

Impression Cytology in the Evaluation of Ocular Surface Squamous Neoplasia- A Retrospective Cohort Study

MEGHANA PATIL¹, TS NAGARAJ², MOHAN KULKARNI³

ABSTRACT

Introduction: Ocular Surface Squamous Neoplasia (OSSN) are a diverse group of lesions varying from Conjunctival Intraepithelial Neoplasia (CIN) to invasive Squamous Cell Carcinomas (SCC). Histopathological examination is the gold standard technique in diagnosing them. However, it is invasive and may not be suitable in recurrent cases. Numerous minimally invasive techniques are available to diagnose OSSN cytologically. This study employs Impression Cytology (IC) to diagnose ocular squamous neoplastic lesions and compare it with the histological diagnosis.

Aim: To utilise IC in the diagnosis of OSSN and to compare its findings with histopathological diagnosis.

Materials and Methods: This was a retrospective cohort study, conducted in a Tertiary Eye care Hospital, Hubli, Karnataka, India during January 2023. The data included was over a period of three years (from July 2016 to June 2019). A total of 44 eyes (34 patients), in which both biopsy and IC done, were included. The patients' age, sex and ocular examination findings were recorded. The cytological and histopathological slides were retrieved from the archives and examined morphologically.

The cytological findings were compared with histopathological diagnosis and the sensitivity and specificity of the IC technique were calculated. The data was analysed using Microsoft excel sheets.

Results: A total of 34 patients were studied ranging from 19 to 85 years. The mean age was 49 years, 21 (61.76%) of them were males and 13 (38.24%) were females. In 24 patients, single eye was affected and in 10 patients bilateral eye involvement was seen. Histology revealed that there were 18 (40.90%) CIN I cases, 4 (9.09%) CIN II, 5 (11.36%) CIN III and 17 (38.65%) SCC. On cytology, 3 (6.82 %) were normal conjunctival epithelium, 30 (68.18 %) were dysplasia and 11 (25.00 %) were SCC. On cyto-histomorphological analysis, 35 (79.55 %) were concordant, out of 44 diagnosis. IC has a sensitivity of 88.89% and specificity of 64.71% to detect dysplasia. To diagnose SCC, it has sensitivity of 64.71% and 100% specificity.

Conclusion: According to the present study observations, IC is an excellent preliminary tool to investigate suspected neoplastic lesions of ocular surface. It has a very good concordance with histopathological diagnosis. IC is highly sensitive to detect dysplasia (CIN) and is very specific to diagnose SCC.

Keywords: Conjunctival intraepithelial neoplasia, Corneal lesions, Dysplasia, Sensitivity

INTRODUCTION

The OSSN comprises of a wide spectrum of corneal and conjunctival lesions, and can vary from mild dysplasia to infiltrating SCC [1]. It is the third most common ocular neoplasia, following retinoblastoma and melanoma [2]. The term OSSN was for the first time coined by Lee GA and Hirst LW [1]. OSSN has a very wide geographical distribution, and incidence ranges from 0.13 to 1.9 per 100,000 population. Males are often affected more than females [3]. The commonest age of presentation is sixth and seventh decades of life [4]. Limbus is the most commonly affected area [5-7]. The principal risk factors are ultraviolet-B light and Human Papilloma Virus (HPV). Other lesser known risk factors are exposure to petroleum products, cigarette smoking, chemicals (trifluridine, arsenicals), ocular surface injury, Vitamin A deficiency, Xeroderma pigmentosum, family origin of British or Austrian ancestry, Human Immunodeficiency Virus (HIV) infection and immunocompromised states [8].

Histopathology is the gold standard method to diagnose OSSN. However, it is invasive and requires Operation theatre set-up to perform the procedure. The other alternative method to evaluate OSSN is IC, which is less invasive and can be performed in Outpatient Department (OPD) itself. IC was first introduced into ophthalmology by Egbert PR et al., in 1977 [9]. Nolan GR et al., employed acetate filter paper for the first time to perform IC to diagnose OSSN [10]. Thiel MA et al., used Biopore membrane to perform IC [11].

