

# Comparison of Microscopy, Culture and Histopathology in the Diagnosis of Onychomycosis

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## ABSTRACT

**Aim:** The study was aimed as comparison of microscopy and culture with histopathologic examination by using Periodic acid –Schiff (PAS) staining of nail clipping in the diagnosis of Onychomycosis.

**Study design:** This was a cross sectional comparative study.

**Setting:** This study was carried out from September 2012 to August 2016, on 300 clinically diagnosed cases of onychomycosis in Department of Microbiology, Quaid-e-Azam Medical College Bahawalpur and Department of Histopathology King Edward medical University Lahore.

**Method:** Specimen was processed by 20% Potassium hydroxide (KOH) mount and mycological culture. Microscopic study on KOH was for the presence of hyphae or spores was considered as a positive test. Mycological culture was done by using Sabouraud's dextrose agar for 4 weeks and pathogen was identified by colony characteristics and microscopy. Presences of intensely stained reddish dots or threads like structures in between the cells of nail plate were considered to be positive results on histopathology with periodic acid Schiff (PAS).

**Result:** out of three hundred cases PAS stain revealed 240(80%) positive specimen. KOH mount showed fungal element in 180 (60%) specimens while in 174(58%) specimen, the culture was positive.

**Conclusion:** Histopathological examination technique PAS was found more effective than other laboratory methods in diagnosis of Onychomycosis.

**Keywords:** Onychomycosis, fungal culture, KOH mount, PAS staining.

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## INTRODUCTION

Onychomycosis is traditionally refers to non dermatophytic infection of the nails but recently it is increasingly used as a general term to denote all fungal infections of the nails. The term Onychomycosis is derived from the Greek word "onyx" a nail and "mykes" a fungus<sup>1</sup>. Clinically Onychomycosis is classified into various types including i) distolateral subungual onychomycosis (DLSO); ii) superficial white onychomycosis (SWO); iii) proximal subungual onychomycosis (PSO); iv) candida onychomycosis (CO); and v) total dystrophic onychomycosis (TDO)<sup>2</sup>. Onychomycosis is a growing global health problem and mainly due to dermatophyte, non dermatophyte, molds or yeast. The term Tinea unguium applied when infection is due to dermatophyte. The prevalence of the disease is rising worldwide and ranges from 2.1% to 9.1%<sup>3,4</sup>.

The general risk factors for any type of onychomycosis are increasing age, male gender, immunodeficiency, diabetes, nail trauma, hyperhidrosis, peripheral vascular diseases, poor hygiene and chronic exposure of nails to water in

candidal onychomycosis<sup>5</sup>. Typically Onychomycosis begins as a yellowish discoloration under the nail. The nail may thicken, become rough and crumbly and separate from the nail bed, and debris may accumulate under the nail. Thickening and dystrophy of the nail result in pressure erosions of the nail bed and Hyponychim<sup>6</sup>. There is an increased prevalence in older adults which is related to peripheral vascular disease, immunologic disorders, and diabetes mellitus. The risk of onychomycosis is 1.9 to 2.8 times higher in persons with diabetes compared with the general population. In patients with human immunodeficiency virus (HIV) infection, the prevalence ranges from 15% to 40%. There is higher prevalence of dermatophyte in temperate zone and moulds such as *Aspergillus* species and *Fusarium* species found in tropical and subtropical countries<sup>7,8,9</sup>.

Direct microscopic of affected nails in KOH does not allow to recognition of type of fungus and culture is needed for specific diagnosis.<sup>10</sup> Routine histopathological examination of nail clippings with standard hematoxylin and eosin (H&E) stained sections are not considered for the diagnosis of onychomycosis. It has been documented that the periodic acid-Schiff (PAS) stain is a sensitive method and has been alternate to be superior to culture and potassium hydroxide preparation for the diagnosis of onychomycosis. Increasing reliance on PAS staining

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makes it a candidate for apparent “gold standard” in diagnosis of onychomycosis<sup>11</sup>.

### MATERIALS AND METHODS

Three hundred patients with clinical diagnosis of Onychomycosis reporting to Dermatology outpatient department of Bahawal Victoria Hospital Bahawalpur, a tertiary care hospital, were included in this study. Non-probability convenience sampling technique was applied. The study was carried out from September 2012 to August 2016. Clinical evaluation included detailed history of trauma, occupation, sharing of common facilities, personal habits such as smoking and drinking, personal hygiene, hyperhidrosis and different predisposing diseases. Patients whose history of antifungal therapy could be known were excluded from the study. The most severely affected nail was selected for specimen collection<sup>12</sup>. Patients presenting for the first time in the outpatient department of Dermatology with clinical diagnosis of Onychomycosis were selected and all the three tests (PAS, KOH microscopy and mycology culture) were included in the study. This study included patients of age group 10 to 70 years and both genders were included with more than one nails effected. Patients already receiving topical or systemic antifungal therapy for fungal infection and those with nail changes due to psoriasis, lichen planus, contact dermatitis and other systemic diseases were excluded from this study<sup>13</sup>.

