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# **Original Research Article**

# A comparison of direct microscopy and culture with periodic acid schiff staining in the diagnosis of onychomycosis



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# ABSTRACT

**Introduction and Objectives:** Onychomycosis, or fungal infection of the nail apparatus by dermatophytes or nondermatophytes, is more than just a cosmetic problem. Dermatophytes are known to cause 90% of toenail and atleast 50% of fingernail onychomycosis. Our study offers insight into the unique current epidemiological aspects of onychomycosis in our region, including the less known nondermatophytemolds;. Since histopathology of nail clippings using Periodic Acid Schiff is clearly an invaluable tool in diagnosing onychomycosis, we integrated and comparatively evaluated it with the tests of routine mycology for the same.

**Materials and Methods:** Patients in Rajarajeswari Medical College and Hospital (a tertiary care hospital) presenting with clinically apparent onychomycosis were included in this study. Each specimen of subungual debris and nail clippings was divided into two portions- one for direct microscopy and the other for culture. Nail clippings alone were used for PAS staining.

**Results:** The present study was carried out on 40 clinically suspected cases of onychomycosis in the Department of Microbiology, Rajarajeshwari Medical College and Hospital over a period of one year. Out of 40 samples, 14(35%) samples showed fungal elements in 20% KOH, in 12(30%) samples fungal culture was positive and 19(47.5%) were PAS stain positive. Out of 12 isolates, 5(41.6%) were dermatophytes and 7(58.4%) were nondermatophytes.

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# 1. Introduction

Onychomycosis, or fungal infection of the nail apparatus by dermatophytes or nondermatophytes, is more than just a cosmetic problem.<sup>1,2</sup> Approximately 10% of the general population, up to 50% of people aged above 70 years and upto one third of diabetic individuals have onychomycosis.Onychomycosis is not cured unless treated.<sup>3</sup> It can cause psychosocial issues, apart from physical constraints on movement and pain from thickened nails.<sup>4</sup> Also, the nails serve as a constant reservoir of fungi for infecting other parts of the skinand contacts. Lifethreatening sequels – foot ulcers in uncontrolled diabetics and cellulitis in diabetics and immunocompromised patients may result.<sup>4</sup>

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Several steps are recognised in the pathogenesis of onychomycosis: contact with arthroconidia, adherence and inv asion of stratum corneum.<sup>5</sup> Depending on the pattern of nail invasion, clinical presentation is categorised into the following subtypes: Distal lateral subungual (DLSO), proximal subungual(PSO), endonyx subungual, superficial (SO), mixed, and secondary onychomycosis. Eventually, the common result is total dystrophic onychomycosis.<sup>6</sup>

Dermatophytes are known to cause 90% of toenail and atleast 50% of fingernail onychomycosis.<sup>4</sup> These are keratinolyticfungi<sup>7</sup> DLSO is the most frequent pattern. Candida yeasts are implicated in immunocompromised individuals and in occupations involving f requent contact with moisture.<sup>4</sup> Nondermatophyte moulds (NDM) lack the enzymes required to digest keratin, and a primary breech by dermatophytes or nail trauma is thought to be necessary for their invasion, however, a recent study



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involving Fusarium species suggests otherwise.<sup>7,8</sup> They are the main pathogens in immunocompromised individuals, including HIV positive patients, exclusively in whom they cause PSO.<sup>6</sup> Diffuse or deep SO and periungual inflammation are also suggestive.<sup>4</sup> However, there is increasing recent evidence of clinically indistinguishable NDM onychomycosis in the absence of predisposing factors; suggesting the importance of geographical variables including climate, and socioeconomic factors. The prevalent pathogen in each region could vary with time.<sup>1,7,9</sup>

Onychomycosis accounts for about 50% of all nail disorders.<sup>4</sup> Many of the gross features are nonspecific, thus laboratory techniques supporting clinical suspicion are needed to establish the diagnosis.<sup>10</sup> Nail plate thickening and the inherently slow growth of nail, makeonychomycosis difficult to treat.<sup>11</sup> Recurrence and relapse are common.<sup>12</sup> Fungal etiology along with clinical subtype and severity must guide choice of treatment as efficacy of systemic antifungal drugs varies with implicated fungi.<sup>13</sup> Delay in diagnosis and treatment may lead to total nail dystrophy.<sup>14</sup>