The IC can also be used for assessing various dry eye disorders such as Vitamin A deficiency, ocular pemphigoid and keratoconjunctivitis sicca [12]. In addition cells retrieved by IC have also been subjected to electron microscopy by Maskin SL and Bodé DD [13]. The present study was undertaken to evaluate the cytological findings of IC in OSSN and compare it with histological diagnosis.

MATERIALS AND METHODS

This retrospective cohort study was conducted in NMR Diagnostics Pvt., Ltd., and MM Joshi Eye Hospital Hubli, Karnataka, India (Department of Cornea and Phaco-refraction) in January 2023. The cases of OSSN were retrieved from the Hospital from July 2016 to June 2019. The study was approved by the Institutional Ethics Committee (IEC Registration No: ECR/928/Inst/KA/2017/RR-20) and adhered to the tenets of declaration of Helsinki.

Inclusion criteria: Patients in whom both Imprint cytology smears and biopsy were done were included in the study.

Exclusion criteria: Patients who were treated for ocular malignancies other than OSSN were excluded.

Study Procedure

A total of 34 patients' case files were obtained. In total there were 44 affected eyes from these patients. Complete ophthalmic examination findings (Visual acuity, anterior segment examination and surgical notes) were recorded from the files along with the slit

lamp microscope findings. Extensions of the lesion, presence of any feeder vessel, or pigmentation were also noted.

Cytological and histopathological slides of these patients were archived from the Diagnostics Pvt., Ltd., The slides were seen by pathologist and the lesions were classified according to Basti S and Mascai MS and American Joint Committee on Cancer (AJCC) staging manual [14,15].

Collection of specimen for IC: After instilling 4% xylocaine into the patients's eye, Nitrocellulose Acetate (NCA) paper (cut into small bits of size 5x5 mm) was pressed firmly against the lesion for about 5 to 10 seconds using sterile forceps. The NCA paper was then transferred immediately into a container with fixative (comprising of glacial acetic acid, 37% formaldehyde and absolute alcohol, mixed in 1:1:20 proportion). After fixation for half an hour, the NCA paper was stained by Haematoxylin and Eosin stain (H&E) and observed under microscope.

Shield's no touch technique [16]: Shield's No Touch Technique was employed to take biopsy from the lesion. Peribulbar anaesthetic injection was given. Then the tumour was resected with a 4 mm margins under aseptic precautions. The excision of lesion was followed by amniotic membrane grafting. The excised tumour was oriented with sutures on Whatman's filter paper No. 16, fixed in 10% formalin and sent to histopathological examination.

Cytology examination: Examination was done under X100 and X400 magnification. The description of normal cells and lesions are as given below.

- Normal Conjunctival epithelium: These cells are small, round, uniform epithelial cells with large basophilic cytoplasm and nucleocytoplasmic ratio of 1:1 or 1:2 [10].
- Metaplasia (Hyperkeratosis): There is loss of goblet cells. Keratinisation is seen which is evident as pinkish cytoplasm, enlarged cells, reduced nucleocytoplasmic ratio (1:4-1:8), pyknotic nuclei and densely packed keratin filaments in the cytoplasm.
- Dysplasia: Cells exhibit pleomorphism, enlarged and hyperchromatic nucleus with irregular nuclear margin, coarse chromatin and prominent nucleoli. Nucleo-cytoplasmic ratio is increased. Mitotic figures are increased and there is loss of normal cellular maturational polarity and syncytial type cellular arrangement [10].
- Invasive SCC: Similar changes that are seen in dysplasia but more severe, more bizarre cell types and patterns along with tumour diathesis (necrotic tumour cells debris), blood and leukocytic exudates [10].

