Leishman staining in field diagnosis of corneal ulcer

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Suppurative corneal ulcers due to bacterial, fungal, protozoal or viral infections are known all over the world.[1] Herpes virus induced keratitis is one of the frequent causes of blindness.[2] A specific diagnosis of the causative microbe is desirable for an effective therapeutic recipe for the patient. A rapid, on-the-spot diagnosis would assist clinicians in several primary health care sites where facilities for bacterial, fungal and viral culture and identification were not available. Corneal scrapings from patients reporting for their outpatients care were examined for any cytological or microbial lesions to obtain a diagnostic clue of the offending microbes.

During the period November 2002 to May 2004, 26 patients with clinical presentation of corneal ulceration were evaluated at the Sant Parmanand Hospital in the Indian Capital Metropolis of New Delhi. The hospital, a private sector tertiary care establishment caters to population in the national capital and adjoining suburbs. The patients pay a nominal charge registration of Rs 20 (US$=0.40) for outpatient ophthalmic consultations. Consent was taken from patients with corneal ulcers for scraping and laboratory investigations. Those who consented and were willing to pay Rs 120 (US$ 3) were investigated.

Cornea was scraped under slit lamp with a number 15 blade under topical anesthesia with 1% proparacaine, and the scrapings transferred on to 2 slides for staining. Scrapings were also transferred onto Blood agar plates and Sabouraud's medium. Plates were incubated at 37^{0}C and the slides, instantly stained in the hospital Microbiology Department, located 20 meters from the outpatient clinics. After staining with Leishman stain, slides were examined under the oil immersion lens. The results were communicated to the clinicians within 30-45 minutes as the patient waited in the clinic.

Among 26 patients, a cytological evidence of herpes simplex virus was evident in 16 patients, fungal infection in 13 cases, dual fungal and viral infection in 5 cases (**Figure 1**), while there was no evidence of fungal or herpes virus infection in 4 cases. The patients were treated with 3% Acyclovir ointment, 5 times a day for 3 weeks for herpes virus, and natamycin 5% eye drops, one drop every 2 hourly for fungal infections. Patients with dual infection received Acyclovir and natamycin. The

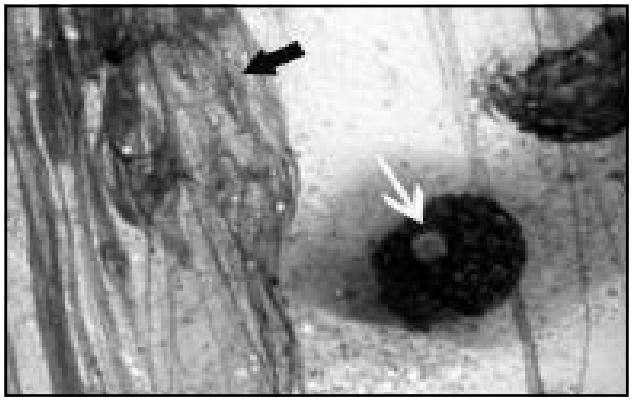

Figure 1 - Leishman stained corneal scraping with white arrow pointing to intraneclear inclusions. The black arrow is directed towards a conglomeration of fungal hyphae.

therapeutic response to the recipe was satisfactory. No pathogenic bacteria were grown in any of the specimens. In only 5 patients, *Aspergillus species* were grown on Sabouraud's plates, with no growth in the remaining samples. Use of transfer of scraped corneal tissue from patients with a clinical evidence of infective keratitis onto slides and almost immediate Leishman staining has been effective in offering a specific rather than empirical treatment in 26 patients. Direct transfer of scraped corneal tissues onto slides rather than the use of cotton swabs prevents any drying of tissues. The intranuclear inclusions in giant cells and fungal hyphae were so distinct in slides stained with Gram stain. Obviously, either of the 2 stains is likely to be available in primary health care establishments in locations lacking facilities for microbial cultures of molecular investigations.[2] Furthermore, absence of cotton fibers in scraped tissue has been an asset during microscopy to eliminate any pseudo-positive reporting attributable to the resemblance among cotton fibers and fungal hyphae. Conventionally, potassium hydroxide (KOH) is employed to liquefy keratin and identify fungi from dermal scrapings during the wet mount microscopy. Nevertheless, that would not appear to be essential for the stained microscopy on corneal scrapings. The oil immersion magnification does assist towards better label of fungal hyphae and fungal chromatin. Stained preparations are examined under oil-immersion magnification when it is possible to identify fungal hyphae including their chromatin. The selection of the above patients is based on their capacity to pay for scraping and laboratory investigations. Failure to isolate any bacterial pathogens in 26 cases appears to be related with self-mediation with antibiotics. In many locations, it is possible to obtain all types of medications without

any prescriptions, and the self-medication with antimicrobial agents is frequent. Most patients tend to self-medicate, and delay seeking professional medical care.[3] In all probability, both the topical and systemic antibiotics had been in use. Growth of *Aspergillus species* in 5 of 13 cases that were positive during microscopy indicates an inadvertent abstinence of the local population towards anti-fungal topical and systemic preparations.

In conclusion, direct staining of corneal scraped tissues for microbes would be universally invaluable. Rapid indication of the offending pathogens would assist in a judicious therapy to limit the scarring and opacity following ulceration of infected corneal ulcers. Such ulcers have been the most frequent cause of monocular blindness, that also relate to use of traditional medicines.[4] Simplified diagnosis of herpes virus associated ophthalmic manifestations of in glaucoma patients on latanoprost and bimatroprost therapy would be so important. Inactivated herpes virus had been reactivated in a 66-year-old woman with bimatroprost. Reactivation resolved after discontinuation of bimatroprost and initiation of acyclovir, ofloxacin and betaxolol.[5] A point-of-care microscopy for the herpes virus intranuclear inclusions should guide the practitioners of ophthalmology towards an antiviral and antibacterial therapy.

Acknowledgment. We would like to thank Ms. Seema George and Ms. Kamini Singh for their technical assistance.

Received 19th May 2004. Accepted for publication in final form 16th August 2004.

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