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ROLE OF IMPRESSION CYTOLOGY IN THE DIAGNOSIS OF OCULAR SURFACE SQUAMOUS NEOPLASIA (OSSN) AT A TERTIARY EYE CARE CENTRE – OUR EXPERIENCE

Pathology

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ABSTRACT

Ocular Surface Squamous Neoplasia (OSSN) is a spectrum of conjunctival and corneal epithelial neoplasms manifesting as Benign, premalignant and malignant lesions. The Invasive and intraepithelial forms of OSSN are difficult to differentiate clinically. Impression cytology has important role in the routine diagnosis of dysplasia and malignancy in OSSN. It is preferred over biopsy when treatment with non surgical intervention is considered. Impression cytology refers to the application of a cellulose acetate filter to the ocular surface to remove the superficial layers of the ocular surface epithelium. These cells can then be subjected to histological, immunohistological, or molecular analysis. The study was carried out from January 2016-June 2017 at Sarojini Devi Eye Hospital under Osmania Medical College.Hyderabad. 35 cases of biopsy proven intraepithelial and invasive OSSN were included in the study. On cytomorpholigical study fallowing Impression Cytology (IC) 31/35 cases correlated with histolopathology . The histopathological spectrum included 14 cases of invasive SCC, 12 cases of In-situ SCC and 9 cases of Dysplasia. IC using Millipore Cellulose acetate paper showed high correlation 88.5% in detecting OSSN.

KEYWORDS

Ocular Surface Squamous Neoplasia(OSSN), Impression cytology(IC), Millipore cellulose acetate filter paper.

INTRODUCTION: Egbert et al., in 1977 introduced the Technique of impression cytology (IC) to study conjunctival goblet cells[1]. They used cellulose acetate filter paper (0.45 micron pore size). Thiel et al. have used of Biopore membrane[2]. Superficial layers of epithelium are removed by applying cellulose acetate filter paper. It is non-invasive, easy to perform, and yields reliable information about the area sampled with minimal discomfort to the patient. This makes it a valuable tool in the understanding of ocular surface disorders. Applications of impression cytology include the aetiological diagnosis of various ocular surface disorders, documenting sequential changes in the conjunctival and corneal surface over time, Nolan et al, : used Impression cytology for diagnosing of conjunctival neoplasms, based on the principle that the abnormal maturation of neoplastic cells is reflected in the morphology of surface cells[3].

The term Ocular Surface Squamous Neoplasia (OSSN) was coined by Lee and Hirst.[4]. It describes a spectrum of conjunctival and corneal epithelial neoplasia manifesting as dysplasia, carcinoma-in-situ (CIS) and invasive squamous cell carcinoma. OSSN is uncommon and it primarily occurs in older males (78.5%). Various authors have placed the incidence between 0.13 to 1.9/100000.[5] It is predominantly seen in dark skinned Caucasians, the age of onset being significantly higher in areas closer to the equator. The average age of occurrence has been noted to be 60 years, ranging from 20 to 88 years] The average age of incidence of carcinoma in situ lesions is 5-9 years lower than invasive OSSN. This difference represents the time taken for progression from intraepithelial neoplasm to invasive carcinoma. Patients of xeroderma pigmentosa and human immunodeficiency virus (HIV) develop OSSN at a younger age.[6] Mutations of p53 gene are the most common genetic anomalies in OSSN. Some have hypothesized that HPV or UV-B may alter p53, resulting in the development of OSSN in susceptible individuals.[7]

OSSN lesions usually presents as gelatinous, leukoplakic or papilleferous lesion most commonly at the limbus, and gives rise to symptoms like foreign body sensation, redness or irritation and rarely, diminution of vision due to high astigmatism or involvement of visual axis. It usually starts in interpalpebral conjunctiva and then grows and straddles the limbus and then may or may not involve the cornea.

Cytological features on IC :

Normal conjunctival epithelium is composed of unkeratinized, stratified squamous epithelium with goblet cells. Cells are small round with basophilic cytoplasm, NC ratio is 1:1 to 1:2. Goblet cells are PAS positive.

Metaplastic features: evidence of keratinazation (pinkish cytoplasm) loss of goblet cells and increased NC ratio.

Dysplastic features : Pleomorphic cells and nuclei, increased NC ratio, prominent nucleoli and increased mitotic figures.

Histopathology of OSSN. The specimens may be obtained from excision biopsies in small lesions which can be removed in toto (curative) or incisional biopsies in cases of large infiltrating lesions planned for eyeball exenteration. **Conjunctival Intraepithelial Neoplasia (CIN)** refers to varying degrees of conjunctival epithelial dysplasia.[8] CIN I represents mild disease dysplastic cells seen in lower third, CIN II refers to moderate disease involving lower 2/3 rd, and CIN III or carcinoma-in-situ suggests near full- thickness epithelial involvement with intact basement membrane.

