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Clinicopathological Correlation and P16 Expressions in Ocular Surface Squamous Neoplasia

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Abstract

Introduction: Ocular Surface Squamous Neoplasia (OSSN) is defined as the range of disease from mild dysplasia to carcinoma in situ and invasive squamous cell carcinoma. OSSN may be located on cornea, conjunctiva and limbus but is mostly found on interpalpebral fissure. OSSN's regional occurrence varies. Previous epidemiological research found that the incidence of OSSN was 0.13 per lakh in Uganda and less than 0.20 per million each year in the UK. Human papillomavirus (HPV), UV exposure, and immunosuppression all have a significant impact on OSSN incidence. Human papillomavirus (HPV), UV exposure, and immunosuppression all have a significant impact on OSSN incidence**.** HPV contributes to cancer pathogenesis by the formation of a protein complex between the host, p53 and HPVE6 protein, resulting in the blockage of p53 supression action. The neutralization of cellular retinoblastoma tumor suppressor (pRB) and the p53 tumor suppressor protein by the HPVE6 and E7 oncogenes induces the expression of p16. Hence p16 expression is considered a marker of high risk HPV serotypes

Objectives

- Clinico pathological correlation in OSSN.
- To evaluate the frequency and pattern of expression of p16 by immunohistochemistry in histopathologically diagnosed samples of OSSN.

Methods: 35 cases of OSSN received in Bangalore Medical College and Research Institute were studied. After routine processing, Hematoxylin and eosin staining and p16 immunohistochemistry was performed.

Results: 35 cases of OSSN cases were studied using H&E for histologic type, grade and stage. Immunohistochemical analysis was done to evaluate p16 expression and its correlation with grade of the tumor were studied. In our present study 27 out of 35 cases showed p16 positivity, suggesting that there is

significant association between HPV and OSSN. In our study no statical significance was found between p16 positivity and different grades of OSSN

Conclusion: 77% of the cases showed positive p16 staining, suggesting that there is a strong correlation between HPV and OSSN in the cases under investigation. P16 came positive more in higher grades such as grade III OSSN and SCC but no statically significant association was found between different grades of OSSN and p16 expression. However, this creates opportunities for more extensive research on p16 in OSSN in the future with more sample size, which could aid in determining a standard cut-off point for OSSN grading.

Keywords: Conjunctiva, Human Papillomavirus, IHC, P16, OSSN, UV Exposure.

Introduction

Ocular Surface Squamous Neoplasia (OSSN) is defined as the range of disease from mild dysplasia to carcinoma in situ and invasive squamous cell carcinoma. OSSN may be located on cornea, conjunctiva and limbus but is mostly found on interpalpebral fissure.

Human papillomavirus (HPV), UV exposure, and immunosuppression all have a significant impact on OSSN incidence. The majority of lesions are found near the limbus, namely in the nasal sector of the interpalpebral fissure. clinically, the diagnosis is hinted to by the emergence of ocular surface epithelial alterations. Gelatinous, leukoplakic, papilliform or nodular lesions during slit-lamp examination.

HPV contributes to cancer pathogenesis by the formation of a protein complex between the host, p53 and HPVE6 protein, resulting in the blockage of p53 supression action. The neutralization of cellular retinoblastoma tumor suppressor (pRB) and the p53 tumor suppressor protein by the HPVE6 and E7 oncogenes induces the expression of p16. Hence p16 expression is considered a marker of high risk HPV serotypes. . p16 (also known as p16 INK4a , cyclin-dependent kinase inhibitor2A, CDKN2A, multiple tumor suppressor 1 and numerous other synonyms), is a protein that slows cell division by slowing the progression of the cell cycle from the G1 phase to the S phase, thereby acting as a tumor suppressor.

Many a times, cases which are clinically diagnosed as OSSN may turn out as Pinguecula, Pterygium etc thus necessitating biopsy. Even though the association of HPV is proved beyond doubt in squamous neoplasia elsewhere, its strong association with ocular neoplasia is not well established.

Objectives

- Clinico pathological correlation in OSSN.
- To evaluate the frequency and pattern of expression of p16 by immunohistochemistry in histopathologically diagnosed samples of OSSN.

Material and Method

Source of Data: All ocular surface squamous neoplasia excision biopsies received in Department of Pathology in BMC&RI and attached hospitals, Bangalore.

