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HISTOPATHOLOGICAL EXAMINATION OF NAIL CLIPPINGS USING PERIODIC ACID SCHIFF STAINING: GOLD STANDARD IN DIAGNOSIS OF ONYCHOMYCOSIS

Pathology

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ABSTRACT

Background: Onychomycosis accounts for approximately 30% of all superficial mycoses worldwide. Conventional diagnostic methods—KOH microscopy and fungal culture—are associated with suboptimal sensitivity, leading to diagnostic delays and empirical antifungal prescribing. Histopathological examination of nail clippings with Periodic Acid-Schiff (PAS) staining has been proposed as a superior alternative. **Objectives:** To confirm clinically suspected onychomycosis histomorphologically and to compare the diagnostic yield of PAS staining with direct KOH microscopy of nail clippings.**Methods:** Hospital-based prospective observational study over one year (November 2021–October 2022) at Government Medical College, Jammu. Nail clippings from 70 clinically diagnosed patients were subjected to both direct KOH mount and PAS-stained histopathological examination, with histopathology serving as the reference standard.**Results:** Males predominated (61.43%); peak age group 41–60 years (44.29%). Distal and lateral subungual onychomycosis (DLSO) was the most common pattern (37.14%). PAS staining demonstrated a sensitivity of 90.16% vs only 14.75% for KOH mount, with both tests showing 100% specificity. The difference was highly statistically significant ($p = 0.0001$).**Conclusion:** PAS-stained histopathological examination of nail clippings is significantly more sensitive than KOH microscopy and should be adopted as a routine first-line diagnostic test for onychomycosis.

KEYWORDS

Onychomycosis, Periodic Acid Schiff Stain, KOH Mount, Nail Clippings.

INTRODUCTION

Onychomycosis is defined as a fungal infection of the nail apparatus and represents the most common global nail disorder affecting toenails and fingernails [1]. The infection is predominantly caused by dermatophytes (60–70%), followed by non-dermatophyte molds (approximately 20%) and yeasts (10–20%) [2]. Approximately 10% of the general population, 50% of individuals aged 70 years and above, and up to one-third of diabetic patients are affected [3]. The incidence in childhood is rare (0.5–2.6%) and uncommon below 6 years of age [4].

Recognized risk factors include nail trauma, diabetes mellitus, immunodeficiency, hyperhidrosis, peripheral vascular disease, male gender, poor personal hygiene, increasing age, and chronic nail exposure to water [5]. Diabetes mellitus doubles the risk of onychomycosis, and HIV-infected patients are 15–40% more susceptible than the general population [6]. Clinically, the condition presents with yellowing, thickening, crumbling, and separation of the nail plate, progressing to accumulation of subungual debris and onycholysis [7].

Five distinct clinical subtypes are recognized: distal and lateral subungual onychomycosis (DLSO), superficial white onychomycosis (SWO), proximal subungual onychomycosis (PSO), endonyx onychomycosis, and total dystrophic onychomycosis (TDO). DLSO, most commonly caused by Trichophyton rubrum, accounts for approximately 90% of cases [8]. SWO is the second most common type (10%), typically caused by T. mentagrophytes var. interdigitale [9]. PSO is the least common pattern and is strongly associated with HIV/AIDS [10]. Endonyx onychomycosis involves nail plate invasion without bed hyperkeratosis or onycholysis [11]. TDO represents end-stage disease characterized by destruction of the nail unit [12].

Delayed diagnosis of onychomycosis can cause irreversible total nail dystrophy. Antifungal treatment is prolonged, expensive, and associated with adverse effects including hepatic enzyme elevation and drug interactions [13]. Accurate early diagnosis is therefore paramount. Conventional diagnostic methods—KOH microscopy and fungal culture—have well-documented limitations. KOH preparation is examiner-dependent and subject to interference from fibers and fat droplets; culture is time-consuming (2–4 weeks) and prone to contamination, yielding suboptimal sensitivity [14, 15].

