Bahrain Medical Bulletin, Vol. 31, No. 2, June 2009

The PAS Stain for Routine Diagnosis of Onychomycosis

Hana Alkhayat, MD, FRCPC* Nourah Al-Sulaili, MD, FRCPC** Elizabeth O'Brien, MD, FRCPC*** Catherine McCuaig, MD, FRCPC**** Kevin Watters, MD, FRCPC*****

Background: Onychomycosis is common, but clinical diagnosis could be difficult. The standard methods of diagnosis are known to have suboptimal yield because of low sensitivity. A more reliable method of detection would improve the diagnosis and facilitate appropriate therapy.

Objective: The purpose of this study is to compare the rate of detection of onychomycosis using PAS stain, KOH microscopy and mycological culture.

Setting: The pathology Department at the Montreal General Hospital, McGill University Health Center.

Design: Retrospective review of all the nail specimens that were submitted to the pathology department between January 1996 and June 2002.

Method: One hundred and forty-one nail specimens submitted from the dermatology clinic or private offices for PAS stain to rule out onychomycosis between January 1996 and June 2002 were reviewed. Only those subjected to all three tests (PAS, KOH microscopy and mycology culture) were included in the study.

Result: Out of the total culture positive cases (N=58), 28 (48.3%) were dermatophyte fungi and 30 (51.7%) non-dermatophytes, (Table 1). The percentage of positive PAS stain was 38.3% (N=54), KOH microscopy 22.7% (N=32) and culture 41.1% (N=58), (Table 2).

The percentage of positivity of combined PAS and culture was 79 (56%) and combined KOH and culture was 64 (45.4%), (Table 2). Comparing PAS test alone as an alternative method of diagnosis to combined KOH and culture (for both culture positive and culture negative cases) showed an overall sensitivity of 60.9% and specificity of 80.3%. When applied specifically to dermatophyte culture positive cases (N=28), the PAS was found to have a sensitivity of 92.9%, compared to 23% for non-dermatophyte positive culture cases (N=30).

For dermatophyte culture positive cases, the PAS and culture tests in combination had an overall specificity of 100% and a sensitivity of 80.3%.

Conclusion: We conclude that the PAS stain shows high reliability and sensitivity for detecting onychomycosis compared to KOH and mycological culture. In addition, the result indicates that PAS and culture combination is superior to combined KOH and culture for detection of dermatophyte infection.

Bahrain Med Bull 2009; 31(2):

* Consultant Dermatologist, McGill University Health Centre
(Currently, Consultant Dermatologist, BDF Hospital, Bahrain)
** McGill University Health Centre
*** Associate professor
Division of Dermatology
Montreal General Hospital, McGill University Health Centre
**** Associate Professor
Division of Dermatology
Centre Hospitalier de l'Université de Montréal
*****Consultant Pathologist, Program Director
Pathology Residency Training Program
Department of Pathology, McGill University Health Centre

Onychomycosis is defined as fungal nail infection. It represents up to 30% of superficial fungal infections and accounts for 40-50% of nail dystrophies. The majority of cases of onychomycosis are due to dermatophyte fungi, mainly T. rubrum, T. mentagrophytes var. interdigitale, and E. floccosum. The most common non-dermatophyte organism found in onychomycosis is Candida, but other organisms include Scopulariopsis, Fusarium, Aspergillus, Alternaria and Acremonium[1-3]. The appearance of onychomycosis could be imitated by a variety of nail disorders, including psoriasis, Reiter's syndrome, Darier's disease, lichen planus, exfoliative dermatitis, paronychia congenital, trauma and hyperkeratotic (Norwegian) scabies[4]. Therefore, the diagnosis should be confirmed before treatment is initiated.

Onychomycosis is a common complaint among many patients. There is a lack of knowledge for the best diagnostic method, few studies compared the efficacy of the available diagnostic methods. PAS stain was evaluated in very few studies and it is still under-utilized.

