

## TOUCH IMPRINT CYTOLOGY- A RAPID INTRAOPERATIVE DIAGNOSTIC METHOD FOR OCULAR SURFACE SQUAMOUS NEOPLASIA

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### ABSTRACT

#### BACKGROUND

The technique of imprint cytology is a simple, cost effective, rapid technique of intraoperative diagnosis of tumours. It plays a significant role in the rapid diagnosis of the lesions in centres with less developed infrastructure.

Objectives of this study were to analyse the accuracy of imprint cytology and thereby to evaluate its diagnostic utility.

#### MATERIALS AND METHODS

The prospective study was carried out in a tertiary care hospital. It included 12 cases of ocular surface squamous neoplasia. The cytodiagnosis was correlated with histopathological (HP) diagnosis to evaluate the accuracy of imprint cytology.

#### RESULTS

Maximum lesions were of invasive squamous cell carcinoma (50%) followed by intraepithelial neoplasms including dysplasias (33.3%) and carcinoma in situ (16.6%). Overall accuracy of detecting type of lesion was 83.33%. Total discordance with HP diagnosis was 16.6%.

#### CONCLUSION

Intraoperative diagnosis using imprint cytology provides a rapid and efficient means of pathological assessment which in experienced hand, is capable of obtaining a high degree of accuracy.

#### KEYWORDS

Ocular Surface Squamous Neoplasia, Imprint Cytology, Histopathology.

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#### BACKGROUND

Ocular surface squamous neoplasia (OSSN) is a broad term encompassing conjunctival intraepithelial neoplastic lesions (CIN) and invasive squamous cell carcinoma (SCC) of conjunctiva and cornea.<sup>1</sup> The term CIN, in vogue today was proposed by Pizarello and Jakobeic, derived from the terminology applied to the intraepithelial cervical malignancies.<sup>2</sup> Clinically, it is difficult to distinguish dysplasia, carcinoma *in situ* and SCC. Conjunctival squamous cell carcinoma (SCC) is uncommon worldwide, and the incidence varies geographically from 0.2 to 3.5/100,000.<sup>3</sup> OSSN clinically manifest with a very wide spectrum. It includes several forms of epithelial, stromal, caruncular, and secondary tumours. In the clinical practice, comprehensive ophthalmologist, cornea specialist, and an ocular oncologist are commonly confronted with ocular surface tumours and

similar looking lesions quite frequently in day to day practice. Predisposing factors for OSSN are exposure to sunlight, HPV type 16 infections, and immunocompromised status such as AIDS. Ocular surface squamous neoplasia is mostly unilateral and rarely bilateral in immunocompromised cases and is seen in middle-aged and older patients. Patient with OSSN often presents with redness and ocular irritation. Vision is affected usually when the lesion encroaches the center of the cornea. The lesion has appearance of being fleshy or nodular, sessile minimally elevated with surface keratin, feeder vessels, and secondary inflammation. The gold standard for the diagnosis of OSSN is the histopathological evaluation of the lesion after an incisional or excisional biopsy. However there are several occasions when the clinician may opt to do diagnostic tests to corroborate the clinical suspicion of OSSN. Histopathologically, mild CIN (dysplasia) is characterized by partial thickness replacement of the epithelium by anaplastic cells that lack normal maturation. Severe CIN is characterized by full thickness replacement of the epithelium by similar cells. A characteristic abrupt demarcation between affected epithelium and normal epithelium is seen in both the variants. Invasive SCC shows a breach in the basement membrane of basal epithelium and is typically a fairly well-differentiated neoplasm composed of abnormal epithelial