Histopathology examination: Histopathological slides were seen under X100 and X400 magnification. The CIN was classified into three categories according to Basti S and Mascai MS and AJCC staging manual [14,15]. In CIN I the dysplastic cells involve the lower third of the epithelium. In CIN II the dysplastic cells extend upto the middle third. In CIN III dysplastic cells extends upto the surface. Invasive SCC shows nests of neoplastic cells that have infiltrated the epithelial basement membrane and spread into the underlying stroma. All the reporting was done by single Pathologist. The above criteria were used in this study to report the studied slides.

STATISTICAL ANALYSIS

The data was collected in Microsoft excel sheet and results were presented as count and percentages.

RESULTS

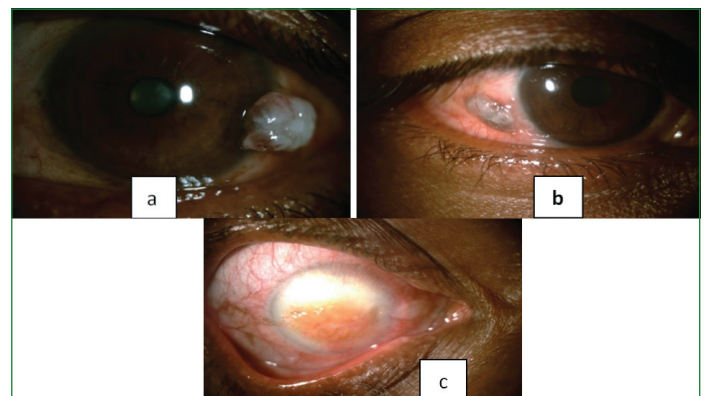
There were 34 patients in total. Twenty one patients (61.76%) were males and 13 (38.24%) were females [Table/Fig-1]. Male to female ratio was 1.6:1. Patients' age ranged from 19 to 85

years, the mean patient age was 49 years. Maximum number of patients 8 (23.53%) were in the age group of 51-60 years, 4 (11.76%) patients were above 71 years and three were 20 years and younger.

Age group	No. of patients	%	Males (n)	Females (n)
≤20	3	8.82	3	0
21-30	4	11.76	3	1
31-40	5	14.70	1	4
41-50	5	14.70	3	2
51-60	8	23.53	5	3
61-70	5	14.70	3	2
>71	4	11.76	3	1
Total	34	100	21	13

[Table/Fig-1]: Age-group and sex-wise distribution of patients.

In 24 patients (70.59%) only one eye was involved and 10 (29.41%) patients had bilateral eye involvement. Nasal side was involved in 30 (88.18%) eyes and temporal side was involved in 14 (41.18%) eyes. In 26 (76.47%) eyes limbus was involved, in 16 (47.06%) eyes conjunctiva was involved and in two (5.88%) eyes cornea was involved [Table/Fig-2].



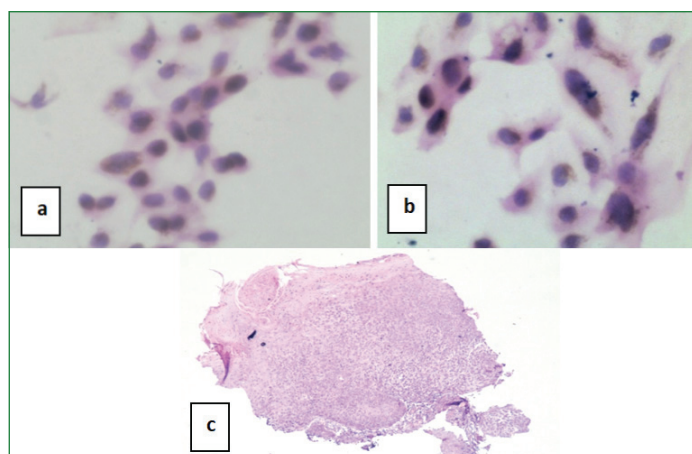
[Table/Fig-2]: Different sites of involvement of OSSN. a) Limbal involvement; b) Conjunctival involvement; c) Corneal involvement.

