

Research Article

Ocular Surface Squamous Neoplasia Impression Cytology V/S Histopathology

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Abstract: Ocular Surface Squamous Neoplasia (OSSN) is spectrum of conjunctival and corneal epithelial neoplasia manifesting as dysplasia, carcinoma-in-situ (CIS) and squamous cell carcinoma (SCC). OSSN has a wide geographical variation, ranging from 0.13 to 1.9 per 100,000 population. Males are affected more often than females. The average age is usually sixth and seventh decade. The abnormal maturation of neoplastic cells is reflected in the morphology of the surface cells and their study forms the underlying principle of cytology. In impression cytology, either cellulose acetate paper or Biopore membrane is used for sampling. The study was conducted between June 2008 and May 2013. The study included 170 eyes of 168 patients. Biopsies from 168 patients found to have histologically proven OSSN are included in the study. On cytomorphology 130/170 specimens (76.4%) correlated with histopathology. Histological spectrum showed well differentiated SCC 34(33.3%) moderately differentiated SCC 53(51.9%) and poorly differentiated SCC 15(14.7%). Recurrence was seen in 31 cases (18.4%) at the limbus. OSSN occurs in the sun exposed areas of the conjunctiva at the limbus in elderly individuals. Impression cytology(IC) employing biopore membrane accurately predicts the histological diagnosis of (OSSN) with a high correlation rate of 76.4%. Histological spectrum showed more of invasive than Non invasive lesions. 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 following excision biopsy.

Keywords: Ocular Surface Squamous Neoplasia, Impression cytology, Limbus, Biopore membrane, Dysplasia, Squamous cell carcinoma

INTRODUCTION

The term Ocular Surface Squamous Neoplasia (OSSN) was coined by Lee and Hirst [1]. It describes a spectrum of conjunctival and corneal epithelial neoplasia manifesting as dysplasia, carcinoma-in-situ (CIS) and invasive squamous cell carcinoma. Incidence of OSSN has a wide geographical variation, ranging from 0.13 to 1.9 per 100,000 population. Males are affected more often than females [2]. The average age of presentation is usually in the sixth and seventh decades. HPV types 16, 18, 31, 33 and 35 are associated with malignant tumors [3].

The exact pathogenesis of OSSN is unknown but is probably multifactorial. These tumors commonly occur at the limbus [4]. It is usually unilateral. The abnormal maturation of neoplastic cells is reflected in the morphology of the surface cells and their study forms the underlying principle of cytology. These cells are studied by impression cytology(IC). Either cellulose acetate filter paper or Biopore membrane is used for sampling. Egbert et al first introduced impression

cytology into Ophthalmology in 1977. They used cellulose acetate filter paper [5]. Thiel *et al.* have described the use of Biopore membrane. The adhered cells are then subjected to haematoxylin and eosin staining or other cytologic stains [6].

Characteristics of cells on cytology:

Normal conjunctiva epithelium: Small, round, uniform epithelial cells with large basophilic cytoplasm, nuclearcytoplasmic ratio of 1:1 or 1:2 along with few plump PAS positive cells.

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

Dysplasia: Pleomorphism, enlarged and hyperchromatic nuclei, irregular nuclear contour with coarse chromatin, prominent nucleoli, increased

nucleocytoplasmic ratio, increased number of mitotic figures [7].

Histopathology

Histopathology is the gold standard for the diagnosis and grading of OSSN. Excision biopsy is both diagnostic and curative. Incision biopsy is undertaken only when the tumor is extensive and orbital exenteration has been planned.

Grades of OSSN on histopathology:

Mild dysplasia: Dysplastic cells restricted to the lower one-third of the epithelial layer.

Moderate dysplasia: Dysplastic cells occupying two thirds of the thickness of epithelium.

Severe dysplasia / Carcinoma-in-situ: Complete involvement of the epithelium including surface layer without breach of the basement membrane.

Invasive squamous cell carcinoma: Breach of the basement membrane with involvement of substantia propria by tumor cells [8]. We report the role of IC in the diagnosis of Ocular Surface Squamous Neoplasia by comparing with histopathology of the excised biopsy specimens

MATERIALS AND METHODS

The study was conducted at Department of Pathology Sarojini Devi Eye Hospital (SDEH), Hyderabad between June 2008 and May 2013. The study included 170 specimens from 168 patients. All patients with clinical suspicion of OSSN who presented to the Department of Oculoplastics SDEH, during this period were included in the study (Fig.1). Detailed history including age, sex, site of lesion, risk factors & HIV status was taken from all these patients. Best corrected visual acuity was recorded. Slit lamp examination, Fundus examination and gonioscopy was performed. Intraocular pressure was measured by applanation tonometry.

