



# International Journal of Dermatology, Venereology and Leprosy Sciences

E-ISSN: 2664-942X

P-ISSN: 2664-9411

[www.dermatologypaper.com](http://www.dermatologypaper.com)

Derma 2021; 4(2): 75-77

Received: 22-07-2021

Accepted: 24-09-2021

**Dr. Saurabh Chhabra**

Junior Resident, Department  
of D.V.L, Maharishi  
Markandeshwar Medical  
College and Hospital,  
Kumarhatti, Solan, Himachal  
Pradesh, India

**Dr. Vinay Shanker**

Professor and Head,  
Department of D.V.L  
Maharishi Markandeshwar  
Medical College and Hospital,  
Kumarhatti, Solan, Himachal  
Pradesh, India

**Dr. Rajwinder Singh**

Professor, Department of  
D.V.L, Maharishi  
Markandeshwar Medical  
College and Hospital,  
Kumarhatti, Solan, Himachal  
Pradesh, India

**Dr. Neelam Gupta**

Professor and Head,  
Department of Pathology,  
Maharishi Markandeshwar  
Medical College and Hospital,  
Kumarhatti, Solan, Himachal  
Pradesh, India

**Dr. Satish Kumar**

Professor and Head,  
Department of Microbiology,  
Maharishi Markandeshwar  
Medical College and Hospital,  
Kumarhatti, Solan, Himachal  
Pradesh, India

**Corresponding Author:**

**Dr. Saurabh Chhabra**

Junior Resident, Department  
of D.V.L, Maharishi  
Markandeshwar Medical  
College and Hospital,  
Kumarhatti, Solan, Himachal  
Pradesh, India

## Comparison of fungal culture with pas staining in diagnosis of onychomycosis

**Dr. Saurabh Chhabra, Dr. Vinay Shanker, Dr. Rajwinder Singh,  
Dr. Neelam Gupta and Dr. Satish Kumar**

**DOI:** <https://doi.org/10.33545/26649411.2021.v4.i2a.98>

### Abstract

Onychomycosis is Infection of the toenails and/or finger nails caused by fungi mainly dermatophytes, non-dermatophyte molds and yeast. Most commonly used diagnostic modalities include KOH mount direct microscopy, Fungal culture and histopathological examination using PAS stain. In our study PAS stain was compared with Fungal culture and PAS was the most sensitive (75%) diagnostic modality for onychomycosis and fungal culture was 31.25%.

**Keywords:** onychomycosis, PAS stain, fungal culture

### Introduction

Onychomycosis is Infection of the toenails and/or finger nails caused by fungi mainly dermatophytes, non-dermatophyte molds and yeast. Five percent of the people are affected worldwide and represent 20-40% of onychopathies<sup>[1]</sup>. It accounts for 30% of the total cutaneous mycotic infections. Toenails are affected more frequently than finger nails.

Clinical picture of onychomycosis is variable and various patterns have been described; namely distal and lateral onychomycosis (DLSOM), proximal white subungual onychomycosis (PWSOM), superficial white onychomycosis (SWOM), Endonyx onychomycosis (EOM) and total dystrophic onychomycosis (TDOM). The most common species among dermatophytes that are the causative agents of onychomycosis are *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum*<sup>[2]</sup>. Amongst yeast group, *Candida albicans* is the most common causative agent. *Acremonium* species, *Alternaria* species, *Aspergillus* species, *Fusarium* species and *Syctalidium* species are the most common non-dermatophytes involved in onychomycosis.<sup>3</sup> However, variation can be as per the geographical location and time.

The diagnostic modalities that are currently available include direct microscopy with potassium hydroxide (KOH), fungal culture, histopathological examination of nail clippings stained with periodic acid-schiff (PAS), nail biopsy, onychoscopy, immunofluorescence microscopy with calcofluor and Polymerase chain reaction (PCR).

Out of all the available diagnostic modalities KOH direct smear, fungal culture and PAS stain are commonly done and are readily available. These investigations are the most used in clinical practice. The gold standard diagnostic modality is fungal culture for onychomycosis, though PAS staining is one of the alternative method, but many studies have concluded that it is the most sensitive diagnostic modality for onychomycosis.

