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Note

Modified PAS stain: A new diagnostic method for onychomycosis

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ABSTRACT

Background: Onychomycosis is the most common nail disease and represents around 50% of nail disorders. Accurate diagnosis with adequate evidence is ideal before starting any treatment. Current diagnostic methods offer low specificity and sensitivity.
Aims: To create a new method for the diagnosis of onychomycosis, and to compare its sensitivity and specificity with the existing methods.
Methods: One hundred and ninety-two samples with clinical suspicion of onychomycosis were included and underwent modified PAS stain (M-PAS), KOH/chlorazol black (KOH/CB) and culture testing. Sensitivity, specificity, positive and negative predictive values were calculated.
Results: In 152 out of 192 samples (79.2%) fungi structures were found in at least one of the three tests performed, and the patients were diagnosed with onychomycosis; 40 samples out of 192 (20.8%) were negative. Using M-PAS, filaments and/or spores were seen in 143 samples from the 152 positive (94%); 39 of them were negative to KOH/CB and positive to M-PAS (25.6%). With KOH/CB, filaments and/or spores were seen in 113 cases from the 152 positive samples (73.8% of the onychomycosis cases). Thirty-five cultures were positive, of which 77% were identified as *Trichophyton rubrum*; 117 onychomycosis cases were diagnosed despite the negative culture (76.9%). M-PAS showed 92.5% sensitivity and 55.55% specificity, a 67.5% positive predictive value and a 81.6% negative productive value.
Conclusions: This procedure, a combination of the existing methods to diagnose onychomycosis, KOH/CB together with a nail clipping biopsy, proved to have high sensitivity, as well as being rapid, easy, inexpensive and readily available in most hospital settings. M-PAS allowed us to diagnose 39 cases (25.6% of the cases of onychomycosis) that were false negative using only KOH/CB and culture.

Examen directo teñido con PAS: un nuevo método diagnóstico de la onicomicosis

Palabras clave:
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RESUMEN

Antecedentes: La onicomicosis es la enfermedad más común de las uñas y representa un 50% del total de las enfermedades que afectan a esta parte del cuerpo. Antes de iniciar un tratamiento, es muy recomendable contar con un diagnóstico preciso y pruebas suficientes. En la actualidad, los métodos diagnósticos ofrecen una sensibilidad y especificidad bajas.
Objetivos: Crear un nuevo método de diagnóstico de la onicomicosis y comparar su sensibilidad y especificidad con los métodos diagnósticos existentes.
Métodos: Se recogieron ciento noventa y dos muestras con sospecha clínica de onicomicosis en las que se aplicaron las pruebas de examen directo con KOH/Negro de clorazol (KOH/CB), cultivo y examen directo teñido con PAS (M-PAS). Se calcularon la sensibilidad, la especificidad, y los valores predictivos positivo y negativo.

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Resultados: En 152 de las 192 muestras (79,2%) se hallaron estructuras micóticas en una de las tres pruebas realizadas como mínimo, y se diagnosticó onicomicosis en dichos pacientes; 40 de las 192 muestras (20,8%) dieron resultados negativos. Mediante M-PAS, se observaron filamentos o esporas en 143 de las 152 muestras (94%); 39 de ellas resultaron negativas con KOH/CB y positivas con M-PAS (25,6%). En el caso de KOH/CB, se observaron filamentos o esporas en 113 de las 152 muestras, (73,8% de los casos de onicomicosis). Treinta y cinco cultivos dieron resultados positivos, conel 77% de los aislamientos obtenidos identificados como *Trichophyton rubrum*; se diagnosticaron 117 casos de onicomicosis a pesar de los resultados negativos en el cultivo (76,9%). La sensibilidad de M-PAS fue del 92,5%, la especificidad del 55,55%, y los valores predictivos positivo y negativo de 67,5% y 81,6%, respectivamente.
Conclusiones: Este procedimiento, una fusión de métodos ya existentes para el diagnóstico de la onicomicosis, que aplica KOH/CB junto con una biopsia de fragmentos de uña, mostró una gran sensibilidad. Es además un método rápido, fácil, económico y disponible en la mayoría de los ámbitos hospitalarios. M-PAS permitió diagnosticar 39 casos (25,6% de los pacientes con onicomicosis) con resultados falsos negativos al utilizar únicamente KOH/CB y cultivo.