Sample Size: All the cases of OSSN operated in the Minto Ophthalmic Hospital, Bangalore and received at the Department of Pathology, Victoria Hospital during the study period between April 2022 to October 2023. A total of 40 cases of OSSN were received and studied.

Study Design: Cross sectional study.

Inclusion Criteria: All clinically diagnosed cases as ocular surface squamous neoplasia which are confirmed by histopathology and those who are willing to participate in the study during the study time.

Exclusion Criteria

1. Scant tissue not sufficient for IHC.
2. Poorly preserved tissue.
3. Clinically suspected ocular surface squamous neoplasia which is diagnosed as benign in histopathology.

Sample Size Estimation

Based on previous study by Tina Shreshta, Won Choi, Ga-eon Kim Jee Myung Yang, Kyung Chulyoon, Human papilloma virus identification in ocular surface squamous neoplasia by p16 immunohistochemistry and DNA chiptest, 31.6% is positive using p16 immunostaining assuming similar results in our study with prevalence 31.6% absolute precision of 16%, the sample size was calculated to be 32. where Zα= Standard table value for 95% ,p = proportion = 31.6,

q = 100 - 31.6 = 68.4

d = precision = 16

Formula Used

q = 100 - 31.6 = 68.4

d = precision = 16

Formula $n = \frac{(Z\alpha)^2 \times p \times q}{d^2}$

$n \frac{(1.96)^2 x 31.6 x 68.}{(16)^2}$

Total sample size = 32

Statistical Analysis

Data was entered in Microsoft Excel 2019 and was analyzed using Med-Calc statistical software. The quantitative variables were expressed in terms of mean, median, range and standard deviation. The categorical variables were expressed in terms of percentages. The association between the categorical variables were assessed using chi-square test and p value of less than 0.05 was considered as significant.

Methodology

After receiving approval from the ethics committee, sample collection got underway. Regional Institute of Ophthalmology at Bengaluru's Minto Ophthalmic Hospital provided the biopsy specimens. Brief clinical information about the patient was recorded, including name, age, sex, symptoms, and clinical diagnosis. The biopsy sample was delivered in a clearly marked jar with 10% formalin. inside the jar is a samples of tissue was labelled by the operating surgeon and placed on a filter paper with the mucosal surface facing up and the lesion's anatomical location noted. When margins weren't sent in separate cassettes or weren't labelled by the surgeon, they were labelled following consultation with ophthalmology residents. The biopsies underwent 10% formalin processing and were paraffin embedded. The paraffin blocks were cut into 4-5 micron-thick sections, which were then transferred to labelled slides for standard hematoxylin and eosin staining. Cases of OSSN that had been histopathologically proven were collected for p16 staining.

Result

Table 1: Age Distribution of Cases

Age Group	No. of case	Percentage
11-20	1	2.9
21-30	4	11.4

31-40	7	20.0
41-50	9	25.7
51-60	7	20.0
61-70	5	14.3
71-80	2	5.7
Total	35	100

The minimum age of presentation was 18 years and the oldest patient was 77 years old. Majority of the patients were in the 41-50 age brackets. The mean age of patientsstudied is 47.9 years.

Graph 1: Distribution of study subjects according to age.

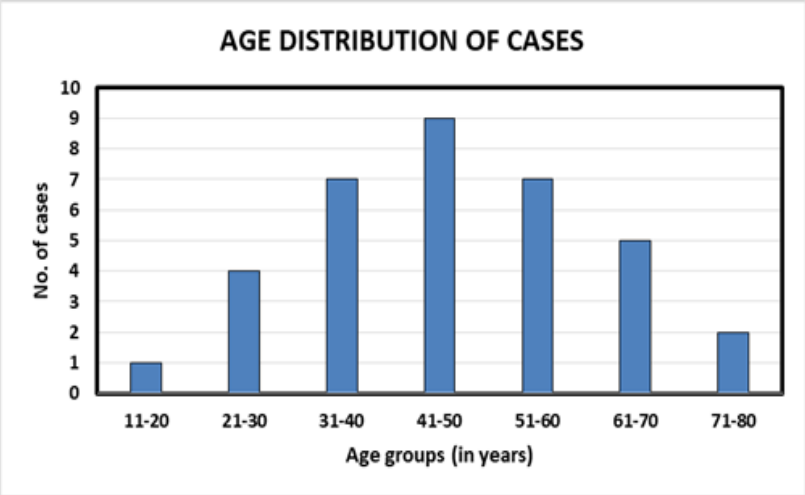


Table 2: Gender wise distribution of cases

Gender	No. of cases	Percentage
Male	17	48.6
Female	18	51.4
Total	35	100

Out of the 35 patients studied 17 (48.6%) were males and 18(51.4%) were females.

