Original Research Article

Comparative evaluation of KOH mount, fungal culture and PAS staining in onychomycosis

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ABSTRACT

Background: Onychomycosis is a common ailment associated with significant physical and psychological morbidity. Increased prevalence in the recent years has attributed to enhanced longitivity, co morbid conditions like diabetes, avid spots participation and emergence of HIV. The objective of the study was to evaluate KOH mount, fungal culture and PAS staining of affected nail plate in diagnosis of onychomycosis.

Methods: The present study was carried out in OPD of Dermatology, PESIMSR, Kuppam, Andhra Pradesh during the period of October 2010 to October 2012 including all patients showing classical clinical features of onychomycosis.

Results: Majority of patients were from 21-40 years age group i.e. 46%. 56% of finger nails were commonly involved than 36% toe nails. DLSO is the most common clinical variant accounting for 80%. Most common isolated species as *Trichophyton rubrum* (31.6%) followed by *T. mentagrophytes* and *Aspergillus niger* 15.8% each. The sensitivity of KOH (81.82%), PAS (84.56%) and culture 57%. Both PAS and KOH were more sensitive than culture (p<0.001). PAS was also more sensitive than KOH. The specificity of KOH 92.86%, culture 92.86% and PAS 57.14%.

Conclusions: Fungal culture though is the gold standard for the identification of pathogens but it takes minimum 2-3 weeks. Histopathological examination of nail clippings with PAS stain is simple, highly sensitive screening test.

Keywords: KOH mount, Fungal culture, PAS, Onychomycosis

INTRODUCTION

Onychomycosis is defined as fungal infection of nail by any fungus including non-dermatophytes and yeasts. Onychomycosis is a common ailment associated with significant physical and psychological morbidity. Increased prevalence in the recent years has attributed to enhanced longitivity, co morbid conditions like diabetes, avid spots participation and emergence of HIV.[1]

Clinically onychomycosis is classified into various types including (i) distolateral subungal onychomycosis (DLSO); (ii) superficial white onychomycosis (SWO); (iii) proximal subungal onychomycosis (PSO); (iv) candida onychomycosis (CO); and (v) total dystrophic onychomycosis (TDO).[2] Onychomycosis is a growing global health problem and mainly due to dermatophyte, non dermatophyte, molds or yeast. The term Tinea unguium applied when infection is due to dermatophyte

The prevalence of the disease is rising worldwide and ranges from 2.1% to 9.1%.[3,4.]

The risk of onychomycosis is 1.9 to 2.8 times higher in persons with diabetes compared with the general population. In patients with human immunodeficiency virus (HIV) infection, the prevalence ranges from 15% to 40%. There is higher prevalence of dermatophyte in temperate zone and moulds such as *Aspergillus* species and *Fusarium* species found in tropical and subtropical countries.[5-7]

Direct microscopic of affected nails in KOH does not allow to recognition of type of fungus and culture is needed for specific diagnosis.[8] Routine histopathological examination of nail clippings with standard hematoxylin and eosin (H&E) stained sections are not considered for the diagnosis of onychomycosis. It has been documented that the periodic acid-Schiff (PAS) stain is a sensitive method and has been alternate to be superior to culture and potassium hydroxide preparation for the diagnosis of onychomycosis. Increasing reliance on PAS staining makes it a candidate for apparent "gold standard" in diagnosis of onychomycosis.

Objective

The objective of the study was to evaluate KOH mount, fungal culture and PAS staining of affected nail plate in diagnosis of onychomycosis.

METHODS

The present study was carried out in OPD of Dermatology, PESIMSR, Kuppam, Andhra Pradesh during the period of October 2010 to October 2012 including all all patients showing classical clinical features of onychomycosis.

Inclusion criteria

Patients of all ages and both sexes with the classical clinical features of onychomycosis and willing to take part in study after written consent.

Exclusion criteria

Patients of peripheral vascular disease, uncontrolled diabetes and connective tissue disorder were excluded. Also those who are already treated with topical or systemic antifungal agents were excluded.

After recording details of the patients, the nail clippings were taken and subjected for KOH mount, PAS staining and culture. The results were recorded and entered in MS excel sheet. Analysis of data was done by using Epi info software. Results were expressed as percentages. Chi square/Fischer's exact test was used to find out significance of study parameters on categorical scale between two or more groups.

RESULTS

Table 1 shows that majority of patients were from 21-40 years age group i.e. 46% followed by 32% from 41-60 years age group. Mean age of study population was 41.34±18.75 years.