In the present study, six patients had systemic associated diseases. Five of them were HIV positive and one was a case of Xeroderma pigmentosum. Out of five HIV positive patients, four were males and one was female. All the lesions were in limbal area, three lesions were 3-4 mm and two lesions were 4 mm. In all five patients, biopsy was done which showed SCC. Cytology showed SCC in four of them and one patient showed dysplasia.

One patient, clinically diagnosed as Xeroderma pigmentosum, was 25-year-old male, presented with limbal lesion in right eye. Both biopsy and cytology of the lesion showed SCC.

On cytology, 3 (8.82%) cases showed normal conjunctival epithelium, 30 (88.18%) showed dysplasia and 11 (32.35%) showed SCC [Table/Fig-3a,b]. Histology revealed there were 18 (52.94%) CIN I cases, 4 (11.76%) CIN II, 5 (14.71%) CIN III and 17 (50.00%) SCC [Table/Fig-3c].

Comparison of cytological findings with histopathological diagnosis showed that 35 (100%) were concordant [Table/Fig-4]. Three CIN I cases showed normal conjunctival epithelium with inflammation on cytology. In six cases, histology showed SCC but cytology showed dysplasia only [Table/Fig-5]. The IC as a diagnostic tool to detect dysplasia has a sensitivity of 88.89%, but specificity is 64.71%. Positive Predictive Value (PPV) is 80% and Negative Predictive Value (NPV) is 78.57%. IC is highly specific to detect SCC and has a specificity and PPV of 100%, but sensitivity is 64.71% and NPV being 81.82% [Table/Fig-6].



[Table/Fig-3]: Cytology and Histology of OSSN. a) Dysplasia: Cells exhibit mild to moderate pleomorphism (H&E, X400); b) Cytology of Squamous Cell Carcinoma (SCC): Cells exhibit severe nuclear pleomorphism admixed with tumour diathesis (H&E, X400); c) Histology of Squamous Cell Carcinoma (SCC) with infiltration of subepithelium (H&E, X40).

Histopathology				
Cytology		Normal	CIN	SCC
	Normal	0	3	0
	Dysplasia	0	24	6
	SCC	0	0	11

[Table/Fig-4]: Concordance between histopathological and cytological diagnosis. NORMAL: Normal conjunctival epithelium; CIN: Conjunctival intraepithelial neoplasia

Sl no.	Cytology	Histopath diagnosis
1	Normal conjunctival epithelium	CIN I
2	Normal conjunctival epithelium	CIN I
3	Normal conjunctival epithelium	CIN I
4	Dysplasia	Squamous cell carcinoma
5	Dysplasia	Squamous cell carcinoma
6	Dysplasia	Squamous cell carcinoma
7	Dysplasia	Squamous cell carcinoma
8	Dysplasia	Squamous cell carcinoma
9	Dysplasia	Squamous cell carcinoma

[Table/Fig-5]: Discordant cases with their cytological and corresponding histopathological diagnosis.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IC to diagnose Dysplasia in OSSN	88.89	64.71	80.00	78.57
IC to diagnose SCC in OSSN	64.71	100.00	100.00	81.82

[Table/Fig-6]: Sensitivity, Specificity, PPV & NPV of impression cytology.

DISCUSSION

The OSSN is predominantly a disease of elderly people. In the present study maximum number of patients 8/34 (23.53%) were in the age group of 51-60 years. Tole DM et al., observed 36% in 60-69 years and 32% cases in 70-79 years, Toopalli K et al., observed maximum number of cases in 6th decade, and Kandyala TP et al., observed maximum number of cases in 61-70 years [2,3,17]. However, in patients with systemic diseases, OSSN can affect younger individuals also. Toopalli K et al., observed that maximum patients with HIV or xeroderma pigmentosum were between 20-30 years. In the present study also, all HIV positive patients were younger, between 20-30 years and patient with xeroderma pigmentosum was 25 years. Males are predominantly affected in OSSN. In the present study, 21 (61.76%) were males and 13 (38.24%) females. Toopalli K et al., observed maximum number of male patients (60%) and females were 40% [17]. Kandyala TP et al., observed 59% males and 41% females [3].

