ORIGINAL ARTICLE

Comparative study for the reliability of potassium hydroxide mount versus nail clipping biopsy in diagnosis of onychomycosis

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ABSTRACT

Introduction: The clinical pictures of nail dystrophies caused by different fungi are seldom diagnostic. Mycological confirmation with appropriate diagnostic techniques including direct microscopy and fungal culture is important to ensure correct diagnosis and treatment. Microscopy can be performed using potassium hydroxide (KOH) mount and histological evaluation of nail plate specimens with the periodic acid - Schiff (PAS) method.

Aim: This study was designed to compare KOH/DMSO (Dimethylsulfoxide) preparation with PAS of nail clippings in the diagnosis of onychomycosis.

Patients and methods: Forty patients with clinically suspected onychomycosis were included. Every patient was subjected to clipping of the distal free edge of the nail plate, along with any attached subungual debris for PAS staining and histopathological examination. Another enough sample of subungual debris to which a drop of KOH/DMSO solution was added on a slide and examined using × 10 and × 40 magnification. Both PAS stained and KOH/DMSO mount samples were evaluated for the presence of hyphae and/or spores.

Results : Among the 40 patients, only 30 proved to be KOH positive while 23 of PAS stained samples were positive. KOH mount has a sensitivity of 75% versus 57.5 % for PAS staining. Statistically, there was no significant differences between the two methods regarding test sensitivity (p=0.098).

Conclusion: KOH test could be considered as a first line preliminary tool in diagnosis of onychomycosis while PAS staining can be used as a confirmatory method.

KEYWORDS: Onychomycosis, KOH mount, periodic acid - Schiff (PAS)

INTRODUCTION

Onychomycosis (OM) is the most common nail disorder and is present in 2% to 13% of general population increasing up to 48% by 70 year of age.[1]

Onychomycosis generally caused by dermatophytes is often symptomatic and can cause functional impairment. The clinical presentation of OM involves hyperkeratosis with thickening and discoloration of the nail plate. Other disorders such as nail psoriasis, lichen planus, OM and nail trauma may yield a nearly identical picture.[2]

An accurate diagnosis of OM is important because systemic antifungal therapy is often indicated which carries an additional expense and is occasionally associated with adverse events and drug interactions.[3]

It is necessary to confirm the clinical diagnosis with sufficient laboratory evidence before initiating oral antifungal treatment. Direct demonstra-

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tion of fungal elements with KOH mount and the isolation of fungus by culture are the routinely done laboratory methods. The method of obtaining nail clippings and the size of specimen are also important factors while considering these tests.[4]

The histopathologic diagnosis often relies on analysis of nail plate specimens with the assistance of special stains. Pathologists utilize periodic acid schiff (PAS) and/or Gomori methenamine silver (GMS) stains to highlight fungi within the nail plate.[5]

The present study was aimed to compare KOH preparation and PAS stained nail clippings to define an easy and reliable method for the diagnosis of OM.

PATIENTS AND METHODS

Forty patients clinically suspected to suffering from OM were included in this study and selected after full history taking, general & local examination. They were 29 males and 11 females aged between 12 to 72 years. Patients that have received systemic antifungal treatment for the last four weeks and/or topical antifungal medications for the last two weeks were excluded.

Every patient was subjected to; 1) full history taking included occupation, special habits, residence and any previous treatment. 2) nail examination for clinical type of OM: discoloration, dystrophy, subungual hyperkeratosis, onycholysis and nail plate thickening. 3) clipping of the distal free edge of the nail plate, along with any attached subungual debris for PAS staining and histopathological examination. 4) Another enough sample of collected subungual debris to which 20% KOH was added on a glass slide and examined under microscope using × 10 and × 40 magnification.

Nail clippings and PAS staining

Nail clippings were fixed in 10% formalin and then treated with 4% phenol for softening. Specimens were processed and embedded in paraffin blocks. About 3-µm thin slices were taken and mounted on glass slides. PAS staining was then performed.