The specimens comprised of nail clipping immersed in 20% KOH were slightly warmed for softening. The softened nail material was examined under both low and high power of direct microscopy. The presence of fungal elements may be hyphae, spores, budding cells and pseudo-hyphae were noted<sup>14</sup>. If fungal elements detected than nail inserted on Sabouraud's dextrose agar were incubated at 25°C and 37°C respectively. After 4 weeks periodically If growth observed follow colony characteristics by cotton blue solution for identification of species. Nail clippings were fixed in 10% formalin then treated with 4% phenol for softening and further processing like dehydration, embedding in paraffin blocks sectioning by microtome machine mounting on slide and finally PAS staining was performed that showed presence of intensely stained reddish dots or threadlike structures in between the cells of the nail plate was considered to a positive results<sup>15</sup>.

### RESULT

Out of three hundred patients, 200 cases were male while 100 were female. Their age ranged between 10

to 70 years. Maximum cases of fungal infection seen in 21 to 30 year age that was 47%. The over all effected age group was 10-50 years while 10% cases seen in above 50 years of age. Direct microscopy with 20% KOH mount, mycological culture and histopathological examination with PAS staining revealed positive results in 180(60%), 159(53%) and 240 (80%) patients respectively (Table 1).

Table 1: Descriptive Analysis of Diagnostic Methods in Comparison (n=300)

Test	Positive	Negative
KOH	180(60%)	120(40%)
Culture	159(53%)	141(47%)
PAS	240(80%)	60(20%)
PAS and Culture	270(90%)	30(10%)
KOH and Culture	210(70%)	90(30%)

Table 2 Distribution of organisms in cultured nails infection (n=159)

Organism	n
<b>Dermatophyte 90(56.60%)</b>	
Trichophyton. Rubrum	62
Trichophyton. Mentagrophytes	28
<b>Non Dermatophytes 69 (43.40%)</b>	
a) Fungi 29(42.03%)	
Fusarium	12
Chrysosporium	04
Aspergillus	03
Aspergillus niger	02
Scytalidium	02
Scopulariopsis	02
Cladosporium	02
Cryptococcus	01
Actinomycete	01
b) Yeast 36(52.17%)	
Candida albicans	31
Candida spp	05
c) Bacteria 4(5.80%)	

Direct microscopy with KOH 20% mount shows 180(60%) specimens having fungal elements while no fungal elements seen in 120(40%) specimens. Mycological culture revealed 159(53%) specimen having positive fungal growth and remaining 141(47%) showed negative culture. The positive culture showed Dermatophytes in 90/159(56.60%) specimens predominately Trichophyton rubrum while 69/159(43.40%), was Non- Dermatophytes. among non-dermatophytes 36 specimens reveal yeast ,predominately 31 Candida albicans while 29 specimens was fungi which includes Fusarium in 12 samples, Chrysosporium spp in 04 samples, Aspergillus in 03 samples, Aspergillus nigar, scytalidium spp, scopulariopsis spp, cladosporium spp in 02 samples each, cryptococcus spp and actinomycete spp in 01 samples each. Trichophyton rubrum was the most common organism isolated

while *Candida albicans* and *Trichophyton mentagrophytes* were the second common isolates in this study. Histopathological examination with PAS staining revealed positive results in 240 (80%) specimens while 60(20%) was negative. A total of 300 suspected patients of onychomycosis specimens carried out in laboratory, direct microscopy with 20% KOH, mycological culture and histopathological examination with PAS staining techniques showed positive results in 180(60%), 159(53%) and 240 (80%) patients respectively. PAS staining was most effective among the diagnostic test irrespective of KOH and culture. It is also observed that the combination of PAS and mycological culture revealed 270(90%) positive results respectively KOH and mycological culture was 210(70%). This study showed PAS staining is more effective either in single or combination of laboratory methods for the diagnosis of onychomycosis.