Several studies were reported in recent years about the treatment of onychomycosis since the advent of more effective oral antifungal agents[1, 5-13]. Topical agents have lower efficacy. Mycological cure rates for ciclopirox nail lacquer applied daily for up to 48 weeks have ranged from 29 to 47%[14]. However, there are conflicting reports regarding the relative efficacy of these medications, partly due to the difficulty in accurately diagnosing onychomycosis[15-20].

The standard methods of diagnosis, KOH examination and mycological culture, are known to have a suboptimal yield due to low sensitivity. Thus, there is a need for a more accurate practical method of onychomycosis detection to recommend antifungal therapy, to determine efficacy of treatment, and to compare different therapeutic agents. These are important considerations because of the prevalence of nail dystrophy, the cost of treatment and the possibility of adverse side effects.

The aim of this study is to compare the rate of detection of onychomycosis using PAS stain, KOH microscopy and mycological culture.

METHOD

All nail specimens submitted from the dermatology clinic or private offices for PAS stain to rule out onychomycosis between January 1996 and June 2002 were reviewed. Only those subjected to all the

three tests (PAS, KOH microscopy and mycology culture) were included in the study, others were excluded.

Nail specimens were treated in a special softening solution called tween solution for 48-72 hours (depending on the degree of nail flexibility). The specimen was then washed in water and mounted in paraffin for routine histopathology examination with periodic acid-schiff (PAS) stain by a pathologist. A positive PAS was defined by the presence of hyphae, see Figure 1.

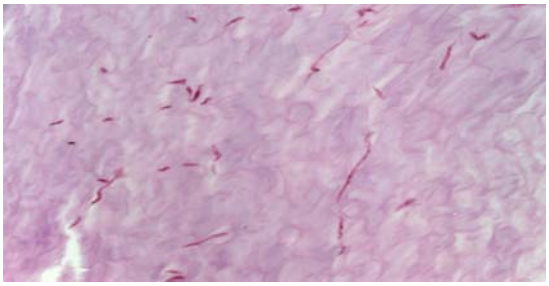

Figure 1: High Power View PAS Stained Hyphae within Nail Tissue

Other diagnostic methods included microscopy with 10% KOH (Potassium Hydroxide) and culture in Sabouraud agar, Littman and Mycosel agar**.** Fungal cultures were routinely kept for three weeks if the KOH was negative and for an additional week if the KOH examination was positive.

There was no need for ethical approval because the study was retrospective.

RESULT

One hundred and forty-one cases were evaluated using all three diagnostic tests. Positive cultures (58) were subdivided into two groups, dermatophyte (DMP) or non-dermatophyte (Non-DMP) according to the culture result. Mycological culture revealed dermatophytes in 48.3% (N=28/58), and non-dermatophytes in 51.7% (N=30/58), see Table 1.

Table 1: Distribution of Organisms in Cultured Nails

Organism	Number
Dermatophyte	**(28)**
T. Rubrum	24
T. Mentagrophytes	4
Non Dermatophytes	**(30)**
Fungi	**13**
Fusarium	2
Acremonium	2
Aspergillus	2
Cryptococcus	1
Rhodotorula	1
Penicillium	1
Scopulariopsis	1
Alternaria	1
Actinomycete	1

Nigrospora	1
Yeast	**15**
Candida albicans	11
Candida parapsilosis	3
Candida krusei	1
Bacteria	**2**

The percentage of positive PAS stain alone was (38.3%), KOH microscopy positive cases were (22.7%) and the positive culture cases were (41.1%). The percentage of positivity of combined PAS and culture was 56% (N=79) and that of combined KOH and culture was 45.4% (N=64), see Table 2.

