COMPARISON OF YIELDS OF KOH MOUNT AND PAS STAINING OF NAIL CLIPPINGS FOR HYPHAE IN CASES OF ONYCHOMYCOSIS

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ABSTRACT

Objective: To compare the diagnostic yields of KOH mount and PAS staining of nail clippings for demonstration of hyphae.

Study Design: Cross sectional study.

Place and Duration of Study: Study was conducted at dermatology department, POFs' Hospital Wah Cantt. The duration of the study was one year, from Aug 2016 to Aug 2017.

Material and Methods: The sample size of 60 patients was calculated using WHO calculator. Non probability consecutive sampling was used for recruitment of participants. Patients of any age and of either sex with clinical suspicion of onychomycosis were included in the study. Nail clippings of affected nails of each subject were examined for hyphae after KOH mount and PAS staining. SPSS software was used for data analysis. Chi-square test and ROC curve analysis were used for diagnostic accuracy measurement.

Results: Out of sixty patients, hyphae were seen in 49 (81.66%) after PAS staining and in 19 (31.66%) after KOH mount. PAS staining is found to be more sensitive (80.3%) for diagnosis of onychomycosis as compared to KOH (29.4%) (p=0.02). ROC curve analysis showed PAS had good diagnostic accuracy (ROC curve area=0.80) while KOH had poor diagnostic accuracy (ROC curve area=0.60).

Conclusion: PAS staining of nail clippings for hyphae is a very sensitive method for diagnosis of onychomycosis as compared to KOH mount. The current gold standard needs to be reviewed.

Keywords: KOH mount, Nail clippings, Onychomycosis, PAS staining.

INTRODUCTION

Onychomycosis is a common dermatological problem worldwide and represents almost half of all nail disease burden[1,2]. A high prevalence has made it an important public health problem and incidence has been on the rise due to an expanding elderly population worldwide and increase in the number of immuno compromised patients[3]. Dermtophytes appear to be the chief organisms responsible for the infection though onychomycosis can be caused by non dermatophyte moulds and yeasts also[4]. It can clinically resemble other diseases involving the nails like psoriasis and lichen planus, onychogryphosis and nail trauma[3]. Particularly nail changes due to psoriasis and those of dermatophyte infection are difficult to differentiate clinically[5]. International guidelines recommend confirmatory testing before initiation of treatment though empirical treatment with certain agents has been found to be more cost effective[6]. Direct microscopic examination of a wet-mount preparation with potassium hydroxide along with fungal culture has been considered the gold standard for diagnosis of onychomycosis[7,8]. However, yield of fungal culture is still low[9]. Most of the dermatologists rely on clinical diagnosis before starting treatment because of their busy OPDs or a poor yield of KOH mount for hyphae. Several studies have suggested that Periodic acid Schiff staining of nail clippings may be a very sensitive method for diagnosis of onychomycosis[7,10,11]. Nail clipping of the distal nail plate is easy to perform and is harmless and painless for the patient[8]. We conducted a comparison of yields of KOH mount

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and PAS staining for hyphae in suspected cases of Onychomycosis in our hospital.

MATERIAL AND METHODS

It was a cross sectional study with duration of one year, from Aug 2016 to Aug 2017. Study was conducted at the department of dermatology, POFs' hospital Wah Cantt. A sample size of 60 patients was calculated with 95% confidence interval, 5% significance level and 8% prevalence using WHO calculator. Patients with the clinical suspicion of onychomycois were selected after an informed consent from the outpatient department. These patients were selected by non-probability consecutive sampling of any age group and of either sex having finger and/or toe nail involvement. Onychomycois was defined as clinical morphologic features suggestive of the disease plus at least one positive test result. Known cases of psoriasis or lichen planus having nail involvement were excluded from the study. Demographic and clinical features of the patients were recorded. Nail clippings were taken with the help of steam autoclaved nail clipper from the diseased free edge of the nail as proximally as possible. Before taking the specimen the area was cleaned with 70% Ethyl Alcohol. The specimen was divided into two parts. For KOH mount, 20% KOH was added to the finely grained specimen and heated to dissolve the keratin and then examined for hyphae by the histopathologist of our hospital. For PAS staining, specimens were first treated with nitric acid to decalcify and soften them and later paraffin embedded sections of nail samples were stained with PAS and then examined for hyphae by the histopathologist. The study was approved by the ethics and scientific committee of the hospital. Computer program SPSS-22 was used to manage and analyze the data. Frequencies and percentages were obtained for the variables where applicable. Mean and standard deviations were calculated for continuous variables. Chi-square test and ROC curve analysis were used for measuring diagnostic accuracies of PAS staining and KOH mount.

