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EVALUATION OF KOH MOUNT, FUNGAL CULTURE, AND PAS STAINING IN ONYCHOMYCOSIS: A COMPARATIVE STUDY

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ABSTRACT:

Background: Onychomycosis is a prevalent condition linked to considerable physical and psychological morbidity. The rise in prevalence in recent years can be linked to increased longevity, comorbid conditions such as diabetes, active participation in certain activities, and the emergence of HIV. The study aimed to assess the efficacy of KOH mount, fungal culture, and PAS staining of the affected nail plate in diagnosing onychomycosis. Approach: One hundred ten patients with clinically suspected onychomycosis were selected for the study. Nail scrapings and clippings underwent KOH mount for direct microscopic examination, culture on Sabouraud's dextrose agar (with and without antibiotics), and histopathologic examination utilising PAS staining (HP/PAS). Results: Direct microscopy with KOH mount, mycological culture, and HP/PAS yielded positive results in 40 (36.36%), 35 (31.81%), and 45 (40.90%) patients, respectively. Fungal infection was confirmed in 45 samples using at least one of the three methods. When using this as the denominator, HP/PAS exhibited a sensitivity of 40.90%, which was significantly greater than that of KOH mount (36.36%) or mycological culture (31.81%). In conclusion, histopathologic diagnosis utilising PAS staining of nail clippings demonstrated the highest sensitivity among the tested methods. The procedure was straightforward, efficient, and resulted in markedly higher detection rates of onychomycosis compared to standard methods, specifically KOH mount and mycological culture.

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

Introduction- Onychomycosis is a fungal infection of the nail, leading to the thickening and discoloration of the impacted nail plate (1). Recent studies indicate that the global prevalence of onychomycosis stands at around 5.5% within the general population (2,3). Onychomycosis represents 50% of all nail diseases and stands as the most prevalent disorder impacting the nail unit (1). Factors that may contribute to the occurrence of this fungal infection include diabetes, human immunodeficiency virus, immunosuppression, obesity, smoking, trauma, tinea pedis, psoriasis, and advancing age (4). Onychomycosis primarily affects the toenails, with the first (great) toenail being the most commonly involved. The condition usually manifests as a white or yellow-brown discoloration of the nail and frequently leads to hyperkeratosis of the nail bed, resulting in different

levels of onycholysis (1,4). Pathogens responsible for onychomycosis encompass dermatophytes, non-dermatophyte moulds (NDMs), and yeasts. Dermatophytes, specifically Trichophyton mentagrophytes and Trichophyton rubrum, account for around 90% of toenail onychomycosis cases. The remaining infections are attributed to Epidermophyton floccosum, Microsporum species, Trichophyton verrucosum, Trichophyton tonsurans, Trichophyton violaceum, Trichophyton soudanense, Trichophyton krajdenii, Trichophyton equinum, and Arthroderma species (5,6). The predominant organisms linked to onychomycosis include Aspergillus spp., Scopulariopsis brevicaulis, Fusarium spp., Acremonium spp., Neoscytalidium spp., and Syncephalastrum spp. (7,8). Onychomycosis induced by yeast is uncommon. Candida albicans is responsible for around 70% of cases of yeast-induced onychomycosis (9). Effective and sensitive diagnostic tests are essential for confirming the diagnosis of onychomycosis prior to the initiation of systemic antifungal therapy. The primary diagnostic techniques for onychomycosis include direct microscopic examination, histological examination, and culture analysis (10). Nonetheless, direct microscopic examination using potassium hydroxide (KOH) and histological examination through periodic acid Schiff (PAS) staining are unable to differentiate fungal species. Consequently, although it is the slowest method, culture analysis offers the advantage of pinpointing the species responsible for onychomycosis (11). This study sought to evaluate the diagnostic efficacy of direct microscopic examination, histopathological examination, and fungal culture analysis in the clinical diagnosis of onychomycosis.

METHODS –The present study was carried out in the World College of Medical Sciences Research and Hospital, Jhajjar, Haryana, during the period of July 2024 to December 2024, including all patients showing classical clinical features of onychomycosis.

Inclusion criteria: Patients of all ages and both sexes with the classical clinical features of onychomycosis and willing to take part in the study after written consent.