Sensitivity of KOH (81.82%), PAS (84.56%) and culture is (57%) whereas specificity is 92.86%, 57.14%. 92.86% respectively<sup>[4]</sup>. In the present study, we will be comparing the most commonly used diagnostic modalities. In the present study we will be comparing fungal culture, histopathological examination with periodic acid-schiff (PAS) stained nail clippings for the diagnosis of onychomycosis.

### Aims and Objectives

1. To determine the sensitivity of fungal culture.
2. To determine the sensitivity of histopathological Periodic acid-schiff examination.
3. To compare the sensitivities of the two different diagnostic modalities

## Materials and Methods

### Inclusion Criteria

All clinically suspected cases of onychomycosis will be included.

### Exclusion Criteria

Patients not willing to be a part of the study.

Patients who have already taken/taking treatment for onychomycosis.

### Fungal Culture

Culture was earlier considered to be the gold standard of diagnosis in onychomycosis. Nail specimens (subungal debris) should be plated on two different medias;

Primary medium- containing cycloheximide against most NDM and bacteria.

Secondary medium such as sabouraud dextrose agar (SDA), littman's oxgall medium and potato dextrose agar (PDA) that are free of cycloheximide and allow isolation of NDM. Antibiotics such as chloramphenicol and gentamicin may be added to SDA or PDA to eliminate bacterial contamination. Cultures will be incubated for three to four weeks at 25 to 30° C and examined weekly. Fungal colonies will be identified on evaluation of their specific growth patterns, colour and microscopic development of macro and microconidia and other characteristic growth characteristics. Lactophenol cotton blue (LPCB) will be done for recognition and identification of fungi.

### Histopathology Using Periodic Acid-Schiff (Pas) Staining

Nail clippings and subungal debris obtained will be fixed in 10% buffered formalin and sections will be made by microtome. Periodic acid Schiff (PAS) staining will then be performed. Presence of intensely stained yeast and hyphae in between the cells of the nail plate will be considered to be a positive result. Periodic acid Schiff (PAS) staining using standard methodology as given below:

In PAS staining the reagents required are:

1. Periodic acid 1%
2. Schiff's reagent
3. Harris haematoxylin.

### Statistical analysis

Data obtained will be analyzed as per the standard statistical method.

Data entry will be done using MS Excel spreadsheet.

Descriptive statistics will be analyzed using statistical software SPSS V.20.

Continuous variables will be presented as mean +/- SD. Categorical variables will be expressed as percentages or frequencies. The chi-square test will be used to determine the relationship between the two categorical variables. Sensitivity will be calculated correlating with fungal culture (gold standard). For all statistical tests, a p value of less than 0.05 will be taken to indicate a significant difference.

### Results

A total of 32 patients were enrolled In the study. After detailed history and examination, samples were collected and sent for histopathological examination with PAS stain and fungal culture. Out of the total 32 patients, 24 patients were positive for PAS stain whereas only 10 were positive for fungal culture.

**Table 1:** Comparison between the sensitivities of the three investigations

Test	Positive	Sensitivity
Fungal Culture	10	31.25%
PAS	24	75.00%

**Table 2:** Collective details of all the tests (PAS & Culture)

	PAS	Fungal culture	No. of patient
All test positive	+ 5	+ 5	5
One test positive (B)	+ 19	-	19
One test positive (C)	-	+ 5	5
All test negative	-	-	3
Total	24	10	32

In table 2, the summary of comparison of the two tests in total, combination and singly. Both the tests were positive in 5 (15.62%) patients. PAS was found to be positive in 24 out of 32 patients whereas only 10 patients had fungal culture positivity. PAS was most sensitive test for onychomycosis with sensitivity of 75%. Fungal culture in our test had the least sensitivity of 31.25%. On comparison of PAS and fungal culture, PAS is more sensitive test than fungal culture (p value  $\leq 0.03$ ).

### Discussion

The study was done from January 2019 to March 2021. Onychomycosis is a fungal infection that affects the toes and finger nails. It is caused by dermatophytes, non-dermatophyte molds (NDM) or yeasts. Aimed in comparing the most commonly used diagnostic modalities i.e., fungal culture, histopathological examination with periodic acid-schiff (PAS) stained nail clippings for the diagnosis of onychomycosis the study was done and analysed.