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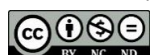
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cells with mitotic activity and keratin. Occasionally, it can be poorly differentiated and shows bizarre, pleomorphic cells, numerous mitotic figures, acanthosis, and dyskeratosis.<sup>4</sup> In 1980 Gelender reported that cytological features can be seen using a cytobrush to obtain a specimen for fixation and Papanicolaou staining.<sup>5</sup> Impression cytology on cellulose acetate paper as described by Nolan et al had a reasonably high predictability rate of 77% (55/71) in diagnosing moderate dysplasia to microinvasive Carcinoma.<sup>6</sup> Diagnosis is based on the presence of the universal cytological criteria which included nuclear enlargement, hyperchromasia, irregular nuclear outline, coarse nuclear chromatin, and prominent nucleoli.<sup>7</sup> Bio pore membrane used for impression cytology was reported to accurately correlate with histological diagnosis in 80% (20/25) by Tole et al. Recent introduction of anterior segment OCT has enabled the assessment of the conjunctiva and cornea with high axial resolution of tissue planes. With the help of technological advances from time-domain to spectral-domain and ultra-high resolution (UHR) OCT, axial resolutions of 2–3 microns allow an optical biopsy of ocular surface tissue. The imprint cytology is one of the upcoming methods that can be used in the diagnosis of malignant and benign lesions in shorter period though the histopathology remains the gold standard. Imprint cytology is a major breakthrough in the field of rapid tissue diagnosis. Besides its speed and enormous simplicity, it provides excellent cellular details.<sup>8</sup> Imprint is a very simple and rapid technique for tissue diagnosis. Imprint is a touch preparation in which tissue is touched on a slide and it leaves behind its imprint in the form of cells on the glass slide. In present study we have correlated the cytological diagnosis by imprint with histological diagnosis and tried to evaluate the accuracy and usefulness of this method in diagnosing OSSN.

MATERIALS AND METHODS

A prospective study including all suspected cases of OSSN attending our outpatient department between august 2015 to July 2017. Imprint smears were prepared from ocular surface squamous neoplastic lesions by touching glass slides on the surface, with special focus on suspicious looking area, preferably under topical anaesthesia. Smears were immediately fixed in 95% alcohol and stained with Papanicolaou’s stain. Air dried smears were stained with May-Grunwald-Giemsa. Reporting on imprint smears was done by a pathologist with good experience in cytology reporting. After reporting of imprint smears, lesions were excised, specimens were sliced and fixed in 40% formalin. After proper fixation samples were processed by routine histopathological processing and sections were stained by Haematoxylin and Eosin. Final reports of both processes were compared to know accuracy of diagnosis by imprint cytology.

RESULTS

A total of 12 patients with suspected OSSN were included in the study. There were female predominance with 7

(58.33%) cases. The majority of the patients (66.6%) were in the age group of >50years of age.

Co-relation between HPE and imprint cytology was most accurate in cases with malignant lesions i.e invasive squamous cell carcinoma (100%), followed by squamous dysplasia (66.6%). Poor correlation was seen in cases of carcinoma in situ which was confirmed mostly on HPE.

On imprint cytology, cases of invasive squamous cell carcinoma showed dysplastic cells with few cells with increased mitotic activity, keratin production, pleomorphic cells, with occasional atypical cells and inflammatory cells. Cases with dysplasia showed atypical dysplastic cells, along with keratin in cases of severe dysplasia. Cases of carcinoma in situ could be differentiated from mild to moderate dysplasia only by HPE in our study. 2 cases suspected of mild to moderate dysplasia by cytology, on HPE were found to involve whole thickness of epithelium pointing towards carcinoma in situ.