Exclusion criteria: Patients with peripheral vascular disease, uncontrolled diabetes, and connective tissue disorder were excluded. Also, those who are already treated with topical or systemic antifungal agents were excluded.

A total of 110 patients, comprising 65 males and 45 females, with clinically suspected onychomycosis and under consideration for treatment, were selected for the study. The participants' ages varied between 21 and 70 years. Nails were decontaminated and subsequently trimmed short using nail clippers. Scrapings were obtained from the affected nail bed and the undersurface of the nail, as close to the cuticle as feasible, using a no. 15 scalpel blade. Nail clippings underwent histopathologic examination utilising PAS staining, while clippings and scrapings were analysed through potassium hydroxide (KOH) mount and mycological culture.

Potassium hydroxide mount -The specimen was mounted on a slide, followed by the addition of a drop of 20% KOH. A cover slip was placed with gentle pressure to remove excess KOH. Incubation was conducted for a duration of 2 hours or more, extending up to 48 hours, until the specimen exhibited softening or digestion (12). Slides underwent microscopic evaluation to identify branching thread-like structures (hyphae) or beaded spherical structures (spores). Their presence was deemed indicative of a positive test result.

Fungal culture-Culture was performed utilising Sabouraud's dextrose agar, both with and without the addition of antibiotics (cycloheximide and chloramphenicol). Growth observation was conducted periodically over a duration of four weeks. Pathogen identification was conducted through cultural characteristics and microscopy in the presence of growth.

PAS staining-Nail clippings were preserved in 10% formalin and subsequently treated with 4% phenol for softening (13). Specimens underwent processing and were embedded in paraffin blocks.

Approximately 3-µm-thick sections were prepared and affixed to glass slides. PAS staining was subsequently conducted. The presence of intensely stained reddish dots or threadlike structures between the cells of the nail plate was deemed a positive result.

RESULT- Out of 110 patients, 65 (59%) cases were male, while 45 (41%) were female shown in fig. 1. Their age ranged between 21 to 70 years. The maximum number of cases of fungal infection was observed in the 21- to 30-year age group, accounting for 31.81%. The overall affected age group was 21-60 years, while 5 % cases were seen in those above 60 years of age shown in Table 1. Direct microscopy with a 20% KOH mount, mycological culture, and histopathological examination with PAS staining yielded positive results in 40 (36.36%), 35 (31.81%), and 45 (40.90%) patients, respectively (Table 2).

Table 1: Distribution of age group

Age group	Number of cases	Percentage (%)
21-30	35	31.81%
31-40	30	27.27%
41-50	25	22.72%
51-60	15	13.63%
61-70	5	4.54%
Total	110	100

