RESEARCH PAPER

A Study on Immunohistochemical Expression of P16 And SOX2 in Preneoplastic and Neoplastic Lesions of Cervix among Patients attending a Tertiary Care Hospital

Dr I R Shinitha[1] , Dr Shobana B [2] , Dr Divyalakshmi. P.K[3]

[1]Postgraduate, Department of Pathology Sree Balaji Medical College & Hospital, Chrompet, Chennai
Mail id: drshinithair@gmail.com
Orchid id: 0009-0006-7444-6664
[2]Professor, Department of Pathology Sree Balaji Medical College & Hospital, Chrompet, Chennai
Mail id: shobdr@gmail.com
orcid - 0000 0003 4880 7279
[3]Assistant Professor, Department of Pathology Sree Balaji Medical College & Hospital, Chrompet, Chennai
Mail id: shiningstar.drdivya@gmail.com
Orchid id: 0000-0003-0648-1530.

ABSTRACT

Background: Cervical cancer remains a major public health concern globally, particularly in low- and middle-income countries, and is strongly associated with persistent infection by high-risk human papillomavirus (hrHPV). Early detection of preneoplastic and neoplastic lesions is critical to improve clinical outcomes, yet conventional cytology and HPV DNA testing have limitations in sensitivity and specificity. Immunohistochemical markers such as p16 and SOX2 have emerged as potential biomarkers for identifying high-grade lesions and invasive carcinoma. This study aimed to evaluate the expression of p16 and SOX2 across preneoplastic and neoplastic cervical lesions and to explore their correlation with histopathological diagnosis and each other.

Methods: A cross-sectional study was conducted at the Department of Pathology, Sree Balaji Medical College and Hospital, Chennai, India, from July 2023 to June 2025. Forty histologically confirmed cervical lesions—including low-grade squamous intraepithelial lesion (LSIL, n = 13, 32.5%), high-grade squamous intraepithelial lesion (HSIL, n = 12, 30%), and squamous cell carcinoma (SCC, n = 15, 37.5%)—were analyzed using immunohistochemistry for p16 and SOX2. Expression was evaluated based on staining intensity and proportion of positive cells. Statistical analysis was performed using Chi-square tests, and $p < 0.05$ was considered significant.

Results: Overexpression of p16 was observed in 14/15 SCC cases (93.3%) and 11/12 HSIL cases (91.7%), while low or negative expression predominated in LSIL (9/13, 69.2%). SOX2 expression increased progressively from LSIL (0/13, 0%) to HSIL (10/12, 83.3%) and SCC (9/15, 60%). Co-expression analysis revealed that among 21 SOX2-positive cases, 20 (95.2%) also demonstrated p16 overexpression, indicating strong concordance ($\chi^2 = 15.60$, $p < 0.001$). Both markers demonstrated statistically significant associations with lesion severity.

Conclusion: The study confirms that p16 and SOX2 are significantly upregulated in high-grade and invasive cervical lesions, with strong concordance between the two markers. Combined assessment of p16 and SOX2 enhances detection of high-risk lesions, aids in risk stratification, and supports improved clinical management of cervical cancer, highlighting their utility as complementary immunohistochemical biomarkers.

Keywords: Cervical cancer, p16, SOX2, Immunohistochemistry, Preneoplastic lesions, Squamous cell carcinoma

How to cite this article: Shinitha IR, Shobana B, Divyalakshmi PK.. A Study on Immunohistochemical Expression of P16 And SOX2 in Preneoplastic and Neoplastic Lesions of Cervix among Patients attending a Tertiary Care Hospital. Int J Drug Deliv Technol. 2026;16(5s): 278-288; DOI: 10.25258/ijddt.16.5s.36

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Cervical cancer continues to represent a formidable public health challenge worldwide, disproportionately affecting women in low- and middle-income countries (LMICs). It ranks as the fourth most commonly diagnosed malignancy and the fourth leading cause of cancer-related mortality among women globally. Despite being largely preventable through vaccination and early detection, cervical cancer remains the most prevalent cancer in 23 countries and the leading cause of cancer mortality in 36 nations, underscoring deep inequities in access to preventive, diagnostic, and therapeutic services across regions. [1,2]

India shoulders a significant portion of this global burden, contributing nearly one-fifth of all new cases and related deaths. The disease accounts for approximately 18% of total diagnoses and mortalities, with a five-year prevalence rate of 18.8%. [3] Although the country has witnessed a modest decline in age-standardized incidence and mortality rates, attributable to improvements in literacy, menstrual hygiene, reproductive health practices, and reduced tobacco use, this decline has been uneven across different geographic

**Author for Correspondence: drshinithair@gmail.com*

regions. [4–7] Since the inception of the National Programme for Prevention and Control of Non-Communicable Diseases (NPCDCS) in 2010, screening uptake has remained disappointingly low, with only 1.2% of women aged 15–49 years and 2.2% of those aged 30–69 years having undergone screening. [8–10] The urban–rural divide further exacerbates access disparities. In alignment with the World Health Organization (WHO)'s Global Strategy for the Elimination of Cervical Cancer, India aims to achieve the targets of 90% HPV vaccination coverage in girls by age 15, 70% screening coverage at ages 35 and 45, and 90% treatment of precancerous and cancerous lesions by 2030 to reduce the incidence to below 4 per 100,000 women-years. [11,12]

Persistent infection with high-risk human papillomavirus (hrHPV) types—particularly HPV 16 and 18, which together account for approximately 71% of cervical cancer cases—is the central etiological factor driving cervical carcinogenesis. The progression from HPV infection to cervical intraepithelial lesion and ultimately invasive carcinoma involves a series of molecular events mediated primarily by the viral oncoproteins E6 and E7. These proteins disrupt key tumor suppressor pathways: E6 promotes degradation of p53, impairing DNA repair and apoptosis, while E7 inactivates the retinoblastoma protein (pRb), resulting in uncontrolled cell proliferation. Additional cofactors, such as early onset of sexual activity, multiple sexual partners, long-term use of oral contraceptives, smoking, and immunosuppression (notably HIV infection), heighten vulnerability to persistent HPV infection and malignant transformation. [13] Because early stages of cervical neoplasia are frequently asymptomatic or present with nonspecific symptoms, diagnosis often occurs at advanced stages, reducing the success of curative interventions.