Direct microscopy of potassium hydroxide mount and fungal culture are the routine techniques of lab diagnosis. KOH mount provides a rapid, simple, inexpensive screening tool.<sup>11</sup> Fungal culture remains the indisputable gold standard, being the only method that identifies the precise identity of causative viable fungi.<sup>15</sup> and the most important test to determine the course of therapy, prognosis and for epidemiology.<sup>7</sup>

The relative difficulty in diagnosing nondermatophyte onychomycosis lies in the wide variety of roles- atleast six ecological subtypes- an isolated nondermatophyte may have. Out of these, only primary and successional invader deserve attention for their implication in treatment.<sup>16</sup> Hence, repeated isolation is recommended along with positive direct microscopy in nondermatophytemolds.<sup>17</sup>

The inclusion of a third diagnostic method- PAS staining of nail sections- has many advantages. It is considered as the gold standard in terms of sensitivity,<sup>14</sup> and is more economical than GMS, which has comparable sensitivity.<sup>18</sup> It is least dependant on sampling methodsdistal clipping suffices. Cases treated prior to diagnosis and efficacy of antifungal treatment can be evaluated<sup>11</sup>. Fungal morphology is better demonstrated than on direct microscopy, and provides results comparable to punch Histopathological demonstration of nail plate biopsy. invasion suffices to positively confirm the pathogenic role of a nondermatophyte repeatedly isolated on culture<sup>19</sup>. Hence, there is a need to comparatively evaluate PAS staining of nail sections against the routinely used methods.

Our study offers insight into the unique current epidemiological aspects of onychomycosis in our region, including the less known nondermatophytemolds. Since histopathology of nail clippings using Periodic Acid Schiff is clearly an invaluable tool in diagnosing onychomycosis, we integrated and comparatively evaluated it with the tests of routine mycology for the same.

# 2. Objectives

- 1. To isolate and identify causative dermatophytes and nondermatophytes in clinically suspected cases of onychomycosis.
- To compare the efficacy of direct microscopy and culture with histologic examination using Periodic Acid Schiff in the diagnosis of onychomycosis.

# 3. Materials and Methods

3.1. Type of study

Laboratory Investigation

#### 3.2. Study design

Comparative Cross-sectional study

#### 3.3. Study population

Patients in Rajarajeswari Medical College and Hospital (a tertiary care hospital) presenting with clinically apparent onychomycosis were included in this study.

# 3.4. Sample size

N = 40

#### 3.5. Subject selection criteria

### 3.6. Inclusion criteria

All clinically suspected cases of onychomycosis were be included in the study, who gave consent to participate in the study.

### 3.7. Exclusion criteria

Fungal infections other than onychomycosis were excluded from the study.

#### 3.8. Duration of study

The duration of study was 8 weeks.

#### *3.9. Data collection procedure*

Data was collected using a specially designed Case Report Form.

- 1. Demographic data-Name, age, sex, occupation, address, phone number, urban/rural
- History: duration of dystrophic nail(s), nail trauma, excessive sweating of hands/feet whichever affected, previous and family history of onychomycosis Disease

data- Presence of HIV, Diabetes mellitus, peripheral vascular disease Treatment data- oral or topical antifungal drugs and their duration of treatment, immunosuppressants Personal history- Smoking.

3. Dystrophic nails shall be classified based on appearance into the five basic types of onychomycosis: distal subungual, proximal subungual, white superficial, Candidial and total dystrophic.

# 3.10. Sample collection

Nail area was thoroughly cleansed with alcohol to remove contaminants like bacteria. Nail clippings will be collected using nail clippers. Specimens like subungual debris and other scrapings were collected using a sharp curetor a no.15 scalpel blade based on the site of maximum localization of the infecting fungi as determined from clinical appearance.<sup>16</sup>

## 3.11. Processing of specimens

Each specimen of subungual debris and nail clippings was divided into two portions- one for direct microscopy and the other for culture. Nail clippings alone were used for PAS staining.

### 3.12. PAS staining and Histologic examination

Nail clippings were fixed in 10% formalin and treated with 4% phenol for softening. The processed specimens were embedded in paraffin blocks, and about 3 micron thin slices will be prepared and mounted on glass slides. PAS staining was then performed. Stained slides were examined microscopically for magenta coloured fungal elements.<sup>16</sup>

# 3.13. Direct microscopy

All specimens were subjected to direct microscopy in 40% KOH solution and examined for the presence of fungal mycelia and spores.

# 3.14. Fungal culture

Nail scrapings and clippings were inoculated on antibiotic containing Sabouraud Dextrose Agar (SDA) with and without cycloheximide. SDA with cycloheximide were incubated at 37°C, and SDA without cycloheximide at 25°C and at 37°C, for 3 weeks aerobically. Isolates were identified by standard laboratory procedures.