Table 1: Age and sexwise distribution of patients.

	Number	Percentage (%)
Age (in years)		
< 20	5	10.0
21-40	23	46.0
41-60	16	32.0
61-80	6	12.0
Sex		
Male	27	54.0
Female	23	46.0

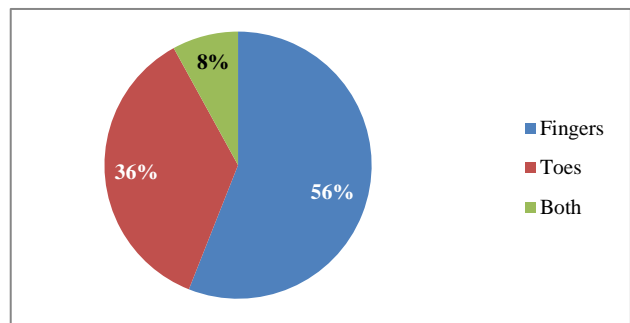In our study 27 i.e. 54% were male and 23 i.e. 46% were females.

Figure 1: Pie diagram showing distribution according to involvement of nail.

In our study, 56% of finger nails were commonly involved than 36% toe nails (Figure 1). This has been attributed with greater burden of wet work with increased trauma facilitating the entry of fungal pathogens.

Table 2: Distribution of clinical variants of the patient studied.

	Number	Percentage (%)
DLSO	40	80.0
PSO	3	6.0
WSO	2	4.0
CO	4	8.0
Mixed	1	2.0
Total	50	100.0

In our study, DLSO is the most common clinical variant accounting for 80% followed by CO 8%, PSO 6%, WSO 4% and mixed 2% (Table 2).

In our study, all three tests were found to be positive in 18 i.e. 36% patients (Table 3).

Table 3: Distribution of mycological findings of the patients.

Mycological findings	Number	Percentage (%)
All 3 tests negative	4	8.0
Only KOH positive	1	2.0
Only culture positive	3	6.0
Only PAS positive	2	4.0
KOH and culture positive	4	8.0
KOH and PAS positive	7	14.0
PAS and culture positive	11	22.0
KOH, culture and PAS positive	18	36.0
Total	50	100.0

Table 4: Distribution of causative organisms isolated from SDA agar culture.

Organisms isolated	Number	Percentage (%)
Trichophyton rubrum	12	31.6
Trichophyton mentagrophytes	6	15.8
Aspergillus fumigatus	3	7.9
Aspergillus niger	6	15.8
Aspergillus flavus	5	13.2
Pencillium species	1	2.6
Microsporum species	1	2.6
Candida albicans	4	10.5
Total	38	100.0

Our study shows that most common isolated species as *Trichophyton rubrum* (31.6%) followed by *T. mentagrophytes* and *Aspergillus niger* 15.8% each. Less commonly isolated species were *Aspergillus flavus* (13.2%), *candida albicans* (10.5%), *Aspergillus fumigates* (7.9%), *Pencillium* species and *microsporum* species 2.6% each (Table 4).

In Table 5, when we compared KOH with culture, KOH has 25 true positive and with PAS, 18 were true positive. When we compared PAS with culture, PAS has 39 true positive.

It is difficult to compare any two tests in our study because we did not consider any one of the three tests as gold standard and we did not have control group to compare. So we can only explain the correlation by comparing two tests with the other when one test is taken as gold standard. For example, when KOH is taken as standard, we can correlate the results of PAS and culture (PAS vs culture), in this, PAS has 39 true positive, 6 false positive, 7 false negative and 8 true negatives.

The sensitivities of each test were as follows: KOH (81.82%), PAS (84.56%) and culture 57%. Both PAS and KOH were more sensitive than culture (p<0.001). PAS was also more sensitive than KOH. The specificities were as follows: KOH 92.86%, culture 92.86% and PAS 57.14%. PPV of the tests were shown as KOH 69.23%, culture 96.15% and PAS 86.67%. NPV of the tests were shown as KOH 83.33%, culture 86% and PAS 69% (Table 6).

Table 5: Correlation of KOH, PAS and culture findings in mubers.