Fig. 1 Distribution of Gender

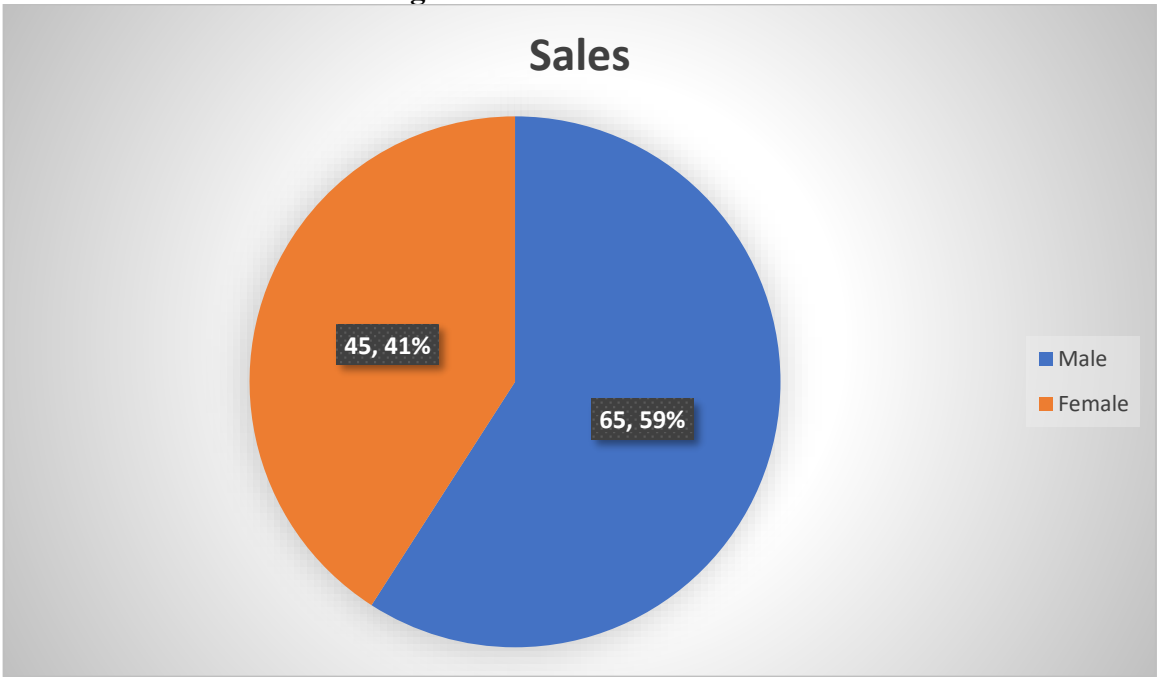

Table 2: Descriptive Analysis of Diagnostic Methods in Comparison (n=110)

Test	Positive	Negative
KOH	40 (36.36%)	70 (63.63%)
Culture	35 (31.81%)	75 (68.18%)
PAS	45 (40.90%)	65 (59.09%)
PAS and Culture	50(45.45%)	60 (54.54%)
KOH and Culture	43 (39.09%)	67 (60.90%)

Table 3: Distribution of organisms in cultured nail infection (n=35)

Organism	**N**
Dermatophytes	
Trichophyton rubrum	12
Epidermophyton	4
Microsporum species	2
Non-Dermatophytes a) fungi	
Aspergillus fumigatus	6
Aspergillus niger	5
b) Yeast	
Candida albicans	6

Direct microscopy using a 20% KOH mount shows that 40 specimens (36.36%) contain fungal elements, whereas 70 specimens (63.63%) exhibit no fungal elements. The mycological culture identified 35 specimens (31.81%) with positive fungal growth, while 75 specimens (68.18%) exhibited negative culture results. The positive culture revealed Dermatophytes in 18 out of 35 (51.42%) specimens, primarily Trichophyton rubrum, whereas 17 out of 35 (48.57%) were Non-Dermatophytes. Among non-dermatophytes, six specimens exhibited yeast, specifically Candida albicans, while eleven specimens contained fungi, including Aspergillus fumigatus in six samples and Aspergillus niger in five samples. Trichophyton rubrum was the predominant organism identified, but Candida albicans and Aspergillus fumigatus were the secondary most prevalent isolates in this investigation. Histopathological analysis utilising PAS staining demonstrated positive results in 45 (40.90%) specimens, whereas 65 (59.09%) yielded negative outcomes. A total of 110 specimens from suspected onychomycosis patients were analysed in the laboratory. Direct microscopy using 20% KOH, mycological culture, and histopathological examination with PAS staining techniques yielded positive results in 40 (36.36%), 35 (31.81%), and 45 (40.90%) patients, respectively. PAS staining showed superior efficacy compared to KOH and culture among the diagnostic techniques. The combination of PAS and mycological culture yielded 50 positive results (45.45%), while KOH and mycological culture produced 43 good results (39.09%). This study showed that PAS staining is more successful, both alone and in conjunction with laboratory procedures, for diagnosing onychomycosis.

DISCUSSION- Onychomycosis is more challenging to cure than most other dermatophytoses due to the intrinsic sluggish growth of the nail (14). Traditional antifungal medications such as griseofulvin are inappropriate for onychomycosis due to their limited efficacy and extended treatment duration. Recent antifungal medications, such as itraconazole and terbinafine, demonstrate elevated cure rates. The abbreviated treatment durations and intermittent dose regimens have been formulated to improve adherence and decrease therapeutic costs. Consequently, numerous individuals with onychomycosis currently get oral antifungal medications. These novel pharmaceuticals also possess the potential for significant adverse consequences. Confirmation of the clinical diagnosis with adequate test evidence is essential before commencing treatment for these patients. Fungal elements are regularly demonstrated using KOH mounts, and fungi are isolated through culture in laboratory procedures. The technique for acquiring nail clippings and the dimensions of the specimen are significant considerations in the evaluation of these tests (15).