KOH mounts

Specimen was placed on a clean glass slide, and a drop of 20 % KOH / 40% Dimethyl Sulfoxide (DMSO) mixture was added (DMSO increases sensitivity of the preparation and softens keratin more quickly than KOH alone in the absence of heat[6]. A cover slip was applied with gentle pressure to drain away excess solution. The sample was kept for 30 minutes and then examined thoroughly for the presence of filamentous, septate, branched hyphae with or without arthrospores. Both PAS stained and KOH/DMSO mount samples were evaluated for the presence of hyphae and/or spores. Cases showing hyphae and/or spores were considered positive. Query cases were repeated for confirmation.

STATISTICAL ANALYSIS

Data were processed and statistically analyzed using SPSS version 17 under the platform of Microsoft windows XP. Sensitivity was defined as the proportion of people with disease who had a positive test result. The different performance tests were compared using the chi-square test. P value < 0.05 was considered statistically significant.

RESULT

From 40 patients with clinically suggestive OM, 29 were men (72.5 %) and 11 were women (27.5 %) with male to female ratio of2.64 : 1. Their ages ranged from 12 to 72 years (mean ± SD; 45.9 ± 18.5).

Fingernail affection constitutes half of cases (50%) followed by toenail affection in 16 cases (40%) and simultaneous fingernail and toenail affection in 4 cases (10%) (Tabel 1).

Distal-lateral subungual OM (DLSO) is by far the dominant presenting pattern; 38 cases out of 40 (95%), while proximal subungual and total dystrophic OM are represented by only 1 case for each (Tabel 2, Fig 1 A, 1B, 1C).

Out of the 40 patients, direct microscopy with KOH/DMSO mount showed positive results in 30 cases (75%) versus 23 (57.5%) with histopathologic PAS staining. All the 23 PAS- positive samples were also KOH positive. Fungal elements were detected by both methods revealing filamentous, septate, branched hyphae with or without arthrospores (Fig. 2A, 2B, 2C and Fig 3A, 3B, 3C, 3D, 3E).

Table 1 Site of nail affection in the studied cases (n=40)

Site of nail affection	Number	Percentage
Fingernails	20	50%
Toenails	16	40%
Finger and toenails	4	10%

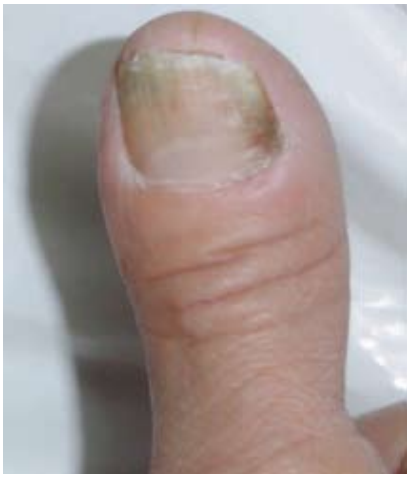

Fig. 1A. DLSO

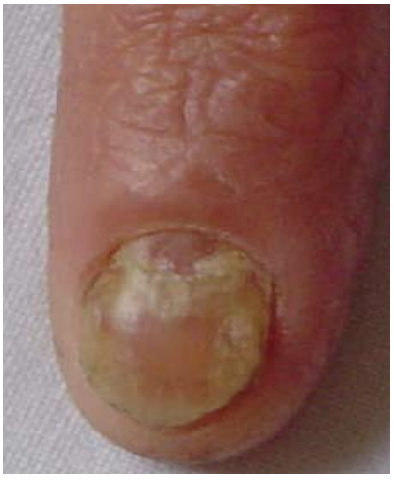

Fig. 1B. PSO

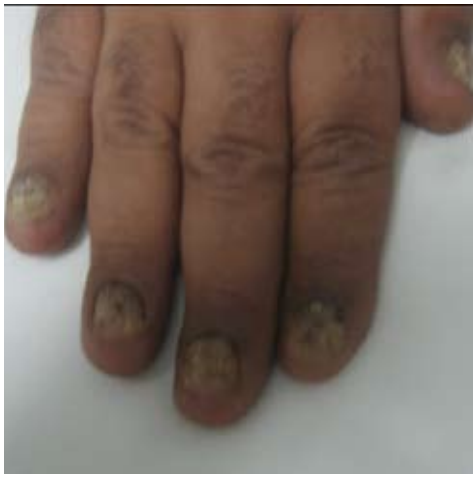

Fig. 1C. TDO of all fingernails

Table 2 Clinical patterns of OM in the studied cases (n=40)