Table 2: Descriptive Analysis of Several Diagnostic Methods by Type of Infection

	Positive (N=141)	Negative	Positive (DMP=28)	Negative	Positive (Non-DMP=30)	Negative
PAS	(54) 38.3%	(87) 61.7%	(26) 92.9%	(2) 7.1%	(7) 23.3%	(23) 76.7%
KOH	(32) 22.7%	(109) 77.3%	(16) 57.1%	(12) 42.9%	(10) 33.3%	(20) 66.7%
Culture	(58) 41.1%	(83) 58.9%	(28) 100%	0	(30) 100%	0
PAS and Culture	(79) 56.0%	(62) 44%	(28) 100%	0	(30) 100%	0
KOH and Culture	(64) 45.4%	(77) 54.6%	(28) 100%	0	(30) 100%	0

N = total, DMP = (dermatophyte), Non-DMP = (non-dermatophyte)

In positive cultures for dermatophytes (N=28), the PAS stain was positive in 92.9% (N=26/28) and KOH was positive in 57.1% (N=16/28). In those cases that showed non-dermatophytes (N=30), the percentage of positive PAS stain was 23.3% (N=7/30), compared to 33.3% (N=10/30) positive with KOH, Table 2.

Comparing PAS test alone as an alternative method of diagnosis to combined KOH and culture (for both culture positive and culture negative cases) showed an overall sensitivity of 60.9% and specificity of 80.3%. When applied specifically to dermatophyte culture positive cases (N=28), the PAS was found to have a sensitivity of 92.9%, compared to 23% for non-dermatophyte positive culture cases (N=30).

The combined PAS and culture tests had an overall specificity of 100% and a sensitivity of 80.3% for dermatophyte culture positive cases.

The results show that while there is an agreement of results between the two test combinations (κ=0.8%), a paired proportional test shows a statistically significant advantage (*p-Value=0.000)* of PAS and culture combined over KOH and culture combined for both dermatophyte and non-dermatophyte culture positive cases.

The positive predictive value and negative predictive value of PAS compared to KOH and Culture was 72.7% and 71.3% respectively. There was moderate agreement between the two diagnostic modalities [kappa=0.42 (95% Confidence interval=0.27-0.57)]. No adverse events were encountered during this study.

DISCUSSION

Successful treatment of onychomycosis usually requires prolonged, often expensive therapy, with potential adverse effects[6]. Although newer antifungal agents offer better response rates than oral Griseofulvin or topical therapy, they still provide suboptimal long-term results in many cases[5,7]. Terbinafine and Itraconazole are the therapeutic agents of choice. Oral Terbinafine therapy is very effective against dermatophytes, which are responsible for the majority of cases, but is less effective against non-dermatophytes. Clinical studies with Terbinafine have shown mycological cure rates of 71-82% and clinical cure rates of 60-70%[5,7,8].

Itraconazole has a broad antifungal spectrum that includes dermatophytes, many non-dermatophytic molds and Candida species. Some studies have demonstrated similar success rates for continuous and pulse therapies, the mycological cure rates was ranging from 45-70% and clinical cure rates from 35-80%[9-12]. Topical agents have lower efficacy.

Confirmation of suspected onychomycosis has customarily relied upon clinical examination and one positive test result by either KOH preparation or fungal culture.

Unfortunately, the yield of positive results from both tests is relatively low, with an average of 10-40% positive for KOH examination and 10-30% for culture[15].

Consequently, efforts have been made to find more reliable diagnostic testing for onychomycosis in order to avoid unnecessary treatment and to be selective in treating those patients[15-17].

Among the diagnostic investigations reported in recent years are confocal microscopy and PAS staining for histopathologic examination of nail specimens[17-23].

The PAS stain (PATHPAS) was reported by Lawry et al to be the most sensitive diagnostic method (90-95%) of onychomycosis detection in distal nail clippings, compared to several methods of KOH examination, and culture[19]. They reported a sensitivity of 85% of the PAS test alone and a sensitivity of combined PAS and culture of 94%. They concluded that PAS staining for histological examination of intact, clipped, distal free part of the nail and attached subungual debris is a simple, non-traumatic, fairly rapid (results within days compared to weeks for culture), and highly sensitive, reliable test for onychomycosis.