Graph 2: Distribution of study subjects according to gender.

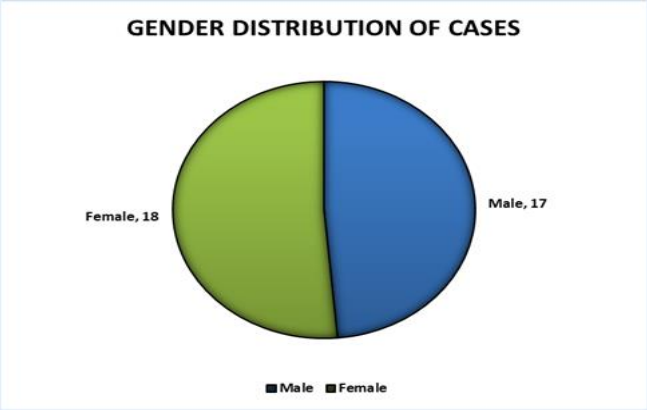


Table 3: Comparison of histological type with clinicomorphological type

Histological grade	Clinico-morphological type					Total
	Leukoplakic	Gelatinous	Diffuse	Papilliform	Nodular	
Grade I	1	2	0	0	0	3
Grade II	1	1	1	1	2	6
Grade III	12	1	0	0	1	14
Carcinoma in-situ	2	0	0	0	0	2
SCC	0	0	0	0	10	10
Total	16	4	1	1	13	35
Chi-square test	P = <0.0001	Significant				

Most of the cases presented with morphological type of leukoplakic, followed by nodular then gelatinous and one case each presented with diffuse and papilliform. Significant association was found between histological type and clinicomorphological type. All of the SCC cases presented with morphological type of nodular type. Both of carcinoma insitu cases presented with leukoplakic lesions. 12 out of 14 cases grade III OSSN presented with leukoplakic lesions, one case presented as nodular and one as gelatinous type. Among 3 cases of grade I OSSN one case presented as Leukoplakic and 2 in gelatinous type. Grade II OSSN presented in all morphological types.

Graph 3: Comparison of histological type with clinic-morphological type

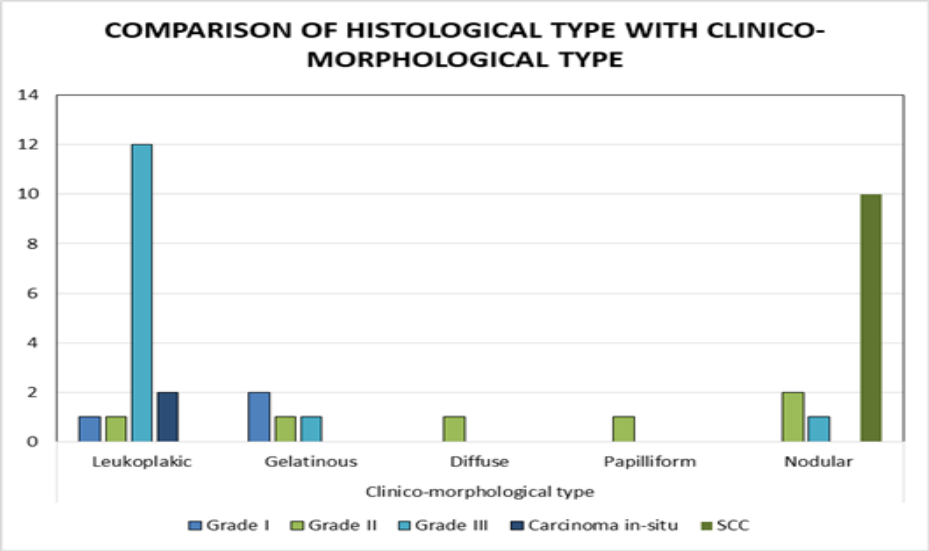


Table 4: Number of HIV infected cases

HIV Infection	No. Of Cases	Percentage
Present	8	22.9
Absent	27	77.1
Total	35	100

8 out of 35 cases were HIV positive (22.9%) with 5 male and 3 female patients P16 expression of the HIV positive cases has been enumerated inTable 2.

Graph 4: Distribution of HIV Positive Patients.