Table-I: Association between disease (Onychomycosis) and tests (KOH & PAS Staining).

KOH Mount	Onychomycosis		Total	*p*-value
	Positive	Negative		
Positive	15(25%)	4(6.7%)	19(31.7%)	0.445
Negative	36(60%)	5(8.3%)	41(68.3%)	
PAS Staining				
Positive	41(68.3%)	8 (13.3%)	49(81.7%)	0.02
Negative	10(16.7%)	1(1.7%)	11(18.3%)	
Total	51(85%)	9(15%)	60(100%)	

Table-II: Diagnostic characteristics of KOH and PAS staining.

Diagnostic Parameters	KOH (C.I 95%)	PAS Staining (C.I 95%)
Sensitivity	29.4%	80.3%
Specificity	55.5%	11.1%
Positive predictive value	78.9%	83.6%
Negative predictive value	12.1%	9%
Accuracy	33.3%	70%

RESULTS

A total of 60 cases of any age group and of either sex were taken. There were 44 (73.33%) toe nail and 16 (26.66%) finger nail samples. There were six patients who were known diabetics. There were 32 males and 28 females. The age group ranged from 17 to 68 years. The mean age was 35.62 years (fig-1).

Among all those who were KOH test negative 41 (68.3%), 36 (60%) were disease positive while 5 (8.3%) were disease negative.

Similarly among all those patients who were KOH positive 19 (31.7%), 15 (25%) were disease positive while 4 (6.7%) were disease negative (p=0.44). Among all PAS negative patients 11 (18.3%), 1 (1.7%) was disease negative and 10 (16.7%) disease positive. Similarly among all PAS positive patients 49 (81.7%), 41 (68.3%) were disease positive and 8 (13.3%) were disease negative (p=0.02) (table-I).

The present study found a sensitivity of 29.4% with KOH mount and 80.3% with PAS staining. Specificity of KOH mount was 55.5% and 11.1% for PAS staining (table-II). Positive predictive value of KOH mount was 78.9% while it was 83.6% for PAS staining. A negative predictive value for KOH was 12.1% and 9% for PAS. However, positive likelihood ratio was 2.01 vs 2.09 and negative likelihood ratio was 0.76 vs 0.001 in KOH and PAS staining respectively (fig-2).

Area under ROC curve of KOH is 0.60 representing poor accuracy while area under ROC curve of PAS staining 0.80 represents good accuracy (fig-3).

DISCUSSION

Onychomycosis constitutes a common dermatological disease. Multiple factors like an increased prevalence of diabetes and peripheral vascular disease and an increasing geriatric population and early participation in sports may increase the burden of onychomycosis in both children and adults[13]. Six distinct patterns of

