Review Article

Role of impression surface cytology in ocular surface disorders

Gupta S

Dr Suruchi Gupta MS, Associate consultant, Centre for sight, Jammu, J&K

Address for Correspondence: Centre for Sight, sec 4A Extension, Sainik Colony, Jammu, J&K, **Email:** gupta.suruchi1@yahoo.com

Abstract

Ocular surface impression cytology is useful, least invasive tool in the diagnosis of various ocular surface disorders like dry eye, xerophthalmia, ocular surface neoplasia etc. Its role in diagnosis of ocular surface squamous neoplasia (OSSN) is well established. This technique has also been shown to have good correlation with histopathological diagnosis. Conjunctival impression cytology involves the removal of superficial epithelial cells of the conjunctiva and their study to make out changes in the conjunctival epithelial cells. These changes can be studied with light microscopy, electron microscopy or using the immunological markers. This article is a retrospective review of English literature articles published on ocular surface impression cytology and its application in various disorders. To achieve this purpose, a detailed online Med line search was done for related publications. The relevant articles were studied and required information was obtained.

Keywords: Squamous, Impression, Cytology, Neoplasia, Ocular

Introduction

Impression cytology refers to the technique of application of cellulose acetate filter paper to the outer layers of the ocular contents like, conjunctiva to remove the superficial layers of epithelium and then to study them. The cells, thus, obtained can be subjected to microscopic examination, fluorescent staining and immunological staining.

Evolution

Impression cytology was started as a technique to study the conjunctival goblet cells. Earlier; this technique was used to study the conjunctival pathological changes in various conditions like dry eye [1]. In1979**,** its use was started to study the mucous network on the surface of conjunctiva [2]. In 1980, this technique was used to describe the changes in the nuclear chromatin in the conjunctival epithelial cells in keratoconjunctivitis sicca (snake-like appearance of the nuclear chromatin) [3]. In 1982 and 1983, this technique was used to describe conjunctival changes in ocular pemphigoid and various causes of dry eye [4, 5]. The role of impression cytology in studying conjunctival changes in vitamin A deficiency was studied by in 1984 [6] and 1986 [7]. In 1985, impression cytology was used for staging the conjunctival epithelial changes in squamous metaplasia [8]. Later, it was used to diagnose mucopolycahharidoses [9].

Material Needed

Various materials like cellophane tape, photographic film etc. for obtaining the sample have been evaluated in a study and it was found that cellulose acetate Millipore filter papers give the best results [1]. MF millipore filter paper of various pore sizes 8.0 to 0.025 micron are available. In a study conducted on rabbit eyes using various pore size filter papers and using various degree of pressure for applanation, it was found that more cells of poor quality are obtained with larger pore size filter papers, whereas fewer but better quality cells are obtained with smaller pore size filter papers. Filter papers with pore size 0.025 micron give the best result. Further, it was established that filter paper with pore size 0.025 micron and 60 gm of pressure give the best results [10].

Manuscript received: 1st Oct 2015
Reviewed: 9th Oct 2015
Author Corrected: 14th Oct 2015
Accepted for Publication: 4th Nov 2015

Procedure: After the instillation of topical anesthetic drops (required according to surgeons' discretion), a piece of filter paper is applied to the conjunctiva with one side turned up to remember the orientation according to the conjunctival area being examined (as shown in fig. 1). The filter paper is left in place for few seconds. The filter paper is removed in the peeling motion after 2-5 seconds to allow recovery of the maximum amount of the sample. The filter papers are placed on the glass slide in the orientation as shown in figure 2 to remember the placement on the conjunctiva in the two eyes. The cells removed are fixed by using various fixatives like formaldehyde (formalin), glutaraldehyde, ethanol, and methanol. The fixation of the sample is followed by staining of the cells removed to enhance the visibility. Various stains used are Periodic Acid-Schiff reagent, Hematoxylin. The protocol used is the **Nelson's protocol** [8]. The specimen is then, examined under the light microscope.