Histopathological examination of nail clippings with Periodic Acid-Schiff (PAS) staining has emerged as a superior alternative. PAS oxidizes glycol groups in fungal cell walls to form aldehydes, which react with Schiff's reagent to produce characteristic magenta-colored fungal structures sharply delineated against the nail plate matrix [16]. The procedure is rapid, non-invasive, and minimally operator-dependent. The combination of KOH with nail clipping PAS reaction (KONCPA) further enhances diagnostic accuracy [17]. The present study was designed to evaluate and compare PAS staining with KOH microscopy as primary diagnostic tools for onychomycosis in a tertiary care hospital setting.

MATERIALS AND METHODS

Study Design and Setting

A hospital-based prospective observational study was conducted at the Postgraduate Department of Pathology, Government Medical College, Jammu, in collaboration with the Department of Dermatology and Venereology, from 1st November 2021 to 31st October 2022.

Study Population and Ethical Considerations

Seventy patients with classical clinical features of onychomycosis presenting to the Dermatology OPD were enrolled after obtaining written informed consent. Detailed clinical history including demographic data, duration of illness, occupational exposure, nail site, and clinical subtype was recorded using a structured proforma. Inclusion criteria: patients of all ages and both sexes with classical clinical features of onychomycosis. Exclusion criteria: patients declining consent; poorly preserved, insufficient, or inadequate nail specimens.

Sample Collection and Processing

Each nail clipping was divided into two portions. The first was subjected to direct KOH examination. The second was fixed in 10% formalin for 24 hours, routinely processed, and paraffin-embedded. Serial sections of 3–5 micrometers were cut, dewaxed, and stained with both H&E and PAS stains.

KOH Examination Procedure

The nail specimen was placed on a glass slide with 20% KOH, coverslipped, and examined at 10× followed by 40× magnification for fungal hyphae or budding yeast indicative of fungal infection [18].

H&E and PAS Staining Procedures

H&E staining: Sections were deparaffinized, rehydrated, stained in alum haematoxylin, differentiated in 1% acid alcohol, blued in

ammonia water, counterstained with 1% eosin Y, dehydrated, cleared, and mounted [19]. PAS staining: After deparaffinization and rehydration, sections were oxidized with periodic acid for 10 minutes, rinsed, treated with Schiff reagent for 15–20 minutes, counterstained with Harris's Haematoxylin for 1 minute, dehydrated, cleared, and mounted. Fungal cell walls, rich in polysaccharides, stain intensely magenta, enabling unequivocal identification of hyphae and spores [19].

Microscopic Evaluation and Statistical Analysis

All slides were examined by a qualified pathologist for features of onychomycosis: fungal hyphae and spores, hyperkeratosis, parakeratosis, spongiosis, acanthosis, neutrophilic infiltration, and hypergranulation. HP diagnosis served as the reference standard. Data were analyzed using IBM SPSS Version 21. Sensitivity, specificity, PPV, NPV, and diagnostic accuracy with 95% CI were calculated. Chi-square test was used for group comparisons ($p < 0.05$ considered significant).

RESULTS AND OBSERVATIONS

Demographic Profile: Age, Sex, Occupation, and Duration of Disease

Of 70 patients, the peak age group was 41–60 years (44.29%), with a mean age of 45.08 ± 18.43 years. Males constituted 61.43% (M: F = 1.59:1). Housewives were the largest occupational subgroup (28.57%), followed by farmers and students (15.71% each), labourers (14.29%), and teachers (12.86%). Most patients presented within 6 months of illness onset (45.71%), while 24.29% had disease of ≥24 months' duration, underscoring the chronic nature of onychomycosis. All demographic data are consolidated in Table 1.