Recently, Reisberger et al and Weinberg et al similarly reported high rates of sensitivity and specificity for the PAS stain. Weinberg et al using the Calcofluor white (CW) stain as their gold standard for statistical analysis concluded that the PAS stain was superior to others as a negative predictive method, and suggested that it is "potentially the single method of choice for the evaluation of onychomycosis"[23].

Although the PAS stain does not allow differentiation of dermatophyte from non-dermatophyte fungi, we noted high rate or correlation between positive PAS stain and dermatophyte growth on culture, with sensitivity rate of 92.9% for dermatophyte growth. Those cases (23.3%), which demonstrated positive PAS with non-dermatophyte yeast or mold consisted mainly of organisms that have been associated with pathogenic infection of nails, although some were likely non-pathogenic[6,11]. It appears that a positive PAS stain, while most often representative of dermatophyte fungus, may indicate a pathogenic non-dermatophyte infection, but it is unlikely to represent mere contamination. This is

important for the treating dermatologist, it provides confirmation of the clinical diagnosis of onychomycosis and may support the recommendation of active treatment, oral or topical.

The specificity rate of PAS alone and PAS combined with culture was 80.3%. This could possibly be further increased by screening for contaminants. Since our study is a retrospective, there was no attempt to screen for contaminant organisms on culture compared to the prospective study by Lawry et al. However, cases which had positive cultures but negative PAS and KOH, showed non-dermatophyte fungi (molds, yeast), suggesting a high negative correlation of PAS stain with non-dermatophyte infection or contamination, as supported by the 100% negative predictive value.

Six cases were positive with both PAS and KOH but had negative cultures. In addition, 15 cases were PAS positive but negative for both KOH and culture. Two cases were reported by the treating dermatologist to have cleared clinically with oral Terbinafine therapy, suggesting that the PAS stain was reliably indicative of onychomycosis. During the study period, we defined only those specimens with identifiable hyphae as PAS positive.

This study have added to the previous studies and supported those showing that PAS stain is more sensitive and rapid method of diagnosis. However, this study has two limitations, one is the small sample size and the second is a retrospective design. Onychomycosis is common and increasing in prevalence worldwide, no region is exempted, and our result is applicable to Gulf countries and worldwide.

CONCLUSION

PAS stain alone shows high reliability and sensitivity for detecting onychomychosis compared to KOH and mycological culture. In addition, results indicate that the combination PAS and Culture is superior to combined KOH and culture for routine diagnosis.

Thus, it appears that although PAS alone is a significantly better diagnostic tool than KOH for detection of dermatophytes, it may be a weaker diagnostic tool for non-dermatophytes.

We suggest that PAS stain of formalin-fixed nail clippings sufficiently reliable and practical to be used routinely as a diagnostic test for Onychomycosis.

Further prospective study is suggested using a bigger sample and follow-up treatment.

REFERENCES

1. Tosti A, Piraccini B, Larenzi S. Onychomycosis Caused by Nondermatophytic Molds: Clinical Features and Response to Treatment of 59 Cases. J Am Acad Dermatol 2000; 42(2): 217-24.
2. Ghannoum MA. A Large Scale North American Study of Fungal Isolates from Nails. J Am Acad Dermatol 2000; 43(4): 641-8.
3. Gupta A, Jain HC, Lynde CW, et al. Prevalence and Epidemiology of Onychomycosis in Patients Visiting Physicians' Offices: A Multicenter Canadian Survey of 15,000 Patients. J Am Acad Dermatol 2000; 43(2): 244-8.
4. Olde Hartman TC, van Rijswijk E. Fungal Nail Infection. BMJ. 2008; 10: 337:a429.doi: 10.1136/bmj.39357.558183.94.