The OSSN commonly affects limbal region in the eyes. In our study, maximum number of lesions (59.09%) had limbal involvement, followed by conjunctival (36.36%) cases and corneal lesions (4.55%). Similar observations were made by Mittal R et al., [8]. They observed that ocular surface lesions had more limbal preponderance than corneal due to presence of transition zone at limbus [8].

Impression Cytology (IC): In the present study, 3 (6.82%) were normal conjunctival epithelium, 30 (68.18%) were dysplasia and 11 (25.00%) were SCC. Dysplasia comprises of wide spectrum lesions, beginning from mild dysplasia to severe dysplasia. However, it is a continuous process in progression to carcinoma. These morphological changes are well-defined in histopathology. But in cytology these lesions are difficult to grade. Hence, in the present study, we have used cytological findings to identify as either dysplasia is present or absent.

Impression Cytology (IC) and histopathological comparison: Cyto-histo comparison showed that 35 cases (79.55%) were concordant and nine cases (20.45%) were discordant. Three CIN I cases showed inflammation on cytology and hence were diagnosed as Normal Conjunctival Epithelium. Review of those three cases showed occasional dysplastic cells. Many such cells were covered by neutrophils and hence resulted in false negative. So caution has to be taken in such inflammatory cases and repeat Imprint cytology or biopsy has to be advised. In six cases, histology showed SCC but cytology showed dysplasia only. Reviewing those six cases again showed severe nuclear pleomorphism in four cases and mild to moderate nuclear pleomorphism in two cases. But tumour diathesis was lacking. So cases with severe pleomorphism definitely warrants further histological examination to rule out malignancy. The two cases with mild nuclear pleomorphism might have been non representational specimen. Hence, in large lesions IC will have to be taken from multiple sites to sample more cells. As illustrated by Tole DM et al., keratinising SCC of the ocular surface may yield very few or rare atypical cells on IC [2]. So a biopsy would be beneficial whenever there are large amount of anucleated squamous cells in cytology.

Studies on IC by other authors also showed similar concordance. Tole DM et al., observed 80% concordance, Kandyala TP et al., observed 86.4% concordance, Nolan GR et al., observed 77% concordance, and Toopalli K et al., observed 76% concordance [2,3,10,17]. Furthermore, all authors have said that there were no false positive cases. Nolan GR et al., has commented that IC is less sensitive for cases of SCC [10].

Limitation(s)

Cytologic grading of OSSN was not done as it has low reproducibility and use of IC in relapse cases was not done.

CONCLUSION(S)

The IC is an excellent preliminary tool to investigate suspected neoplastic lesions of ocular surface. It has a very good concordance with histopathological diagnosis. IC is highly sensitive to detect Dysplasia (CIN) and is very specific to diagnose SCC. Further studies may be done to include other ocular surface tumours apart from SCC. In future, studies may be done to detect relapse of tumour following surgery, using IC.

REFERENCES

- Lee GA, Hirst LW. Ocular surface squamous neoplasia. *Surv Ophthalmol.* 1995;39(6):429-50.
- Tole DM, McKelvie PA, Daniell M. Reliability of impression cytology for the diagnosis of ocular surface squamous neoplasia employing the Biopore membrane. *Br J Ophthalmol.* 2001;85(2):154-58.
- Kandyala TP, Dayasagar Reddy K, P Modini TK. Impression cytology in the diagnosis of ocular surface squamous neoplasia (OSSN). *Int J Recent Trends Sci Technol.* 2015;2(September):253-57.
- Sharmiladevi V, Radha PR, Anandababu M, Meenakshi B SJM. Correlation of impression cytology with histopathology in the diagnosis of ocular surface squamous neoplasia. *TNOA J Ophthalmic Sci Res.* 2017;55(4):267-72.
- Ateenyi-Agaba C, Weiderpass E, Smet A, Dong W, Dai M, Kahwa B, et al. Epidermodysplasia verruciformis human papillomavirus types and carcinoma of the conjunctiva: a pilot study. *Br J Cancer.* 2004;90(9):1777-79.