Table 1: Combined Demographic Profile — Age, Sex, Occupation, and Duration of Disease (n=70)

Parameter	Category	Frequency (n)	Percentage (%)
Age Group (years)	< 20	12	17.14
	21–40	13	18.57
	41–60	31	44.29
	> 60	14	20.00
	Mean ± SD: 45.08 ± 18.43	Total: 70	100.00
Sex	Male	43	61.43
	Female	27	38.57
	M : F Ratio = 1.59:1	Total: 70	100.00
Occupation	Housewife	20	28.57
	Farmer	11	15.71
	Student	11	15.71
	Labourer	10	14.29
	Teacher	9	12.86
	Others (Businessman / Health Worker / Professional / Govt. / Driver)	9	12.86
Duration of Disease	< 6 months	32	45.71
	6–12 months	13	18.57
	12–24 months	8	11.43
	≥ 24 months	17	24.29

Figure 1 illustrates the age and sex distribution graphically. A rising incidence trend with age was evident, peaking in the 41–60-year group. Male predominance was consistent across all age groups, most pronounced in the> 60-year cohort, consistent with cumulative occupational nail trauma and environmental exposure.

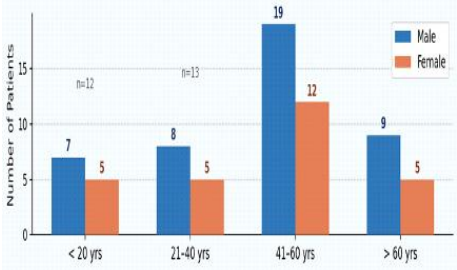

Figure 1: Grouped bar diagram showing age-wise and sex-wise distribution of 70 onychomycosis patients. Males predominated across all age groups; peak incidence was in the 41–60 years group (n=31).

Site of Nail Involvement

Toenails were more frequently involved (41.43%) than fingernails (38.57%), with both sites affected in 20% of cases. In males, toenail involvement predominated (19/43), whereas in females, fingernail involvement was slightly more common (14/27), consistent with chronic wet work exposure. These findings are detailed in Table 2.

Table 2: Site of Nail Involvement by Sex (n=70)

Site of Involvement	Male (n)	Female (n)	Total (n)	Percentage (%)
Toenail only	19	10	29	41.43
Fingernail only	13	14	27	38.57
Both Toenail & Fingernail	11	3	14	20.00
Total	43	27	70	100.00

Clinical Patterns and Predisposing Factors

DLSO was the predominant clinical pattern (37.14%), followed by PSO (28.57%), TDO (20.00%), and WSO (14.29%). Figure 1 shows the typical clinical presentation of a DLSO case from this study—a 61-year-old female with distal yellowing, subungual hyperkeratosis, and partial onycholysis, characteristic features that prompted nail clipping and histopathological evaluation.

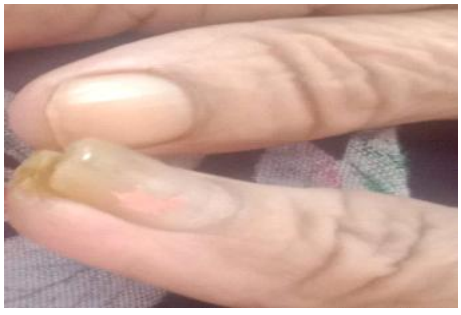

Figure 2: Clinical photograph of a 61-year-old female patient showing Distal and Lateral Subungual Onychomycosis (DLSO) — distal yellowish-brown discolouration, subungual hyperkeratosis, and early onycholysis of the fingernail, the most common clinical pattern observed in 37.14% of cases in the present study.

Nail discolouration was the leading predisposing factor (41.43%), followed by diabetes mellitus (27.14%), nail trauma (17.14%), hypertension (7.14%), and psoriasis with paronychia (7.14%). Notably, in 5 psoriasis patients (7.14%), PAS examination confirmed superimposed fungal infection, underscoring the critical role of histopathology in detecting co-existing onychomycosis in psoriatic nail disease. The distribution of clinical patterns and predisposing factors is displayed graphically in Figure 3.

