

Comparison of potassium hydroxide mount and mycological culture with histopathologic examination using periodic acid-Schiff staining of the nail clippings in the diagnosis of onychomycosis

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ABSTRACT

Background: Onychomycosis is a common problem noticed in clinical practice. Currently available standard laboratory methods show inconsistent sensitivity; hence there is a need for newer methods of detection. **Aims:** This study involves comparison of standard laboratory tests in the diagnosis of onychomycosis, namely, potassium hydroxide mount (KOH mount) and mycological culture, with histopathologic examination using periodic acid-Schiff (PAS) staining of the nail clippings. **Methods:** A total of 101 patients with clinically suspected onychomycosis were selected. Nail scrapings and clippings were subjected to KOH mount for direct microscopic examination, culture using Sabouraud's dextrose agar (with and without antibiotics) and histopathologic examination with PAS staining (HP/PAS). Statistical analysis was done by McNemar's test. **Results:** Direct microscopy with KOH mount, mycological culture, and HP/PAS showed positive results in 54 (53%), 35 (35%), and 76 (75%) patients respectively. Laboratory evidence of fungal infection was obtained in 84 samples by at least one of these three methods. Using this as the denominator, HP/PAS had a sensitivity of 90%, which was significantly higher compared to that of KOH mount (64%) or mycological culture (42%). **Conclusions:** Histopathologic diagnosis with PAS staining of nail clippings was the most sensitive among the tests. It was easy to perform, rapid, and gave significantly higher rates of detection of onychomycosis compared to the standard methods, namely KOH mount and mycological culture.

Key Words: Culture, Onychomycosis, Periodic acid-Schiff staining, Potassium hydroxide mount

INTRODUCTION

Onychomycosis is a common problem in clinical practice, accounting for up to 50% of all nail diseases.^[1] It is caused by a variety of fungal agents and presents in several clinical forms. It can mimic several diseases in the field of dermatology and internal medicine. It is necessary to diagnose the infection with some laboratory evidences before treating them with antifungal drugs, whose duration of treatment is long and may have some serious side effects. Currently available routine laboratory methods are direct microscopy with potassium hydroxide mount (KOH) and mycological culture. These traditional methods give inconsistent results, and the

results may be altered depending on the method of collection and preparation of specimen.^[2] There are many reports in the recent literature on histopathologic diagnosis using periodic acid-Schiff (PAS) staining of nail plates as a useful diagnostic test.^[2-6] This study was carried out to determine the sensitivity of this test in the diagnosis of onychomycosis as compared to that of KOH mount and mycological culture.

METHODS

A total of 101 patients (61 males and 40 females) with clinically suspected onychomycosis under consideration for the treatment were selected for the study. Their ages ranged

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from 16 to 80 years (mean, 45.2 years). Affected nails were cleaned to remove contaminants and were clipped short with nail clippers. Scrapings were collected from the involved nail bed and from the undersurface of the nail as proximal to the cuticle as possible with a no. 15 scalpel blade. Nail clippings were sent for histopathologic examination with PAS staining, and clippings and scrapings were subjected to potassium hydroxide (KOH) mount and mycological culture.

Potassium hydroxide mounts

Specimen was placed on a slide, and a drop of 20% KOH was added. A cover slip was applied with gentle pressure to drain away excess KOH. Incubation was done for 2 h or more (up to 48 h) until softening or digestion of the specimen occurred.^[7] Slides were microscopically evaluated for the presence of branching thread-like structures (hyphae) or beaded spherical structures (spores). When they were present, it was considered to be a positive test.

Mycological culture

Culture was done using Sabouraud's dextrose agar with and without antibiotics (cycloheximide and chloramphenicol). Observation for growth was done periodically for 4 weeks. If there was growth, pathogen was identified by cultural characteristics and microscopy.

PAS staining

Nail clippings were fixed in 10% formalin and then treated with 4% phenol for softening.^[8] 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. Presence of intensely stained reddish dots or threadlike structures in between the cells of the nail plate was considered to be a positive result.

Results so obtained were tabulated, and statistical evaluation of the results was done.