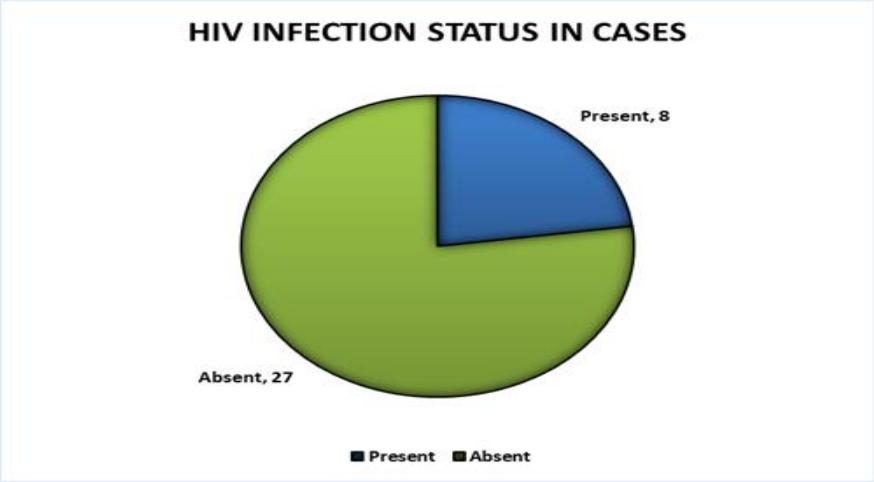


Table 5: Histological grades of OSSN

Histological grade	No. of cases	Percentage
Grade I	3	8.6
Grade II	6	17.1
Grade III	14	40.0
Carcinoma in-situ	2	5.7
SCC	10	28.6
Total	35	100

Out of the 35 cases studied there were 3 case of Grade I OSSN, 6 cases of Grade II OSSN, 14 cases of Grade III OSSN,2 cases of carcinoma insitu and 10 cases of Squamous cell carcinoma . Majorityof cases presented with Grade III OSSN (40%)

Graph 5: Distribution of study subjects according to histopathologicaldiagnosis

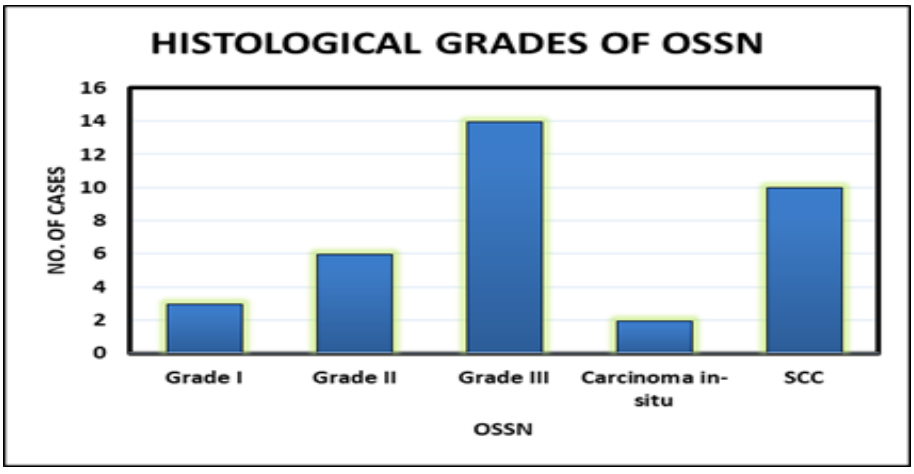


Table 6: P16 Expression in Cases

P16 expression	No. of cases	Percentage
Positive	27	77.1
Negative	8	22.9
Total	35	100

Among 35 cases studied 27 cases showed positive staining for P16 (77.1%) and 8 cases were showing negative staining (22.9%).