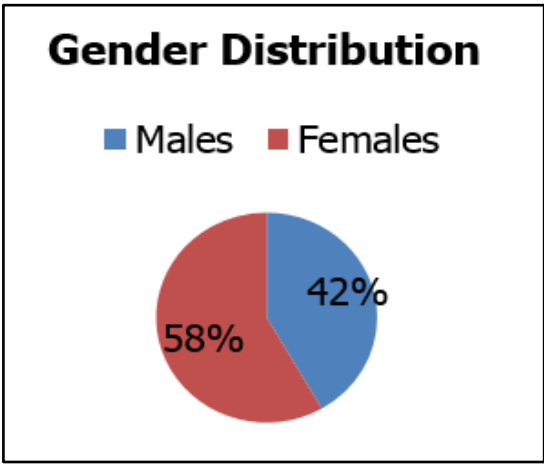


Figure 1. Gender Distribution

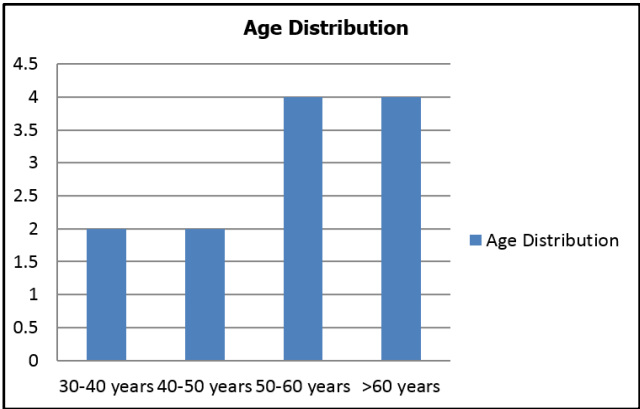
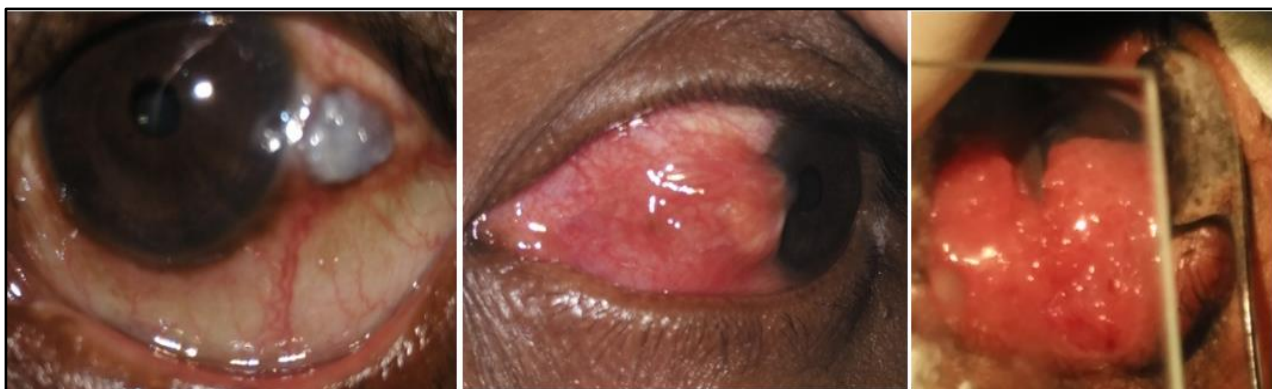


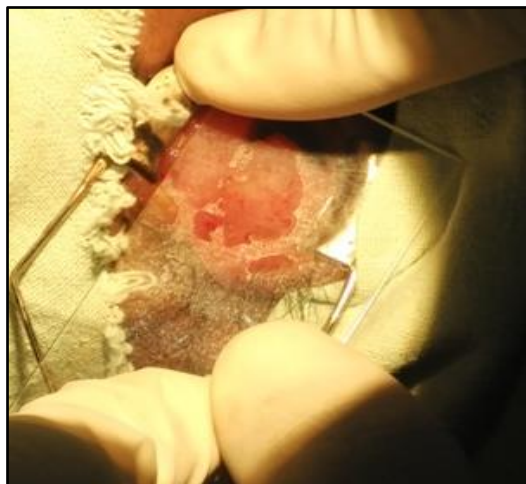
Figure 1. Gender Distribution

Final Diagnosis	Histopathology	Cytology
Dysplasia	4	6
Carcinoma in situ	2	0
Invasive squamous cell carcinoma	6	6

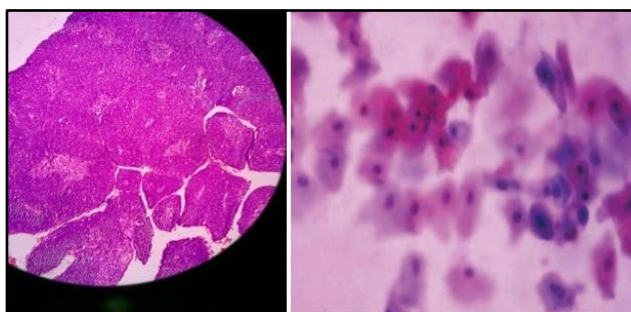
Table 1. Correlation between HPE and Imprint Cytology (12 Cases)