Conventional screening methods—such as cytology (Pap smear) and HPV DNA testing—are widely implemented but exhibit notable limitations in sensitivity, specificity, and reproducibility, especially for identifying high-grade lesions. Their performance is further compromised by operator dependence and infrastructural constraints, particularly in resource-limited settings. Consequently, late-stage detection and underdiagnosis remain common. Dual-stain immunocytochemistry (p16/Ki-67) has emerged as a more specific approach, detecting cell-cycle deregulation associated with hrHPV and thereby improving diagnostic accuracy while minimizing overdiagnosis and overtreatment.

Among molecular markers, p16INK4a and SOX2 have gained prominence due to their strong associations with the molecular mechanisms underlying cervical neoplasia. The p16INK4a protein, encoded by the *CDKN2A* gene, is a cyclin-dependent kinase inhibitor that regulates the G1–S phase transition of the cell cycle. Under normal conditions, p16 suppresses the cyclin D–CDK4/6–pRb–E2F axis, acting as a tumor suppressor. In hrHPV infection, E7-mediated pRb inactivation removes this regulatory control, leading to compensatory overexpression of p16. This aberrant expression, easily detectable through immunohistochemistry (IHC), serves as a reliable surrogate marker for oncogenic HPV activity. Numerous studies have established p16 as a highly sensitive and specific biomarker for high-grade intraepithelial lesions and invasive carcinoma, effectively distinguishing premalignant or malignant transformations from benign reactive changes. [14] Notably, p16 expression is absent or minimal in normal cervical epithelium, making its presence an indicator of underlying oncogenic stress.

SOX2 (SRY-box transcription factor 2) is another crucial molecular player implicated in cervical carcinogenesis. A key regulator of pluripotency and self-renewal in embryonic and cancer stem cells, SOX2 functions as a nuclear transcription factor that promotes tumorigenic potential. Aberrant SOX2 overexpression has been documented in a wide range of cancers, including squamous cell carcinoma of the cervix, where it correlates with enhanced tumor invasiveness, chemoresistance, and poor prognosis. [15,16] In cervical lesions, SOX2 expression is minimal in normal epithelial tissues but increases progressively in preneoplastic (LSIL and HSIL) and neoplastic conditions. Mechanistically, it may facilitate epithelial–mesenchymal transition (EMT), metastasis, and recurrence, emphasizing its potential as both a diagnostic and prognostic biomarker.

Rationale and Significance of the Present Study

Despite the established diagnostic roles of p16 and SOX2 individually, comparative assessments of their expression patterns across the spectrum of cervical preneoplastic and neoplastic lesions remain limited. A combined evaluation could yield deeper insights into the molecular interplay driving cervical carcinogenesis and provide adjunctive evidence to histopathological interpretation, particularly in morphologically ambiguous cases. Moreover, understanding the co-expression dynamics of p16 and SOX2 may aid in risk stratification, prognostication, and therapeutic decision-making.

Therefore, the present study aims to investigate the immunohistochemical expression of p16 and SOX2 in preneoplastic and neoplastic cervical lesions and to explore their associations with clinicopathological parameters. This dual-marker analysis seeks to enhance molecular characterization, improve diagnostic precision, and ultimately contribute to more informed clinical management strategies for cervical cancer.

METHODOLOGY

Study Design and Setting

This research was designed as a cross-sectional study conducted in the Department of Pathology, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India. The study spanned a period of two years, from July 2023 to June 2025. It aimed to assess the immunohistochemical expression of p16 and SOX2 in preneoplastic and neoplastic lesions of the cervix and to correlate their expression patterns with histopathological findings and clinicopathological parameters.

Study Population and Sample Size

A total of forty (n = 40) histopathologically confirmed cases were included in the study. The study population consisted of thirteen cases of low-grade squamous intraepithelial

lesion (LSIL), twelve cases of high-grade squamous intraepithelial lesion (HSIL), and fifteen cases of squamous cell carcinoma (SCC). Among these, twenty-seven were cervical biopsy specimens and thirteen were hysterectomy specimens. The cases were retrieved from the archives of the Department of Pathology and included only those with adequate formalin-fixed, paraffin-embedded (FFPE) tissue for immunohistochemical analysis. The sample size was determined based on the availability of histologically proven cases within the study period and represented the distribution of preneoplastic and neoplastic lesions diagnosed in the institution.

Inclusion and Exclusion Criteria

The study included histologically confirmed malignant and premalignant lesions of the cervix, such as cervical intraepithelial lesion (LSIL and HSIL) and invasive squamous cell carcinoma. Exclusion criteria comprised non-neoplastic lesions, benign cervical pathologies, and histologically proven adenocarcinomas of the cervix, as these do not fall within the squamous lineage under investigation. This inclusion framework ensured the selection of representative cases across the continuum of squamous cervical carcinogenesis for immunohistochemical evaluation.

Materials and Reagents

Formalin-fixed, paraffin-embedded tissue blocks of LSIL, HSIL, and SCC were used for the study. The materials required for histopathological and immunohistochemical processing included a microtome for sectioning, positively charged hydrophobic glass slides, hematoxylin and eosin (H&E) stains, mounting medium, and coverslips. Supporting equipment included a water bath, slide warmer, tissue processor, light microscope, and microscope camera for image documentation.

The immunohistochemical (IHC) analysis was carried out using ready-to-use primary antibodies. The anti-p16 antibody was a mouse monoclonal antibody (Clone G175-405; PathnSitu, PM143-3ml RTU), and the anti-SOX2 antibody was a rabbit monoclonal antibody (Clone EP103; PathnSitu, PR071-3ml RTU). The detection system utilized the PolyExcel Target Binder and PolyExcel Poly-HRP secondary antibody (PathnSitu). The chromogenic reaction was visualized using Diaminobenzidine (DAB) substrate (PolyExcel Stunn DAB; PathnSitu). Hydrogen peroxide was used to block endogenous peroxidase activity, while antigen retrieval was achieved using a citrate-based retrieval solution in a pressure cooker. Harris hematoxylin served as the nuclear counterstain.