Onychomycosis accounts for 50% of all nail diseases.[1,14] It affects 2–13% of the general population, and this percentage increases with age, reaching up to 40% in the elderly.[4,7] The main differential diagnosis is with psoriasis, lichen planus and traumatic onychodystrophy.[1,2,9,11]

KOH/CB is the most used diagnostic tool because it is fast, non-expensive, and non-invasive. The sample is obtained by scraping the subungueal hyperkeratotic area of the affected nail, placed on a slide with KOH/CB and then observed with light microscopy (10× and 40×).[6,14]

The rest of the scales are placed in a Sabouraud with cycloheximide medium for culture. This procedure is considered the gold standard for species identification.[5,15,16] The most widely used diagnostic methods are summarized in Table 1.

Other available diagnostic methods are biopsies in different variants such as nail clipping, nail plate punch or keratin biopsy, where the collected samples are processed in the same way as skin samples, and stained with PAS. These are excellent methods but not easy to perform.[3]

Diagnostic accuracy is important because oral antimycotic treatment is lengthy, expensive and significant drug interactions can occur. The currently employed methods do not offer enough specificity and sensitivity for the diagnosis.[7,15] The objective of this study was to create a new method for the diagnosis of onychomycosis, and compare its sensitivity and specificity with those of the current methods.

Materials and methods

We designed a new diagnostic procedure that consists of taking a sample obtained by scraping the subungueal hyperkeratotic area of the affected nail, fixating it on a slide with nitrocellulose–toluene–formaldehyde (transparent nail polish), and then staining the sample with PAS (M-PAS) in the histology department. PAS stain was performed in the usual manner: the slides were oxidized in 0.5% periodic acid solution for 5 min, rinsed in distilled water, placed in Schiff reagent for 15 min, washed in lukewarm tap water for 5 min, counterstained in hematoxylin for 1 min, washed in tap water for 5 min, and finally coverslipped using a synthetic mounting medium.

Table 1
Comparison of traditional diagnostic methods.

	Sensitivity (%)	Specificity (%)
KOH/CB[8,15]	75–80	72
Culture[15,16]	53	82
Nail clipping biopsy[8,15,16]	80–92	72
Nail plate punch biopsy[12]	100	NA
Keratin biopsy[3]	NA	NA

NA, not available.

These slides were observed in light microscopy at 10×, 20× and 100× by an experienced mycologist that was blinded to the results obtained in the routine tests, KOH/CB and culture.

We considered as inclusion criteria samples of patients that had the enough material for the three studies (M-PAS, KOH/CB, and culture), with clinical suspicion of onychomycosis that were referred to the mycology section of our hospital for a routine workup for onychomycosis diagnosis.

The exclusion criteria included those patients that had received topical or systemic treatment 1 or 3 months before taking the sample, respectively, and those with other ungueal diagnoses such as lichen planus, psoriasis, or traumatic dystrophy.

The elimination criteria were the loss of a sample during any of the proceedings involved.

Every culture was performed on BBL Mycosel Agar (Becton Dickinson and Company, Sparks, MD, USA), and incubated up to 4 weeks. If a colony developed, the macroscopic and microscopic characteristics were analyzed to determine the fungal species. We considered a positive result when fungal structures were identified.

The KOH/CB test was accomplished in the usual manner. This test was considered positive when fungal structures such as hyphae or spores were visualized.

The M-PAS stain exam was performed as follows: (i) the slide was properly marked with an identification number; (ii) nitrocellulose–toluene–formaldehyde was applied to the slide, in a enough amount for the scale to adhere; (iii) part of the subungueal hyperkeratotic sample was placed on top of the nitrocellulose–toluene–formaldehyde; (iv) after the sample had dried (about 5 min) it was sent to the histopathology department for PAS staining; (v) the sample was considered positive if hyphae or spores were observed.

For the evaluation of the sensitivity and specificity, we considered culture together with KOH/CB as the gold standard for comparison, based on the fact that these two methods are sensitive and specific when performed in combination and evaluated by trained personnel. Moreover, these are the two methods we use as routine diagnostic tests for onychomycosis.

The diagnosis of onychomycosis was made when one or more of the three diagnostic tests were positive.

Results

We included 192 samples of patients with clinical suspicion of onychomycosis. All of them underwent the three diagnostic methods. One hundred and fifty-two (79.2%) were positive in at least one of the tests.