The mean age of patients was found to be  $47.34 \pm 15.28$  yrs of age, with majority of them in age group of 40-50 yrs (45.7%) followed with age group of 20-39 years of age (28.6%). This data is consistent with the findings of Gupta *et al.*, study, in which the most prevalent age group of presentation was between 21 and 60 years old, with a mean age of 41.35 years<sup>[5]</sup>. In a research done by Yadav *et al.*, the mean age of patients in study was 42.4 years and 40% of patients were between the ages of 31-45 yrs<sup>[6]</sup>. Another study done by Kayarkatte M.N. *et al.*, the mean age of  $40.48 \pm 15.47$  years was seen in the study group. The findings in our study were consistent with the studies done earlier.

The rise in incidence with the age is most likely due to repetitive nail injuries, greater occupational exposure, venous insufficiency, and increased susceptibility to pathogenic fungus. Children have a lower prevalence of onychomycosis than adults due to differences in nail plate structure, being less prone to damage, and having a considerably quicker development rate of the nail plate, which results in faster clearance of fungus from the nail in case of infection.

The differences in the incidence of onychomycosis in males and females is because of the differences in occupational exposure. Males are involved in outdoor occupations resulting in increased chances of nail trauma at work. That coupled with the frequent use of closed footwear by men in a tropical country like India is the reason why men develop

onychomycosis more than females. Lesser incidence of onychomycosis in females in comparison to males may probably be because of underreporting of ailment to the hospital.

Toe nails were involved frequently when compared to the finger nails in the present study. This is in consonance with earlier study carried out by Gupta *et al.* (2004) [7]. In our study exclusive finger nails were involved in 5 (14.28%) patients where as exclusive toe nail involvement were seen in 24 (68.57%) patients. 6 (17.14%) patients had involvement of both the finger and toe nails in our study. In females, fingernails were affected more frequently than toenails, which could be because most females in our study were housewives and they indulged more in household chores which involved excessive exposure to water. Toenail infection was more common in males and can be possibly be attributed to the use of occlusive footwear [4]. Another reason of increased toe nail involvement in our study can be due to geographical area. People in Himachal Pradesh tend to wear shoes more frequently because of the difficult terrain and a lower ambient temperature.

In our study, the sensitivity of PAS, KOH and fungal culture was derived and it was 75%, 59.37% and 31.25%. this is in accordance to the studies done earlier. Study by M karimzadegan-Nia *et al* in 2007 found the sensitivities of PAS, KOH and fungal culture to be 80.8%, 76.5% and 53.2% respectively [8]. Shenoy *et al* and Reisbeger *et al* reported that fungal culture was found to be positive in 35% the patients they enrolled in their studies [9].

The false negativity on fungal culture can be found in cases where the nail clipping specimen have only dead or non-living fungal organism. If there is insufficient sample collection then also the fungal culture can turn out to be false negative. If the nail specimen is not crushed before adding in the specimen that can also be another reason for a false negative result.

In the present study, PAS was the most sensitive (75%) diagnostic modality for onychomycosis and it was significantly more sensitive than any other single diagnostic modality. Fungal culture had a sensitivity of 31.25% which is significantly lower than that of PAS stain.

When the sensitivities of the three tests were compared, PAS was significantly more sensitive test ( $p$  value  $<0.05$ ) than any other single test. It was also seen that direct microscopy was more sensitive than culture with sensitivity of 31.25% respectively. Other studies done by Lawry MA *et al* had a similar finding in which PAS was the most sensitive test ( $p \leq 0.05$ ) when compared to direct microscopy and culture [10].

### Conclusion

Histopathological examination using PAS stain is the most sensitive test and time efficient test. However, it requires special expertise and equipment to be carried out so it cannot be done easily at all the hospital settings. On the other hand, fungal culture is considered as a gold standard test for onychomycosis but the chances of false negativity with this diagnostic modality is high. It requires proper collection of the sample along with proper inoculation. It takes 3-4 weeks for the test results and requires expertise. Even the highly trained operator can deem the culture as negative. So a combination of these tests is must to rule out the disease. Furthermore, there are newer diagnostic modalities that enhance the sensitivity of the existing

investigations. More detailed studies are required to validate the results.

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