Histopathological Examination

Paraffin-embedded tissue blocks were sectioned at a uniform thickness of 3.5 µm using a rotary microtome. The sections were mounted on positively charged slides and stained with hematoxylin and eosin (H&E) following standard protocols. Each stained slide was examined under a light microscope to confirm the histopathological diagnosis. Based on microscopic evaluation, cases were categorized as LSIL, HSIL, or SCC. Representative tissue sections containing well-preserved lesional areas were selected from each block for immunohistochemical analysis.

Immunohistochemistry (IHC) Procedure

Immunohistochemical staining was performed on two serial sections for each case—one for p16 and the other for SOX2 expression. The sections were deparaffinized in xylene, rehydrated through descending alcohol grades, and subjected to antigen retrieval using a pressure cooker for twenty minutes. Endogenous peroxidase activity was quenched by incubating the sections with hydrogen peroxide for ten minutes. After washing with phosphate-buffered saline (PBS), the slides were incubated with the primary antibodies (p16 or SOX2) for forty-five minutes at room temperature.

Following primary antibody incubation, the sections were treated with PolyExcel Target Binder and PolyExcel Poly-HRP secondary antibody, each for ten minutes. The antigen–antibody reaction was visualized using the DAB chromogen for five minutes, resulting in brown-colored deposits at antigenic sites. The slides were counterstained with Harris hematoxylin for nuclear contrast, dehydrated, cleared, and mounted with a permanent medium.

Each IHC batch included positive controls—glial tissue for SOX2 and squamous cell carcinoma for p16—to ensure the validity of staining. Normal cervical epithelium present in each section served as an internal control. The stained slides were observed under a light microscope, and both the distribution and intensity of staining were analyzed to determine marker expression.

Evaluation of Immunohistochemical Expression

The immunohistochemical expression of SOX2 and p16 was evaluated semi-quantitatively, taking into account both the staining intensity and the percentage of positively stained cells.

For **SOX2**, only nuclear staining was considered positive. The staining intensity was graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The percentage of positive cells was scored as 0 (<10%), 1 (10–25%), 2 (26–50%), 3 (51–75%), and 4 (>75%). The final histological score (H-score) was calculated by multiplying the intensity and percentage scores. Based on the H-score, the expression was classified as 0 (negative), 1–4 (weakly positive), 5–8 (positive), and 9–12 (strongly positive). For analytical purposes, negative and weakly positive scores (0–4) were grouped as negative expression, while positive and strongly positive scores (5–12) were grouped as positive expression.

For **p16**, both nuclear and cytoplasmic staining patterns were assessed. The staining intensity was graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The percentage of positive cells was scored as 1 (<1%), 2 (1–10%), 3 (11–33%), 4 (34–66%), and 5 (>66%). The histological score was derived by summing the intensity and percentage scores. Expression was classified as low (0–2), moderate (3–5), or overexpression (6–8). This semi-quantitative approach facilitated the comparison of expression profiles across the histopathological spectrum.

Quality Control

All staining procedures were conducted under standardized laboratory conditions at room temperature. Each batch of slides included known positive and negative controls to ensure procedural accuracy. Two independent pathologists, blinded to clinical data, evaluated the slides to minimize

observer bias. Discrepancies in scoring were resolved through consensus review.

Ethical Considerations

The study was approved by the Institutional Ethics Committee of Sree Balaji Medical College and Hospital, Chennai (IEC Reference No: SBMC/IHEC/2023/PA-014). All procedures were conducted in accordance with the ethical standards of the institutional and national research committees and the 1964 Declaration of Helsinki and its later amendments. As the study utilized archived formalin-fixed, paraffin-embedded tissue specimens with anonymized patient data, the requirement for individual informed consent was waived by the Ethics Committee. Confidentiality of patient information was strictly maintained throughout the study.

Statistical Analysis

All collected data were entered into Microsoft Excel and subsequently analyzed using the Statistical Package for the Social Sciences (SPSS) software version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize categorical variables in terms of frequencies and percentages, and continuous variables were expressed as mean ± standard deviation (SD). The Chi-square test (χ^2) or Fisher's exact test, as appropriate, was employed to evaluate the association between p16 and SOX2 expression and the histopathological categories (LSIL, HSIL, and SCC). Correlation analysis was also performed to assess the relationship between p16 and SOX2 expression. A p-value of <0.05 was considered statistically significant.

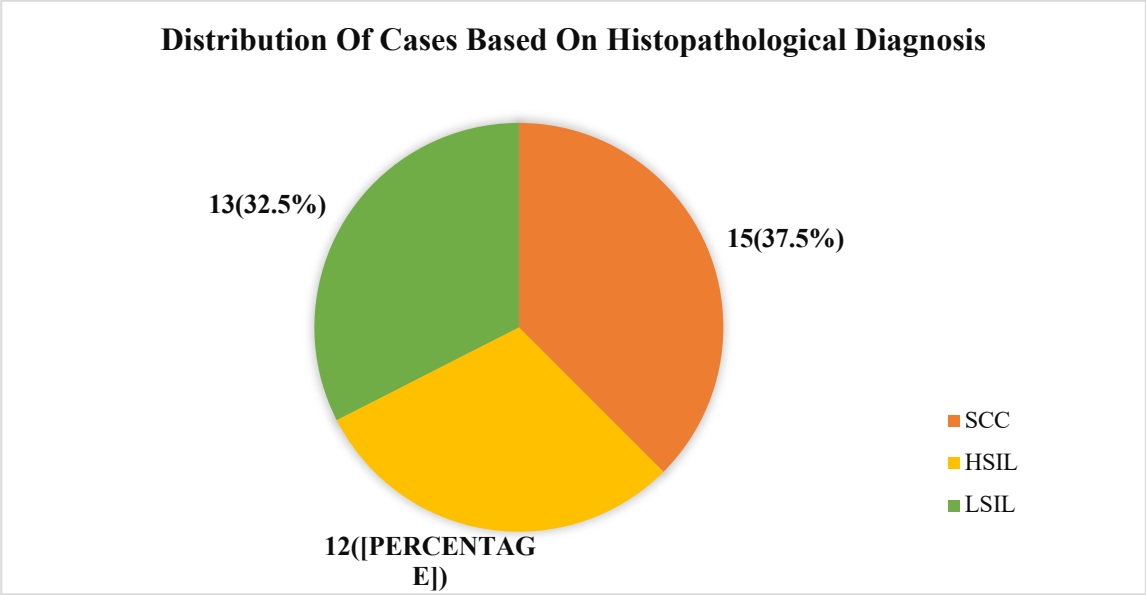
Results:

Among the 40 study participants, the majority belonged to the 41–50 years age group (12 cases, 30%), followed by 51–60 years (11 cases, 27.5%) and 61–70 years (10 cases, 25%) with mean age of 55.13+ 16.4 years (. A smaller proportion were aged 31–40 years (4 cases, 10%), 71–80 years (2 cases, 5%), and above 80 years (1 case, 2.5%). Most of the women were postmenopausal (23 cases, 57.5%), while 17 (42.5%) were premenopausal. Regarding specimen type, cervical biopsy specimens constituted the majority (27 cases, 67.5%), whereas hysterectomy specimens accounted for 13 cases (32.5%). (Table 1)

Table 1: Distribution of Study Participants According to Age, Menopausal Status, and Type of Specimen (n = 40)

Variable	n	%
Age Group (years)		
31–40	4	10.0
41–50	12	30.0
51–60	11	27.5
61–70	10	25.0
71–80	2	5.0
>80	1	2.5
Menopausal Status		
Premenopausal	17	42.5
Postmenopausal	23	57.5
Type of Specimen		
Hysterectomy	13	32.5
Cervical Biopsy	27	67.5
Total (all variables)	40	100.0

Out of the 40 cases analyzed, squamous cell carcinoma (SCC) constituted the largest diagnostic category, comprising 15 cases (37.5%). High-grade squamous intraepithelial lesions (HSIL) accounted for 12 cases (30.0%), while low-grade squamous intraepithelial lesions (LSIL) represented 13 cases (32.5%). Thus, approximately two-thirds of the study population presented with preneoplastic lesions (LSIL and HSIL), whereas one-third demonstrated invasive carcinoma, reflecting a balanced representation across the cervical neoplastic spectrum suitable for comparative immunohistochemical analysis. (Figure 1)



A
mong the 40 cervical tissue samples analyzed, SOX2 immunohistochemical expression showed variable intensity across the spectrum of preneoplastic and neoplastic lesions. Sixteen cases (40%) were completely negative for SOX2 expression, while 6 cases (15%) exhibited weak positivity. Moderate to strong expression was observed in 18 cases (45%), of which 5 (12.5%) were positive and 13 (32.5%) strongly positive, indicating upregulation of SOX2 in more advanced lesions.

For p16, overexpression was predominant, seen in 27 cases (67.5%), followed by low expression in 10 cases (25%) and moderate expression in 3 cases (7.5%). The predominance of p16 overexpression correlates with the presence of high-grade lesions and invasive carcinomas, consistent with its role as a surrogate marker of high-risk HPV-mediated oncogenic transformation. (Table 2)

Table 2: Distribution of SOX2 and p16 Expression in Cervical Lesions (n = 40)

Expression of SoX2		Frequency	Percentage
Negative	Negative (0)	16	40
	Weakly Positive (1-4)	6	15
Positive	Positive (5 - 8)	5	12.5
	Strongly Positive (9-12)	13	32.5
Expression of p16			
Low Expression		10	25
Moderate Expression		3	7.5
Over Expression		27	67.5

A statistically significant association was observed between both **p16** and **SOX2** expression patterns and the histopathological spectrum of cervical lesions ($p < 0.001$ for both). For p16, overexpression was seen in the vast majority of squamous cell carcinoma (SCC) cases (14/15, 93.3%) and high-grade squamous intraepithelial lesions (HSIL) (11/12, 91.7%), whereas low or negative expression predominated in low-grade lesions (LSIL) (9/13, 69.2%). Conversely, SOX2 expression demonstrated a progressive increase from LSIL to invasive carcinoma. While 14 of 13 LSIL cases (approximately 93%) were negative for SOX2, strong positivity was evident in 9 SCC (60%) and 10 HSIL (83.3%) cases. These findings highlight the stepwise upregulation of both p16 and SOX2 with advancing dysplasia, reinforcing their potential roles as molecular markers of cervical carcinogenesis and their utility in differentiating preneoplastic from malignant lesions. (Table 3)

Table 3: Association of p16 and SOX2 Expression with Histopathological Diagnosis in Cervical Lesions (n = 40)

Expression Type	Expression Category	SCC (n)	HSIL (n)	LSIL (n)	Total (n)	Chi-square (χ^2)	p-value
p16	Low/Negative	0	1	9	10	28.15	<0.001
	Moderate/Weak +	0	0	3	3		
	Over/Positive	14	11	2	27		
SOX2	Negative	5	2	14	21	20.43	<0.001
	Positive	9	10	0	19		

A statistically significant association was observed between **p16** and **SOX2** expression in cervical lesions ($\chi^2 = 15.60$, $p < 0.001$). Among cases with positive SOX2 expression, the majority (20/21, 95.2%) demonstrated p16 overexpression, whereas only 1 case showed low p16 expression. In contrast, cases with negative SOX2 expression primarily exhibited low or moderate p16 expression (12/19, 63.2%), and 7 cases showed overexpression. These results indicate a strong concordance

between p16 and SOX2 upregulation, particularly in higher-grade lesions and invasive carcinoma, supporting their combined diagnostic and prognostic relevance in cervical carcinogenesis. (Table 4)

Table 4: **Association Between p16 and SOX2 Expression among the study population (n=40)**

p16 and SOX2 Expression		p16 Expression			Chi Square	p-value
		Low Expression	Moderate Expression	Over Expression		
SOX2 Expression	Positive	1	0	20	15.60	<0.001
	Negative	9	3	7		