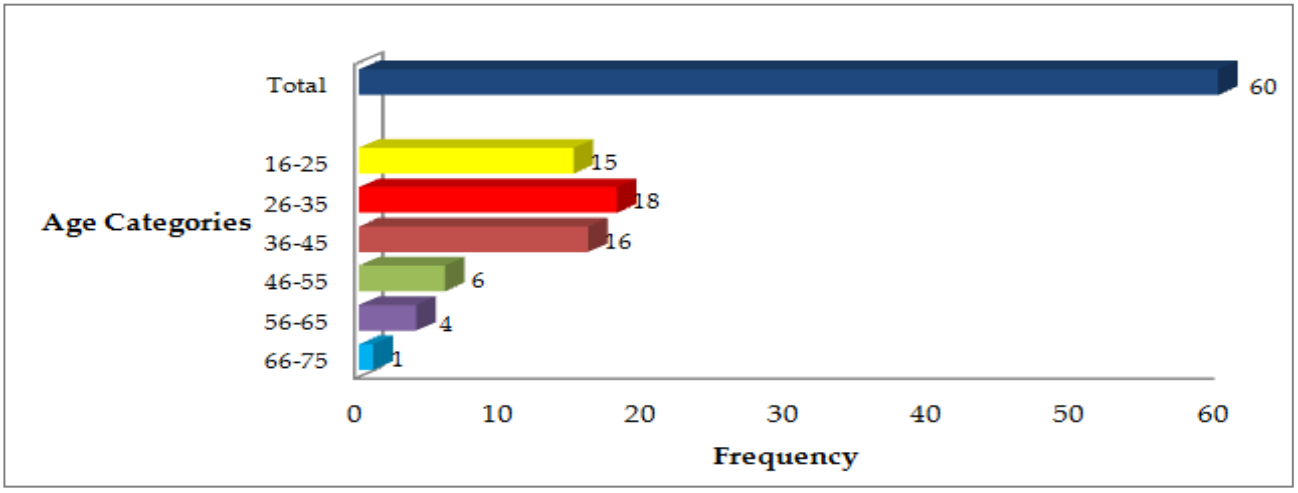

Figure-1: Age distribution of participants.

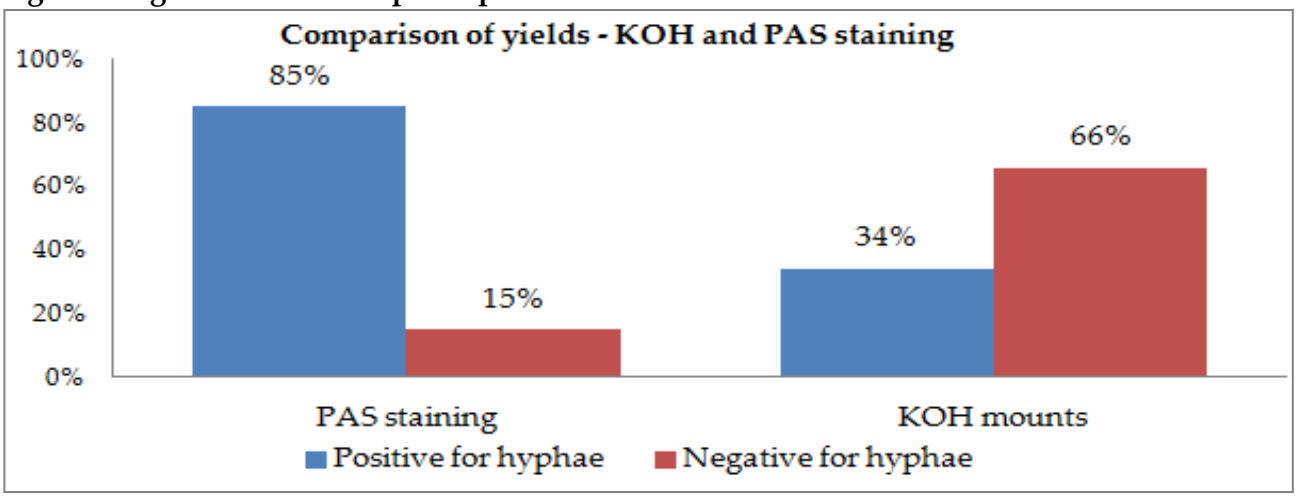

Figure-2: Comparison of yields of KOH mount and PAS staining for hyphae.

onychomycosis caused by dermatophytes have been described[14]. Distal and lateral subungual onychomycosis (DLSO), proximal subungual onychomycosis (PSO), superficial onychomycosis (SO), endonyx onychomycosis, total dystrophic onychomycosis (TDO) and mixed onychomycosis. DLSO is the commonest pattern seen[15,16,17]. The most frequent clinical features are thickening and opacification of the nail with greyish white and yellow brown discolouration as clinical hallmarks[4,18]. The non dermatophyte moulds should be considered in culture negative cases or those with treatment failure[19]. Some newer diagnostic modalities like confocal laser scanning microscopy, conventional PCR and real time PCR

