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ORIGINAL RESEARCH

Immunohistochemical expression of p16 & SOX2 in preneoplastic& neoplastic lesions of cervix

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ABSTRACT

Introduction: Cervical cancer is the fourth most prevalent cancer among women worldwide and second most common cancer among Indian women. P16INK4a (p16), a tumor suppressor protein analysed by immunohistochemical and its overexpression is utilized as a surrogate biomarker for high-risk human papillomavirus (HPV) infection. SOX2 (sex-determining region Y-box 2), is a transcription factor essential for sustaining the self-renewal and pluripotency of undifferentiated embryonic stem cells (ESCs). Elevated expression of SOX2 has been observed in cervical cancer. **Aim:** To analyse the expression patterns of p16 and SOX2 in various preneoplastic and neoplastic cervical lesions. **Materials & methods:** This study was conducted in the Department of Pathology, MKCG Medical College, Berhampur, Odisha, between June 2023 to February 2025. A total of 79 cases of cervical lesion, were studied and IHC was performed on each case for both p16 and SOX2. p16 and SOX2 expressions in different lesions were assessed using chi-square analysis, Fishers exact test and all the data were tabulated and analysed using SPSS version 23.0. **Result:** Among the 79 cases, squamous cell carcinoma accounted for 64 cases (81%), followed by high-grade squamous intraepithelial lesions with 12 cases (15.2%), and rest 3 cases were of low-grade squamous intraepithelial lesion. Significant overexpression of SOX2 (p-value = 0.00035) and p16 (p-value = 0.00028) was observed in both SCC and HSIL. In HSIL cases, the expression of SOX2 and p16 extended to two-thirds or even the full thickness of the epithelium. In contrast, SCC demonstrated immunohistochemical expression that reached the full thickness consistently. **Conclusion:** Both Sox2 and p16 show increasing expression as the lesion progresses from low grade dysplasia to high grade dysplasia and invasive cancer and can complement each other to make a definitive diagnosis

Keywords: Cervical cancer, High-grade squamous intraepithelial lesion, Immunohistochemistry, Low-grade squamous intraepithelial lesion, p16, SOX2.

INTRODUCTION

Cervical cancer ranks as the fourth most common malignancy affecting women globally, with an estimated 660,000 new cases reported in 2022, according to GLOBOCAN. Notably, approximately 94% of the 350,000 cervical cancer-related deaths that year occurred in low- and middle-income countries, highlighting a significant disparity in disease burden and access to care.[(1)]Incidence rate of cervical cancer in India is 17.70 per 100,000 population, with a mortality rate of 11.2 per 100,000. Cervical cancer disproportionately affects younger women, positioning it as a major cause of maternal mortality, nearly 20% due to cancer. [(2)]

Approximately 5.0% of women in general population have chronic infection with high-risk HPV types 16 or 18, resulting in 83.2% of invasive cervical cancer cases[(3)].

P16INK4a (p16) is a tumor suppressor protein that inhibits cyclin-dependent kinases 4 and 6, responsible for phosphorylating the retinoblastoma protein (pRB). pRB regulates p16 levels via negative feedback mechanism. P16 overexpression occurs when pRB is functionally inactivated by the HPV E7 oncoprotein. P16 immunohistochemistry overexpression serves as a surrogate biomarker for HPV infection.[(4)]

SOX2 (sex-determining region Y-box 2) is a transcription factor, a member of SOXB1 family, essential for sustaining the self-renewal and pluripotency of undifferentiated embryonic stem cells (ESCs). [(5)]Researcheshave shown SOX2 involvement in the carcinogenesis of various malignancies along

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with its involvement in tumor invasion and metastasis. SOX2 levels are elevated in cervical cancer and are associated with poorer prognostic outcomes, such as reduced survival rates and resistance to chemotherapy.[6,7]

The objective of this study was to evaluate the distribution pattern and intensity of SOX2& p16 immunostaining in pre-neoplastic & neoplastic conditions of cervix.