RESULTS

Out of the 101 patients, direct microscopy with KOH mount, mycological culture, and histopathologic examination with PAS staining showed positive results in 54 (53%), 35 (35%), and 76 (75%) patients respectively [Table 1]. Hence mycological culture was the least sensitive, and HP/PAS was the most sensitive among the tests. Culture yielded growth in 35 (35%) patients, with dermatophytes in 11 (31%), *Candida* in 2 (6%), and non-dermatophyte moulds (NDM) in 22 (63%) patients [Table 2]. Similar significantly high non-dermatophyte isolation has been reported earlier.^[9,10]

Table 1: Positive results and sensitivity

Test	Positive (Total No. of samples = 101)	Sensitivity* %
KOH mount	54 (53%)	42
Culture	35 (35%)	64
HP/PAS#	76 (75%)	90

*Calculated using samples showing at least one laboratory test positive (i.e., 84 samples as the denominator). #Histopathologic examination using PAS staining

Table 2: Results of mycologic culture

Fungal agent		Number (Total-26)
Dermatophyte	<i>Trichophyton rubrum</i>	6
	<i>Trichophyton mentagrophytes</i>	4
	<i>Epidermophyton floccosum</i>	1
<i>Candida</i>	<i>Candida albicans</i>	2
Non-dermatophyte moulds (NDM)*	<i>Aspergillus</i>	6
	<i>Scopulariopsis brevicaulis</i>	3
	<i>Scytalidium dimidiatum</i>	2
	<i>Fusarium</i>	2

*Positive NDM isolates (corrected)

Aspergillus species were the leading NDM isolates (15), and *Scopulariopsis* (3), *Scytalidium* (2), and *Fusarium* (2) were also isolated [Table 2]. It is difficult to interpret the role of non-dermatophyte mould because the same fungi that can be laboratory contaminants are also occasionally found to be pathogens.^[11] Hence it is suggested that if agents other than dermatophytes are isolated, they are probably laboratory contaminants unless KOH or microscopy demonstrates atypical hyphae associated with non-dermatophyte moulds or if the same organism is repeatedly isolated.^[11] Using these criteria, only 13 (*Aspergillus*, 6; *Scopulariopsis*, 3; *Scytalidium*, 2; and *Fusarium*, 2) out of the total 22 non-dermatophyte moulds isolated qualify as true pathogens.

In 20 patients, PAS staining was the only evidence of fungal infection [Table 3]. Therefore, if this test was not done, all these samples would have been falsely reported as negative. Laboratory evidence of fungal infection was obtained in 84 out of the 101 samples by at least one of these three methods being positive. Using this as the denominator, HP/PAS had a sensitivity of 90%, which was significantly higher compared to that of KOH mount (64%) or mycological culture (42%). Statistical analysis of the result was done using McNemar's test. Comparison of HP/PAS results with those of KOH mount and culture was done, and the variations were statistically significant (*P* values < 0.001). It was difficult to comment upon the specificity of these tests since we did not consider any single test as the gold standard.

Table 3: Details of test results

	HP/PAS [#]	KOH mount	Mycologic culture	No. of patients	Total positive
All tests positive	+	+	+	23	23
Two tests positive	+	+	-	24	35
	+	-	+	09	
	-	+	+	02	
One test positive	+	-	-	20	26
	-	+	-	05	
	-	-	+	01	
All tests negative	-	-	-	17	-
Total	76	54	35	101	84

[#]Histopathologic examination using PAS staining

DISCUSSION

Onychomycosis is difficult to treat as compared to most other dermatophytoses because of the inherent slow growth of the nail.^[11] Older antifungal agents like griseofulvin are unsuitable for onychomycosis because of their relatively poor efficacy and longer duration of treatment. Newer antifungal agents like itraconazole and terbinafin offer higher cure rates. The shortened treatment durations and intermittent (pulsed) dosing schedules have been designed to enhance compliance and reduce the cost of therapy. Because of all these reasons, nowadays a number of patients with onychomycosis receive oral antifungal drugs. These newer drugs also have some potential for serious side effects. It is necessary to confirm the clinical diagnosis with sufficient laboratory evidence before initiating treatments in these patients. Direct demonstration 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. Reported sensitivity of fungal culture for identifying dermatophytes varies from 25% to 80% with an approximately 30% false-negative results with culture and KOH studies.^[12] These high false-negative results are unacceptable; hence there is need for a test with higher sensitivity that also gives an early diagnosis. DNA-based techniques are currently being used to identify a number of pathogens and may soon be used regularly in the diagnosis of onychomycosis.^[13] These tests are likely to be more expensive and may not be available in all centers dealing with nail infections. In the literature, there are reports on histopathologic examination with PAS staining (HP/PAS) of nail clips as a highly reliable diagnostic tool for onychomycosis.^[2,6] Periodic acid-Schiff (PAS) stain demonstrates the presence of certain polysaccharides present in the walls of fungal hyphae. We carried out