	True positive	False positive	False negative	True negative	Total
KOH vs culture	25	1	11	13	50
KOH vs PAS	18	8	4	20	50
PAS vs culture	39	6	7	8	50
PAS vs KOH	18	8	20	4	50

Table 6: Diagnostic accuracy of KOH, PAS and culture findings.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy(%)	P value
KOH vs culture	69.44	92.86	96.15	54.17	76	0.0001
KOH vs PAS	81.82	71.43	69.23	83.33	76	0.0001
PAS vs culture	84.56	57.14	86.67	53.33	70.33	0.0001
PAS vs KOH	71.43	81.82	83.33	69.23	76	0.0001

DISCUSSION

Onychomycosis affects all age groups. In our study the major age group affected is 20-40 years. The incidence may be higher in elderly in our country but the disease being mostly asymptomtic and elderly being dependent upon others for medical help, they may not be presenting to the hospitals.

Age and sex

In a study by Taniwala, 39 (97.5%) of the 40 cases of onychomycosis occurred in patients older than 20 years with peak incidence of 11 (27.5%) cases each during 4th and 5th decade.[8] 22.5% cases aged between 21-30 years and 20% between 41-50 years age. In our study the distribution of onychomycosis in less than 20 years age was 10%. 21-40 years was 46%, 41-60 years was 22%.

Adhikari et al also found higher prevalence in 3rd decade i.e.58.8%.[9] This could be due to increased exposure to occupation related trauma.[8]

Grover reported 56% cases of onychomycosis between 20-40 years age in his study, 16% were in 6th decade and 14% in 5th decade.[10]

Out of 50 cases of onychomycosis in our study (Table 1), 27 i.e. 54% were male and 23 i.e. 46% were females in comparison with Taniwala which stated 82.5% males and 17.5% females.[8] Majority of Indian studies support male preponderance. Onychomycosis in our study was found to be more in males than in females. However candidial onychomycosis was more common in females. This can be attributed to greater burden of wet work with increased trauma facilitating easy entry of pathogens.

Nail involvement

In our study, 56% of finger nails were commonly involved than 36% toe nails (Figure 1). This has been attributed with greater burden of wet work with increased trauma facilitating the entry of fungal pathogens. Most of the studies from India showed that finger nail onychomycosis is more common than toe nail onychomycosis. Our observation correlates with Madhuri et al who also reported an increased involvement of finger nails.[11] The low incidence of toe nail onychomycosis in our study may be attributed to open footwear and lesser concern for appearance of feet and toenails in our population.

Clinical variant

In our study as given in Table 2, DLSO is the most common clinical variant accounting for 80% followed by CO 8%, PSO 6%, WSO 4% and mixed 2%. In a study conducted by Ram Taniwala[8], DLSO was reported to be most common variant (65%). Ahmed also reported DLSO as common variant with 67% patients.[12]

Culture isolates

Our study (Table 4) shows that most common isolated species as *Trichophyton rubrum* (31.6%) followed by *T. mentagrophytes* and *Aspergillus niger* 15.8% each. Less commonly isolated species were *Aspergillus Flavus* (13.2%), *Candida Albicans* (10.5%), *Aspergillus Fumigates* (7.9%), *Pencillium* species and *Microsporum* species 2.6% each. Altough *T. mentagrophyte* was reported to be usually associated with toenail but *T. rubrum* is still more common than *T. mentagrophytes* in toenail onychomycosis.[12]

Diagnostic accuracy

The sensitivities of each test were as follows: KOH (81.82%), PAS (84.56%) and culture 57%. Both PAS and KOH were more sensitive than culture (p<0.001). PAS was also more sensitive than KOH. The specificities were as follows: KOH 92.86%, culture 92.86% and PAS 57.14%. PPV of the tests were shown as KOH 69.23%, culture 96.15% and PAS 86.67%. NPV of the tests were shown as KOH 83.33%, culture 86% and PAS 69%. (Table 5 and 6).

Hassab et al reported the sensitivity of KOH as 75% and PAS as 57.5% with no statistical significance between both.[13] On the other hand, Haiso et al reported the sensitivity of KOH as 87% and PAS as 81%.[14] Weinberger et al investigated 105 patients with KOH mount, culture and PAS staining and reported sensitivities to be 80%, 59% and 92% respectively.[15]

CONCLUSION

KOH mount can be done as screening test and routine investigation in the diagnosis of onychomycosis since it simple, time saving and cost effective when compared with PAS. PAS is more sensitive than KOH and culture, but by this test it is not possible to find the specific pathogen or species. Fungal culture though is the gold standard for the identification of pathogens but it takes minimum 2-3 weeks and false positive results are common with fungal culture. Histopathological examination of nail clippings with PAS stain is simple, highly sensitive screening test.

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