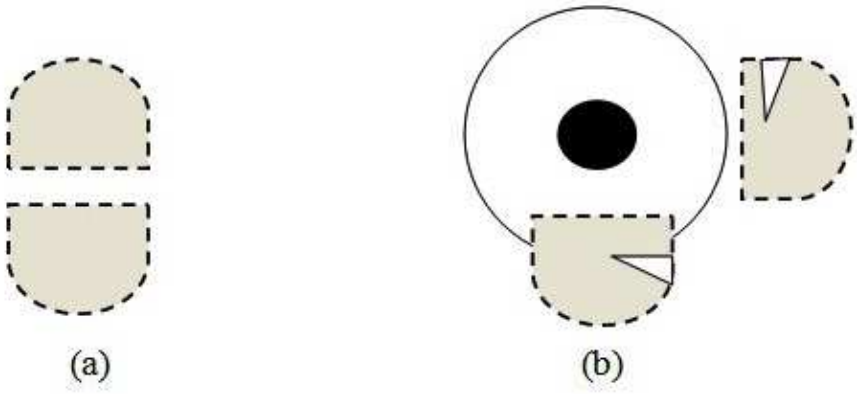

Figure 1

(a) Filter paper strips cut into two disc shaped halves. (b) Discs are turned on one side to recognize orientation. For conjunctival impressions, discs are placed parallel to the limbus to remember part of conjunctiva examined. For taking impression cytology for limbal stem cell assessment, discs are placed half on cornea and half on conjunctiva to remember orientation

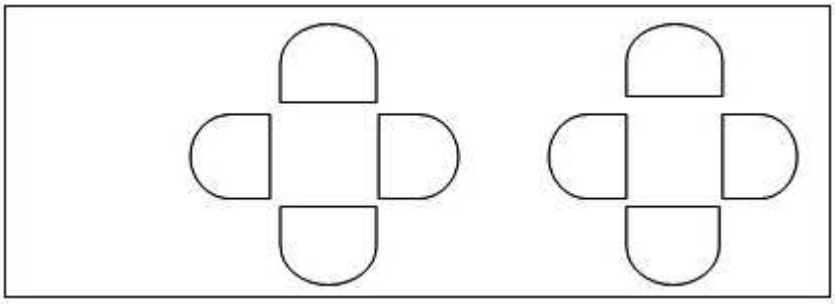

Figure 2

The sample, thus, obtained is placed on the glass slide in the orientation depicted. The discs obtained from nasal bulbar conjunctiva are placed medially and discs obtained from temporal bulbar conjunctivas are placed laterally

Special staining techniques- Special staining techniques have been devised for studying the impression cytology specimens with electron microscopy and immune-cytochemistry. For electron microscopy, the sample is fixed with 4% phosphate buffered formaldehyde with 1% glutaraldehyde and ruthenium red dye, post fixed in buffered osmium fixative, dehydrated, and fixed in resin [9]. Krenzer and Freddo devised a technique for staining the sample for immune-cytochemical study [11]. The specimen collected on a pure nitrocellulose membrane was fixed with a spray fixative and transferred to a poly-L-lysine coated glass slide and dried. This slide was placed in acetone with continuous agitation for 1 hour, washed with tap water for 5 minute followed by cellulose digestion for 2 hours at 37 º C. Various factors studied are- goblet cell density and morphology, nuclear/cytoplasmic ratio, nuclear morphology and inclusions, color of the

cytoplasm, emergence of keratinization, epithelial cell morphology, epithelial cell size, presence of inflammatory cell and cell sheet quality.

Indications:

Table I: Indications of ocular surface impression cytology

Definite indications
Studying conjunctival cellular morphology in normal ocular surface
Impact of various conditions like dry eye, chronic conjunctivitis, vitamin A deficiency, contact lenses on ocular surface
Detect the presence of micro-organisms, like Chlamydia
Diagnosing and staging ocular surface Neoplasia

Relative indications
Evaluate the effect of therapeutic interventions
Effect of systemic diseases like diabetes, renal failure, anorexia and thyroid disorders
Conjunctival changes in conditions like cystic
Fibrosis
Conjunctival melanosis

Grading system: Various grading systems have been devised for morphological assessment of the changes in conjunctiva on impression cytology. Grading system using goblet cell number and appearance of epithelial cells as devised by Nelson JD [4] is given below:

Grade 0- The epithelial cells are round and small with eosinophilic staining cytoplasm. The nuclei are large, basophilic with N/C ratio of 1:2. The mean individual epithelial cell area (MIECA) is <1000 μm^2. The goblet cells are abundant (>500 cells/mm^2), plump, and oval and have intensely PAS- positive cytoplasm.

Grade 1- The epithelial cells are larger and polygonal (MIECA<1000 μm^2) and have eosinophilic staining cytoplasm. The nuclei are smaller with N/ ratio of 1:3. The goblet cells are decreased in number (350-500 cell/mm^2). However, shape remains unchanged.

Grade 2- The epithelial cells are larger and polygonal (MIECA>1000 μm^2), occasionally multi-nucleated with variable staining. The N/C ratio varies from 1:4-1:5. The goblet cells are markedly decreased (100-350 cells/mm^2 and, are smaller, less intensely PAS-positive, with poorly defined cellular borders.

