton floccosum	1
2	Non dermatophyte mould (NDM)	Scopulariopsis	7
		Scytalidium dimidiatum	4
		Fusarium	4
		Aspergillus	12
3	Yeast	Candida albicans	5



Out of the 59 culture positive 27 were positive for Dermatophyte, 27 were non dermatophytic mould and 5 were Candida. Among non dermatophytic mould, Aspergillus was the most common isolate followed by Scopulariopsis.

Table 3

Method	Microscopy & culture positive	Microscopy +ve & culture negative	Microscopy -ve culture + ve	Microscopy& culture - ve	Total
KOH	46	29	09	66	150
CFW	53	36	06	55	150

DETAILS OF TEST RESULTS

Table 4: KOH and culture

	KOH	Culture	No of patients
Two test positive	+	+	44
One test positive	+	–	29
One test positive	–	+	09
Two test negative	–	–	68
Total	**73**	**53**	**150**

Table 5: CFW and culture

	CFW	Culture	No of patients
Two test positive	+	+	53
One test positive	+	–	36
	–	+	06
Two test negative	–	–	55
Total	**89**	**59**	**150**

Table 2,3,4 and 5 shows the fungal culture and direct microscopy methods and sensitivity & specificity of staining methods relative to fungal culture results

Table 6: KOH mount and culture

	Gold std positive (culture)	Gold standard (Negative)
Test positive	TP = 44	FP=29
Test negative	FN=9	TN=68

Table 7: CFW and culture

	Gold std positive (culture)	Gold standard (Negative)
Test positive	TP = 53	FP=36
Test negative	FN=6	TN=55

Table 8

S. No.	Test	Sensitivity (TP/ TP+FN)	Specificity (TN/TN+FP)
1	KOH	83.02 %	70.1 %
2	CFW	89.83 %	60.44 %

Culture was taken as the gold standard and when KOH mount microscopy was compared to the culture, sensitivity of KOH microscopy was 83.02% and specificity was 70.1%. When calcofluor white microscopy was compared to the culture, sensitivity of calcofluor white microscopy was 89.83% and specificity was 60.44%.

Table 9

S. No.	Test	PPV (TP/ TP+FP)	NPP (TN/TN+FN)
1	KOH	60.27 %	88.31%
2	CFW	59.6%	90.16%

Positive predictive value (PPV) of KOH and cacoflour was found to be 60.27, 59.6% respectively when compared to culture. Negative predictive value (PPV) of KOH and calcoflour was found to be 88.31, 90.16% respectively when compared to culture.

RESULT

Table 10: Accuracy (TN+TP/TN+TP+FN+FP)

Comparison of Results of Microscopic Methods		
Method		
	KOH%	Calculuor%
Sensitivity	83.02 %	89.83%
Specificity	70.1%	60.44%
Positive Predictive Value	60.27%	59.6%
Negative Predictive Value	88.31%	90.16%
Accuracy	74.7%	72%

DISCUSSION

Onychomycosis is one of the most common fungal infections which pose difficult to treat using antifungal agents because of the inherent slow growth of the nail.[11]

Mycological isolation of Anthropophilic or zoophilic dermatophyte or Neoscytalidium dimidiatum from clinical specimen like skin, hair and nail clippings have good correlation with clinical condition irrespective of negative direct microscopic examination. Trichophyton tonsurans (anthropophilic fungus); an agent of tinea capitis is an exception because of carrier state.

Out of the 150 patients, direct microscopy with KOH mount, calcofluor mount and mycological culture showed positive results in 84(56%), 95(63.33%), 59(39.33%) respectively

Culture is 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 were reported in various studies and this may vary depending on the experience on the mycology laboratories and their isolation methods. High false negative results in mycological culture may be due to various other factors.

This may be due to

1. Non viability of certain fungal agents
2. In appropriate or insufficient sample collection (Nail clippings collected distal to the fungal disease)
3. Insufficient crushing of clinical material before subjecting for test

Direct KOH mount is a simple, rapid inexpensive point of care test, which requires minimum technical aids. Interpretation of KOH smears require some amount of experience.[7] When compared to mycological culture, KOH mount had the ability to detect more fungal agents from clinical specimens. In our study direct KOH mount showed 56% positivity which was higher than fungal culture (39.9%). False-negative rates are relatively high in KOH mount.[10] When KOH mount was compared with culture sensitivity and specificity of KOH mount was 83.02 and 70.1% respectively.

Calcofluor white showed 89.83% sensitivity and specificity was 60.44% when compared to mycological culture. Calcofluor white 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.

Key Advantages of Calcofluor over KOH mount

1. Minimum time required for screening the sample. KOH mount can take 3-4 minutes for screening each slide. CFW preparation can be

viewed or screened easily on low power objective (10X) and it can be completed within 1 minute.
2. Quick, reliable, more sensivity and specificity than KOH mount.
3. Background or debris material can be easily differentiated using CFC.

Mycological culture was the least sensitive method and CFW was the most sensitive method among the 3 methods.

Positive predictive value (PPV) of KOH and calcoflour was found to be 60.27, 59.6% respectively when compared to culture. Negative predictive value (PPV) of KOH and calcoflour was found to be 88.31, 90.16% respectively when compared to culture. Because of the high negative predictive value false positivity seen in other methods can be reduced by employing this method.

Trichophyton rubrum was the most common isolate. T. rubrum is the commonest cause of human fungal infection of skin and nail. The identification of the mold has important implication in patient management. This result is similar to previous studies where Trichophyton rubrum has been the most common Dermatophyte.[11,12,13] The increase in Trichophyton rubrum may be due to the affinity of fungus towards the keratin layer of nails. Anthropophilic dermatophytes tends to grow well at 37 0 C. The hot and humid environment within the shoe may have favored the fungus to cause infection in toe nail.

Non-dermatophyte moulds can often be recovered as contaminants from nail tissue, they should only be considered significant causes of infection if hyphae are seen on direct microscopy and a mould is isolated in pure culture from a significant number of inoculum points in the absence of a dermatophyte.

Delay in diagnosis or detection of onychomycosis can result in dystrophy of nail plate that may not be regaining its normal structure even after successful therapy.

CONCLUSION

It is important to recognize, identify and confirm onychomycosis due to non dermatophyte filamentous fungi as some of them may require a longer duration of treatment as compared to dermatophytes and a few others may not be responsive to even newer antifungal agents. Correct identification of the causative pathogen is imperative to aid clinicians in choosing the appropriate therapy. In conclusion Calcofluor white is an excellent method to detect fungal agents from clinically suspected onychomycosis cases with high sensitivity and negative predictive value. It can be done in all clinically suspected onychomycosis in any laboratory having adequate technical aids. In resource poor settings, KOH mount serve as an alternative method with comparable sensitivity and negative predictive value.

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