Graph 6: p16 expression in cases

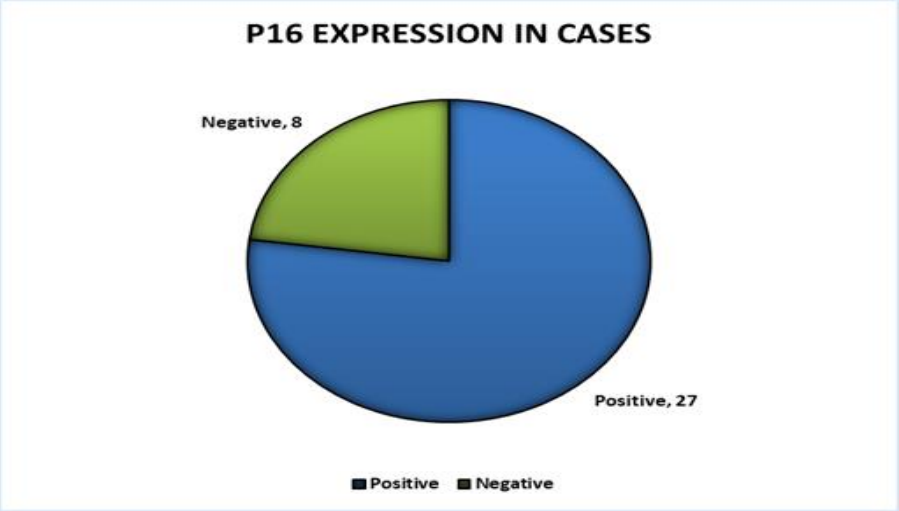


Table 7: p16 expression in different grades of OSSN

P16 expression	Grade I	Grade II	Grade III	Carcinoma in-situ	SCC	Total
Positive	2	6	9	1	9	27
Negative	1	0	5	1	1	8
Total	3	6	14	2	10	35
Chi-Square Test	P = 0.28	Not Significant				

Immunohistochemical stain p16 was applied to all the cases and the results have been compiled in the table above . Out of 3 cases of grade I OSSN 2 were positive. All 6 cases of grade II OSSN turned out to be positive. 9 out of 14 cases were positive for grade III OSSN. One among 2 carcinoma insitu came positive. In case of squamous cell carcinoma 9 out of 10 cases became positive. Applying the chisquare test p value came as 0.28 showing that there is no significant correlation between different grades of OSSN and p16 expression.

Graph 7: P16 expression in each histopathological grade.

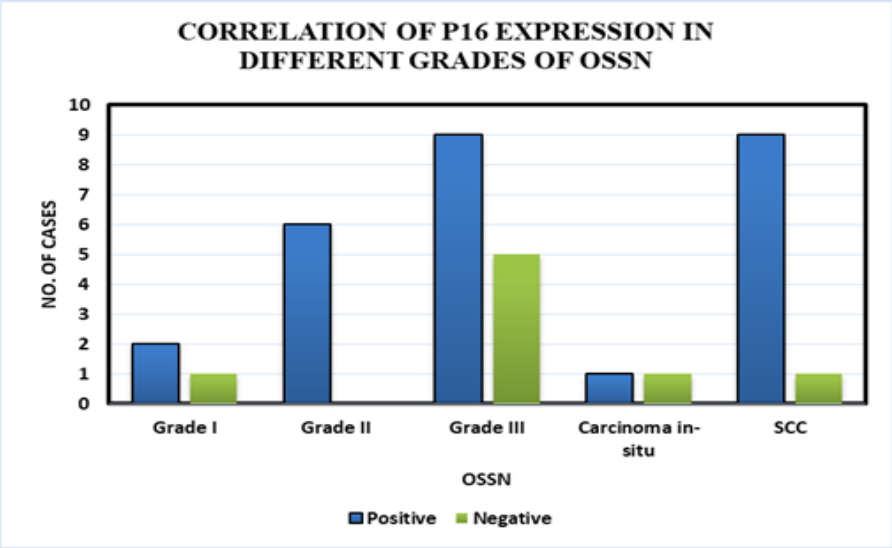


Table 8: Intensity of p16 expression in different grades of OSSN

Intensity of P16 expression	Grade I	Grade II	Grade III	Carcinoma in-situ	SCC	Total
Moderate	3	6	13	2	10	34
High	0	0	1	0	0	1
Total	3	6	14	2	10	35
Chi-Square Test	P = 0.81	Not Significant				

Among 35 cases studied, 34 cases showed moderate degree of intensity of expression including 3 grade I OSSN, 6 cases of grade II OSSN ,13 cases of grade III OSSN , 2 cases of carcinoma insitu. 10 squamous cell carcinoma cases. One case of grade III OSSN showed high intensity of expression. Since the p value came above 0.05 no significant association was found out between different grades of OSSN and intensity of p16 expression.