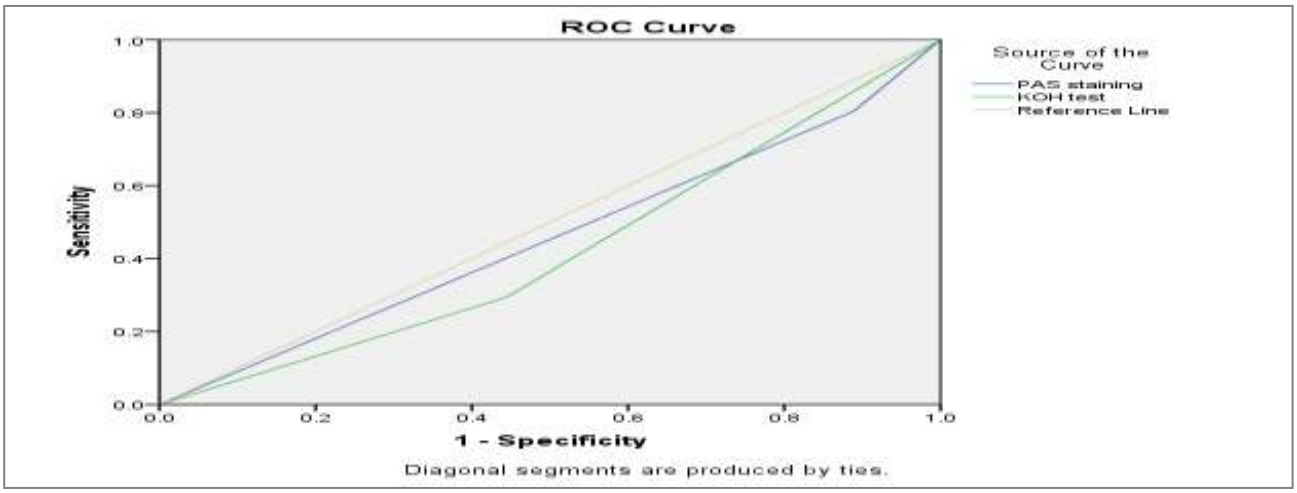

Figure-3: ROC curve analysis.

though have been found to be effective but are very expensive, have limited availability and can only be performed by highly skilled staff[20,21,22]. Dermoscopy has been used as a diagnostic aid but cannot be relied upon as the only diagnostic criterion[1]. It has also been used to identify the best location on the nail for a mycological sample[23].

In our study yield of PAS staining (80.3% sensitivity) was comparable to other studies[7,11,12] but the yield of KOH mount was low, which is an indicator that this simple office based procedure is practiced less often resulting in low level of expertise. PAS staining though is a more useful test with a high yield but needs the help of a histopathologist and can only be done in an established histopathology department. In a study by Jung et al[7] the sensitivities for KOH mount and PAS staining were 55.9% and 88.2% respectively. In another study by Jeelani et al[12] direct microscopy with KOH and DMSO was positive in 63.9% and PAS staining in 76% of the samples.

In a study by Mayer et al[10] out of 100 consecutive cases of suspected onychomycosis which didn't show any fungal element on direct microscopy or cultures showed hyphae in 38% after PAS staining. In yet another study by Wilsman Theis et al sensitivities of PAS staining, KOH and fungal culture were 82%, 48% and 53% respectively[11]. In another study by Machler et al routine histologic examination with PAS stain was found to be equal to culture and superior to KOH in leading to a diagnosis of dermatophyte infection of nail[5].

LIMITATION OF STUDY

The study sample size was small that may have over estimated the sensitivity of PAS staining. Study was conducted at single center that limits its generalizability.

CONCLUSION

PAS staining of nail clippings for hyphae is a very sensitive method for diagnosis of

onychomycosis as compared to KOH mount. The current gold standard needs to be reviewed.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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