Invasive squamous cell carcinoma: Breach of the basement membrane is seen with involvement of substantia propria by tumor cells.

Mucoepidermoid carcinoma represents a aggressive variant of SCC. On pathology, dysplastic squamous cells and malignant goblet cells are seen with mucin stains.

The aim of the study was to emphasize the role of Impression cytology in the diagnosis of dysplastic and malignant Ocular Surface Squamous Lesions.

MATERIALS AND METHOD:

This study was carried at Sarojini Devi Eye Hospital under Osmania Medical College Hyderabad during January 2016-June 2017. Total of 35 patients who underwent impression cytology and have biopsy proven intraepithelial and invasive OSSN are included in the study group. Clinical details were collected from the patients sent for impression cytology from the Department of Oculoplasty, SDEH.

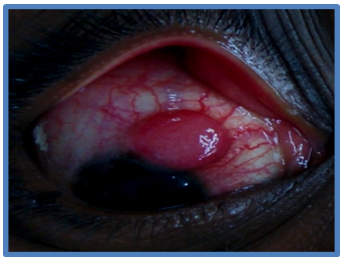
Cytological sampling was done using Millipore cellulose acetate filter

paper which was cut into small asymmetrical strips. After instilling 4% xylocaine drops into the eye, strip was applied rough surface down on lesion using blunt ended forceps. Gentle pressure was applied and strip was carefully peeled off the epithelial surface. After fixing the strips in 90% alcohol, staining with H & E was done and after the final dehydration step, xylene is used to make the filter paper transparent. Before mounting, the filter paper is placed with the epithelial cells facing up. The completed slides are examined by light microscopy.[3]
Histopathological study: The specimens may be obtained from excision biopsies in small lesions which can be removed in toto or incisional biopsies in cases of large infiltrating lesions.Excision biopsy of lesions sent in formalin were routinely processed and stained with H & E. Examined under Microscope and cytohistological correlation was done.

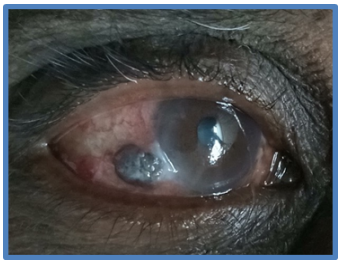
Fig: 1 Procedure of Impression Cytology using Millipore Cellulose Acetate filter paper.

RESULTS:

A total of 35 patients who had biopsy proven intraepithelial and invasive OSSN and had preoperative Impression cytology were included in the study . Out of 35 cases there were 23 males (65.72%). aged between 15 and 75 with a median of 56 years, and 12 females (34.28%) aged between 18 and 67 years (median 50 years). Patient age according to decade included: 0–20 (four); 21–40 (seven); 41–60 (20); 61–80 (four). There was Cytohistological correrelation in 31 out of 35 cases . 31 cases showed OSSN on cytology. Histopathology of 35 cases showed dysplasia 9 cases (25.71%) , carcinoma in situ 12 cases (34.28%)and squamous cell carcinoma in 14 cases(40%). OSSN predominantly affects limbus of elderly males .Impression cytology employing millipore filter paper accurately predicted the histological diagnosis of dysplasia/malignancy in OSSN with high correlation rate of 88.5%(31/35) in our study. Nolan et al., study had predictability rate of 77%(55/71) [9]and Derek et al had Predictability rate of 80%(20/25).[10]. We categorized lesions on cytology according to Nolan et al who subdivided intraepithelial OSSN into high grade (moderate dysplasia, severe dysplasia, and carcinoma in situ) or low grade (mild dysplasia) in a manner similar to that used in the Bethesda System for reporting cervical cytologic diagnoses.[9]
The causes of poor correlation in 4 cases in our study is due to presence of abundant surface keratin (2) which showed squamous cell carcinoma on histology and scanty cellularity (2), which showed dysplasia and SCC on histology .There are no false positives in our study similar to the study done by Nolan et al [9]and Tole et al.[11]

