



Original Research Article

Role of impression cytology in ocular surface disorders

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ABSTRACT

Purpose: To study the role of Impression cytology in various ocular surface disorders and to evaluate its safety and compatibility.

Materials and Methodology: A case control study was done of which 25 patients who had clinically obvious conjunctival.

Involvement served as cases and 25 who were without any symptoms served as control. All the patients were subjected to tests of dry eye evaluation such as Schirmers test and Tear Film Break Up time (TFBUT) test. Conjunctival impressions were obtained using millipore cellulose acetate paper strips. Cytological samples were graded according to Nelson and Wright classification.

Results: Patients with bitots spot had large goblet cells and squamous metaplasia. All of the patients showed Grade I cytological changes (34.61% of all samples) Patients with severe chemical burns showed Grade III cytological changes i.e 100% of samples and patient with mild chemical burn showed grade II cytological change, i.e 99% of samples.

Conclusion: Impression cytology is a simple and non-surgical technique for diagnosis and therapeutic control of ocular surface disorders. It helps in identifying those at imminent risk of developing symptoms but also helps in assessing the severity of cytological changes in symptomatic subjects.

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1. Introduction

Ocular surface is comprised of the cornea, conjunctiva and the limbus comprise the tissues at the ocular surface. The primary function of the ocular surface is to provide clear vision when the eye is open. To achieve this while maintaining comfort and preventing microbial invasion, the ocular surface must be covered by a stable tear film.

Ocular surface disorders are a group of diverse pathogenesis in which the disease results in failure of mechanisms that maintain a healthy ocular surface. Two major types have been identified by impression cytology based upon resultant epithelial phenotype. The first type shows pathologic transition of normal non-keratinized ocular surface epithelia into keratinized epithelia (squamous metaplasia). The second type is characterised by the

replacement of the normal corneal epithelial phenotype with an invaded conjunctival epithelium (limbal stem cell deficiency).

Conjunctival impression cytology (CIC) refers to the application of cellulose acetate filter paper to the ocular surface to remove the superficial layers of the conjunctival epithelium. These cells can then be subjected to histological, immunohistological or molecular analysis. Application of impression cytology includes documenting dry eye, chronic infections and inflammations conjunctival scarring as in Steven-Johnson syndrome, Vitamin A deficiency etc. The morphology of the conjunctival ocular surface can be studied and the degree of squamous metaplasia can be assessed. This technique is noninvasive, economical, fast and easy to perform, causes minimal discomfort to the patient and can be used as follow up tool for changes in the conjunctival ocular surface over time. Hence, it is the ideal

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test for screening ocular surface disorders.

Other methods of conjunctival study are conjunctival biopsy and conjunctival scraping. They are invasive and hence cause patient discomfort. Limited sample of tissue are available for examination and samples cannot be flat mounted over slide.

Materials and Method

A total of fifty patients were selected from the Outpatient department of Ophthalmology of a tertiary care hospital in Maharashtra. Out of which 25 patients had clinically obvious conjunctival involvement and 25 were without any symptoms and signs served as control with age match. Patients who were included in the study were both male and female of age 20-60 years with chemical burns, thermal burns, Vitamin A deficiency and contact lens users. Patients with age less than 20 years and with conjunctival papillae and follicles were excluded from the study.

Cellulose acetate filter paper (sheet) was used for the study. (Cat# HAWP 304 FO from Millipore was trimmed into 3 x 10 mm size with a diagonal edge) Other materials used for the study were sterile Schirmer and fluorescein stain strips, fixating solution (95% Ethanol & 1% Formalin), staining reagents (Periodic Acid Schiff & Hematoxylin-Eosin), teflon container, glass slides, cover slips, stainless steel tweezer, simple microscope, slit lamp (Five step magnification, Appasamy).

Date of sample collection and the impression site for both eyes was noted. Impression site was according to the four quadrants of bulbar conjunctiva from where the sample was taken.

The patients were explained the procedure and their written consents were taken. After obtaining a detailed history of symptoms like redness, itching or discharge, thorough slit lamp examination of the anterior surface was performed. In addition, tear film evaluation was done by Schirmer test and tear film break up time (TBUT). Schirmer test was performed using Whatman No. 41 filter paper strips (5x35mm) after anaesthetising with 4% xylocaine. Tear film break up time was evaluated. Normal varies between 15 and 35 sec.