MATERIALS & METHODS

A total of 79 cases were studied over a period of twenty one months, from June 2023 to February 2025, at MKCG Medical College, Berhampur, Odisha. This prospective cross-sectional study was carried out after obtaining the Institutional Ethics Committee clearance (as per letter no.1252). Among these, 3 were LSIL, 12 were HSIL & 64 cases were of SCC of cervix. Biopsy-proven cases of cervical squamous intraepithelial lesion and squamous cell carcinoma of the cervix. Cases without proper consent, improperly fixed specimens, adenocarcinoma of the cervix, and specimens deemed inadequate for the immunohistochemical procedure were excluded from the study.

All hysterectomy specimens were grossed as per the protocol and fixed in formalin for 24–48 h and were routinely processed.Deparaffinized sections were stained with hematoxylin andeosin (H&E). After reviewing the H&E sections, the paraffin blocks wereselected for IHC study.

Immunohistochemistry

Thin sections of 4 microns thickness were taken on poly-L-lysinecoated slides and air-dried. Two sections were allocated for immunohistochemistry targeting SOX2 and p16, respectively. These slides were then incubated at 60–70°C for one hour, followed by deparaffinization and subsequently rehydration. Antigen retrieval was performed at 150°C using citrate buffer for 15 minutes, followed by a 20-minute wash in Tris Buffer Solution. The slides were then allowed to cool to room temperature and rinsed twice in distilled water for 5 minutes each. To block endogenous peroxidase activity, 1% hydrogen peroxide was applied to the sections and incubated for 5 minutes. Finally after another wash in buffer solution for 2 minutes, slides were treated with primary antibodies of p16INK4a and SOX2seperately (both the products of PathnSitucompany) and incubated for 30 minutes at room temperature. After incubation, the slides were washed twice in buffer solution, each wash lasting 2 minutes. Secondary antibody was added and incubated for 15 minutes, followed by two washes in buffer for 2 minutes each. This was followed by incubation with horseradish peroxidase for 15 minutes.Colour development was achieved by incubating the sections with diaminobenzidine (DAB) for 5 minutes. The sections were then rinsed with distilled water and counterstained with hematoxylin for 2 seconds.Finally, the slides were washed under running tap water for 3 minutes, air-dried, cleared in xylene, and mounted using DPX mounting medium.

For each staining batch, a histological section of cervical squamous cell carcinoma with established p16 positivity was incorporated as a positive control. For the negative control, phosphate buffer solution was substituted in place of the primary antibody.

Scoring system for p16 and SOX2[8, 9]

The p16 shows nuclear and cytoplasmic positivity. The intensity of the staining was classified as strong (3), moderate (2), weak (1), and negative (0).Percentage of positive tumor cells were scored as; score 1 (<1%), score 2 (1-10%), score 3 (11-33%), score 4 (34-66%) and score 5 (>66%). The histological score was obtained by adding the percentage of positive cells score and the intensity of the staining score and was categorised as low expression (Histological score 0-2), moderate expression (Histological score 3-5) and over expression (Histological score 6-8).

The SOX2 shows nuclear positivity. The intensity of the staining was classified as strong (3), moderate (2), weak (1), and negative (0).The percentage of positive tumor cells were scored as 0 (<10%), 1 (10-25%), 2 (26-50%), 3 (51-75%) and 4 (>75%). The histological score was defined as the percentage of positive cells score multiplied by the intensity of the staining score. Histological score was described as following categories: negative (score 0), weakly positive (score 1-4), positive (score 5-8) and strong positive (score 9-12). Negative & weakly positive was evaluated as Negative expression whilepositive & strongly positive is assessed as Positive expression for SOX2 marker.

Statistical analysis

p16 and SOX2 expressions in different lesions were assessed using chi-square analysis, Fishers exact test and all the data were tabulated and analysed using SPSS version 23.0.

The frequency and percentage analyses of various parameters were performed using SPSS software. A p-value of <0.05 was considered statistically significant.

RESULTS

In the present study, squamous cell carcinoma was the most common tumor, representing 81% (n=64) of the cases. Followed by high-grade squamous intraepithelial lesions (HSIL), accounting for 15.20% (n=12) of the cases. Low-grade squamous intraepithelial lesions (LSIL) had the lowest prevalence, making up just 3.80% (n=3) of the cases [Table-1].