Clinical patterns of OM	Number	Percentage
DLSO	38	95%
PSO	1	2.5%
TDO	1	2.5%

DLSO: Distal-lateral subungual OM, PSO: Proximal subungual OM, TDO: Total dystrophic OM.

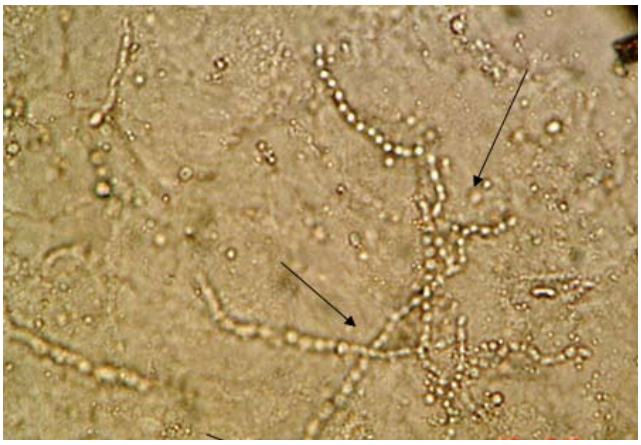

Fig. 2A. Nail KOH mount showing interlacing branching hyphae (KOH mount x 400).

The KOH mount has a sensitivity of 75% while PAS staining has a sensitivity of 57.5%. Statistically, there was no significant differences between the two methods regarding test sensitivity (p=0.098) (Table 3).

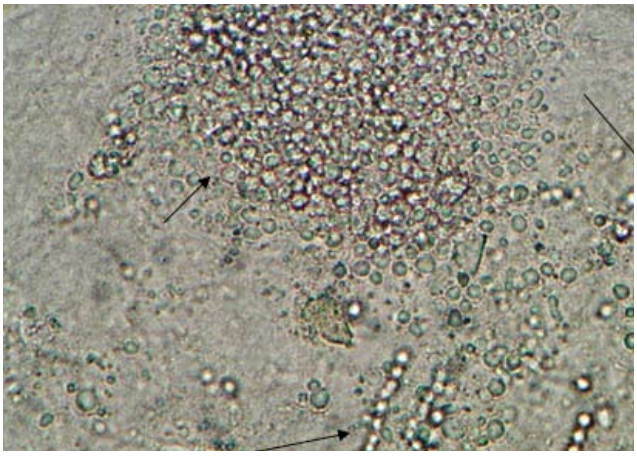

Fig. 2B. Nail KOH mount showing hyphae and arthrospores (KOH mount x 400).

DISCUSSION

The reported sensitivity of fungal culture varies from 25% to 80% with approximately 30% false-negative results with culture and KOH studies[7]. In fact, false-negative results are obtained in about 10% of the nail specimens under direct micro scopic examination of KOH preparations, while the culture test suffers from a very high level of misleading results with a false-negative results represent at least 20% of the cases and may rise up to 35%[8].

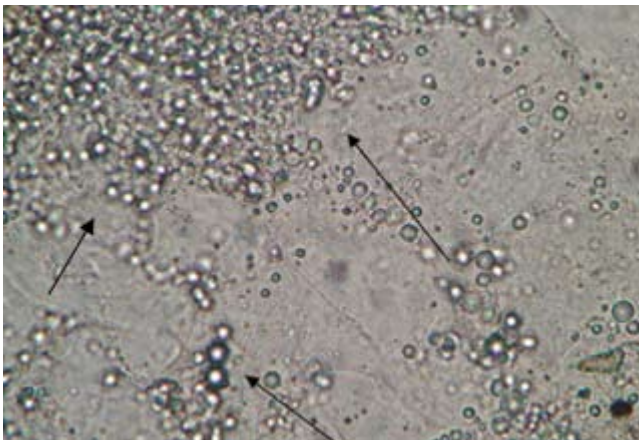

Fig. 2C. Nail KOH mount showing arthrospores (KOH mount x 400).

