Research Article

Impression cytology in the diagnosis of ocular surface squamous neoplasia (OSNN)

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Abstract

Introduction: Ocular Surface Squamous Neoplasia (OSSN) describes a spectrum of conjunctival and corneal epithelial neoplasia manifesting as dysplasia, Carcinoma-In-Situ (CIS) and Squamous Cell Carcinoma (SCC). Impression cytology refers to the application of cellulose acetate filter to the ocular surface to remove superficial layers of the ocular surface epithelium. The cells thus removed are subjected to haematoxylin and eosin staining. **Objectives:** To evaluate the accuracy of impression cytology in the diagnosis of Ocular Surface Squamous Neoplasia by comparing it with the histopathology of the excised specimens. **Methods:** It was a prospective, interventional case series conducted from December 2007 to June 2009 at Sarojini Devi Eye Hospital. 50 eyes of 48 patients with clinical suspicion of OSSN were included. Impression cytology was done using biopore membrane and reported by a single Pathologist, following which excision biopsy was performed. Cytology and histopathology reports were compared and correlation was said to be present if dysplastic cells were present on cytology in a patient found to have neoplasia on histopathology. **Results:** Out of 50 eyes with clinical suspicion of OSSN, 44 eyes were found to have neoplasia on histopathology. Correlation between impression cytology and histopathology was present in 38 eyes (86.36%). **Conclusion:** Impression cytology is a simple, non-invasive technique with a high correlation rate in the diagnosis of OSSN. It is especially useful in patients with suspected recurrence. Presence of hyperkeratosis on cytology has a poor correlation with histopathology.

Keywords: Histopathology, impression cytology, biopore membrane, OSSN.

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INTRODUCTION

The term **Ocular Surface Squamous Neoplasia** (OSSN) was coined by Lee and Hirst. It describes a spectrum of conjunctival and corneal epithelial neoplasia manifesting as dysplasia, carcinoma-in-situ (CIS) and squamous cell carcinoma (SCC). Incidence of OSSN has a wide geographical variation, ranging from 0.13 to 1.9 per 100,000 population. Worldwide it is the third most common tumour after retinoblastoma and melanoma. Males are affected more often than females. The average age of presentation is usually in the sixth and seventh decade. The exact **pathogenesis** of OSSN is unknown but is probably multifactorial. These tumors commonly occur at the limbus, a dynamic transitional zone containing stem cell population that has lifelong potential for clonagenic expansion. Ultraviolet-B rays cause formation of pyramidine dimmers that damage DNA strand and result in delay or failure in DNA repair. This may lead to somatic mutations and development of neoplasia. Phenotypic features such as fair skin, pale irides, propensity to sunburn, history of actinic skin lesions before fifty years of age are known **risk factors** for development of OSSN. Xeroderma pigmentosum has a predilection for the development of OSSN due to an abnormality in DNA repair. A close association between OSSN and Human Papilloma Virus (HPV) has been observed with HPV being detected in 39-88% of patients. OSSN is also associated with HIV. In HIV patients, the tumor occurs in a younger age group, tends to be bilateral and multifocal, exhibits rapid growth and is of greater severity with a higher recurrence rate. These tumors are

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characteristically located at the limbus ecncroaching onto the corneal surface but may occur elsewhere in the conjunctiva. It is usually unilateral. OSSN on the cornea presents as an elevated gray intraepithelial plaque with fimbriated margin and clusters of isolated gray spots that have a beaten metal sheen under retroillumination. **Morphologically** there are three types of lesions:

1. Gelatinous (leukoplakic or papilliform)
2. Nodular
3. Diffuse

Characteristics of cells on **Cytology**:

Normal

Conjunctival epithelium: Small, round, uniform epithelial cells, large basophilic cytoplasm, nucleocytoplasmic ratio of 1:1 or 1:2, plump PAS positive cells (>500/sq.mm).

Metaplasia

Loss of goblet cells, keratinization evident as pinkish cytoplasm, enlargement of cells, nucleocytoplasmic ratio reduced (1:4-1:8), pyknotic nuclei with densely packed keratin filaments in the cytoplasm.

Dysplasia

Pleomorphism, enlarged and hyperchromatic nuclei, irregular nuclear contour with coarse chromatin, prominent nucleoli, nucleocytoplasmic ratio increased, increased number of mitotic figures, loss of normal cellular maturational polarity.

Invasive squamous cell carcinoma

Severe changes than dysplasia, more bizarre cell types and patterns, tumor diasthesis – necrotic tumor cells, debris, blood and leukocytic exudates.

Histopathology is the gold standard for the diagnosis and grading of OSSN. Excision biopsy is both diagnostic and curative.

Grades of OSSN

Mild dysplasia: Dysplastic cells restricted to the lower one-third of the epithelial layer. Moderate dysplasia: Dysplastic cells occupying three-fourth the thickness of epithelium. Severe dysplasia/Carcinoma-in-situ: Complete involvement of the epithelium including surface layer but no breach of basement membrane. Invasive squamous cell carcinoma: Breach of the basement membrane with involvement of substantia propria.