The prevalence of onychomycosis among individuals aged 21 to 30 years was found to be 31.81% in our study. Individuals aged 21-40 years represent 27.27% of the population, while those aged 41-50 years account for 22.72%. Adhikari et al. also reported a higher prevalence in the third decade, specifically 58.8%. This may result from heightened exposure to occupational trauma (16). Grover reported that 56% of onychomycosis cases occurred in individuals aged 20-40 years, while 16% were in the sixth decade and 14% in the fifth decade (17). A study by Taniwala found that 39 (97.5%) of the 40 cases of onychomycosis were in patients over 20 years old, with the highest incidence of 11

(27.5%) cases occurring in the 4th and 5th decades of life. 8 cases, representing 22.5%, were aged between 21 and 30 years, while 20% were aged between 41 and 50 years (18).

In our study of 110 cases of onychomycosis, 65 (59%) were male and 45 (41%) were female. This contrasts with Taniwala's findings, which reported 82.5% males and 17.5% females, indicating a higher male prevalence compared to our study (18). Most Indian studies indicate a predominance of males. Our study revealed a higher prevalence of onychomycosis in males compared to females. Candidal onychomycosis exhibited a higher prevalence in females. This is due to the heightened burden of wet work, which increases trauma and facilitates the entry of pathogens.

In this study, KOH yielded a positive result in 40 cases (36.36%), culture was positive in 35 cases (31.81%), and PAS stain was positive in 45 cases (40.90%). A study by Gianni et al. reported that direct microscopy was positive in 59.3% of nail specimens, histological examination was positive in 54.6% of samples, and fungal culture yielded positive results in 90 cases (52.9%), which is higher than the findings in our study (19). Conversely, an alternative centre may exhibit superior proficiency in direct microscopy, leading to an elevated positivity rate for this method, as demonstrated in the study by Hajar et al. (59% for direct microscopy and 18% for culture) (20). Shenoy et al. demonstrated that the KOH mount yielded a positive result in 53% of cases, while culture showed positivity in 35%. The sensitivity was 64% for the KOH mount and 42% for mycological culture, aligning with the findings of our study (21).

In our study, the most commonly isolated fungi were dermatophytes, found in 18 (51.42%) samples, and non-dermatophytes in 17 (48.57%) samples. Among these, Trichophyton species were the most prevalent, aligning with Grover et al., who identified fungi in 44% of 120 cases, with 70.2% of these positive cases attributed to Trichophyton spp. Hajar et al. reported the presence of Trichophyton spp. in 80% of positive cultures (17&20). Jung et al. conducted a study in which Trichophyton spp. were identified as the most frequently isolated organisms (22).

In our study, the most commonly isolated species was Trichophyton rubrum (12 isolates, 34.28%), followed by Aspergillus fumigatus (6 isolates, 17.14%) and Candida albicans (6 isolates, 17.14%). Research conducted by Begari et al. indicates that the predominant isolated species is Trichophyton rubrum (31.6%), followed by T. mentagrophytes and Aspergillus niger, each at 15.8%. Infrequently isolated species included Aspergillus flavus (13.2%), Candida albicans (10.5%), Aspergillus fumigatus (7.9%), as well as Penicillium species and Microsporum species, each at 2.6%, consistent with our findings (23).

Conclusion- Delayed diagnosis of onychomycosis can result in total nail dystrophy, which may not restore its normal architecture even with appropriate treatment. Therefore, we underscore the importance of early diagnosis of this clinical condition. Early diagnosis requires a straightforward laboratory test that can be conducted in a standard hospital environment and demonstrates high sensitivity. The histopathologic examination of nail clippings using periodic acid-Schiff staining is a straightforward and highly sensitive screening test applicable in any laboratory equipped for histopathology. This test can serve as a complementary assessment to mycological culture and KOH mount, thereby addressing the diagnostic gap. This procedure may be implemented routinely in all instances of nail dystrophy where there is a strong clinical suspicion of onychomycosis.

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