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Table 1: Distribution of Histological Types of Cervical Lesions (n=79)

Histological type	Frequency	Percentage (%)
LSIL	3	3.80
HSIL	12	15.20
Squamous cell carcinoma	64	81.00
Total	79	100

In this study, large cell non-keratinizing squamous cell carcinoma (SCC) was the predominant histological pattern, accounting for 89% of cases. Keratinizing SCC was observed much less frequently, representing 9.4% of the samples. A single instance of papillary SCC was also noted [Table-2].

Table 2: Histological patterns of squamous cell carcinoma (n=64)

Histological pattern	Frequency	Percentage (%)
Papillary	1	1.6
Keratinizing	6	9.4
Non- keratinizing	57	89.0
Total	64	100

In this study, the ages of patients diagnosed with cervical carcinoma ranged from 35 to 78 years, with a mean age of 56.01 years. The youngest patient was 35 years old and the oldest was 78. The age group most commonly affected was 51–60 years, accounting for 31 cases (39.20%). Further analysis revealed that within the 51–60 year age group, there were 27 cases of squamous cell carcinoma (SCC) and 4 cases of high-grade squamous intraepithelial lesion (HSIL), together making up 39.24% of the total cases. This was followed by the 61–70 year age group, which comprised 26.58% of cases, and the 41–50 year age group, accounting for 22.80% of cases. These findings underscore that cervical carcinoma predominantly affects women in their fifth and sixth decades of life, with a marked peak in the 51–60 year age range.as showed in [Table-3].

Table 3: Age wise distribution of cervical squamous lesions (n=79)

Age (years)	LSIL	HSIL	SCC	Total	Percentage (%)
31-40	3	0	3	6	7.59
41-50	0	6	12	18	22.80
51-60	0	4	27	31	39.24
61-70	0	2	19	21	26.58
71-80	0	0	3	3	3.79
Total	3	12	64	79	100%

Per vaginal bleeding was the predominant clinical presentation, observed in 58.22% of cases (46 out of 79 cases), followed by abdominal pain 24.05%. Other presentations included whitish discharge (15.20%), and least commonly, post-coital bleeding (2.53%).

Analysis of p16 expression revealed that 59 cases (74.68%) exhibited overexpression, as summarized in [Table-4].

Table 4: Expression of p16 in all cervical lesions.

p16 expression	Total	Percentage
Low expression	6	7.60%
Moderate expression	14	17.72%
Over expression	59	74.68%
Total	79	100%

When lesions were evaluated for SOX2 expression, a majority demonstrated positivity, with 62 cases (78.50%) testing positive, as illustrated in [Table-5].

Table 5: Expression of SOX2

SOX2 expression	Total	Percentage
Negative	17	21.50%
Positive	62	78.50%
Total	79	100%

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Histopathological and respective IHC microphotographs of various lesions: fig 1. fig 2. and fig 3.

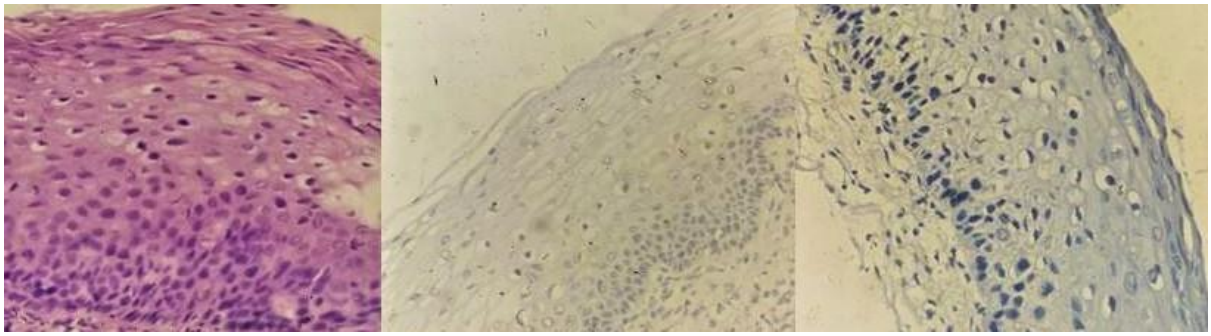

Fig 1: p16 & SOX2 expression in LSIL; A-LSIL, H&E(x400), B- Low p16 expression, IHC (x200), C- Negative expression of SOX2, IHC (x200).

