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Histopathological diagnosis of onychomycosis using calcofluor white (CW) stain: comparison with PAS staining of nail sections

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Objective: The aim of this work is to compare results from PAS routine staining and CW stain on histopathological evaluation of onychomycosis.
Methods: Ninety six nail clippings from patients clinically suspected of having onychomycosis were used. All patients were attending the Podology Service in the Hospital of Guimarães (Centro Hospitalar do Alto Ave) between January and October 2008. Nail clippings were obtained by non-invasive means and were fixed with 10% formalin, embedded in liquid paraffin at 60 °C, cut into 5–6 thin slices (3 μm) of the paraffin blocks and dried onto slides. A fully automated embedding process provided histological slides within 24 h. After deparaffination and hydratation in descending alcohol solutions (100% × 2, 95% × 2, 80%, 70%, 50%) half of the slides were stained with periodic acid-Shiff (PAS) reagent for 10 min, washed in deionized water and stained with Shiff Reagent for another 5 min. Slides were washed with three exchanges of deionized water and dehydrated in ascending alcohol solutions (50%, 70%, 80%, 95% × 2, 100% × 2) before mounting in an organic mounting medium. The remaining slides were stained with one drop of Calcofluor White Stain 1% (CW) for 3–5 min, followed by washing in deionized water to remove excess fluorochrome. Slides were covered with Vectashield mounting medium for delay fluorescence quenching. Histological diagnosis was performed using conventional methods. Samples were first screened with a low magnification objective (10 ×). Upon localization of a site suspicious of fungal infection a high magnification (40 × or 100 ×) was used to confirm the diagnosis. All randomized samples were observed twice in a blind assay.
Results: Calcofluor white (CW) stain is a fluorescent brightener, with a high affinity for chitin and cellulose, which are major components in the cell walls of fungi, so is particularly useful in the detection of fungal elements in specimens. CW was used as an alternative of PAS staining in histological nail sections. On 96 samples analysed and stained with PAS, a percentage of 34.4% nails were found positive for fungi, 62.5% were negative and 3.1% gave an inconclusive diagnosis. Results from CW staining showed 46.9% of positive cases, 52.1% of negative cases, and it was not possible to establish a conclusive diagnosis in 1.0% of cases.
Conclusion: Results of the present study indicate that the histological examination of nail clipping specimens with CW stain is an easily and rapid performed procedure that represents an alternative to routine periodic acid-Shiff (PAS) staining of nail clipping sections, principally in non-massive infection cases where fungal elements are scarce.