Impression Cytology refers to the application of cellulose acetate filter to the ocular surface to remove the superficial layers of the ocular surface epithelium. The cells thus removed are subjected to haematoxylin and eosin staining. Our study compares the impression cytology findings of patients with clinical suspicion of OSSN with the histopathology of the excision biopsy specimens.

METHODS

The study was conducted at Sarojini Devi Eye Hospital, Hyderabad between December 2007 and June 2009. It was a prospective, interventional case series. The study included 50 eyes of 48 patients. All patients with clinical suspicion of OSSN who presented to the department of Oculoplastics during this period were included in the study. Patients were explained about impression cytology and consent was taken. Exclusion criterion: Patients who refused surgery were excluded from the study. Methodology: Detailed history was taken from all these patients. Best corrected visual acuity was recorded. Slit lamp and fundus examination was performed. Intraocular pressure was measured by applanation tonometry. Gonioscopy was performed. All patients were subjected to impression cytology. Impression cytology: A drop of 4% xylocaine was instilled into the eye of the patient. Biopore membrane was cut into small bits of size 5×5mm. Eyelids of the patient were held wide apart. The biopore membrane bit was held with a sterile forceps and pressed firmly against the lesion for about 5 to 10 seconds. The membrane was then transferred into a container of 95% alcohol for about half an hour for fixation. It was then mounted onto a slide, stained with haematoxylin and eosin and observed under light microscope. All the slides were examined and reported by a single Pathologist. The slides were observed for the presence of dysplastic cells, hyperkeratosis, inflammatory cells or other associated findings. Surgery: Wide excision biopsy with cryotherapy of the conjunctival margins were performed under local anaesthesia. In patients with extensive intraocular involvement enucleation was done. In patients with extraocular spread exenteration was performed. Excision biopsies and enucleated specimens were subjected to histopathology. Cytology and histopathology reports were compared. Correlation between cytology and histopathology was said to be present if dysplastic cells were present on cytology in a patient found to have neoplasia on histopathology.

Figure 1

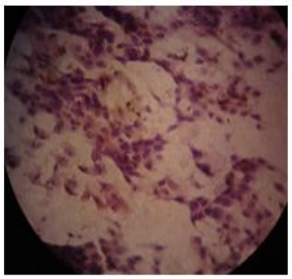


Figure 2

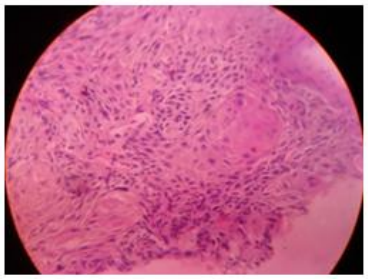


Figure 3

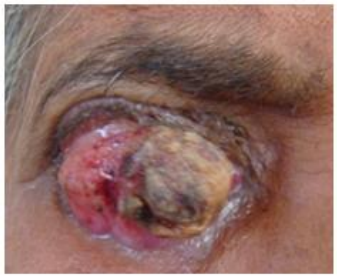


Figure 4

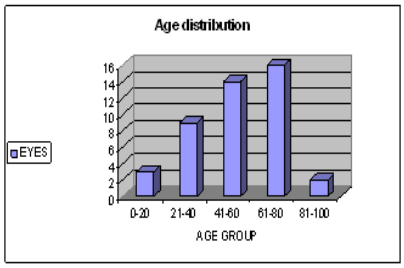


Figure 5

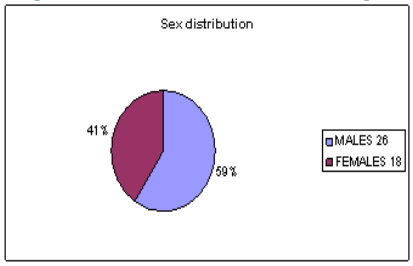


Figure 6

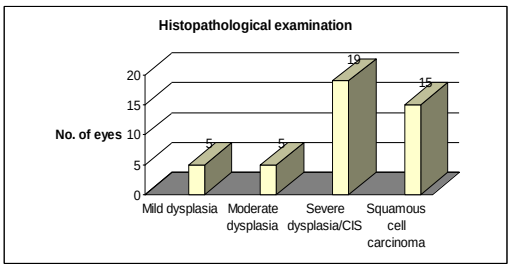


Figure 7

Legend:

Figure 1: Showing photograph of a patient with right eye OSSN

Figure 2: Showing dysplastic cells on impression cytology

Figure 3: Showing moderately differentiated squamous cell carcinoma

Figure 4: Showing late presentation of a patient with squamous cell carcinoma with destruction of eyeball.

