Evaluation of impression smears in the diagnosis of fungal keratitis

Dear Editor,

We read with interest the article by Jain *et al.*¹ and although their results are impressive we have certain observations to make.

The surface of any ulcer is coated with exudates, necrotic debris and drug deposits besides microorganisms. Histopathologically it has been shown that the fungal filaments are usually absent from the surface but present within the corneal stroma.² Therefore techniques which are superficial, including swabbing and impression smears do not reach the necessary depth to collect samples for which scraping is indicated.² Multiple samples should be collected from the advancing edge of the corneal ulcer,3 difficult with impression smears. The usefulness of impression cytology in various disorders of the ocular surface epithelia is beyond refute but these results cannot be extrapolated to fungal or bacterial keratitis which also involve the deeper layers. The authors state that impression smears are particularly helpful in small lesions. No data is presented in the study to support this statement. Direct visualization under good illumination and magnification will enable better sampling of a small lesion, particularly if it is deep, with corneal scraping rather than with impression smears where diffuse pressure needs to be applied. The authors state that in conventional scraping smears may be inadequate, cells undergo distortion and lose spatial relation. While such spatial relation may be relevant in conditions like dysplasia/neoplasia it is of little relevance in fungal keratitis where the goal is to visualize the fungal filament.

The authors state that impression cytology is safe, simple, atraumatic and precludes the use of slit-lamp or operating microscope. Impression cytology is definitely an office procedure yet it requires dexterity and expertise to handle a 3x3 mm filter paper. Cells adhere to paper on the basis of adequate pressure and such pressure can lead to perforation in the presence of corneal thinning or descemetocele. The authors' contention that impression smear is safer in comparison to scraping in preventing perforation is difficult to understand as with direct visualization thinned areas can be avoided which is not possible when the ulcer itself may be covered with a 9 mm² piece of paper.

The standard recommendation is to directly inoculate the media or make smears as material collected from corneal ulcers are less in amount.^{2,3} Impression smear technique described

here involves retransferring of material from the filter paper onto a glass slide which would further reduce the yield. Improved material like specialized Biopore membranes⁴ are being advocated now over conventional cellulose acetate paper for impression cytology, particularly for better adherence and better direct examination of the paper/membrane itself. In Fig. 3 of the article the KOH wet mount preparation shows pink staining of fungal filaments although authors mention no additional staining.

Conventional scraping debulks the ulcer of fungal load and removal of surrounding epithelium improves penetration of antifungal drug.³ In addition smears can be made for Gram and Giemsa stain and various culture media can be inoculated at the same time. All these are unlikely with impression smears and thus make the process an additional superfluous procedure in the workup of a case of corneal ulcer.

Although the results are comparable the study lacks convincing arguments for switching from the established procedure of scraping or for incorporating the new technique as an additional tool in the existing protocol, hence the clinical usefulness of impression smears in the diagnosis of fungal keratitis is questionable.

Samrat Chatterjee, MS

MGM Eye Institute, 5th Mile, Vidhan Sabha Road, Raipur - 492 007 (Chhattisgarh), India. E-mail: drsamrat@yahoo.com

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