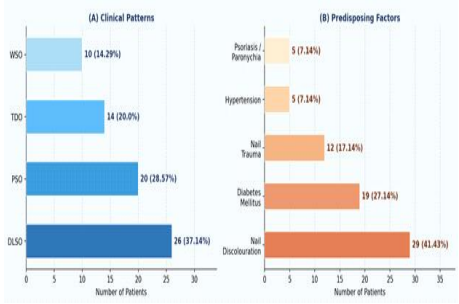

Figure 4: Horizontal bar diagrams illustrating (A) distribution of clinical patterns and (B) predisposing factors among 70 onychomycosis patients. DLSO was the most common pattern; nail discolouration and diabetes mellitus were the leading predisposing conditions. (DLSO = Distal Lateral Subungual; PSO = Proximal Subungual; TDO = Total Dystrophic; WSO = White Superficial Onychomycosis)

Comparison of Diagnostic Tests: KOH Mount vs PAS Staining

Of 70 cases, HP examination confirmed onychomycosis in 61 patients and was negative in 9. KOH mount was positive in only 9 of 61 HP-positive cases (sensitivity 14.75%) and negative in all 61 specimens, including all 9 HP-negative cases (specificity 100%). PAS staining was positive in 55 of 61 HP-positive cases (sensitivity 90.16%) and negative in all 9 HP-negative cases (specificity 100%). The association between HP and PAS was highly statistically significant (p = 0.0001), while the KOH-HP association was non-significant (p = 0.26). Tables 3 and 4 detail the contingency analysis and diagnostic performance parameters.

The stark diagnostic contrast is illustrated in Figures 2A and 2B. Figure 4A shows a KOH mount from a case of proximal subungual onychomycosis—the preparation is negative for identifiable hyphal elements despite confirmed fungal infection on histopathology, representing one of the 52 false-negative KOH results in this cohort. Figure 4B is the PAS-stained section from a corresponding case, demonstrating abundant magenta-staining fungal pseudohyphae and budding spores within the nail plate matrix—a diagnostic finding that would have been entirely missed on KOH preparation alone.

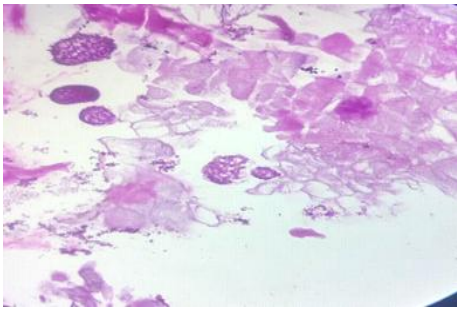

Figure 4A: Photomicrograph of a KOH mount preparation (×400) from a case of Proximal Subungual Onychomycosis in the present study showing a NEGATIVE result — absence of identifiable fungal hyphae or spores. This case was confirmed positive for onychomycosis on PAS-stained histopathology, representing one of 52 false-negative KOH results (KOH sensitivity 14.75%, p = 0.26, non-significant).

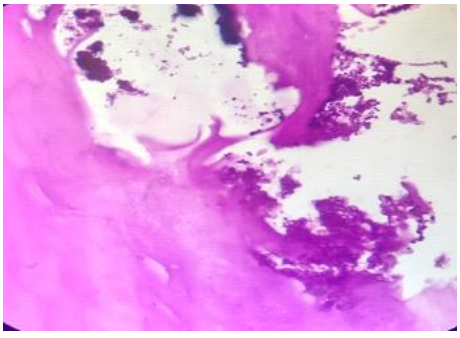

Figure 4B: Periodic Acid-Schiff (PAS) stained section (×400) of a nail plate clipping showing intensely magenta-staining fungal pseudohyphae and abundant budding spores within the nail plate matrix — a clearly positive PAS result confirming onychomycosis. PAS staining demonstrated a sensitivity of 90.16% (95% CI: 79.81%–96.30%) compared to 14.75% for KOH mount; the difference was highly statistically significant (p = 0.0001).