The high false-negative with the possibility of false-positive results of these standard diagnostic procedures are unacceptable; hence there is need for a diagnostic test with higher sensitivity and accuracy. There are multiple reports on histopathologic examination with PAS staining of nail clippings as a highly reliable diagnostic procedure for OM.[7, 9-12]

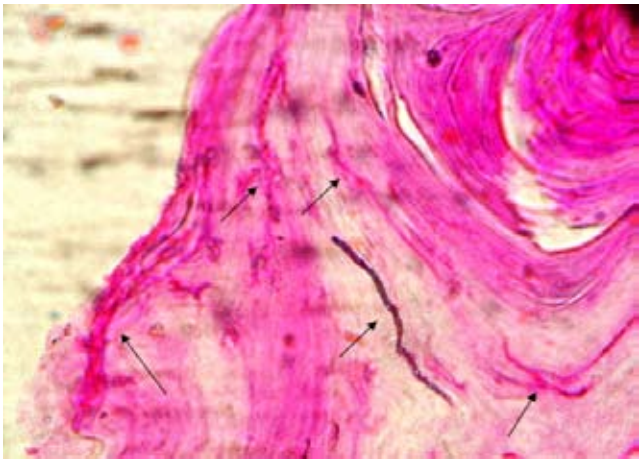

Fig. 3A. PAS-positive branching intensely stained reddish hyphae within the nail (PAS staining x 200).

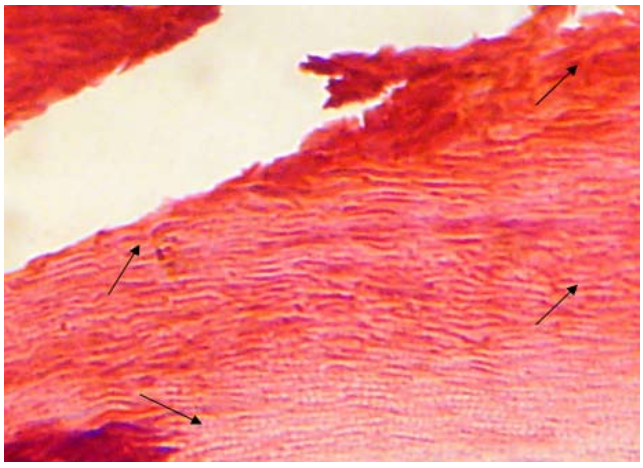

Fig. 3B. PAS-positive regular, septate, homogeneous pink-coloured hyphae invading the entire nail substance (PAS staining x 200).

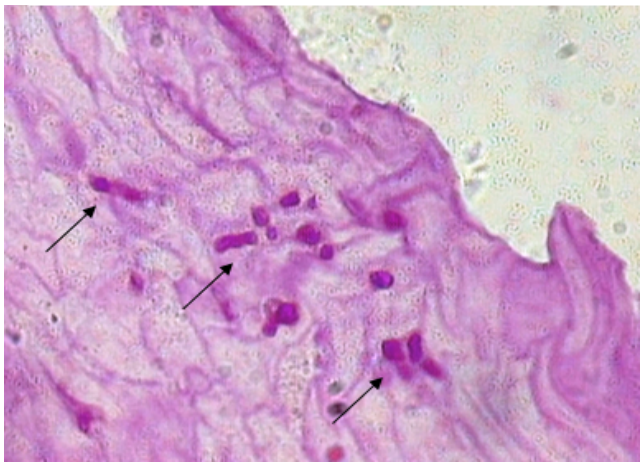

Fig. 3D. PAS-positive short hyphae and arthrospores within the infected nail bed (PAS staining x 400).