- [6] McKelvie PA, Daniell M, McNab A, Loughnan M, Santamaria JD. Squamous cell carcinoma of the conjunctiva: a series of 26 cases. *Br J Ophthalmol*. 2002;86(2):168-73.
- [7] Waddell KM, Downing RG, Lucas SB, Newton R. Corneo-conjunctival carcinoma in Uganda. *Eye* [Internet]. 2006;20(8):893-99. Available from: <https://doi.org/10.1038/sj.eye.6702043>.
- [8] Mittal R, Rath S, Vemuganti GK. Ocular surface squamous neoplasia- Review of etio-pathogenesis and an update on clinico-pathological diagnosis. *Saudi J Ophthalmol Off J Saudi Ophthalmol Soc*. 2013;27(3):177-86.
- [9] Egbert PR, Lauber S, Maurice DM. A simple conjunctival biopsy. *Am J Ophthalmol*. 1977;84(6):798-801.
- [10] Nolan GR, Hirst LW, Wright RG, Bancroft BJ. Application of impression cytology to the diagnosis of conjunctival neoplasms. *Diagn Cytopathol*. 1994;11(3):246-49.
- [11] Thiel MA, Bossart W, Bernauer W. Improved impression cytology techniques for the immunopathological diagnosis of superficial viral infections. *Br J Ophthalmol*. 1997;81(11):984-88.
- [12] Tseng SC. Staging of conjunctival squamous metaplasia by impression cytology. *Ophthalmology*. 1985;92(6):728-33.
- [13] Maskin SL, Bodé DD. Electron microscopy of impression-acquired conjunctival epithelial cells. *Ophthalmology*. 1986;93(12):1518-23.
- [14] Basti S, Macsai MS. Ocular surface squamous neoplasia: a review. *Cornea*. 2003;22(7):687-704.
- [15] Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, et al. *AJCC cancer staging manual*. Vol. 1024. Springer; 2017.
- [16] Rao R, Shields CL. *Surgical Techniques for Conjunctival and Corneal Tumours*. In: Chaugule SS, Honavar SG, Finger PT, editors. *Surgical Ophthalmic Oncology: A Collaborative Open Access Reference* [Internet]. Cham: Springer International Publishing; 2019. pp. 75-83. Available from: https://doi.org/10.1007/978-3-030-18757-6_7.
- [17] Toopalli K, Namala V, Pandharpukar M, Bharadwaj N, Jayalaxmi S, Vivekanand N. Ocular surface squamous neoplasia impression cytology V/S histopathology. *Int J Recent Trends Sci Technol*. 2015;2(September):253-57.

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Ophthalmology, KAHERS JGMMMC, Hubballi, Karnataka, India.
2. Associate Professor, Department of Pathology, KAHERS JGMMMC, Hubballi, Karnataka, India.
3. Consultant Pathologist, Department of Pathology, NMR Diagnostics Pvt Ltd, Hubballi, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

TS Nagaraj,
S/o S M Bhat, House No. 33, Radhakrishna Nagar, Dharwad-580003,
Karnataka, India.
E-mail: tsnagaraj18@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Mar 14, 2023
- Manual Googling: May 22, 2023
- iThenticate Software: May 24, 2023 (12%)

ETYMOLOGY: Author Origin**AUTHOR DECLARATION:**

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Mar 07, 2023**Date of Peer Review: **Apr 15, 2023**Date of Acceptance: **May 25, 2023**Date of Publishing: **Jul 01, 2023**