

**CONJUNCTIVAL IMPRESSION CYTOLOGY (CIC)- TECHNIQUE AND INTERPRETATION**Muralidharan P<sup>1</sup>, Amudha Vadivu Subramaniyan<sup>2</sup><sup>1</sup>Assistant Professor, Department of Ophthalmology, Government Thiruvavur Medical College, Thiruvavur.<sup>2</sup>Associate Professor, Department of Ophthalmology, Government Thiruvavur Medical College, Thiruvavur.**ABSTRACT****BACKGROUND**

To outline the technique and interpretation of Conjunctival Impression Cytology (CIC). 20 normal subjects between the age of 20-40 years with normal Schirmer's basic secretion test (>10 mm) and Tear Breakup Time (TBUT >10 secs) were included in this study. Subjects with a history of contact lens use, ocular surgery, degenerative corneal disease, autoimmune and metabolic disease and subjects on systemic or topical medications were excluded from the study.

**MATERIALS AND METHODS**

The Conjunctival Impression Cytology (CIC) sample was collected from superotemporal quadrant of bulbar conjunctiva using cellulose acetate filter paper 0.45 µm (millipore), stored and stained with PAS and Hematoxylin stain. The sample so obtained was then analysed for epithelial cells, goblet cells, mucin spots, mucin strands, mucus debris and other cells, e.g. neutrophils, mast cells, etc. Goblet cell density was then calculated as number of goblet cells multiplied by 100 number of epithelial cells and expressed as % per HPF. Squamous metaplasia was observed and graded based on goblet cell density and morphology, morphological changes of the nucleus and epithelial cells, metachromatic changes of cytoplasm and keratinisation and Nuclear-to-Cytoplasmic Ratio (N/C).

**RESULTS**

The GCD in the superotemporal quadrant of bulbar conjunctiva ranged from 24.2% to 36.5% with a mean of 30.60%. None of the eyes showed any squamous metaplasia. The technique of CIC, staining, calculation of goblet cell density and grading of squamous metaplasia will be discussed.

**CONCLUSION**

CIC is a simple, rapid, reliable, reproducible, noninvasive and effective technique, which can be used for diagnosis, treatment and prognosis of ocular surface disorders.

**KEYWORDS**

Conjunctival Impression Cytology, Ocular Surface Disorder, Schirmer's Test, Tear Film Breakup Time.

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**BACKGROUND**

Conjunctival Impression Cytology (CIC) is a minimally-invasive technique used to study conjunctiva and any changes if any at the cellular level. Egbert et al (1977)<sup>1</sup>; Tseng (1985)<sup>2</sup> impression cytology refers to the application of cellulose acetate filter paper to the conjunctival surface to remove the superficial layers of the ocular surface epithelium and subjected to histological analysis. It is noninvasive, easy to perform and provides reliable information about the goblet cells and cellular structure of the conjunctiva. This technique preserves the limbal stem cells also. This technique was first described by Egbert et al (1977).<sup>1</sup> This technique has been used to evaluate several ocular surface

disorders and several modifications to the original technique and interpretation have been introduced. CIC is 100% sensitive and 87% specific (Rivas et al 1993) Gill GW, Frost JK, Miller KA (1974).<sup>3</sup>

**Purpose-** To outline the technique and interpretation of Conjunctival Impression Cytology (CIC).

**AIMS AND OBJECTIVES**

20 normal subjects between the age of 20-40 years with normal Schirmer's basic secretion test (>10 mm) and Tear Breakup Time (TBUT >10 secs) were included in this study. Subjects with a history of contact lens use, ocular surgery, degenerative corneal disease, autoimmune and metabolic disease and subjects on systemic or topical medications were excluded from the study. The conjunctival Impression Cytology (CIC) sample was collected from superotemporal quadrant of bulbar conjunctiva using cellulose acetate filter paper 0.45 µm (millipore) after taking written consent.

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## MATERIALS AND METHODS

**Materials Needed for Collection and Staining CIC Sample are Millipore-** Type HA, pore size 0.45, 25 mm in diameter, Xylocaine 4%, straight non-toothed forceps, fixative solution in glass vials, cachets, micropore/leucoplast, Schiff's reagent, Haematoxylin stain, distilled water ethanol 50%, 60%, 70%, 80%, absolute 100%, xylene, glass slides, coverslip, oil for immersion slides and microscope.

Sample collection is done by a drop of Xylocaine 4% is installed in each eye and fornices are completely dried for any extra wet areas with cotton swab after closing the eyes. Round sheets of cellulose acetate filter paper millipore- type HA, pore size 0.45, 25 mm in diameter are cut into triangular pieces by dividing the circumferences into areas of 10 mm cut radially.

The pointed tip facilitates holding and transferring of the filter paper with the forceps, which is applied to the superotemporal area of the bulbar conjunctiva. The filter paper is marked for the side the smear is taken with lead pencil. The filter paper strip is then held with forceps. The subjects is said to look inwards and down to expose superotemporal bulbar conjunctiva.