**Figure 3. (a) Moderate Dysplasia, (b) Carcinoma in Situ, (c) Invasive Squamous Cell Carcinoma**



**Figure 4. Imprint Cytology Method**



**Figure 5. (a) HPE of Squamous Cell Carcinoma, (b) Imprint Cytology**

## DISCUSSION

Although histopathological (HP) appearance of a lesion in any organ is considered to be the final arbiter of its diagnosis, yet the delay involved may at times affect the course of treatment in certain situations. Imprint cytology intends to overcome this delay and save valuable time. Imprint cytology serves surgeons in identifying lesions whether it is malignant or not. This technique was first utilized by Forkner.<sup>9</sup> in 1927 for lesions of excised lymph node. Dudgeon and Barret, Tribe et al, Pilar & Rubenston and Amarjeet Singh et al suggested some points to improve the accuracy especially.<sup>10-13</sup>

1. The tissue surface to be imprinted should be flat and there should be no portion of fat protruding from the edges as these tend to smudge the imprint.
2. Sometimes the first imprint contains excess tissue fluid

and blood and it was found that subsequent imprints give better cytological results.

3. The ease with which any tumor gets imprinted varies considerably. In order to obtain imprint smears of one cell thickness, the amount of pressure applied at the time of imprinting varies. Benign looking lesions usually require more pressure in order to obtain sufficient cells for diagnosis while malignant tumours get imprinted more easily.
4. Malignant tissue imprints were more cellular than those of benign looking lesions.

Though this method is being used by surgeons worldwide for diagnosing various tumours intraoperatively but there are not many literatures pointing towards its application in ophthalmic tumours. In present study we focussed on how accurate is imprint cytology in diagnosing OSSN intraoperatively and observed that accuracy was more for invasive carcinomas (100%) as compared to intraepithelial neoplasms (66.6%). The imprint smears from cases of mild to moderate dysplasias were found to be hypocellular and required more pressure while imprint from malignant invasive lesions were hypercellular and required less pressure. Dudgeon and Barret, Tribe et al, Pilar and Rubenston, Solanki et al, Suen et al and Helpap et al.<sup>8,11-15</sup> found in their studies on imprint cytology in non-ophthalmic surgical cases, such as breast, lymph node etc soft tissue tumours, that in benign condition cells appear in group and do not separate readily so the accuracy in benign lesions was low than malignant lesions. Probably, in our study too, the readily separable tumor cells of invasive carcinoma gave more accurate results in imprint cytology compared to intraepithelial neoplasms with less no of dysplastic cells.

Advantages of touch imprint cytology is that the procedure for imprint cytology can be done even in underdeveloped infrastructure. With this technique, immediate result with minimal artefacts is achieved, is cheaper and provides analysis of individual cells. A precise diagnosis is received through this technique.<sup>16</sup> Disadvantage of this technique is that the depth of infiltration cannot be assessed through this technique.<sup>17</sup>

## CONCLUSION

Ophthalmic pathology is unique as it encompasses wide range of tissues - epithelia, connective tissue and specialized

tissue and is therefore unique. It offers exposed surfaces and fluid filled chambers for diagnosis. It shows wide range of infections and neoplasia-some are similar to or variants of tumours that present elsewhere and others are unique to eye. Many neoplastic conditions masquerade as or mimic other less aggressive neoplastic or non-neoplastic inflammatory conditions and needs differentiation before definitive therapy is planned. Ophthalmic Cytology as a special procedure has been in use since many years. Impression cytology, scrape cytology and Fine needle aspiration cytology have improved diagnosis of these lesions over years. But, in poor and underdeveloped nations, in hospitals with underdeveloped infrastructure, sometimes these procedures may take a lot of time to detect the disease. Without any specialized instruments required and diagnosing within less time, touch imprint cytology proves very useful as an intra-operative diagnostic procedure in less equipped centers without facilities of frozen section or in centres with no readily available biopore membranes or cellulose acetate filter papers. In spite of drawbacks it can be concluded that imprint is a simple, fast, easy and reliable technique for the diagnosis of malignant ocular surface squamous neoplasms and can suffice well to histopathological diagnosis, though cannot substitute it.

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