Table 3: Association Between Histopathological Diagnosis and Diagnostic Tests (n=70)

Diagnostic Test	Result	HP Positive (n=61)	HP Negative (n=9)	Total	p-value
KOH Mount	Positive	9	0	9	0.26 (NS)
	Negative	52	9	61	
PAS Staining	Positive	55	0	55	0.0001 (S)*
	Negative	6	9	15	

*Chi-square test; S = Significant (p<0.05); NS = Not Significant; HP = Histopathology (reference standard).

Table 4: Diagnostic Performance Characteristics of KOH Mount vs PAS Staining (n=70)

Diagnostic Parameter	KOH Mount (95% CI)	PAS Staining (95% CI)
Sensitivity	14.75% (6.98%–26.17%)	90.16% (79.81%–96.30%)
Specificity	100% (66.37%–100%)	100% (66.37%–100%)
Positive Predictive Value (PPV)	100%	100%
Negative Predictive Value (NPV)	22.68% (20.90%–24.56%)	71.76% (54.31%–84.46%)
Diagnostic Accuracy	31.80% (21.18%–44.02%)	90.20% (83.17%–97.22%)
P-value (Chi-square)	0.26 — Not Significant	0.0001 — Significant*

Histopathological Patterns Observed on PAS Staining

Beyond fungal detection, PAS staining provided a comprehensive histomorphological profile of each case. Fungal spores and hyphae (magenta on PAS) were identified in 41.43% of specimens. Other features included neutrophilic infiltration (21.43%), hyperkeratosis (15.71%), spongiosis (12.86%), parakeratosis (4.29%), acanthosis (2.86%), and hypergranulation (1.43%). H&E staining alone was insufficient for definitive diagnosis, as the pale pink nail plate did not afford the diagnostic contrast achieved by PAS.

Figure 5 illustrates one of the most instructive histopathological findings of the study—a PAS-stained nail plate section from a case of DLSO demonstrating intensely magenta-staining arthrospores within the nail plate layers. The characteristic magenta signal of the fungal cell wall glycoproteins against the pale nail plate background exemplifies the diagnostic precision of PAS staining that is unachievable by KOH preparation or H&E staining alone.

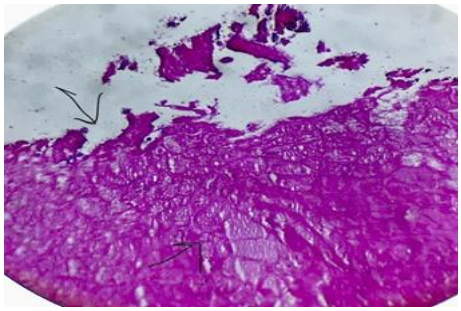

Figure 5: Photomicrograph of a Periodic Acid-Schiff (PAS) stained section (×400) of a nail clipping from a case of Distal and Lateral Subungual Onychomycosis (DLSO) in the present study. The section demonstrates intensely magenta-staining arthrospores (arrows) aggregated within the nail plate material. The vivid colour contrast of PAS-positive fungal cell walls against the pale background of the nail plate enables rapid and unequivocal identification of fungal elements — the hallmark of PAS staining as the gold standard diagnostic technique for onychomycosis.

DISCUSSION

Onychomycosis represents approximately 30% of all superficial mycoses and is a recalcitrant condition whose management is complicated by diagnostic inaccuracy. This study evaluated and compared PAS-stained histopathology and direct KOH microscopy in 70 clinically diagnosed patients, confirming the markedly superior diagnostic sensitivity of PAS staining and establishing it as the gold standard diagnostic technique.