The patient was asked to lie down in supine position. The eyes were topically anaesthetized with 4% xylocaine drops. A speculum was inserted and the lacrimal lake at the inner canthus was dried with a swab. Conjunctival impressions were obtained using millipore cellulose acetate paper strips (3 x 10 mm size with a diagonal edge). A blunt smooth edged forceps was used to grasp the filter paper strip at one end and the paper was applied on the temporal bulbar conjunctiva. A smooth glass rod held in the other hand was used to press the paper gently. The paper strip was then removed with a peeling motion after 2-3 seconds. The strips were then applied to clean glass slides and were transferred to the pathology laboratory for fixing and staining. Fixing was done using 95% ethanol and 1%

formalin for one hour in Teflon container. The slides were stained with Periodic acid Schiff and Haematoxylin-Eosin. The slides were studied under low and high power with a light microscope

The cytology was graded according to the scheme suggested by Nelson.

2. Result and Observations

This study was conducted on 50 patients out of which 25 were diagnosed cases of ocular surface disorders on the basis of history, symptoms and clinical examination and 25 served as control. They were subjected to tear film evaluation and impression cytology in the affected eye. Tear film break up time was found to be abnormal in all cases and Schirmer's test was positive in chemical and thermal burn patients.

The impression cytology specimens were graded and classified according to Nelson and Wright classification. (Table 1)

Findings

Table 2: Schirmer Test Values

Disease	No. of patients	Schirmer test (mm)	
		Mean	Range
Chemical burns	04	11.5	10-15
Contact lens users	10	15.2	12-20
Vit A deficiency	09	13.22	11-15
Thermal burns	02	13	11-15
Normal Values of Schirmer test: 15 mm with topical anaesthesia			

Table 3: TFBUT Test

Disease	No. of patients	TFBUT test (sec)	
		Mean	Range
Chemical burns	04	5.25	4-6
Contact lens users	10	7.8	6-10
Vit A deficiency	09	4.2	3-5
Thermal burns	02	9	8-10
Normal Values of TFBUT test: 15-35 seconds			

Table 4: Findings of impression cytology

GRADING	No. of patients	Percentage
GRADE 0	18	36%
GRADE I	26	52%
GRADE II	03	06%
GRADE III	03	06%

3. Discussion

Conjunctival impression cytology was found to be suitable technique to obtain information of conjunctival surface. To

Table 1: Cytological graduation as per nelson & wright

Grade	Epithelial Cells	Nuclear to cytoplasm ratio	Goblet cells	Cytoplasmic cells
0	Small round	1:2	Abnormal	Eosinophilic
1	Larger	1:3	Abnormal	Eosinophilic
2	Larger	1:4 (Multi nuclear)	Decrease	Eosinophilic
3	Larger polygonal	1:6 or 1:7	Absent	Basophilic

Table 5: Findings of impression cytology: squamous metaplasia

Disease	Squamous metaplasia		
	Mild	Moderate	Severe
Chemical burns	-	-	04
Contact Lens users	-	19	-
Vit A deficiency	-	09	-
Thermal burns	-	-	02
Control group	18	07	-

Table 6: Findings of impression cytology: goblet cell count

Disease	Goblet cell count		
	Abnormal	Scanty	Absent
Chemical burns	-	01	03
Contact Lens users	10	-	-
Vit A deficiency	09	-	-
Thermal burns	-	02	-
Control group	25	-	-

study the conjunctiva in patients, previous investigators had used excised pieces of tissue or scrapings of conjunctiva which was traumatic to the patients. Conjunctival impression cytology was used for the first time in 1970.

The present study was undertaken on 50 patients out of which 25 served as control and 25 as cases and included both sex between age group 20-60 years. Diagnosis of the ocular surface was made depending upon history of redness of eyes, itching or discharge and a thoroughness of the anterior surface., Schirmer break up time (TFBUT).

Alterations of the ocular surface usually start with minor changes such as shortening of Tear film break up time (TFBUT) which subsequently progresses to squamous metaplasia. The end stage involves corneal neovascularization and persistent epithelial defects as a result of stem cell dysfunction. Although a decrease in tear production is a common condition in many ocular surface disorders, the severity of ocular surface lesions varies greatly from disease to disease. A clear correlation has not been found between tear functions tests such as the Schirmers test and TFBUT and ocular surface disorders. In present study, Schirmers test showed abnormal results only in 3 cases of alkali burns and one case of thermal burns. The TFBUT was found to be abnormal in all the cases. Decreased break-up time is associated with the decreased goblet cell density.¹

Subsequently, these patients were subjected to conjunctivacytology (CIC). Since the cell density and characteristics of the conjunctival surfacemay differ according to localization and the changes in ocular surface disorderfirst observed in bulbar then palpebral conjunctiva. In the present study, all samples were taken from the bulbar conjunctiva. The cytological features of epithelialcells as well as goblet cells were studied. The conjunctival impressions showed thatthe entire layer of superficial cells maintain their normal relationship to eachother. The conjunctival features of epithelial as well goblet cells were graded on basis of Nelson's classification. The goblet cells are identified by PAS positive cytoplasmwhich is dark pink in colour or by their eccentrically placed nuclei and plump shapeand large size. The epithelial cells are small and round with eosinophiliccytoplasm and larger basophilic nuclei with nucleo cytoplasmic ratio 1:2 in grade 0. As the grade increases the number of goblet cells reduce. The nuclei of epithelial cells go on reducing in size and there occurs change in nucleocytoplasmic ratio.