Filter strip is applied onto the conjunctiva and gentle pressure is applied to the strip with forceps to adhere to conjunctiva so as to take care that no wetting of strip occurs on reflex tearing; otherwise, we have to repeat again, filter paper is taken from the conjunctival surface with forceps to give it a onion peel like feel and transferred onto the fixative containing vials.

### Inclusion and Exclusion Criteria

Fixative preparation is freshly prepared by mixing- 75 mL of ethanol + 5 mL of glacial acetic acid + 5 mL of formalin dehydrate (37%) + 25 mL distilled water and stored in small vials (marked separately for right or left eye) for collection of CIC samples, which have to be stained as soon as possible as they soon gets destroyed on long storage and not give desired results.

Staining technique of the CIC sample is by transferring of perforated rectangular boxes known as cachets, which are sealed on edges with leucoplasts or micropore, so the CIC sample does not get washed off on washing procedures. To remove all acid, water and fixative from CIC samples- 10 dips in distilled water done with 2 changes of distilled water (extensive washing must be done to get desired results).

### Adams AD (1979), Marner K (1980), Sommer A (1996)<sup>4-7</sup>

1% PAS staining (cytoplasmic stain) is done by dipping the cachets in 1:1 distilled water- PAS, which is later shifted to Schiff's reagent stain (which is standardised first by staining with known tissue slides for staining consistency) for 5 minutes only so that the wrap must be red practically. Later put it under running tap water after 15 minutes to wash out stain, which again must be extensive so as to remove extra stain to get desired staining results.

After 15 minutes of wash, nuclear stain done with harer haematoxylin stain, 10 dips of 3 changes done and left under

running tap water to wash out stain for 15 minutes, which must be extensive for to remove extra stain to get staining results.

Later CIC smear is dehydrated with 50%, 70%, 80%, 95% ethanol of 10 dips each. Later leucoplast tags are removed from the edges of the cachets before dipping it into xylene.

Clearing done by putting the CIC smears in (1:1) xylene:ethanol for 15 minutes and later shifted to pure xylene for half to 2 hours before mounting is done.

## RESULTS

Mounting is done later after clearing of the CIC sample had occurred. They are transferred with forceps onto glass slides taking care correct side of smears is mounted upside looking for pencil lead mark side and cover slip applied without any air bubble being trapped in between glass slide and cover slide.

Then, oil mounting is done and examined under oil immersion and high magnification for cellular morphology.

### Grading of CIC Changes

Various staging systems are defined according to the following cytological features-

- Goblet cell density and morphology.
- Morphological changes of the nucleus and epithelial cells.
- Nucleocytoplasmic ratio (N/C).
- Metachromatic changes of cytoplasm and keratinisation.

$$\text{GCD} = \frac{\text{Goblet Cells} \times 100}{\text{Epithelial Cells}}$$

**Tseng et al, Adam et al and Nelson et al (1988)<sup>2-9</sup>** had studied goblet cell density in normal subjects and proposed staging systems for goblet cell density and squamous metaplasia of CIC.

The scheme of both Nelson and Tseng et al had the limitation of assigning a single or dominant grade to each specimen. However, with the modification of Nelson's technique, the sensitivity to variations in the distribution of squamous metaplasia can be increased.

### Modified Staging System

Grade	Cell Size	Cytoplasm Edge	N/c Ratio
Mild (Gr. 1)	Slight increase	Slight coagulation	1:3
Moderate (Gr. 2)	Two times	Market coagulation	1:4 to 1:5
Severe (Gr. 3)	Four times	Rolling of edges	>1:6

## OBSERVATIONS

The GCD in the superotemporal quadrant of bulbar conjunctiva ranged from 24.2% to 36.5 with a mean of

30.60%. None of the eyes showed any squamous metaplasia.

### DISCUSSION

CIC is a useful technique to evaluate squamous metaplasia and goblet cell changes in any ocular surface disease. There are numerous applications of impression cytology like in dry eye syndrome to diagnose and identify goblet cell density and squamous metaplasia, ocular surface neoplasia, vitamin A deficiency, diagnosis of limbal deficiency, monitoring of microorganisms and monitoring of topical medications.

The most common applications in diagnostic ocular pathology are- (i) Primary diagnosis and followup of ocular surface squamous neoplasia including after therapy. The sensitivity is high (78-87%); and (ii) Dry eye syndrome where squamous metaplasia and/or hyperkeratosis are noted.

There are limitations of the technique as dysplasias are often keratinising and may yield very few or even no dysplastic cells with impression cytology. Secondly, no definite cytological criteria reliably distinguish invasive neoplasia of ocular surface from in situ disease.

### CONCLUSION

CIC is a relatively simple, reproducible technique. It can be used as an adjunct to Schirmer's and tear film studies for evaluation of ocular surface disorder. The ability to obtain multiple sample of the ocular surface at one sitting with minimal discomfort to the patient makes it an ideal method of investigating ocular surface disorders.

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