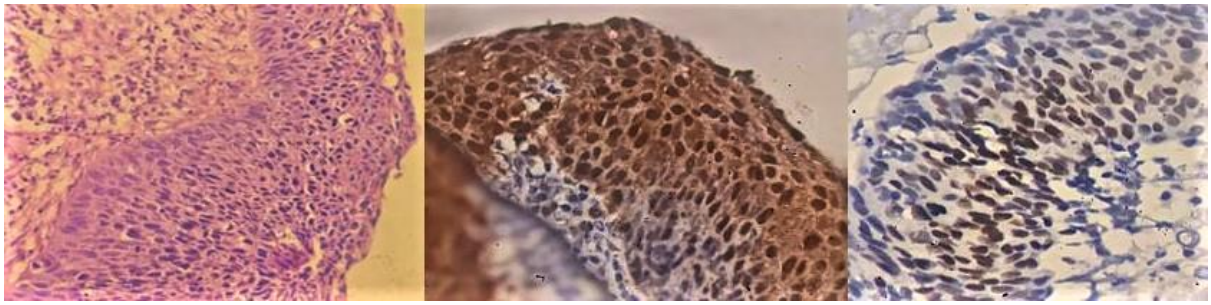

Fig 2: p16 & SOX2 expression in HSIL; A-HSIL, H&E(x400), B- p16 expression in full thickness of epithelium, IHC (x200), C- Expression of SOX2 in two-thirds of epithelium, IHC (x200)

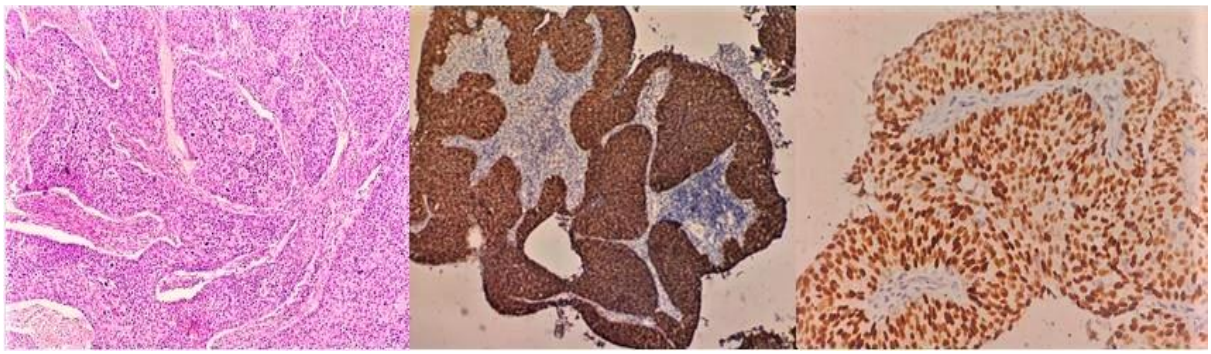

Fig 3: p16 & SOX2 expression in SCC; A-SCC, H&E(x200), B- p16 expression in full thickness of epithelium, IHC (x100), C- SOX2 expression in full thickness of epithelium, IHC (x200)

Correlation between histopathological characteristics and the expression patterns of SOX2 and p16

Regarding p16, overexpression was detected in 52 of 64 SCC cases, 7 of 12 HSIL cases, and none of the 3 LSIL cases. Conversely, low p16 expression was present in 2 of 64 SCC, 1 of 12 HSIL, and 3 of 3 LSIL cases. Moderate p16 expression was found in 10 of 64 SCC cases, 4 of 12 HSIL cases. There was a significant correlation between p16 expression levels and histopathological findings [Table-6].