this study in order to reaffirm whether the probability of pathogen identification by HP/PAS differs significantly from that by KOH mount and mycologic culture. Our results are at par with the results obtained by other authors like Weinberg *et al.* and Lawry *et al.*^[2,6] Weinberg JM *et al.* evaluated 105 patients with onychomycosis with four diagnostic tests, namely, KOH preparation, culture, HP/PAS, and calcofluor white stain. They obtained sensitivities of 80% for KOH mount, 59% for culture, and 92% for HP/PAS as compared with calcofluor white staining, which was chosen as the gold standard for statistical purposes.^[2] Lawry *et al.* found HP/PAS to be 85% sensitive. In their study, sensitivities for potassium hydroxide dissolution and centrifugation, combined with PAS, fluorescent brightener, or chlorazol black E, were 57%, 53%, and 53% respectively for the three combinations.^[6] Culture on Sabouraud's agar with chloramphenicol and cycloheximide (Mycosel agar) was 32% sensitive; and on Littman-oxgall agar, it was 23% sensitive.^[6] It may hence be concluded that HP/PAS undoubtedly has a higher sensitivity compared to KOH mount or mycological culture.

KOH mount is a simple, rapid, inexpensive test to perform, which requires minimum infrastructure but some amount of experience to interpret the smears.^[7] Reported false-negative rates are relatively high, but it may score over mycologic culture in its ability to detect fungal agents.^[10] Culture is generally considered as the gold standard since it is the only test in routine use that can identify the species of fungus causing onychomycosis. Reported false-negative results are high and may vary based on the experience of the laboratories.^[12] False-negative results in culture may arise if the nail sample contains only nonviable organisms, when insufficient sample is collected, when the nail sample is clipped distal to the fungal growth, or if the sample is not crushed before subjecting to the test.^[2] A modification of standard method of culture (Trypsin treatment) has also

been suggested to improve sensitivity.^[14] Identification of fungal elements on microscopy after PAS staining is simple since the spores and mycelia can be seen as conspicuous intensely stained reddish structures. However, precise identification of pathogen and determination of its vitality is not possible. It is more expensive compared to the KOH mount; however, the cost is not high when compared to the total cost of the treatment of onychomycosis. For all the reasons mentioned below, HP/PAS can be considered as an invaluable test in the evaluation of onychomycosis and may also score over the other tests.

1. It has a proven high sensitivity.
2. It is simple and results can be obtained early (in 2 to 7 days depending on the thickness of nails).
3. Outcome of the test is least likely to be influenced by the sampling methods.
4. It is possible to morphologically distinguish the group of pathogens,^[6,15] although the present study did not focus on this aspect.
5. It can determine precise location of the pathogen.
6. It can also help in evaluating the efficacy of antifungals by reevaluating after treatment.^[15]
7. Histopathologic slides can serve as records for future reference.

Delay in the diagnosis of onychomycosis can lead to total nail dystrophy, which may not regain its normal architecture in spite of adequate treatment. Hence we emphasize on the early diagnosis of this clinical condition. Early diagnosis needs a laboratory test that is simple, can be performed in a regular hospital setup, and has a high sensitivity. We consider histopathologic examination of the nail clippings with periodic acid-Schiff staining as a simple, highly sensitive screening test that can be performed in any laboratory having infrastructure for histopathology. It can be performed as a complementary test to mycological culture and KOH mount to close the diagnostic gap. It can also be done as a routine in all cases of nail dystrophy where clinical suspicion of onychomycosis is high.

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