

## SENSITIVITY OF IMPRESSION CYTOLOGY IN DIAGNOSING OCULAR SURFACE SQUAMOUS NEOPLASIA

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**ABSTRACT:** OSSN describes a spectrum of neoplastic lesions, originating from squamous epithelium ranging from simple dysplasia to invasive squamous cell carcinoma (SCC), involving the conjunctiva, limbus and the cornea. Impression cytology refers to the technique by which superficial layers of the ocular surface are removed through application of cellulose acetate filter material onto the ocular surface. **AIM:** To Assess the Sensitivity of Impression Cytology in the Diagnosis of Ocular Surface Squamous Neoplasia Using Millipore Filter Paper. **MATERIALS AND METHODS:** This is a Prospective Observational and Interventional Study conducted at Sarojini Devi Eye Hospital and Gandhi Medical College/Hospital, Hyderabad. Study was conducted from July 2013 to Feb 2015 and included 50 patients presenting with conjunctival mass at the limbus or on the conjunctiva. Hospital ethics committee approval was obtained. No financials involved in the study. **RESULTS:** A total of 50 excision biopsies of 50 patients suspected for OSSN were performed. There were 40 males and 10 females. Excision biopsy confirmed the Impression Cytology results in 44 cases. In 6 cases there was poor correlation among which 4 cases showed mild dysplasia in Impression Cytology while HPE showed Invasive Squamous cell carcinoma in 2 cases. Few dysplastic cells were noted in Impression cytology but HPE showed Carcinoma in situ. **CONCLUSION:** This study shows that Impression Cytology has a promising role in diagnosing Ocular Surface Squamous Neoplasia for its high positive predictive accuracy (95.65%) compared with tissue histology.

**KEYWORDS:** Impression Cytology, Ocular surface squamous neoplasia.

**INTRODUCTION:** OSSN describes a spectrum of neoplastic lesions, originating from squamous epithelium ranging from simple dysplasia to invasive squamous cell carcinoma (SCC), involving the conjunctiva, limbus and the cornea.

**Predisposing factors:** Genetic injury to a proliferating cell disturbs the basic cellular functions like division and differentiation and these cells become neoplastic. OSSN may arise from dysfunctional limbal stem cells having been altered by various mutagenic agents, such as UV radiation. It starts in the interpalpebral conjunctiva and then straddles the limbus and may involve cornea.

The technique of impression cytology was first described by Egbert et al in 1977.<sup>(1)</sup> Adams described the use of this technique to study the mucous network on the conjunctival surface in 1979. Hatchell and Sommer suggested that impression cytology could be used for early detection of Vitamin A deficiency.<sup>(2)</sup> Tseng SCG has done staging of conjunctival metaplasia by impression cytology in 1985.<sup>(3)</sup>

Nolan et al first reported application of impression cytology using acetate filter paper for the diagnosis of conjunctival neoplasm.<sup>(4)</sup> Thiel et al described the use of new Biopore membrane device for obtaining conjunctival cells for the diagnosis of superficial infections.<sup>(5)</sup>

Derek M Tole studied the reliability of impression cytology for the diagnosis of ocular surface squamous neoplasia employing the Biopore membrane. Impression cytology has been found to be

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useful in assessing the ocular surface in various dry eye disorder such as keratoconjunctivitis sicca, ocular pemphigoid, vitamin A deficiency and ocular surface squamous neoplasia.<sup>(6)</sup>

The technique is non-invasive and easy to perform, causes minimal discomfort to the patient and can be used to follow changes in the conjunctival ocular surface over time.

### MATERIALS AND METHODS:

#### CRITERIA FOR SELECTION OF PATIENT:

**Inclusion Criteria:** Patients with conjunctival limbal lesions presenting as an elevated, variably shaped, accompanied by feeding blood vessels are included.

**Exclusion Criteria:** Patients already undergone treatment were excluded from the study Limbal mass with feeder vessel.