Grade 3- The epithelial cells are large and polygonal (MIECA>1000 μm^2) with basophilic staining cytoplasm. The nuclei are small, pyknotic and in some cells may be absent. The N/C ratio is 1:6. The goblet cell number is markedly reduced (<100 cell/mm^2).

This is the first morphological system of grading based on the appearance of the conjunctival epithelial and goblet cells. It provides quick means of gross assessment of the morphological appearance of the ocular surface thus, being a useful for the diagnosis and follow up.

Impression cytology in various ocular conditions:
OSSN: Impression cytology using the cellulose acetate membranes accurately predicts the diagnosis of neoplasia. In a study, it was proven to have 80% accuracy for predicting the subsequent histological diagnosis of OSSN The presence of characteristics like hyperkeratosis, inflammatory cells with only few dysplastic cells might indicate high-grade keratinizing dysplasia [12]. In another study, conjunctival impression cytology was shown to have 77% predictability rate for moderate dysplasia [13]. Impression cytology has also been used to study the effects of mitomycin C on OSSN. In one study, it was established that mitomycin therapy is beneficial in OSSN. Impression cytology serves as beneficial tool in studying the effects of mitomycin therapy on OSSN and thus, help in following the course of therapy and any recurrence [14].

Impression cytology in **keratoconjunctivitis sicca**: In KCS, with no inflammatory component like blepharitis, drug toxicity, the changes in the conjunctival goblet cell and epithelial changes occur in the inter-palpebral

conjunctiva in the early stages of the disease. As the disease severity progresses; the palpebral conjunctiva in the inferior fornix also shows the changes. The presence of inflammatory cells in the impressions confirms the complicated KCS, like drug toxicity states. In severe dry eye, the ocular surface may show keratinization (figure 3) of squamous epithelium

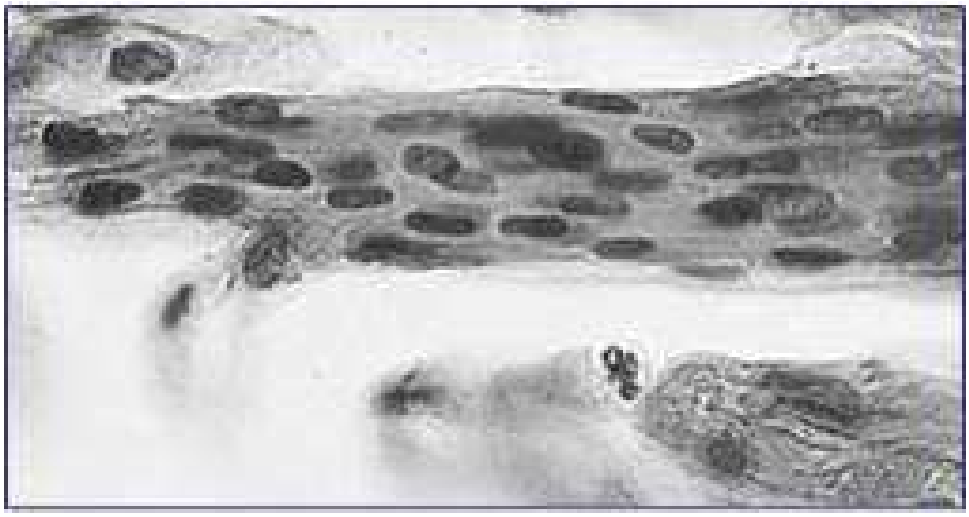

Figure 3: Microscopic picture of ocular surface impression cytology showing keratinized squamous epithelium

Impression cytology in intrinsic diseases like pemphigoid, chemical burns: In such conditions, both the palpebral and bulbar conjunctiva are involved. If the goblet cell density is <500 cell/mm^2 (grade 2 or 3), the presence of intrinsic diseases should be suspected.

Nelson and Wright [15] in a study showed that the goblet cell loss in keratoconjunctivitis sicca is 77% in the inter-palpebral bulbar conjunctiva and 60% in the inferior palpebral conjunctiva. The goblet cell density loss in ocular pemphigoid is 96% in the inter-palpebral conjunctiva and 95% in the inferior palpebral conjunctiva. In Stevens-Johnson syndrome, the goblet cell density loss is 99% in the inter-palpebral bulbar conjunctiva and 98% in the inferior palpebral conjunctiva.

Impression cytology in mucopolysaccharidoses IV: In MPS IV, impression-acquired epithelial cells showed cytoplasmic granularity, which stained positively with Alcian blue. Transmission electron microscopy shows sub cellular vacuoles bound by a single membrane containing fibrillogranular conclusions.