5. Hofmann H, Brautigam M, Weidinger G, et al. Treatment of Toenail Onychomycosis. A Randomized, Double-blind Study with Terbinafine and Griseofulvin. LAGOS II Study. Arch Dermatol 1995; 131: 919-22.
6. Trepanier EF, Amsden GW. Current Issues in Onychomycosis. Ann Phamacother 1998; 32: 204-13.
7. Crivier BJ, Paul C. Long-term Efficacy of Antifungals in Toenail Onychomycosis: A Critical Review. Br J Dermatol 2001; 145(3): 446-52.
8. Goodfield MJ, Andrew L, Evans EG. Short-term Treatment of Dermatophyte Onychomycosis with Terbinafine. BMJ 1992; 304: 1151-4.
9. De Doncker P, Decroix J, Pierard GE, et al. Antifungal Pulse Therapy for Onychomycosis. A Pharmacokinetics and Pharmacodynamic Investigation of Monthly Cycles of 1-Week Pulse with Itraconazole. Arch Dermatol 1996; 132: 34-41.
10. Havu V, Brandt D, Heikkila H, et al. A Double Blind, Randomised Study Comparing Itraconazole Pulse Therapy with Continuous Dosing for the Treatment of Toe Nail Onychomycosis. Br J Dermatol 1997; 136: 230-4.
11. De Doncker PD, Gupta AK, Marynissen G, et al. Itraconazole Pulse Therapy for Onychomycosis and Dermatophytosis: An Overview. J Am Acad Dermatol 1997; 37: 969-74.
12. Gupta AK, De Doncker P, Scher RK, et al. Itraconazole for the Treatment of Onychomycosis. Int J Dermatol 1998; 37: 303-8.
13. Nunley KS, Cornelius L. Current Management of Onychomycosis. J Hand Surg [Am] 2008; 33(7): 1211-4.
14. Gupta AK, Fleckman P, Baran R. Ciclopirox Nail Lacquer Topical Solution 8% in the Treatment of Toenail Onychomycosis. J Am Acad Dermatol 2000; 43(4 Suppl): 570-80.
15. Ellis DH. Diagnosis of Onychomycosis Made Simple. J Am Acad Dermatol 1999; 40(6): 53-58.
16. Meireles TE, Rocha MF, Brilhante RS, et al. Successive Mycological Nail Tests for Onychomycosis: A Strategy to Improve Diagnosis Efficiency. Braz J Infect Dis 2008; 12(4): 333-7.
17. Hongcharu W. Confirmation of Onychomycosis by In-Vivo Confocal Microscopy. JAAD 2000; 42(2 pt1): 214-16.
18. Singh G, Lavanya MS. The Reliability of Periodic Acid-Schiff Staining in the Diagnosis of Onychomycosis. Indian J Dermatol Venereol Leprol 2009; 75(1): 73-4.
19. Lawry M, Hancke E, Strobeck K, et al. Methods for Diagnosing Onychomycosis. Arch Dermatol 2000; 136: 1112-16.
20. Shenoy MM, Teerthanath S, Karnaker VK, et al. Comparison of Potassium Hydroxide Mount and Mycological Culture with Histopathologic Examination Using Periodic Acid-Schiff Staining of the Nail Clippings in the Diagnosis of Onychomycosis. Indian J Dermatol Venereol Leprol 2008; 74(3): 226-9.
21. Zabawski EJ Jr., Styles AR, Cockerell CJ. Routine Periodic Acid-Schiff Staining of Nail Plate Fragments in Fungal Cultures for Onychomycosis: A Method to Increase the Sensitivity of Diagnosis. Cutis 2000; 66(6): 456-8.
22. Reisberger E-M, Abels C, Landthaler M, et al. Histopathological Diagnosis of Onychomycosis by Periodic Acid-Schiff-Stained Nail Clippings. Br J Derm 2003; 148(4): 749-54.
23. Weiberg JM, Koestenblatt EK, Tutrone WD, et al. Comparison of Diagnostic Methods in the Evaluation of Onychomycosis. J Am Acad Dermatol 2003; 49: 193-7.