The sensitivity of SOX2 and p16 expression was assessed across different histopathological categories, with corresponding 95% confidence intervals (CI). In low-grade squamous intraepithelial lesions (LSIL), SOX2 showed 0.00% sensitivity (95% CI: 0.00% to 21.53%), while p16 demonstrated 14.29% sensitivity (95% CI: 4.01% to 39.94%). In contrast, high-grade squamous intraepithelial lesions (HSIL) exhibited a high sensitivity for both markers — SOX2 at 83.33% (95% CI: 55.20% to 95.30%) and p16 at 91.67% (95% CI: 64.61% to 98.51%). For squamous cell carcinoma (SCC), SOX2 sensitivity was 64.29% (95% CI: 38.76% to 83.66%), whereas p16 expression showed 100% sensitivity (95% CI: 78.47% to 100%). (Table 5)

Table No 5: Sensitivity of SOX2 and p16 expression across Histopathological Diagnosis.

HISTOPATHOLOGY	**SOX2 EXPRESSION**		**P16 EXPRESSION**	
	Sensitivity	95% CI	Sensitivity	95% CI
LSIL	0.00%	0.00 % to 21.53 %	14.29%	4.01 % to 39.94 %
HSIL	83.33%	55.20 % to 95.30 %	91.67%	64.61 % to 98.51 %
SCC	64.29%	38.76 % to 83.66 %	100%	78.47 % to 100 %

Figure 2 illustrates the histomorphological and immunohistochemical features of a low-grade squamous intraepithelial lesion (LSIL). The H&E section shows squamous epithelium with koilocytosis characterized by hyperchromatic nuclei, irregular nuclear membranes, and prominent perinuclear halos in the superficial layers. Corresponding immunohistochemistry demonstrates low p16 expression and negative SOX2 expression, supporting the diagnosis of LSIL and indicating limited disruption of cell cycle regulation and stemness-associated pathways at this stage.

Fig.No.2: p16 and SOX2 expression in Low Grade Squamous Intraepithelial Lesion (LSIL); A) H&E with squamous epithelium showing koilocytes in the superficial layer with hyperchromatic nuclei, irregular nuclear membrane and perinuclear halo, 40x; B) p16 IHC showing low expression, 40x; C) SOX2 IHC showing negative expression, 40x (H312/24)

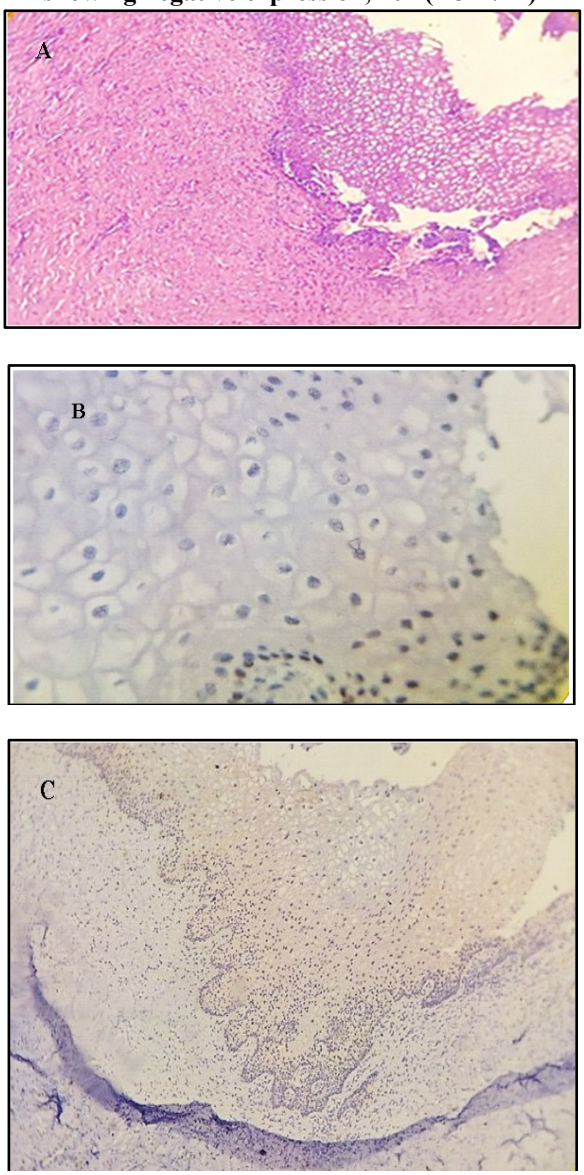


Figure 3 depicts a high-grade squamous intraepithelial lesion (HSIL) with characteristic histological and immunohistochemical findings. The H&E section reveals full-thickness epithelial dysplasia with involvement of

endocervical glands. Immunohistochemistry shows strong overexpression of p16 and robust nuclear positivity for SOX2, reflecting high-risk HPV-associated oncogenic transformation and increased cellular proliferation and stemness, consistent with a high-grade preinvasive lesion.