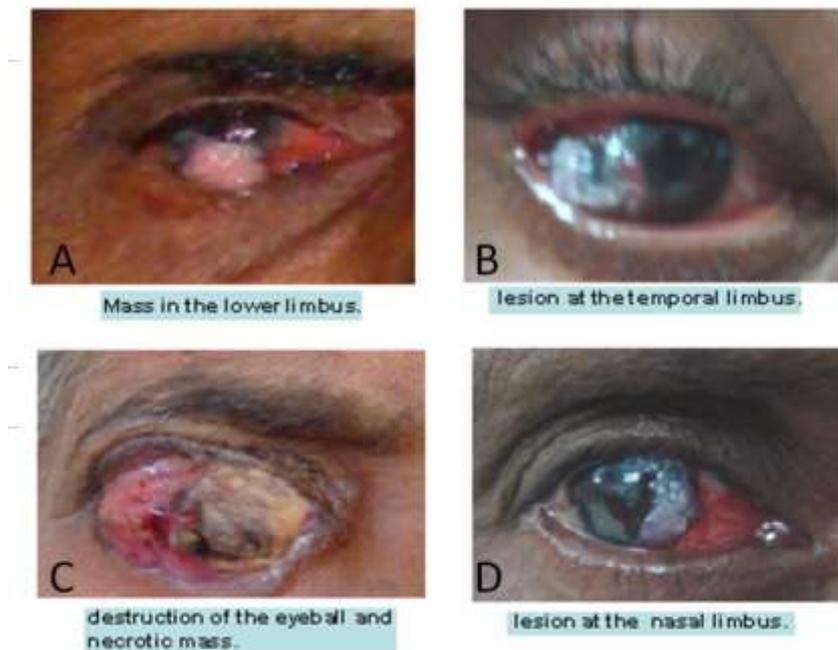


Fig.1:A.mass in lower limbus, B. Lesion at temporal limbus, C. destruction of eye ball and necrotic mass, D. Lesion at nasal limbus

All patients were subjected to IC Procedure: A drop of 4% xylocaine was instilled into the eye of the patient. 5mm millipore filter paper was held with a sterile forceps and pressed firmly against the lesion for about 5 to 10 seconds, 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 observed for the presence of dysplastic cells, hyperkeratosis, inflammatory cells or any other associated findings.

RESULTS

Biopsies from 168 patients found to have histologically proven OSSN are included in the study. Two patients had bilateral tumor making a total of 170 specimens.

There were 100 (60%) men and 68 (40%) women. Age of patients, ranged from 7-85yrs with a mean age of 46yrs. Maximum number of cases occurred in 6th decade. Nineteen patients were HIV positive. One HIV positive patient had tumor in both eyes. Two patients had Xeroderma pigmentosa. One of them presented with tumor in both the eyes. Recurrence was seen in 31 cases (18.4%) at the limbus.

On cytomorphology 130/170 specimens (76.4%) correlated in the comparison of cytology with histopathology. IC showed poor correlation or non correlation with histopathology in 40 specimens. However there were no false positives on cytomorphology (Fig. 2).

Histological spectrum showed 68(40%) non invasive lesions and 102(60%) invasive malignancies. Of the 102 invasive malignancies, well differentiated squamous cell carcinoma constituted 33.3%, moderately differentiated 51.9% and poorly differentiated 14.7% (Fig. 3).

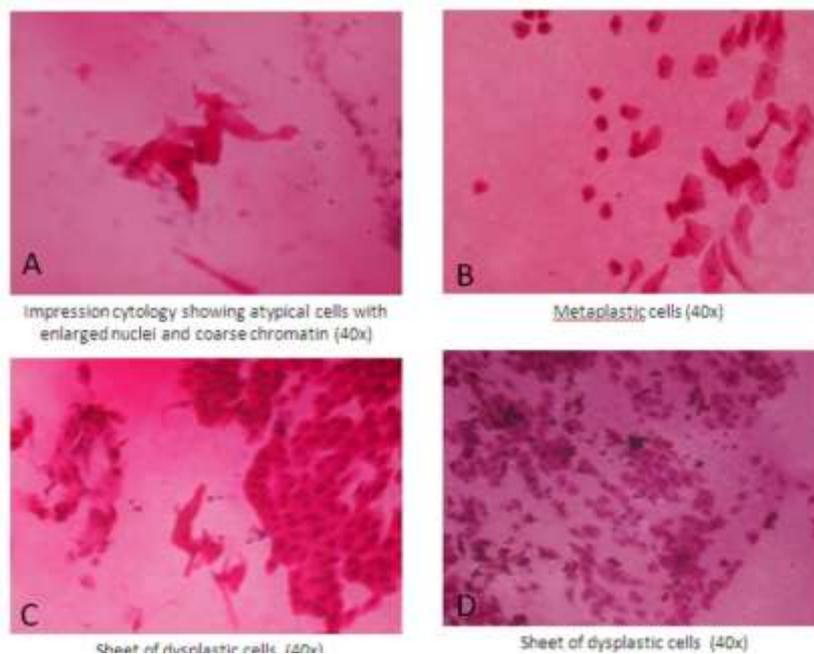


Fig. 2: A.40x.IC showing atypical cells with elongated nuclei and coarse chromatin, B.40x Metaplastic cells, C. 40x Sheet of dysplastic cells, D.. 40x Sheet of dysplastic cells