Graph 8: Intensity of p16 expression in different grades of OSSN

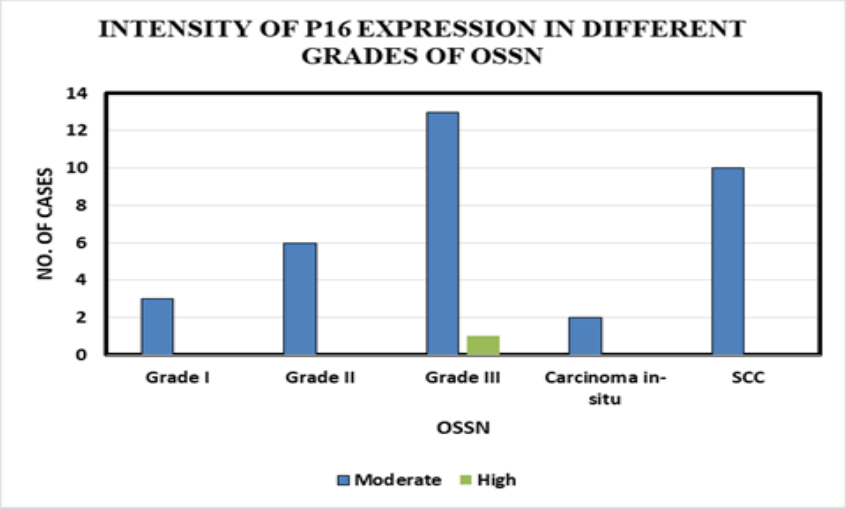
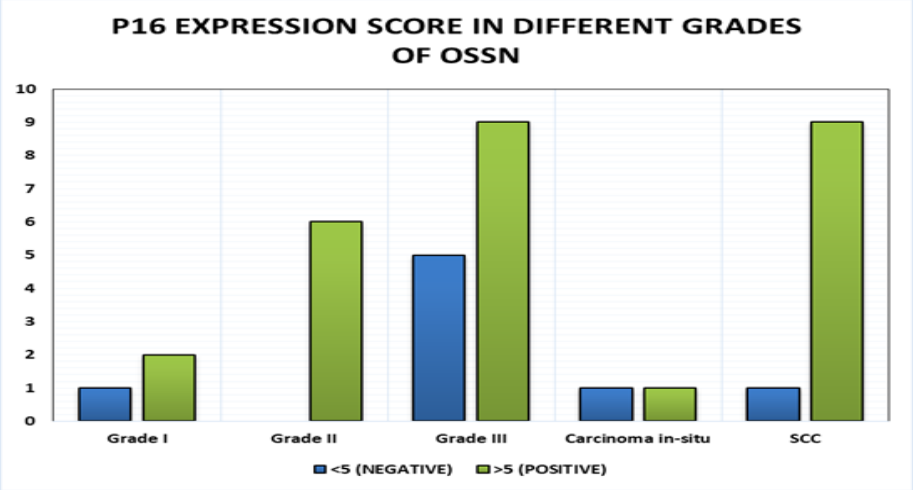


Table 9: p16 expression scores in different grades of OSSN

Total Score Of P16 Expression	Grade I	Grade II	Grade III	Carcinoma in-situ	SCC	Total
<5 (Negative)	1	0	5	1	1	8
>5 (Positive)	2	6	9	1	9	27
Total	3	6	14	2	10	35
Chi-Square Test	P = 0.28	Not Significant				

Graph 9: p16 expression score in different grades of OSSN



Discussion

Ocular surface squamous neoplasia is the most common tumor of the ocular surface involving the limbus, cornea or conjunctiva. It is defined as a range of disease from mild dysplasia to carcinoma insitu and invasive squamous cell carcinoma. This study was undertaken to examine

1. Clinico pathological correlation in OSSN.
2. To evaluate the frequency and pattern of expression of p16 by immunohistochemistry in histopathologically diagnosed samples of OSSN.

35 cases of OSSN that the Department of Pathology received and that met the study's inclusion and exclusion criteria were examined in total. The patients' ages ranged from 18 to 77 years, with a mean of 47.9 years. Males and females were equally involved with males: females ratio 1:1. This is concurrence with study done in Africa which records the maximum incidence of OSSN in the world.

Both males and females are equally exposed to sunlight leading to similar risk of developing OSSN. Occupational factors such as outdoor work may contribute to OSSN risk, and both genders can be exposed to similar occupational hazards.