Fig. 1 Distribution of age group

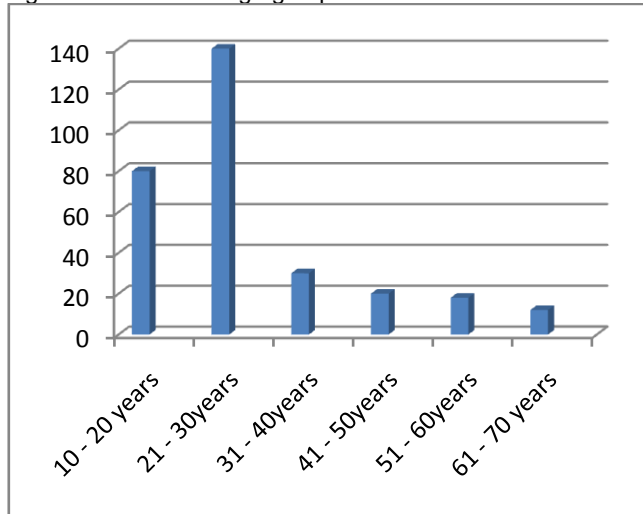
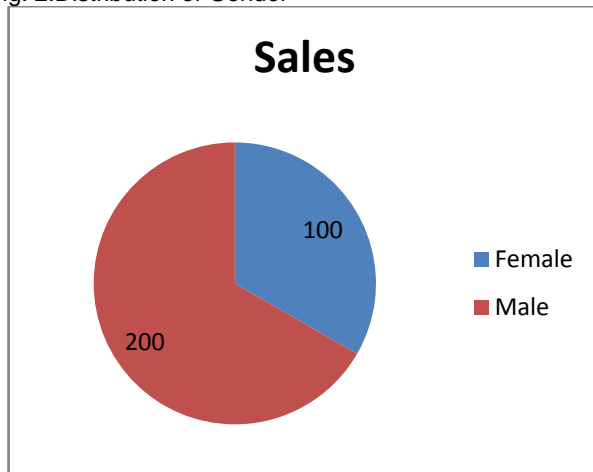


Fig. 2. Distribution of Gender



## DISCUSSION

Onychomycosis is a common disorder that difficult to treat as compared to other fungal diseases due to rational use of drugs without proper isolation of microorganism and laboratory investigation. It is a global health problem and prevalence of the disease is rising worldwide and ranges from 2.1% to 9.1%. The cause of onychomycosis based on many contributing factors like trauma to nail bed as biting increased age, diabetes mellitus, peripheral vascular disease, immunodeficiency disease, prolong chemotherapy, contaminated swimming pools and foot wears. It may be rarely caused by genetic alteration of immune system. The direct microscopy with KOH serves as screening for the presence of fungi or not while mycological culture reveals the isolation of pathogens at species level for proper selecting drugs and PAS histopathological staining give results within 12 hours. In this study our results PAS histopathological staining reveal 80%, KOH 60% and mycological culture was 53% which is consistent with Shenoy et al<sup>15</sup> who showed PAS 75%, KOH mount was positive in 53% and culture in 35%. Our results are at par with another authors obtained results like Alkhayat et al<sup>16</sup> that showed PAS stain was positive in 79% KOH was positive in 57% and culture in 41%. A study done by Weinberg et al<sup>17</sup> that evaluated known onychomycosis cases from different diagnostic tests and found PAS 92%, KOH 80%, culture 59% , PAS/HP staining the accurate and timely results available for the diagnose the suspected disease. PAS staining by Ahmad R et al<sup>18</sup> showed 77% positive, KOH in 59% and Culture in 40% is consistent with our study PAS staining was 80% positive, KOH 60% and mycological culture were 53%. Comparing the present gold standard direct microscopy and fungal culture with histological examination with periodic acid-Schiff staining (PAS) by Wilsmann-Theis D et al<sup>19</sup> showed in 1146 nail clippings samples in the diagnosis of onychomycosis and concluded that the sensitivity of culture was 53% followed by 82% PAS staining positive are very much consistent with our study that showed culture was revealed 53% and PAS was 80% alone. An investigating different techniques for the diagnosis of onychomycosis by Gianni C et al<sup>20</sup> found fungal culture was positive 52.9% , showing a dermatophyte 50% and KOH microscopic was positive in 59.3% is closely match with our study that showed mycological culture positive in 53% and among them 56.60% dermatophyte, and KOH direct microscopic was revealed 60%. Lilly KK<sup>21</sup> compared the different Laboratory techniques and found PAS stain was the most sensitive test 98.8% positive with KOH in

combination was 94.3% sensitivity and mycological culture was showed 57.3% results which is very much consistent with our study PAS 80%, culture 53% and KOH was 60%. Jung MY<sup>22</sup> et al compared the different routine mycological diagnosis by using culture media, KOH and PAS histopathological staining for onychomycosis in nail clippings. In the study it was concluded KOH was alone 55.9% positive results which was consistent with our study showed 60%. The PAS and culture showed 94.1%, KOH and culture showed 70% that was much more consistent with our study 90% and 70% respectively. Our study is compared with the study of Lawry MA<sup>23</sup> et al who showed PAS was positive in 85%, PAS and culture in combination results in 94% positive that was concluded by our study was 80% and 90% respectively.

## CONCLUSION

PAS (Periodic acid –Schiff) staining is more efficient than mycological techniques in the diagnosis of Onychomycosis than other methods adapted routinely in pathology laboratory. PAS in combination with mycological culture and KOH with mycological culture is much more sensitive that compared with KOH and Mycological culture alone.

**Recommendation:** PAS histopathological staining can be performed regularly and routinely as a diagnostic tool in pathology laboratory for improving accuracy of diagnosis of onychomycosis.

**Conflict of Interest:** All the cost of the study was born by the Dr Muhammad Wajid Khurshid Sipra. The authors had no affiliations with any pharmaceuticals/private organizations during the course of this project.

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