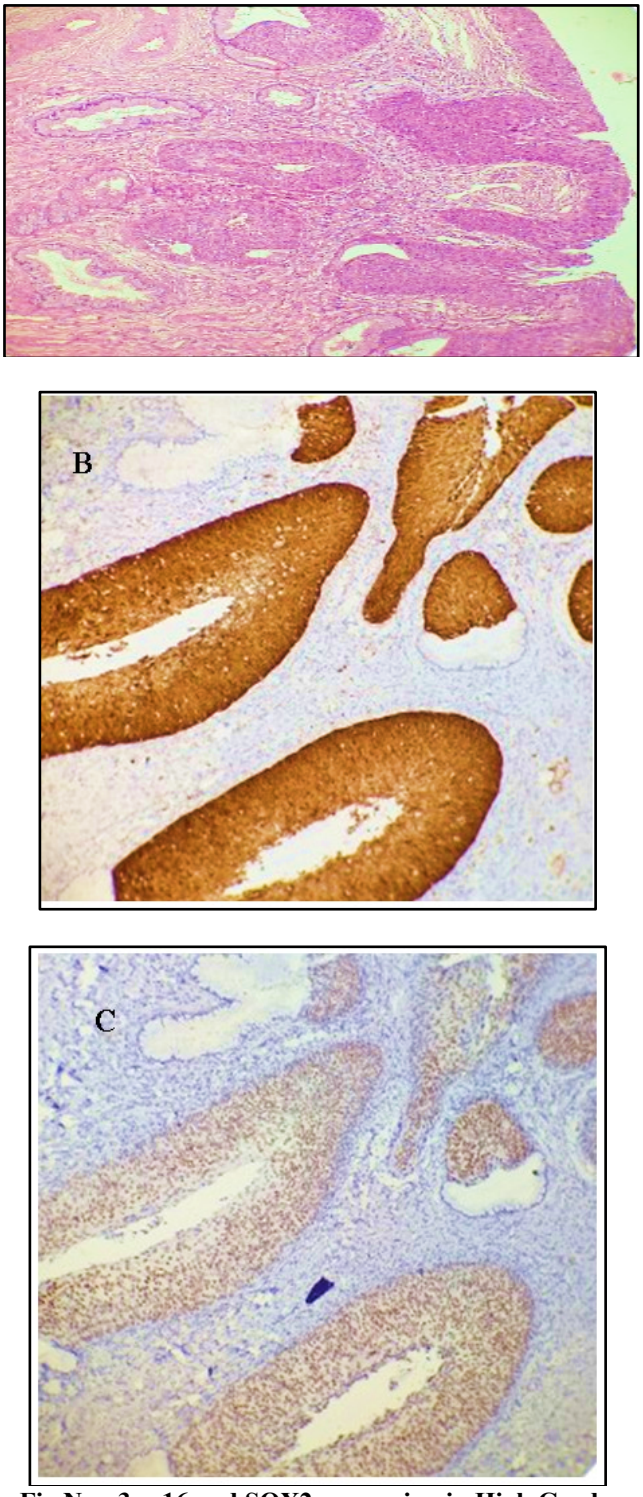


Fig.No.: 3: p16 and SOX2 expression in High Grade Squamous Intraepithelial Lesion (HSIL); A) H&E showing full thickness dysplasia also involving endocervical glands, 10x; B) p16 IHC showing over expression, 40x; c) SOX2 IHC showing strong positivity, 40x (H862/24)

Figure 4 demonstrates the features of squamous cell carcinoma of the cervix on biopsy. Histopathology shows nests and sheets of malignant squamous cells exhibiting marked nuclear pleomorphism. Immunohistochemical staining reveals diffuse p16 overexpression and strong SOX2 positivity, indicating high-risk HPV-driven malignancy and activation of stem cell–related transcription pathways, which are associated with invasive behavior and tumor progression.

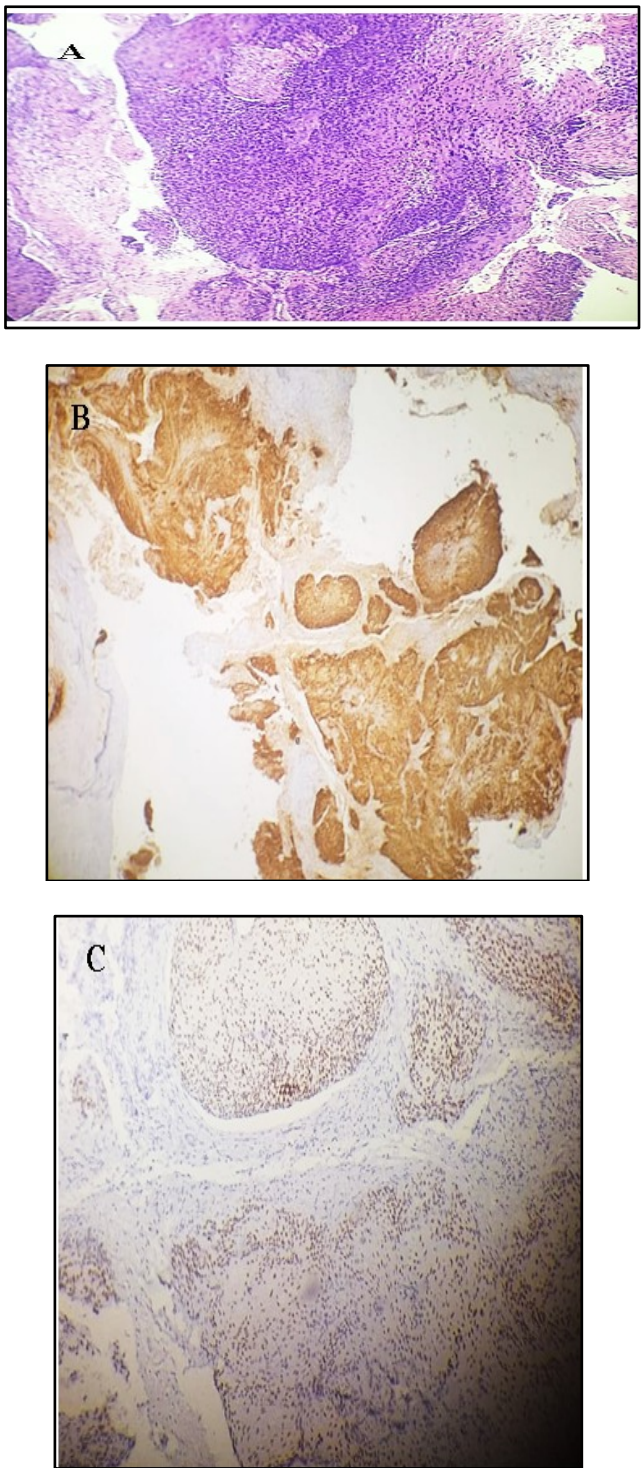
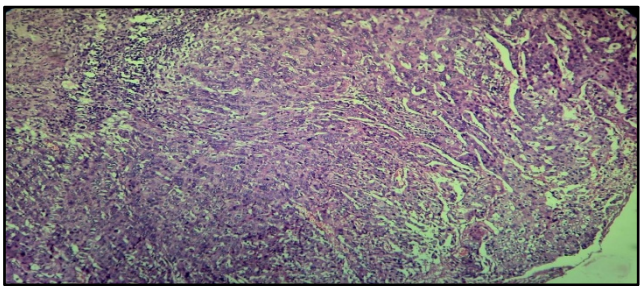
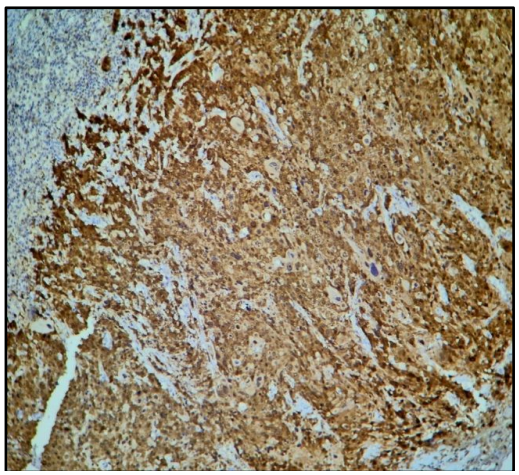