Table 10: Comparison of Mean Age at Presentation and Gender of Patients with OSSN in Various Studies with Present Study

Study	Place of Study	Mean Age	Male	Female	M:F
Tina Shreshta 2018	Chonnam	69.1	27	11	1:0.04
Ateenyi-Agaba et al 1995	Uganda	33	52	48	1:0.9
Sheetal Chauhan	New delhi	64	50	14	1:0.28

Spitzer et al, 2008	Malawi	33	42	58	1:2.1
Simbiri et al. 2010	Botswana	39	39	61	1:1.6
Pola et al. 2003	Zimbabwe	35	30	70	1:1.4
Mahomed&Chetty,2002	South Africa	37	20	20	1:1
Chisi et al. 2006	Kenya	38	50	50	1:1
Makupa et al. 2012	Tanzania	39	32	32	1:1
Present study		47.9	17	18	1:1

The majority of research conducted in Africa attribute the lower age of onset in their population to HIV infection. In the current study, the mean age of onset for HIV positive cases was 32 years, compared to 47.9 years for total cases. Therefore, it is also comparable to the current study.

Table 11. Comparison of P16 Positivity In Cases.

Study	Positive	Negative	Total
Shreshta et al	12	26	38
Carla carrilho et al	14	61	75
Present study	27	8	35

p16 expression can be used as a surrogate marker of HPV infection.p16 positive cases showed koilocytic changes in lower grades of OSSN such as grade I and II , but these changes were seen decreased in higher grades.p16 positive cases showed more dysplastic features such as increased nuclear and cellular size with irregular nuclear contours and hyperchromasia than p16 negative cases. However some p16 negative cases still showed features of more dysplasia ,albeit with different molecular mechanisms involved. As per shreshta et al's study conducted in 38 cases p16 expression came positive in minority of cases , that is in 12 (31%). As per Carla carrilho et al 's study also 14 out of 75 cases showed positive staining that is in 14% cases. But in our present study 27 out of 35 cases showed p16 positivity.77% of the cases showed positive p16 staining, suggesting that there is significant association between HPV and OSSN. Our study proves beyond doubt that HPV plays a major role in the pathogenesis of OSSN.

Table 12: Comparison of P16 Expression in Different Grades of OSSN.

Study	Grade I	Grade Ii	Grade Iii	Carcinoma Insitu	SCC
Tina shreshta et al	2(16.6%)	0(0%)	2(16.6%)	5(41.8%)	3(25%)
Carillho et al				1(7.1%)	13(92.8%)
Present study	2(7.4%)	6(22%)	9(33%)	1(3.7%)	9(33.3%)

As per the study p16 immunochemistry came positive more in higher grades such as grade III OSSN (33%) and in SCC (33%).This is in comparison with Carrillho et al where p16 immunochemistry came positive more in Squamous cell carcinoma (92.8%) then in carcinoma insitu (7.1%).According to Tina shreshta et al maximum p16 positive was shown by carcinoma insitu (41.8%) then by squamous cell carcinoma (25%).Less positivity of carcinoma insitu in the present study may be due to less representation of the CIN cases in the present study.

Conclusion

Ocular surface tumours are a group of diseases with a variety of clinical manifestations. Histopathology aids in early diagnosis and is necessary for confirmation. The goal of this study was to investigate OSSN clinicopathological corelation. P16 is a potential surrogate marker for HPV infection. Since P16 showed positive in the majority of the cases, we can draw the conclusion that there is a strong correlation between HPV and OSSN in the cases under investigation. Without a doubt, HPV has been shown to be a significant factor in the pathophysiology of OSSN. Additionally, it aimed to assess, using immunohistochemistry, the frequency and pattern of p16 expression in samples of OSSN that had been histopathologically diagnosed. P16 came positive more in higher grades such as grade III OSSN and SCC but no statically significant association was found between different grades of OSSN and p16 expression. However, this creates opportunities for more extensive research on p16 in OSSN in the future with more sample size, which could aid in determining a standard cut-off point for OSSN grading. This will support the pathologist and treating physician in deciding on the patient's course of treatment and prognosis.