Table 6: p16 expression in different cervical lesions (n=79)

p16 expression	LSIL	HSIL	SCC	Total	Fisher's exact test & p value
Low expression	3	1	2	6	
Moderate expression	0	4	10	14	
Over expression	0	7	52	59	p value 0.00028
Total	3	12	64	79	

SOX2 immunostaining staining was observed in 55 of 64 SCC cases and 7 of 12 HSIL cases. None of LSIL cases showed SOX2 positivity. A significant correlation was observed between SOX2 expression and the histopathological findings [Table-7].

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Table 7: SOX2 expression in different cervical lesions (n=79)

SOX2 expression	LSIL	HSIL	SCC	Total	Chi-square test & p-value
Negative	3	5	9	17	x^2: 15.93
Positive	0	7	55	62	p value: 0.00035
Total	3	12	64	79	

DISCUSSION

p16 expression in various cervical lesions

p16 expression is markedly increased in squamous cell carcinoma compared to both high-grade and low-grade squamous intraepithelial lesions, with LSIL typically exhibiting low levels of p16 in this study. In HSIL, p16 staining often extends up to two-thirds or even the full thickness of the epithelium, reflecting a higher degree of dysplasia. In the present study, p16 overexpression was observed in the majority of SCC cases, with an incidence rate of 81.26%, highlighting a strong association between p16 positivity and increasing lesion severity. This pattern aligns with previous researches, which consistently demonstrates a progressive increase in p16 expression from LSIL to HSIL and finally to invasive carcinoma. The findings are in agreement with earlier studies by SowmyaDayalan et al[8]. andLesnikova et al[9].

Usha Sharma et al [10], Kaur S et al [11] , Abha Pandey et al[12] and Anu Anna Jacob et al[13] all support the observed pattern of increasing p16 expression correlating with higher grades of cervical epithelial dysplasia. Their findings consistently demonstrate that p16 positivity rises as the severity of dysplastic changes progresses, reinforcing the role of p16 as a biomarker for stratifying cervical dysplastic and neoplastic lesions.

A small subset of squamous intraepithelial lesion cases exhibited negative p16 expression, which may be attributed to the potential for spontaneous regression observed in some lesions. Similar observations were reported in a previous study by SowmyaDayalan et al., indicating that not all lesions consistently express p16, possibly reflecting their variable biological behaviour and likelihood of regression.

Overall, the evidence strongly supports the utility of p16 as a biomarker for distinguishing between grades of cervical lesions, particularly when used alongside traditional histopathological evaluation.

SOX2 expression in various cervical lesions

In this study, a notably high prevalence of positive SOX2 expression was identified predominantly in cases of squamous cell carcinoma, followed by high-grade squamous intraepithelial lesions. These findings align with previous reports by Chun-Fang Cai et al[14], Jobran M. Moshi et al[15]., and SowmyaDayalan et al[8]. Furthermore, data from Yuan D et al[16]. demonstrate that SOX2 expression is significantly elevated in cervical cancer cases, supporting the association of SOX2 with cervical carcinogenesis and progression.

Most HSIL lesions in the current study displayed SOX2 expression extending into the lower two-thirds or the full thickness of the epithelium, similar to the separate studies by SowmyaDayalan et al[8], Jobran M. Moshi et al[15], Wolsky, R. J. et al[17] &Atigan et al[18].

Whereas all LSIL cases in present study were negative for SOX2 expression. This observation aligns with the findings of Chun-Fang Cai et al[12] and SowmyaDayalan et al[8], who also reported predominantly negative SOX2 expression in LSIL cases.

CONCLUSION

The expression of SOX2 and p16 is notably elevated in squamous cell carcinoma (SCC) of the cervix, with a progressive increase in expression as the grade of dysplasia advances from low-grade to high-grade precancerous lesions and ultimately to SCC. The combined heightened immunoexpression of p16 and SOX2 in cervical malignancies suggests an aggressive tumor behavior, requiring appropriate management strategies. However, due to the limited duration and sample size of the current study, a more comprehensive investigation is needed to further evaluation.

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