Fig. No.4: p16 and SOX2 expression in Squamous Cell Carcinoma of the Cervix (SCC) (cervical biopsy specimen). (A) H&E showing nests and sheets of malignant squamous cells with nuclear pleomorphism, 40x; (B) p16 IHC showing over expression, 10x; (c) SOX2 IHC showing strong positivity, 40x (H1222/24)

Figure 5 presents squamous cell carcinoma of the cervix involving the endometrial cavity in a hysterectomy specimen. The H&E section shows malignant squamous cells arranged in solid sheets with pronounced pleomorphism, hyperchromatic nuclei, prominent nucleoli, and an increased nuclear-to-cytoplasmic ratio. Immunohistochemistry demonstrates strong and diffuse p16 overexpression along with intense SOX2 positivity, reflecting advanced disease with extensive spread and reinforcing the role of these markers in identifying high-grade, invasive cervical carcinoma.





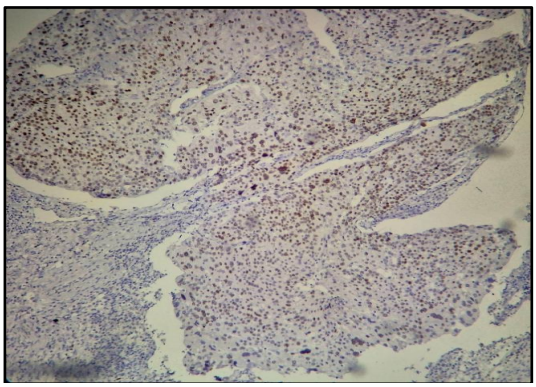


Fig. No.5: p16 and SOX2 expression in Squamous Cell Carcinoma of the Cervix involving the endometrial cavity (Hysterectomy specimen). (A) H&E showing malignant squamous cells arranged in solid sheets exhibiting pleomorphism, hyperchromatic nuclei with prominent nucleoli and an increased nuclear to cytoplasmic ratio, 40x; (B) p16 IHC showing over expression, 40x; (c) SOX2 IHC showing strong positivity, 40x (H1490/24)

DISCUSSION:

Cervical cancer remains one of the leading gynaecologic malignancies worldwide and is particularly burdensome in developing countries like India, where delayed diagnosis and limited screening contribute to high mortality. Persistent infection with high-risk human papillomavirus (HR-HPV) plays a central role in carcinogenesis, targeting basal cells of the transformation zone and driving oncogenic transformation through E6 and E7 oncoproteins. These viral proteins inactivate tumor suppressor genes such as p53 and Rb, resulting in deregulated cell cycling and neoplastic progression. Biomarkers such as p16 (a surrogate marker for HR-HPV integration) and SOX2 (a transcription factor related to cancer stem cell renewal) are increasingly recognized as adjunct diagnostic and prognostic tools in cervical lesions. The present study examined their expression across low-grade (LSIL), high-grade (HSIL), and invasive squamous cell carcinoma (SCC) lesions to assess diagnostic relevance.

The mean age of patients in this study was 55.13 years, with most cases occurring between 41–50 years (30%), consistent with observations by Jadhav A et al. [17]. This reflects the long latency between HPV infection and malignant transformation. These age trends indicate that middle-aged women form the predominant at-risk population. LSIL and HSIL were more frequent among premenopausal women, while invasive carcinoma predominated in postmenopausal women, findings comparable to those of Choi MS et al [18]. The hormonal milieu and cervical epithelial atrophy in postmenopausal women may facilitate deeper stromal invasion once dysplasia develops.

In this study, 67.5% of cases were diagnosed on cervical biopsies, while 32.5% were hysterectomy specimens, underscoring the increasing preference for minimally invasive diagnostic approaches. In contrast, Nayak K et al. and Upadhyay et al. reported higher proportions of hysterectomy specimens (60–80%) [19]. This difference likely reflects improved screening uptake and early detection through outpatient colposcopy-directed biopsy at our institution, whereas referral of advanced cases to higher centres limited the number of hysterectomies.

Among the 40 evaluable cases, SCC constituted 37.5%, HSIL 30%, and LSIL 32.5%. The predominance of SCC indicates that many women still present at advanced stages. In comparison, Pandya M et al. found lower SCC frequencies (1.8% and 0.2%, respectively) with LSIL predominance [20]. The disparity likely reflects differences in screening coverage and healthcare-seeking behavior. Strengthening community-based screening through Pap

smear and HPV DNA testing could enable earlier detection and reduce progression to invasive cancer.

In our cohort, 67.5% of all lesions demonstrated p16 overexpression, with 100% of SCC and 91.7% of HSIL cases showing strong, diffuse staining. LSIL exhibited only low to moderate expression in 64.3% of cases. The strong correlation between p16 expression and lesion grade (χ^2 = 28.15, $p < 0.001$) mirrors previous report by Sarma U et al., who documented progressive p16 up-regulation from SIL 1 to SIL 3 [21]. Gupta R et al. similarly noted that high p16 expression reliably distinguishes transforming HPV-related lesions from transient infections [22]. The limited p16 expression in LSIL supports the concept that many low-grade lesions represent transient HPV infections rather than true precancer.