Nelson et al² have successfully counted the goblet cells using a calibrated grid at 200 or 400 over an area of 0.03 or 0.08 sq.mm respectively. The mean total of each such 10 areas was recorded for each specimen. Unfortunately such a grid was not available to us at the time of study. In our study, although the control group (n=25) was made up of healthy individuals of similar ages. Grade II or III metaplasia was not observed in healthy subjects but 72% were found to have Grade 0 and 28% showed Grade 1 cytological changes. Maumenee³ suggests that conjunctival keratinization may even be encountered when the tear film is normal. It has been reported that Schirmer 1 and TFBUT tests may reveal normal results in spite of the formation of metaplasia in conjunctiva. Thus diagnosis of the ocular surface was made depending upon thorough history and positive findings seen on examination.

In cases (n=25), following results were observed: Grade I changes in 76%, Grade II changes in 12% and Grade III changes in 12% The patient distribution in percentage was calculated to be; contact lenses users 40%, chemical burns 16%, Vitamin A deficiency 36% & thermal burns 8%.

In the present study, contact lens wearers safely underwent CIC without any pain or discomfort post procedure, unlike excision biopsy wherein even cessation of lens wear may be needed. All the 10 patients wearing contact lens showed grade 1 cytological changes. Both squamous metaplasia and goblet cell loss was observed in

our study and in the study conducted by Knop & Brewitt.^{4,5}

Contact lens wear is known to induce inflammation and tear film instability. Mechanical irritation by contact lens may cause squamous metaplasia and goblet cell loss, resulting in dry eye symptoms. Thus, CIC should be advised in those wearing contact lens wearers, whether symptomatic or not so that patients with CIC changes can be identified at an early stage and followed up regularly. This information can be used to advise the patient whether to continue or discontinue the lenses. Also, long term studies using CIC should be conducted to evaluate whether squamous metaplasia and goblet cell loss associated with contact lens use continues or plateaus with time.

Yet another interesting ocular disorder in our study is Vitamin A deficiency which may occur due to malnourishment or malabsorption. Wolbach⁶ has demonstrated that Vitamin A is essential for the normal differentiation and survival of the mucosal epithelium, and that its deficiency causes a metaplasia of mucosal epithelium to keratinized squamous epithelium. It shows a dull irregular surface due to squamous metaplasia with enlargement of cells subsequent keratinisation. This process is observed diffusely in bulbar conjunctiva (bitot spots) In our study, we had selected patients with bitots spot and we observed large goblet cells and squamous metaplasia. All of the patients showed Grade 1 cytological changes. Thus, it is suggested that CIC should be included as a routine screening test in children as well as patients of malabsorption, so that the deficiency gets detected and steps can be taken as early as possible.

In chemical injury, there may be mild to severe squamous metaplasia with decreased goblet cell count, depending upon the chemical and the time of contact. Alkali burns are usually more dangerous as they saponify the lipids in the cell membrane and this reaction often reaches the basal layer. The process is augmented by effects of collagenase and inflammatory mediators, loss of conjunctival stem cells, chronic inflammation and subconjunctival fibrosis resulting in goblet cell loss and mucin deficiency.⁷

In our study, there were 4 patients with alkali burns graded according to Hughes-Ropes-Hall⁸ classification of chemical burns All samples of patients with severe chemical burns showed Grade III cytological changes and 99% of the samples of patients with mild chemical burn and 2 patients with thermal burn showed grade II cytological changes. By applying CIC, we can detect and quantify the loss of goblet cells and thus think of conjunctival transplantation either from same eye, contralateral eye or buccal or nasal mucosal graft. It is advisable doing CIC rather than conjunctival scraping or biopsy which will be highly traumatic in such patients. Thus the method enables us to study and standardize the state of superficial conjunctival cells in these ocular surface disorders. While our series is too small to comment conclusively further research needs to be done.

This study has opened new avenues in early detection of changes in subjects of ocular surface disorders, utility in advanced investigations like transmission electron microscopy to demonstrate features of mucopolysaccharidosis and detection of viral particles in dreaded diseases like AIDS. Recently it has been adapted in immunohistochemistry as major advantage when compared with conventional tests for diagnosis of superficial viral infection. All these methodologies have been invaluable as research tools. Thus, it is only the beginning for further research in this field.