**Fig. 1**

**METHODOLOGY:** After instilling topical anaesthetic drop in to the eye, impressions were obtained using Millipore filter paper pre-operatively from all the patients undergoing Excision biopsy for suspected OSSN.

The Millipore filter paper with pore size 0.22 micrometre is cut in to 3mm bits by holding it carefully with the forceps, to avoid sticking of desquamated cells from the hands.

Under topical anaesthesia these strips were placed on the ocular surface lesion and pressed gently, until the filter paper becomes wet, this takes on an average 10-20 sec.

Then the filter paper is carefully peeled off from the lesion and transferred into a container of 90% alcohol immediately without air-drying for 15-20 min.

Millipore membrane



**Fig. 2**

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**In the laboratory the filter paper are stained with Haematoxylin and Eosin stain as follows:**

1. Remove the strips from 90% alcohol and dry them.
2. Stain with Haematoxylin solution for 1 hr. and wash under tap water.
3. Quickly dip in Acid alcohol followed by a dip in Ammonia for 1 sec.
4. Stain with Eosin solution for 1 sec.
5. Dehydrate in graded alcohol's 70%, 90% and absolute alcohol with dip in each container for 1 sec.
6. Dry the paper thoroughly and dip in Xylene for 10-15 min and mount them on glass slides using cover slip.
7. The Millipore filter paper at this stage is fully transparent and allows cytological examination.

**Cases were then posted for surgery for wide excision with 3mm normal margin of tumour:**

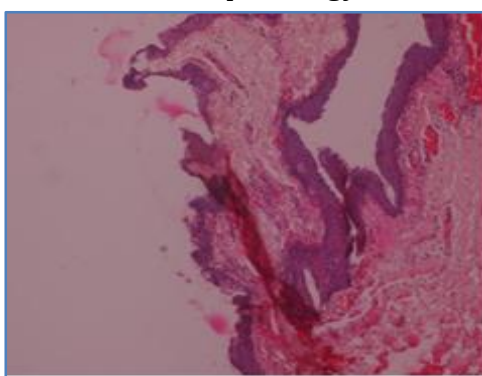
- Freeze – thaw – Freeze cryo application was done to bare sclera.
- Samples were sent for Histopathological examination.