Figure 5: Showing age distribution of patients

Figure 6: Showing sex distribution: There were 26 males (59%) and 18 females (41%) in our study

Figure 7: Showing histopathology findings

RESULTS

50 eyes of 48 patients were included in the study. Of these eyes with clinical suspicion of OSSN, 44 eyes were confirmed to be Ocular Surface Squamous Neoplasia on histopathology. **Age distribution** of patients with OSSN was as shown in table 1 and figure 1.

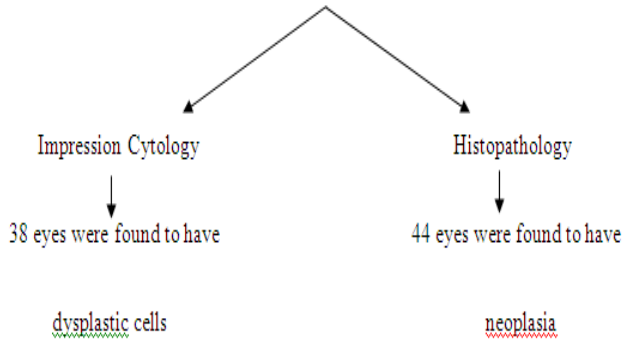
Table 1: Age distribution of patients with OSSN

Age group	Number of eyes
0-20	3
21-40	9
41-60	14
61-80	16
81-100	2
Total	**44**

Site: Initial site of appearance of the mass was as follows:

- Bulbar conjunctiva: 42 eyes (95.5%).
- Palpebral conjunctiva: 2 eyes (4.6%)

Pigmented OSSN was present in 4 eyes (9.1%). Destruction of eyeball with **fungating mass** was noted in 2 eyes (4.6%). **Bilateral** sequential involvement was seen in 2 eyes (4.6%). 50 eyes with clinical suspicion of OSSN



Impression Cytology Histopathology 38 eyes were found to have 44 eyes were found to have dysplastic cells neoplasia

Table 2: Showing positive and negative cases on cytology and histopathology

	Positive	Negative
Positive	38	0
Negative	6	6
Total	**44**	**6**

Histopathology Impression Cytology

Sensitivity of impression cytology: 86.4%
Specificity of impression cytology: 100%
Correlation between impression cytology and histopathology was present in 38 eyes (86.36%). There was no correlation in 6 eyes.

Table 3: Showing eyes with non-correlation

Sr. No.	Cytology	Histopathology
1.	Hyperkeratosis	Squamous cell carcinoma.
2.	Hyperkeratosis	Carcinoma-in-situ
3.	Hyperkeratosis	Carcinoma-in-situ
4.	Normal squamous cells with no dysplastic cells	Moderate dysplasia
5.	Normal squamous cells with inflammatory cells	Squamous cell carcinoma
6.	Scanty cellular yield with normal squamous cells	Carcinoma-in-situ

Table 4: Showing Histopathological examination findings of OSSN

Histopathology	No. Of eyes
Mild dysplasia	5 (11.4%)
Moderate dysplasia	5 (11.4%)
Severe dysplasia/Carcinoma-in-situ	19 (43.2%)
Invasive squamous cell carcinoma	15 (34.1%)
Total	**44**

DISCUSSION

50 eyes of 48 patients were included in the study. 44 eyes turned out to be Ocular Surface Squamous Neoplasia on histopathology. Commonest age group of presentation was between 60 and 70 years with male preponderance (59%). Most of the patients were farmers and labourers involved in outdoor occupation thereby exposed to UV radiation for long hours. Limbus was the predominant site of involvement in these patients. There were no false positives on impression cytology. The sensitivity of impression cytology was 86.4% and specificity was 100%. There was high correlation between cytology and histopathology (86.4%) in our study.

Table 5: Showing comparison with other studies

	Impression cytology	Correlation
Our study	Biopore membrane	86.4%
Tolea *et al*[1]	Biopore membrane	80%
Nolan *et al*[5]	Cellulose acetate filter paper	77%

The higher correlation rate in our study could be because of presentation of patients at a more advanced stage of the disease. The percentage of invasive squamous cell carcinoma in our study was 34.1% whereas in a study by Tolea *et al*[1] it was 4%.In our study we found that impression cytology had a poor correlation with histopathology in eyes with hyperkeratosis. This could be due to scanty cellular yield on cytology in patients with hyperkeratosis.

CONCLUSIONS

Impression cytology is a simple, non invasive technique with a high correlation rate in the diagnosis of Ocular Surface Squamous Neoplasia. It can be used as a routine investigation in patients with suspected Ocular Surface Squamous Neoplasia. It is especially useful in patients with suspected recurrence following excision biopsy because repeated biopsies can lead to stem cell deficiency. It is also useful in patients who are not fit for surgery and those who refuse surgery. It can be used for follow up in patients on Mitomycin C therapy. Presence of hyperkeratosis on cytology has a poor correlation with histopathology.

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