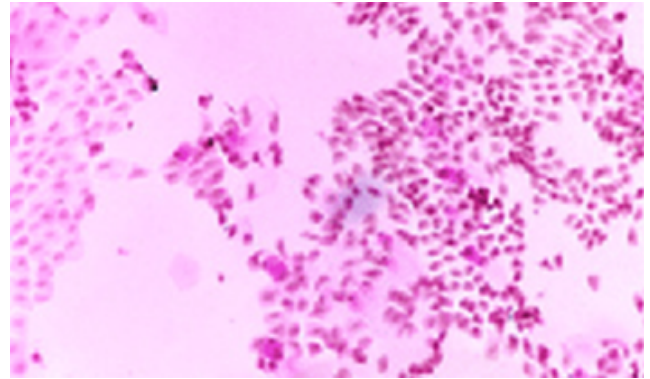


Fig. 1: Impression cytology of normal conjunctiva (X100, periodic acid schiff)

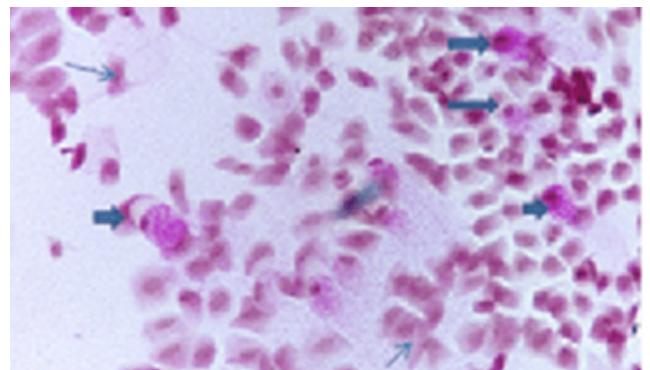


Fig. 2: impression cytology of normal conjunctiva showing goblet cells (X400, periodic acid schiff)

4. Conclusion

Our study revealed that impression cytology is a simple and non-surgical technique which allows qualitative and quantitative assessment of conjunctiva and provides better understanding of the pathological behaviour of the tissue. The method is both rapid and easy to perform in routine clinical practice. It helps in identifying those at imminent risk of developing symptoms of ocular surface disorders and helps in assessing the severity of cytological changes in



Fig. 6: Schirmers Strips



Fig. 5: 5:Fluorscein Strip

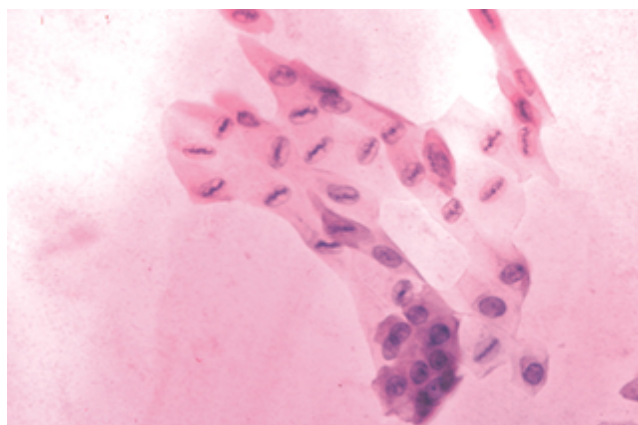


Fig. 3: impression cytology ofconjunctival surface in keratoconjunctivitis SICCA

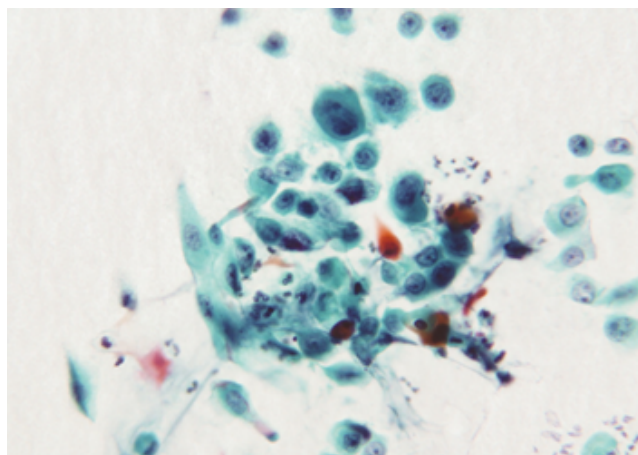


Fig. 4: Impression cytology of ocular surface with dysplastic squamous cells

symptomatic subjects. To conclude, it is a valuable adjunct for diagnosis of ocular surface disorders and provides reliable results but further research is required.

5. Source of funding

None.

6. Conflict of interest

None.

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