The peak incidence in the 41–60-year age group (44.29%), mean age 45.08 ± 18.43 years, is consistent with age-related factors—cumulative nail trauma, venous insufficiency, and immunosenescence. This contrasts with Gautam M et al. (2021) and Jesudanam et al. (2003) who reported peak incidence in the 21–40-year group, differences attributable to demographic and geographic variation [20, 21]. The male predominance (M: F = 1.59:1) reflects greater outdoor exposure and occupational trauma, consistent with reports by Yadav P et al. (2015), Kour T et al. (2012), and Kumar RS et

al. (2018) [22, 23, 24], although Das NK et al. (2008) noted female predominance in their cohort [25].

Housewives forming the largest occupational subgroup (28.57%) is consistent with findings by Kaur et al. (2008) and Bokhari et al. (1999), attributed to chronic wet work and minor digital trauma [26, 27]. Farmers and labourers represent elevated risk from soil exposure and nail trauma, as reported by Gupta A et al. (2000) [28]. DLSO was the predominant clinical pattern (37.14%), consistent with its established status as the most common subtype attributable to T. rubrum, as documented by Garg et al. (2004), Grover S et al. (2003), and Sen et al. (2018) [29, 30, 21]. Toenail predominance (41.43%) is attributable to tight footwear and slower toenail growth [32, 33].

Nail discolouration (41.43%) and diabetes mellitus (27.14%) were the leading predisposing factors. The strong association with diabetes reflects impaired peripheral circulation, neuropathy, and immunosuppression, consistent with Yadav P et al. (2015), Garg et al. (2004), and Dogra S et al. (2002) who showed that diabetics are 2.5 times more likely to develop onychomycosis [22, 29, 35]. The 5 psoriasis patients (7.14%) with confirmed superimposed onychomycosis on PAS staining highlight the indispensability of PAS evaluation in psoriatic nail disease to exclude or confirm co-existing fungal infection—as demonstrated by Grover C et al. (2005) and Kacar et al. (2007) [36, 37].

The central finding—PAS sensitivity of 90.16% versus KOH sensitivity of 14.75% ($p = 0.0001$)—is the most clinically significant result of this study and is graphically demonstrated in Figures 2A and 2B above. This is consistent with Weinberg JM et al. (2003) who reported 92% sensitivity for HP/PAS versus 59% for fungal culture [38], Lawry et al. (2000) who found 85% versus 53% for PAS and KOH respectively [39], Wilsmann-Theis et al. (2011) who confirmed PAS as the most sensitive single test (82%) against culture (53%) and direct microscopy (48%) [40], and Shenoy et al. (2008) in an Indian cohort [41]. The 100% specificity of both tests ensures no false-positive diagnoses before committing patients to prolonged antifungal therapy. The superior NPV of PAS (71.76% vs 22.68%) means a negative PAS result is far more reliable in excluding onychomycosis.

The histological diversity observed—including neutrophilic infiltration, hyperkeratosis, parakeratosis, and spongiosis—provides additional diagnostic information beyond mere fungal detection, aiding in characterization of the host tissue response. As demonstrated in Figure 5, PAS staining renders even fine fungal arthrospores unmistakably visible within the nail plate lamellae, a feat not achievable by KOH preparation alone. This combination of high sensitivity, specificity, detailed tissue characterization, and clinical relevance firmly establishes PAS histopathology as the gold standard diagnostic method for onychomycosis.

CONCLUSION

This prospective study conclusively demonstrates that PAS-stained histopathological examination of nail clippings is significantly more sensitive (90.16%) than KOH microscopy (14.75%) for diagnosing onychomycosis, with identical specificities of 100% ($p = 0.0001$). As illustrated in the clinical and photomicrographic evidence presented (Figures 1, 2A, 2B, and 5), PAS staining rapidly and unambiguously identifies fungal elements—hyphae, arthrospores, and budding spores—within the nail plate matrix, while simultaneously characterizing the associated tissue response, information that KOH preparation cannot provide.

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