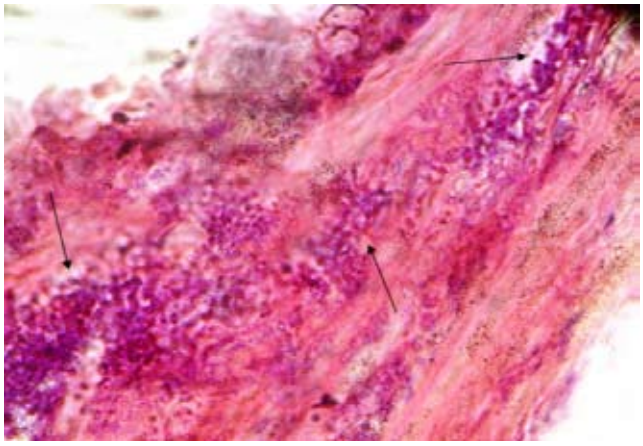

Fig. 3C. PAS-positive arthrospores within the nail (PAS staining x 200).

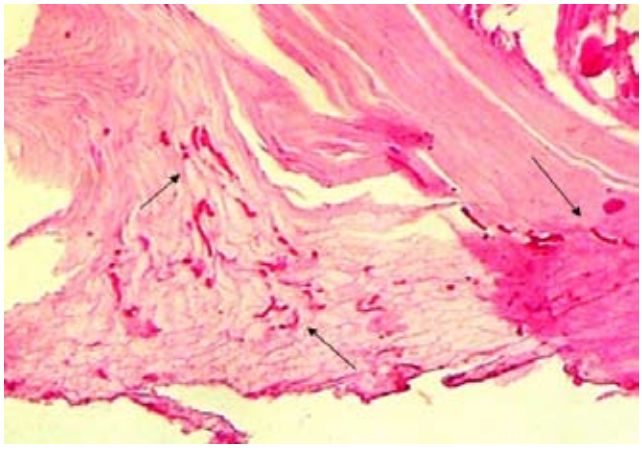

Fig. 3E. PAS-positive hyphae within the nail (PAS staining x 200).

Table 3 Sensitivity of performed mycological investigations

Test		**Investigated**	**Positive**	**Sensitivity**
KOH		40	30	75.0 %
PAS		40	23	57.5 %
Chi-square test	X2	2.74		
	P	0.098		

When the KOH and fungal cultures are negative but the clinical picture is highly suggestive of OM, a nail incisional/punch biopsy is recommended. However, this procedure is often painful and can cause permanent alteration of the nail plate.[12] In such circumstances, the histological study of full-thickness nail clippings can help to confirm or refute both the clinical diagnosis and the results obtained with standard microscopy and culture tests.[13]

In the present study, we compared KOH mount versus PAS stained nail clipping to refine both methods in the diagnosis of OM. Only 30 cases out of 40 (75% sensitivity) proved to be KOH positive while 23(57.5% sensitivity) of PAS stained samples were positive. There was no statistically significant difference between both test sensitivities.

Variable results obtained with these procedures are reported in the literatures. Agreeing with our results, Gianni et, al [10] showed that 102/112 (91%) patients were positive with KOH while 94/112 (84%) proved positive with PAS. Also, our results were parallel to Hsiao et, al[14] who showed that sensitivities of KOH and PAS were 87% and 81% respectively. On the other hand, Machler et, al[15] found that 100% of patients got positive results with both PAS staining and KOH mounting, Our results are on the other side of the results obtained by Weinberg et, al[11] and Lawry et, al.[9]

Weinberg et, al[11] invesigated 105 patients with KOH preparation, culture and PAS staining. They reported sensitivities of 92% for PAS, 80% for KOH mount and 59% for culture. Lawry et, al[9] found PAS to be 85% sensitive while sensitivity for KOH was 53%.

Supporting these results, other authors reported superior PAS positivity versus KOH. Shenoy et, al[4] reported positive PAS in 76/101 (75%) versus 54/101 (53%) for KOH. Liu et, al[16] reported positive PAS in 26/43 (61%) versus 19/43 (44%) for KOH. Reisberger et, al[17] reported 182/387 (47%) versus156/387 (40%).