Impression cytology in chlamydial conjunctivitis: In inclusion conjunctivitis, impressions from the palpebral conjunctiva show the presence of basophilic inclusions in the cytoplasm of the epithelial cells, which are suggestive of elementary bodies.

Impression cytology in herpes zoster ophthalmicus: Impression cytology from cutaneous vesicular lesion shows the presence of multinucleated giant epithelial cells.

Allergic eye disorders: impression cytology from the conjunctiva in allergic eye disorders shows the presence of eosinophils/eosinophilic granules.

Conjunctival changes in contact lens wearers: In contact lens wearers, squamous metaplasia is seen on impression cytology, which has been defined by Tseng as -the pathological transition of a non-keratinized, stratified epithelium either secretory or non-secretory to a non-secretory keratinized epithelium [8]. Various changes seen in the conjunctival epithelial cells which indicate squamous metaplasia are- alteration in the shape (pyknosis, snake like chromatin) and density of the nucleus (altered N/C ratio), increase in the size of the epithelial cells and decrease in the goblet cell density [16]. Other changes seen are- greater expression of HLA DR and CD23 [17] and decreased expression of three out of five anti-oxidant enzyme genes [18]. However, these changes are reversible, not related to the material of the contact lenses and the duration for which the contact lenses are worn [8].

Impression cytology in Vitamin A deficiency: In some of the studies, it was shown that impression cytology

can be used to study the conjunctival changes in the avitaminosis [19, 20]. The changes seen were increased epithelial cell size and reduced goblet cell density in xerophthalmia. However, in another study, it was observed that impression cytology is not useful in detecting the changes in the conjunctiva in conditions like vitamin A deficiency [21].

Impression cytology in systemic diseases: In psoriasis, impression cytology showed squamous metaplasia as well as increase in goblet cell density [22]. Sixty-six percent of the patients with chronic renal failure showed Grade 3 changes of squamous metaplasia on impression cytology [23]. Acne vulgaris also sows the changes of squamous metaplasia [24].

Impression cytology in acanthamoeba keratitis: In a study, it was established that conjunctival impression cytology gives a high yield (94.6%) for the diagnosis of acanthamoeba keratitis. This demonstrates the presence of acanthamoeba trophozoites and cysts in the conjunctival epithelial cells [25].

Immunocytochemistry findings on impression cytology in various ocular surface disorders [26]:

Normal conjunctiva: In normal conjunctiva, immunological study for class II antigen HLA DR and IGE receptor CD23 are negative.

Chronic conjunctivitis: In chronic conjunctivitis, both the monoclonal antibodies were positive. However, use of topical steroids prevents the expression of these markers in the conjunctival cells.

Dry eye conditions: In Sjogren's syndrome, conjunctival cells are positive for HLA DR, but not for IGE receptors. In Steven-Johnson syndrome, both the antibodies were strongly positive.

Conclusion

For diagnosing the ocular surface disorders, various techniques available are conjunctival swabs/ biopsy/scraping etc. Impression cytology in comparison to all these techniques has the advantages like ease of manipulation, less traumatic to the patient; it has attracted the ophthalmologists' attention in the diagnosis and study of various ocular surface disorders. It is non-invasive and reproducible technique. Ocular surface impression cytology has been found immensely helpful in diagnosing dry eye states, following their course with treatment. It has been of great help in diagnosing and staging squamous metaplasia as well as follow up following resection or therapy with mitomycin C. Immunological markers like HLA DR and IGA receptor CD23 also help in diagnosis and differentiation of various ocular surface disorders. However, the full potential of ocular surface impression cytology remains to be explored.

Funding: Nil. **Permission for IRB**: Yes

References

1. Egbert PR, Lauber S, Maurice DM. A simple conjunctival biopsy. Am J Ophthalmol. 1977 Dec;84(6):798-801.

2. Adams AD. The morphology of human conjunctival mucus. Arch Ophthalmol. 1979 Apr;97(4):730-4.

3. Marner K. "Snake-like" appearance of nuclear chromatin in conjunctival epithelial cells from patients with keratoconjunctivitis sicca. Acta Ophthalmol (Copenh). 1980 Oct;58(5):849-53.

4. Nelson JD. Ocular surface impressions using cellulose acetate filter material: ocular pemphigoid. Surv Ophthalmol. 1982 Jul-Aug;27(1):67-9.

5. Nelson JD, Havaner VR, Cameron JD. Cellulose acetate impressions of the ocular surface: dry eye states. Arch Ophthalmol. 1983 Dec;101(12):1869-72.

