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Fungal keratitis: Rapid diagnosis using methylene blue stain

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ABSTRACT

Earlier and accurate diagnosis of the fungal infection in the cornea is necessary for effective treatment. In developing countries, microscopical evaluation is the most valuable and rapid diagnostic tool. Therefore we aimed to investigate the efficacy of methylene blue (MB) staining in comparison with potassium hydroxide (KOH) and calcofluor white (CW) stain. Corneal scraping from 48 cases with suspected fungal keratitis were included in the study from January 2014 to December 2014. The specimens were subjected to direct examination by MB, 10% KOH and CW stain. The staining results were confirmed with fungal culture and strain identification. Topical amphotericin B was started for all positive fungal cases; 39 (81.25%) were proven fungal cases. Positive rate of calcofluor white, MB and 10% KOH staining were 79.2%, 75% and 68.75% respectively. CW showed higher sensitivity and specificity (99.44% and 90.91% respectively), followed by MB (92.31% and 80.0% respectively) and lastly KOH 10% (84.62% and 71.43% respectively). 71.8% of cases had healed scars and only 4 patients (10.3%) required keratoplasty (PK). Direct microscopic detection of fungal structures by MB staining in corneal scrapes is a fast and effective method for the early diagnosis of fungal keratitis.

1. Introduction

Fungal keratitis is a leading cause of serious ocular morbidity and blindness. It is worldwide in distribution, but is more common in the tropics and subtropical regions [1]. It was reported that the incidence of fungal keratitis in Egypt is increasing, correlating with the climatic changes (rises in minimum temperature and the maximum atmospheric humidity) in the region [2].

In fungal keratitis, early diagnosis and antifungal therapy is necessary in preventing further complications such as hypopyon formation, endophthalmitis, or loss of vision [3]. The diagnosis of fungal keratitis remains dependent upon staining smear and fungal cultures [4,5]. Fungal culture is the 'gold standard' for the diagnosis of fungal keratitis [6]; however, this process takes time (2–21 days), which delays clinical treatment [7,8]. Therefore although culture helps in definite diagnosis and identification, direct microscopic detection of fungal structures in corneal scrapes permits a rapid presumptive diagnosis

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[1]. In developing countries, microscopic evaluation is the most valuable and rapid diagnostic tool for detection of fungal elements in corneal scrapings. Thus, there is a need to identify a staining method for fungal keratitis diagnosis that is rapid, easy to perform and has a high sensitivity in the clinical setting.

Methylene blue (MB) is widely used in clinics as a dye and a biological staining agent. MB detects gonococci, early cancer cells, metastasized cancer cells in lymph nodes in early-stage breast cancer, and is also used to diagnose pre-cancerous pathological changes and early gastric cancer [9,10]. The efficacy of MB has been investigated for the diagnosis of fungal keratitis in one study, and has been found to be a fast and effective method for the early diagnosis of fungal keratitis [11].

Therefore we aimed in the current study to confirm the efficacy of MB staining for the rapid detection of fungal keratitis and to compare the positive rates, sensitivity and specificity with those of a 10% KOH-based smear and calcofluor white (CW) stain.

2. Materials and methods

2.1. Patients

Patients with clinically suspected fungal keratitis who attended the outpatient clinic of Mansoura ophthalmic center (Dakahlia, Egypt) from January, 2014 until December, 2014 were included in this study. For all patients, a detailed medical history was taken including duration of the symptoms, presence of predisposing factors (e.g. presence and nature of trauma, contact lens usage, previous history of ocular surgeries, history of diabetes, and usage of topical or systemic steroids), type of immediate treatment administered. Full clinical examination was done with assessment of elevation of slough (raised or flat), texture of slough (wet or dry), ulcer margins (serrated or well defined), size of the abscess, pigmentation, Descemet's folds, satellite lesions, dendritic lesions, immune ring, hypopyon, fibrin, flare or cells in the anterior chamber, deep lesions and endothelial plaque. Clinical photographs were taken using the Haag Streit slit, with photo slit attachment. Clinically suspected cases of fungal keratitis were diagnosed on the basis of the presence of the following: (i) a history of trauma with plant origin to the eye; (ii) the presence of clinical signs such as ulcers with irregular and feathery margins, satellite lesions or dry eye, and mild discomfort due to mild photophobia, tears, or mild irritation [12]. Patients were excluded from the study if they had proven bacterial keratitis or had received antifungal drugs. This study was conducted with approval from the Medical Research Ethics Committee, Mansoura University. Written informed consents were obtained from all participants.

2.2. Sample collection

Following topical anesthesia of the eye with topical benoxinate eye drops and under slit-lamp magnification, corneal scrapings were taken from the base and edge of each ulcer. This procedure was carried out aseptically with a special triangle ended sterilized scalpel. Two scrapings were obtained for each patient, the first scraping was tapped over 3 slides for direct microscopic examination using MB, 10% KOH and CW stain. The second scraping was inoculated on Sabouraud dextrose agar (SDA) media using C shaped slit technique.

2.3. Detection of the fungus by KOH-based smears

One drop of 10% potassium hydroxide was added to the first slide, a cover slip was applied and the wet mount was examined by direct microscopy.

2.4. Detection of the fungus by MB staining

One drop of MB (20 μl) was added to the second slide and a cover slip was applied and the slide was examined by direct microscopy.

2.5. Detection of the fungus by CW stain

One drop of CW (comprising 1 g/l Calcofluor White M2R and 0.5 g/l Evans blue; Sigma-Aldrich, St Louis, MO, USA) was then added to the third slide at one edge of the cover slip and a filter paper was placed at the opposite edge to draw the stain over the smears between the slide and cover slip. The slide was then left to stand for 1–2 min before being examined by fluorescence microscopy using blue light excitation (300–400 nm for the emission wavelength with excitation at around 355 nm).

2.6. Fungal cultivation

Inoculated SDA media were incubated at 27 °C for up to 3 weeks. The cultures were observed at intervals for fungal growth. The strains of fungi were identified according to the colony characters, growth rate and the morphology of the hyphae and spores.

2.7. Antifungal topical therapy

Antifungal topical therapy with amphotericin B in concentrations of 5 mg/ml was started for all cases immediately on receiving a positive report of fungal filaments by microscopic examination of the corneal scraping. The product was prepared from the intravenous formulation (Fungizone, Bristol-Myers Squibb, New York, NY) diluted in distilled water. One hourly topical drops was applied for a week, then each two hours for three weeks and then continued on a tapering basis depending on the activity of keratitis till resolution of the ulcers. Additional surgical procedures were undertaken for patients not responding to medical therapy and these procedures included therapeutic penetrating keratoplasty (PK), anterior chamber wash with amphotericin B or evisceration if needed.

2.8. Statistical analysis

Statistical analyses were carried out using the SPSS statistical package, version 10.0 (SPSS Inc., Chicago, IL, USA) for Windows. The results obtained from examination of the smear