Recently, in a study on 10 patients, Moreno-Coutino et, al[18] found that PAS staining was positive in all cases while KOH was positive in 8 patients.

In this study, the investigators used punch biopsy to collect the affected sample from cases of white OM only. Wilsmann-Theis et, al[19] reported that the most sensitive single test for the diagnosis of OM was PAS (82%) followed by culture (53%) and direct microscopy (48%).

Using nail clipping histopathology, Gianni et, al[10] were able to identify typical aspects of a dermatophyte (regular, septated, homogeneously pink-coloured hyphae) in 5 cases which were negative at culture examination and again in other 3 samples which were negative at both direct microscopy and culture.

These results may hence conclude that PAS undoubtedly has a higher sensitivity compared to KOH mount as identification of fungal elements with PAS staining is simple since the spores and hyphae can be seen as conspicuous intensely stained reddish structures.

Shenoy et, al[4] documented that PAS can be considered as an invaluable test in the evaluation of

OM and may also score over the other tests for some reasons; 1) it has a proven high sensitivity, 2) it is simple and results can be obtained early (2-7 days), 3) test outcome is least likely to be influenced by the sampling methods, 4) it is possible to morphologically distinguish the group of pathogens, 5) it can determine precise location of the pathogen, 6) it can also help in evaluating the efficacy of antifungals by reevaluating after treatment, and moreover 7) histopathologic slides can serve as records for future reference.

Despite its high sensitivity, Singh and Lavanya[20] highlighted certain fallacies of PAS staining in this context. They judged it is not an invaluable test in the diagnosis of OM owing to its ineffectiveness in identifying the causative pathogen, which would aid in advocation of correct treatment. Also, false positivity may occur with other inflammatory nail dermatoses as they may be indistinguishable histologically as also clinically.[21]

Singh and Lavanya [20] relied in their judgment (in part) on Pierard et, al[22] who commented that "morphological differentiation of dermatophytes from nondermatophytes is not always feasible with PAS staining and erroneous false PAS positivity is seen with psoriasis, starch particles, and serum parakeratotic cells". As a result, a patient may be mistakenly diagnosed as a case of OM resulting in erroneous diagnosis and inappropriate treatment.

Also, it should be kept in mind that the false negative results of PAS staining (as in this work) is not a rare finding as demonstrated by the study of Reza Kermanshahi and Rhatigan[23] who found that 4 out of 30 cases were negative with original PAS stain, but stained positive both with repeated PAS (cutting in a deeper level in the paraffin block) and Grocott methenamine-silver (GMS) stains.

KOH mount is a simple, rapid, inexpensive test to perform, which requires minimum infrastructure but some amount of experience to interpret the smears.[24] Reported false-negative rates are relatively high and may vary based on the experience of the laboratories.[7] Accordingly, if investigators depended solely upon the results of KOH preparation, they would have missed a good number of patients.[25] Feuilhade de Chauvin[26] mentioned that direct microscopy must always be coupled with fungal culture for accurate diagnosis and allowing correct species identification.

Multiple negative KOH preparations must be obtained before the suspected nail is considered not to be mycotic. A negative KOH does not in any way rule out OM but only prompts further testing (ie, culture or biopsy). Poor sampling, poor microscopy techniques, and examiner bias can cause false-negative results. Therefore, a KOH preparation is best conducted by a physician trained in specimen collection and fungal microscopy. [27]

To improve the sensitivity of a KOH test and to avoid/reduce false negative result, repeat the KOH several times, select a new lesion or area for collection, and reexamine the specimens already collected and examine the entire cover-slipped area. To avoid/reduce false positive result, an examiner experienced in interpretation may be needed to distinguish hyphal elements from various artifacts.

CONCLUSION

KOH examination could be used as a first line preliminary routine test in diagnosis of OM as it is relatively simple and rapid than PAS staining which can be used as a confirmatory method.

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