6. Hatchell DL, Sommer A. Detection of ocular surface abnormalities in experimental vitamin A deficiency. Arch Ophthalmol. 1984 Sep;102(9):1389-93.

7. Wittpenn JR, Tseng SCG, Sommer A. Detection of early xerophthalmia by impression cytology. Arch Ophthalmol. 1986 Feb;104(2):237-9.

8. Tseng SCG. Staging of conjunctival squamous metaplasia by impression cytology. Ophthalmol. 1985 Jun;92(6):728-33.

9. Maskin SL, Bode DD. Electron microscopy of impression-acquired conjunctival epithelial cells. Ophthalmol. 1986 Dec;93(12):1518-23.

10. Martinez AJ, Mills MB, Jaceldo KB et al. Standardization of conjunctival impression cytology. Cornea. 1995 Sep;14(5):515-22.

11. Krenzer KL, Freddo TF. Cytokeratin expression in normal human bulbar conjunctiva obtained by impression cytology. Invest Ophthalmol Vis Sci. 1997 Jan;38(1):148-52.

12. Tole DM, McKelvie PA, Daniell M. Reliability of impression cytology for the diagnosis of ocular surface squamous neoplasia employing the bio pore membrane. Br J Ophthalmol. 2001 Feb;85(2):154-8.

13. Nolan GR, Hirst LW, Wright RJ, et al. Application of impression cytology to the diagnosis of conjunctival neoplasms. Diagn Cytopathol. 1994;11(30:246-9.

14. McKelvie PA, Daniell M Impression cytology following mitomycin C therapy for ocular surface squamous neoplasia Br J Ophthalmol 2001;85:1115-9

15. Nelson JD, Wright JC. Conjunctival goblet cell densities in ocular surface disease. Arch Ophthalmol. 1984 Jul;102(7):1049-51.

16. Tomatir DK, Erda N, Gurlu VP. Effects of different contact lens materials and contact lens-wearing periods on conjunctival cytology in asymptomatic contact lens wearers. Eye Contact Lens. 2008 May;34(3):166-8. doi: 10.1097/ICL.0b013e31815788ea

17. Albeitz JM. Conjunctival histologic findings of dry eye and non-dry eye contact lens wearing subjects. CLAO J. 2001 Jan;27(1):35-40.

18. Galarreta DJ, Corrales RM, Herreras JM et al. Levels of anti-oxidant enzyme genes in conjunctival impression cytology specimens from hydrogel contact lens wearers. Inv Ophthal Vis Sci. 2003;44:e abstracts 3682.

19. Singh M, Singh G, Dwewedi S, Kumar D, Tiwari A, Aggarwal M. Conjunctival impression cytology in xerophthalmia in rural children. Indian J Ophthalmol. 1997 Mar;45(1):25-9.

20. Chowdhary S, Kumar R, Ganguly NK, Kumar L, Naik CK, Walia BNS. Conjunctival impression cytology with transfer (CICT) to detect pre-clinical vitamin A deficiency among slum children in India. Br J Nutr. 1996 May;75(5);785-90.

21. Rahman MM, Mahalanabis D, Wahed MA, Islam M, Habte D, Khaled MA, Alvarez JO. Conjunctival impression cytology fails to detect sub clinical vitamin deficiency in young children. J Nutr. 1995 Jul;125(7):1869-74.

22. Soker S, Nergiz Y, Cakmak S, Aytekin S. Conjunctival impression cytology and bulbar surface epithelium changes in patients with psoriasis. Dicle Tip Dergisi. 2007;34:102-6.

23. Dursun D, Demirhan B, Oto S, Pinar A. Impression cytology of the conjunctival epithelium in patients with chronic renal failure. Br J Ophthalmol. 2004 Nov;84(11):1225-7.

24. Alp BN, karabas L, Bilen N, Talu H, Kaur A, Yanyali A. Evaluation of ocular surface changes on impression cytology in acne vulgaris. Tr J Med Sci. 1999;29:693-6.

25. Kanavi MR, Hosseini B, Javadi M, Rakhshani N, Javadi MA. Impression cytology in eyes with clinical and confocal scan features of acanthamoeba keratitis. J Ophthalmic Vis Res. 2013 Jul;8(3):207-12.

26. Baudouin C, Haouat N, Brignole F, Bayle J, Gastaud J. Immunopathological findings in conjunctival cells using immunofluorescence staining of impression cytology specimens. B J Ophthalmol. 1992 Sep;76(9):545-9.

.....

How to cite this article?

Gupta S. Role of impression surface cytology in ocular surface disorders. *Int J Med Res Rev* 2015;3(10):1228-1233. doi: 10.17511/ijmrr.2015.i10.223.

……