### 3.15. Criteria for nondermatophyte onychomycosis

A nondermatophytemold /yeast isolated on culture had to show corresponding findings- atypical hyphae or yeasts and pseudohyphae respectively- on direct microscopy or PAS stained nail sections to be considered significant. In addition, no dermatophyte must have been concurrently isolated in case of nondermatophytemolds if PAS stained nail sections were negative for fungal elements.

# 3.16. Statistical tools

SPSS software was used for statistical analysis of the final data.

# 3.17. Ethical considerations

Only patients who give informed consent were included in the study. Institutional Ethical Committee approval was obtained prior to starting the study.

### 4. Results

The present study was carried out on 40 clinically suspected cases of onychomycosis in the Department of Microbiology, Rajarajeshwari Medical College and Hospital over a period of one year to compare the efficiency of KOH, culture and PAS stain in the diagnosis of onychomycosis.

The results are analysed as follows

Table 1: Results by different diagnostic methods

Results	n(%)
КОН	14 (35)
Fungal Culture	12(30)
PAS	19(47.5)

### 5. Results

Out of 40 samples, 14(35%) samples showed fungal elements in 20% KOH, in 12(30%) samples fungal culture was positive and 19(47.5%) were PAS stain positive.

Culture results	n(%)
Dermatophytes	5(41.6)
Non-Dermatophytes	7(58.4)
Total	12(100)

Out of 12 isolates, 5(41.6%) were dermatophytes and 7(58.4%) were nondermatophytes.

### 6. Discussion

The routine diagnostic tests in onychomycosis are direct microscopy of KOH mount and fungal culture. KOH mount is a rapid, simple, inexpensive screening test. Potassium hydroxide is used to digest, soften and clear the keratin of nail plate and subungual debris; thus making fungal elements more visible. However, its sensitivity is low, as fungal elements require experience to be identified. Cotton fibres may stimulate fungal elements. The sensitivity of KOH mount can be improved by performing centrifugation

Organism	Number
Dermatophyte	5
Trichophyton rubrum	3
Trichophyton mentagrophytes	1
Epidermophytonflocosum	1
Non-dermatophyte	7
Fungi	6
Curvularia	2
Alternaria	2
Fonscesea	1
Epicoccumpurpurascens	1
Yeast	1
Candida albicans	1

Table 3: Organisms isolated

of KOH treated nail clippings followed by staining with chitin specific Chlorazol Black E, fluorescent brightener or PAS. The class of pathogenic fungi can be delineated through morphology. However, these procedures may not be feasible for routine use, and fluorescent brightener requires a special microscope to be visualized.

Fungal culture is the only test that can identify the causative agent at a genus and species specific level. However, up to 4 weeks may be required, and culture does not distinguish pathogens from contaminants. The major disadvantage of these two tests is their high false negative rates. Delayed or false negative diagnosis may result in total nail dystrophy due to inadequate or delayed treatment.

Mayer et al in 2012, evaluated HPE-PAS as a second line diagnostic tool in onychomycosis by subjecting 100 direct microscopy and fungal culture negative nail samples to HPE-PAS. 38% of these turned out positive. They also observed parakeratosis and globules of plasma significantly more when fungal elements were present, indicative of ongoing inflammatory reaction.<sup>20</sup>

M.Shenoy et al noted a significantly higher sensitivity for PAS staining of nail sections (90%) over KOH mount (64%) and fungal culture (42%).<sup>11</sup> Jeelani et al observed a similar sensitivity for HPE-PAS(91.6%), while fungal culture (88%) was found to be more sensitive than KOH mount (77%), both showing considerably higher sensitivities.<sup>14</sup>

In a similar study in 2011, Wilsmann-Theis et al evaluated nail samples from a total of 851 clinically suspected patients and found HPE-PAS to be most sensitive (82%), followed by fungal culture (53%) and direct microscopy (48%). In our study KOH showed 14(35%) sensitivity, culture 12(30%) sensitivity and PAS showed 19(47.5%) sensitivity.<sup>21</sup>

### 7. Conclusion

Both dermatophytes and nondermatophyticmolds are causative agents of onychomycosis and sometimes yeasts are encountered. KOH mount and fungal culture are routinely used for diagnosing onychomycosis which has lesser sensitivity compared to PAS. PAS staining helps in rapid identification and helps in s tarting treatment early.

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None.

### **10.** Conflict of Interest

None.

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