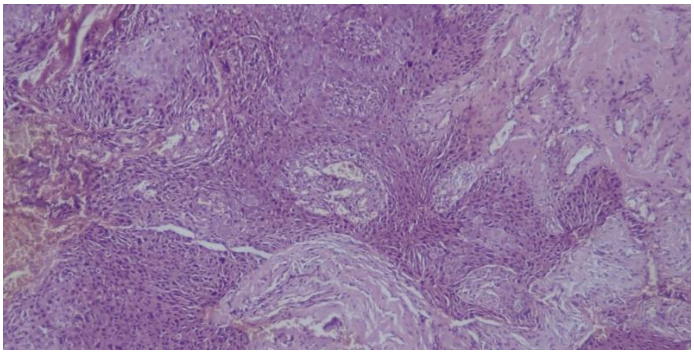


Figure 1: Hematoxylin and eosin stained section showing tumour cells in sheets with invasion (SCC)

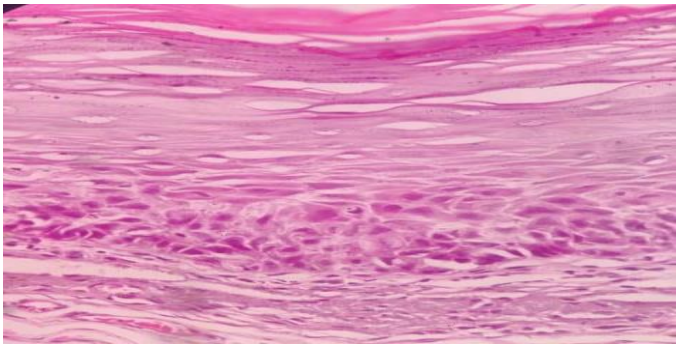


Figure 2: Hematoxylin and eosin stained section showing dysplasia involving 2/3rd of epithelium – GRADE II OSSN.

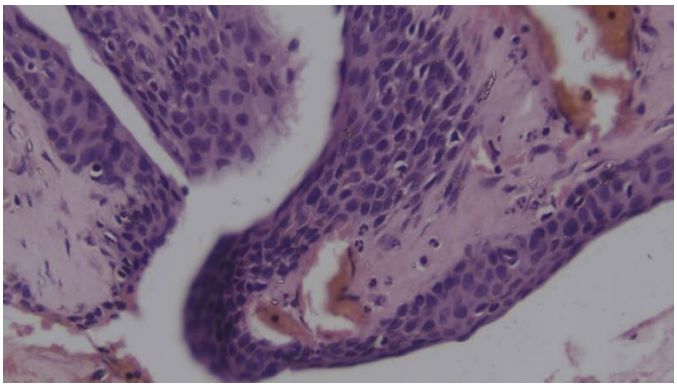


Figure 3: Hematoxylin and eosin stained section showing full thickness dysplasia of epithelium- GRADE III OSSN

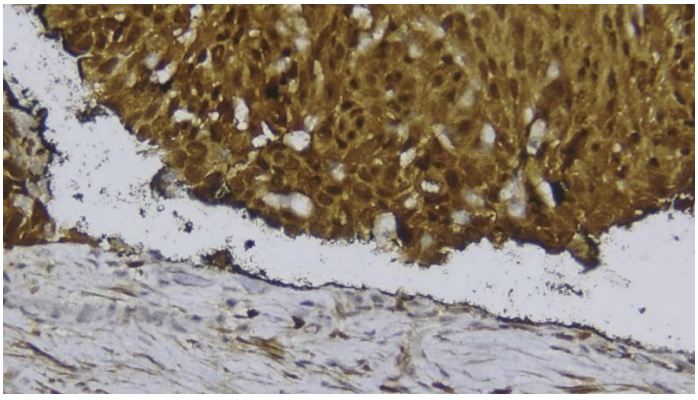


Figure 4: P16 showing strong intensity of staining

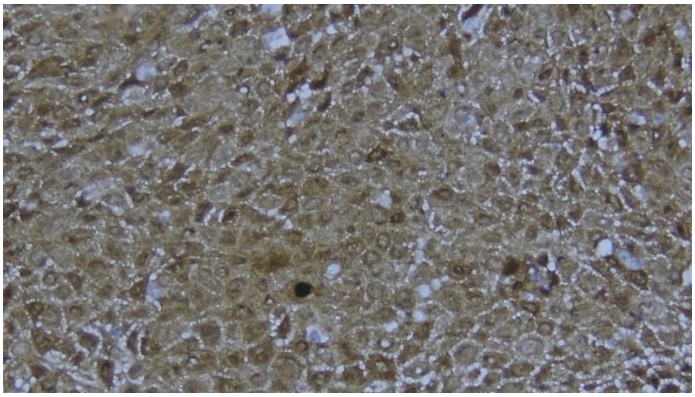


Figure 5: P16 showing moderate intensity of staining

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