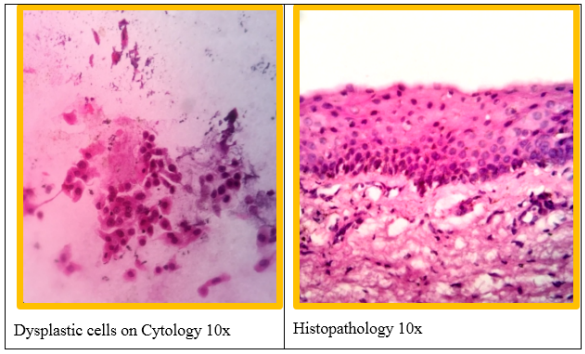


Ossn lesion upper canthus



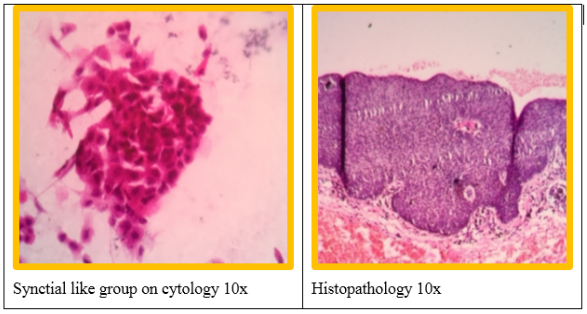
Ossn lesion temporal canthus

Fig: 2 OSSN lesions



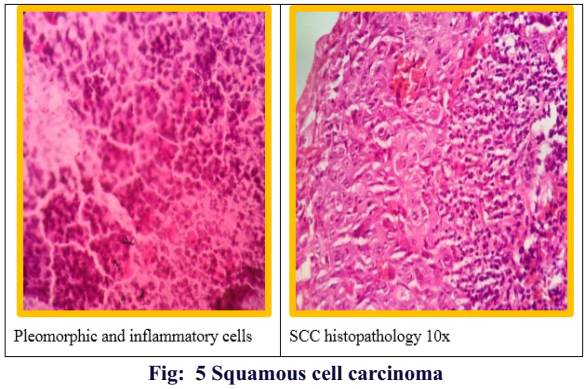
Dysplastic cells on Cytology 10x | Histopathology 10x

Fig: 3 Dysplasia



Synctial like group on cytology 10x | Histopathology 10x

Fig: 4 Carcinoma in situ



Pleomorphic and inflammatory cells | SCC histopathology 10x

Fig: 5 Squamous cell carcinoma

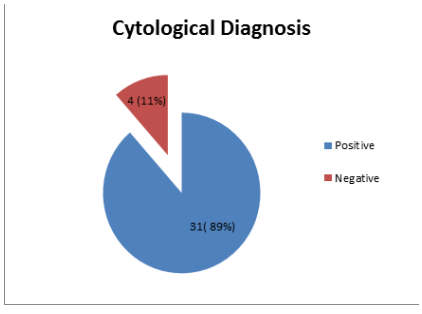


Chart: 1 pie chart showing positive cytological correlation

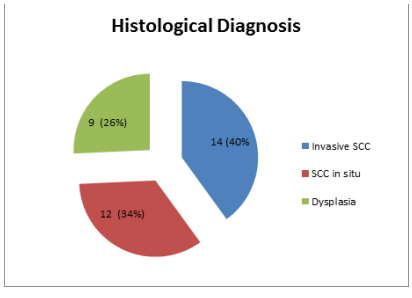


Chart: 2 showing percentages of various lesions on histology.

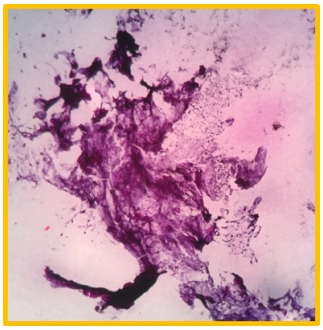


Fig: 6 False negative on cytology – Keratinous strands on 10x

DISCUSSION :

Impression cytology can play a valuable role in assisting with the diagnosis and follow-up of OSSN lesions in which recurrence is common. The size of the filter paper pores affects the consistency of epithelial cells collected and the resolution of cell detail[12]. Larger pore sizes collect cells better, but the cell detail is less well preserved. Most authors use filter paper of a pore size between 0.22 µm and 0.44 µm.6An impression cytology usually removes only 1–3 cell layers, It is therefore ideal for studying the surface epithelium). However, using multiple impressions of the same area the morphology of the basal limbal epithelium can also be studied. Impression cytology has advantages over biopsy that samples only a very small surface area to minimize scarring. It also has advantages over spatula scrapes that can be difficult to interpret,are more traumatic for the patient, and give little information about cell to cell relations. Impression cytology readily lends itself to the collection and study of multiple samples from an eye, and the "geographic" location of abnormal areas can be determined.[3] 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[3].

SSN has a tendency for recurrence. IC is safer than biopsy for follow up in patients with recurrent OSSN [10]Since the limbal stem cells, which are responsible for renewal of the corneal epithelium throughout life, are potentially reduced in number with each surgical biopsy in these patients, impression cytology offers a safer alternative to diagnosis than repeated biopsies. In cases of recurrent dysplasia where mitomycin C therapy is being contemplated, a biopsy would also delay institution of therapy, as the site would have to heal before the drops could safely be given[10].

CONCLUSION

Impression cytology is useful in the initial diagnosis and follow up of OSSN. Impression cytology is a simple non invasive, repeatable, OP basis test that causes minimum discomfort to the patient. Impression cytology employing cellulose acetate filter paper accurately predicts the histological diagnosis of dysplasia/malignancy in OSSN with high correlation rate

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