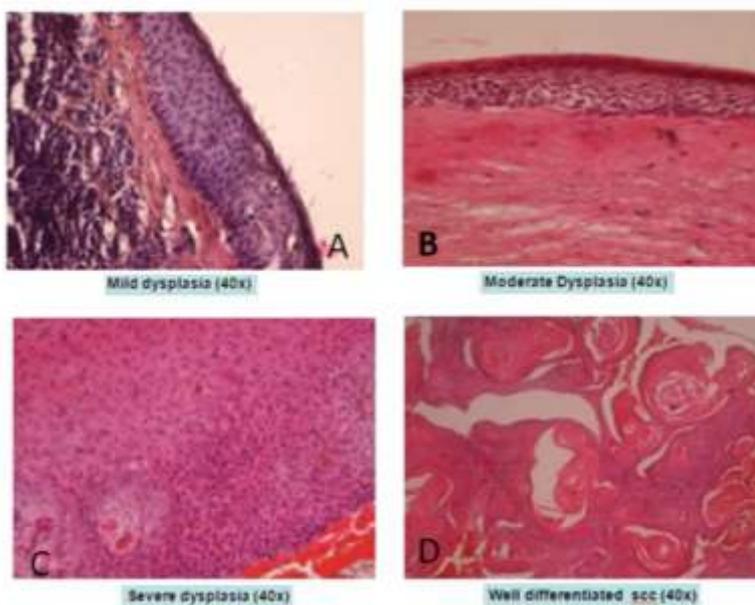


Fig. 3:A.40x Mild dysplasia, B.40xModerate dysplasia, C.40xSevere dysplasia, D.40xwell differentiated SCC

DISCUSSION

OSSN occurs in the sun exposed areas of the conjunctiva at the limbus in elderly individuals. Excessive exposure to U-V light causes DNA damage. A failure in DNA repair may lead to fixed somatic

mutations and cancerous transformation. It presents as a mass in inter palpebral fissure usually at the nasal or temporal limbus. It may have gelatinous, velvety, papilleferous, or leukoplakic appearance. Both

intraepithelial and invasive OSSN are sometimes difficult to identify clinically [9].

The surface layers of normal conjunctival epithelium, a stratified cuboidal to columnar epithelium with mucin secreting goblet cells, are usually readily removed by IC and are often seen as large cohesive sheets. The conjunctival epithelium merges into the nonkeratinized stratified squamous epithelium of the cornea at the limbus. Because healthy corneal cells are tightly bound to each other and to their basement membrane, only a few ragged intermediate squamous cells are released usually. In contrast, neoplastic cells usually are readily obtained from both areas of the ocular surface [7].

In the present study 168 patients were studied with a clinical suspicion of OSSN. Impression cytology and histopathological examination was done. Age of patients ranged from 7 to 85 years with a mean age of 46 years. 56 years is the mean age in other study [9].

Out of 168 patients 100 (60%) were males and 68 (40%) were females. Two patients had tumor in both eyes making a total of 170 specimens. 130/170 specimens (76.4%) correlated in the comparative studies. IC employing millipore filter paper accurately predicts the histological diagnosis of OSSN with a high correlation rate of 76.4%. Nolan *et al* had almost similar correlation in their study using cellulose acetate filter paper [10].

Table 1: Comparison with other studies

	Impression cytology	Correlation with HP
Our study	Millipore filter paper	76.4%
Tolea <i>et al.</i> [17]	Biopore membrane	80%
Nolan <i>et al.</i> [10]	Cellulose acetate filter paper	77%

IC and histopathology showed poor correlation or non correlation in 40 specimens. The causes of poor correlation or non correlation in our study may be due to sampling error, non representative sample, uncooperative patients and difficulty in interpretation of lesions close to the cornea. Cellular yield is scant in leucoplakic lesions. IC offers a safer tool for diagnosis than repeated biopsy. Moreover, IC can be used in the follow-up of patients after treatment to determine the recurrence of the disease, as well as the effects of treatment such as topical chemotherapy [11]. Recurrence of OSSN was seen in 31 cases (18.5%). Recurrences occurred in 22 patients with CIN and 9 patients with invasive neoplasia. Intraocular or orbital extensions or both occurred in four patients and metastatic disease in two patients [12], 27% in other studies [13]. Bilateral OSSN is rare [14, 15]. Twelve (70.6%) of the 17 patients tested were HIV positive, and they were all younger than 50 years. [16]

Summary

OSSN occurs in the sun exposed area at the limbus in elderly males.

IC with millipore filter paper v/s histopathology had a high correlation of 76.4%. Histological spectrum showed more of Invasive than Non invasive lesions. In HIV positive patients, tumor presents at a younger age and tends to recur after excision. OSSN is a slow growing tumour capable of causing extensive tissue destruction but metastasis is rare. No metastasis or deaths are reported in our study.

CONCLUSION

IC 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.

Expertise with the specialized cytology of the ocular surface and awareness of the limitation of the technique in dysplastic lesions with hyperkeratosis helps in achieving a higher degree of accuracy in diagnosis of OSSN.

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