**Normal conjunctival epithelium:**

*Impression cytology*



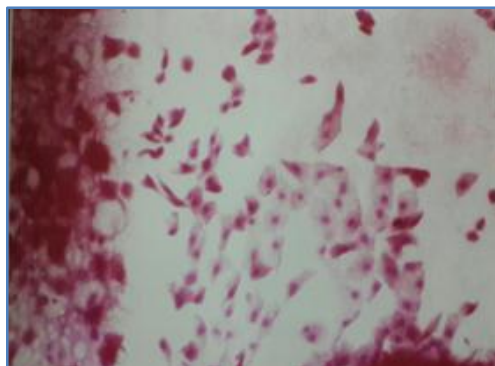
*Histopathology*



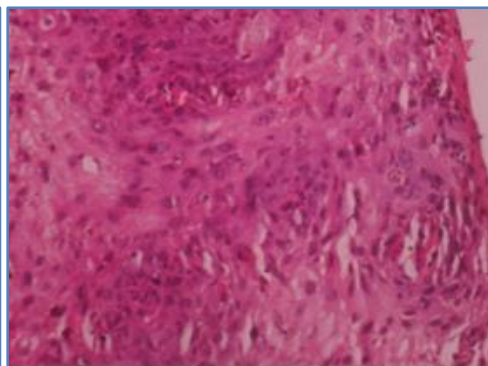
**Fig. 3**

**Dysplastic Epithelium:**

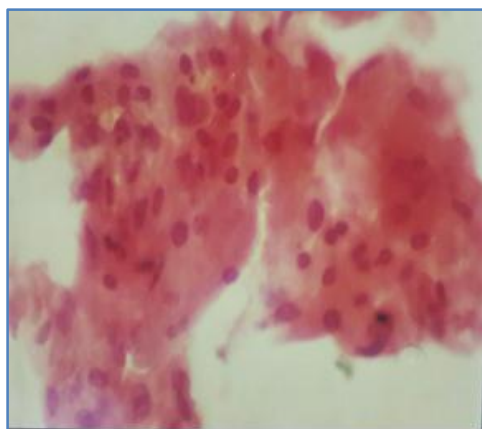
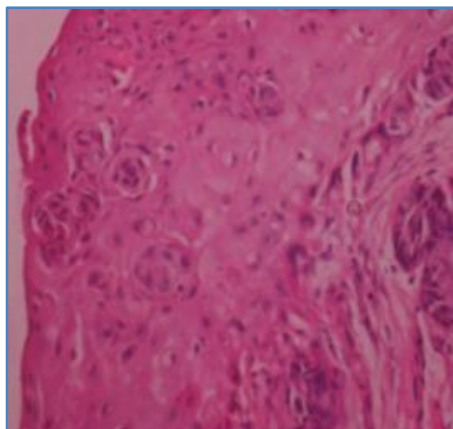
*Impression cytology*



*Histopathology*



**Fig. 4**

**Squamous Metaplasia:***Impression cytology**Histopathology***Fig. 5**

**RESULTS:** A total of 50 Excision biopsy of 50 patients suspected for OSSN were performed. There were 40males and 10 females. Excision biopsy confirmed the Impression Cytology results in 44 cases In 6cases there was poor correlation, among which 4 cases showed mild Dysplasia in Impression Cytology while HPE showed Invasive Squamous cell carcinoma. In 2 cases few Dysplastic cells were noted in Impression cytology but HPE showed Carcinoma in situ. On analysing data Impression Cytology sensitivity comes to 91.6%, High correlation rate of 88% with histopathology Positive predictive value 95.65%, and Negative predictive value 25%.

**CONCLUSION:** This study shows that Impression Cytology has a promising role in diagnosing Ocular Surface Squamous Neoplasia for its high positive predictive accuracy (95.65%) compared with tissue histology. However, a fair negative predictive accuracy (25%) indicates that Impression Cytology is a Valuable screening technique, but it is not a “Gold standard”.

**DISCUSSION:** Impression cytology employing millipore membrane accurately predicts the histological diagnosis of ocular surface squamous neoplasias with a high correlation rate of 88%. Before the introduction of millipore membrane for ocular surface impression cytology, cellulose acetate membranes were used which were time consuming and required a technical person to perform the procedure.

This laborious procedure has restricted many ophthalmologists from employing this technique. By comparison new millipore membrane device is easy and rapid to use in routine clinical practice. The kit contained sterile filter paper strips, xylocaine topical eye drops and 20ml jar filled with 95% alcohol, which can be carried easily and transport the specimens to the laboratory for cytological assessment.

It is found in the study that impression cytology with millipore membrane has a role in the assessment follow up of patients with suspected ocular surface squamous neoplasia.

The reason for developing the technique of impression cytology is that it is very simple, practical and noninvasive technique with a high level of sensitivity for diagnosis of ocular surface squamous neoplasia. OSSN is predominantly a disease affecting limbus in elderly individuals.

Squamous cell carcinoma of ocular surface is a low grade malignancy. Elderly patients are not

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always fit for biopsy and refuse biopsy for a disease that is not going to reduce their life expectancy.

Impression cytology offers a safer alternative to diagnosis than biopsy in older patients. Impression cytology with millipore membrane may also be used in confirmation of diagnosis, where excision biopsy is not required, such as follow up in those patient who have been treated with alternative methods to excision biopsy such as mitomycin.

### Sex distribution of cases

Sex	Number of cases	Percentage%
Male	40	80%
Female	10	20%

### Cytological correlation of cases

Number of cases with good correlation	Predictability%
44	88%

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