These findings reinforce recommendations of the Lower Anogenital Squamous Terminology (LAST) project, which advises p16 immunostaining in morphologically equivocal cases or when histologic–cytologic correlation is discordant. Routine p16 testing is unnecessary in clearly benign biopsies because focal staining may occur in reactive epithelia. When interpreted with morphology, "block-positive" p16 is a robust indicator of high-grade disease and HPV-driven transformation.

SOX2 was positive in 32.5% of cases, absent in all LSIL, but detected in 83.3% of HSIL and 64.3% of SCC. The statistically significant association ($p = 0.0000367$) demonstrates progressive up-regulation with lesion severity. These results align with Dayalan S et al., who reported strong SOX2 expression in HSIL and SCC but minimal expression in LSIL [23], and with Yang Z et al., who found SOX2 positivity in 74.5% of cervical carcinomas [24]. Up-regulation of SOX2 promotes self-renewal, epithelial–mesenchymal transition, and treatment resistance, linking it to cancer stem-cell phenotypes. Huo T et al. reported that high SOX2 expression correlated with recurrence (90.9% vs 58.0%, $p = 0.003$) and poorer survival [25]. Similarly, Kim BW et al. found high SOX2 expression in 77.6% of cervical cancers [26]. Our results corroborate these trends, suggesting that SOX2 may serve not only as a diagnostic adjunct but also as a potential prognostic biomarker identifying lesions with greater invasive potential.

A significant correlation was observed between p16 and SOX2 expression ($\chi^2 = 15.60$, $p = 0.00041$). Of 21 SOX2-positive cases, 20 also demonstrated p16 overexpression, whereas SOX2-negative cases showed variable or low p16 levels. Similar findings were reported by Wolsky RJ et al., who demonstrated co-expression of SOX2 and p16 in HSIL/SCC but limited basal staining in LSIL [27]. This relationship suggests that SOX2 up-regulation occurs predominantly in HPV-driven lesions with established oncogenic integration (p16-positive), further enhancing proliferative and invasive capacity.

From a diagnostic perspective, dual evaluation of p16 and SOX2 increases specificity for high-grade lesions. While p16 alone is highly sensitive for detecting HSIL/SCC, concurrent SOX2 positivity identifies lesions with a stem-cell–like phenotype and greater malignant potential. Therefore, combined p16–SOX2 immunoprofiling may assist in refining grading, distinguishing equivocal HSIL from reactive atypia, and predicting progression risk.

Within preneoplastic lesions, LSIL exhibited uniformly negative SOX2 staining and low p16 expression, consistent with transient HPV infection without viral integration. HSIL, in contrast, demonstrated strong p16 overexpression (91.7%) and SOX2 positivity (83.3%), indicating integration-associated transformation. These patterns parallel molecular evidence that disruption of the Rb pathway by HPV E7 increases p16 accumulation, while activation of stemness pathways (SOX2, NANOG, OCT4) drives progression to carcinoma. Consequently, dual negativity for both markers may indicate reactive or regressive lesions, whereas dual positivity signifies transformation.

The complementary expression patterns of p16 and SOX2 strengthen the diagnostic continuum from early HPV infection to invasive carcinoma. p16 is an early biomarker of viral integration, while SOX2 marks downstream oncogenic activation. Incorporating both into diagnostic protocols enhances accuracy in differentiating LSIL from HSIL and identifying morphologically ambiguous lesions. Clinically, patients with strong dual positivity could benefit from closer surveillance or early intervention, while marker-negative LSIL could be managed conservatively.

Moreover, SOX2's link to recurrence and poor prognosis suggests potential for risk stratification and targeted therapy research. Its stem-cell–related properties may help explain resistance to conventional therapy, highlighting the importance of incorporating SOX2 into prognostic models for cervical cancer.

Few limitations must be acknowledged. This was a single-centre study with a modest sample size, limiting generalizability across diverse populations. The small number of confirmed high-grade and invasive cases reduces precision for sensitivity and predictive value estimates. Although all immunostaining was performed by a single experienced pathologist to maintain consistency, ultrasound and histopathologic grading remain operator dependent. The study also reflects a tertiary referral bias, potentially enriching for more severe cases. Furthermore, potential confounders such as prior uterine surgery, comorbidities, and variations in sampling technique or fixation time were not fully controlled. These factors may have influenced marker expression intensity. Finally, advanced molecular confirmation (e.g., HPV genotyping or viral load quantification) was not performed, limiting direct correlation between biomarker expression and viral integration status.

Despite these limitations, the study reinforces the diagnostic and prognostic value of p16 and SOX2 in cervical epithelial lesions. p16 remains a robust surrogate marker for HR-HPV-driven transformation, while SOX2 complements it by identifying lesions with stem-cell activation and higher malignant potential. Integration of these markers into routine diagnostic algorithms could improve lesion classification, reduce interobserver variability, and guide individualized management strategies. Larger multicentre studies incorporating molecular assays and longitudinal follow-up are

recommended to validate these findings and explore their role in predicting recurrence and treatment response. Ultimately, combined biomarker panels such as p16 + SOX2 may help bridge histopathology with molecular pathology, advancing precision diagnostics and improving outcomes for women with HPV-related cervical disease.

CONCLUSION:

This study demonstrates that both **p16** and **SOX2** are significantly upregulated in high-grade cervical intraepithelial lesions and invasive squamous cell carcinoma compared to low-grade lesions, reflecting their critical roles in cervical carcinogenesis. p16 overexpression reliably serves as a surrogate marker for high-risk HPV-mediated oncogenic activity, while SOX2 expression correlates with tumor progression and aggressiveness. The strong concordance between p16 and SOX2 expression suggests that their combined assessment can enhance diagnostic accuracy, particularly in differentiating preneoplastic from neoplastic lesions and identifying high-risk cases. These findings underscore the potential utility of p16 and SOX2 as complementary immunohistochemical biomarkers for early detection, risk stratification, and improved clinical management of cervical cancer.

REFERENCE

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2021: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249.
2. World Health Organization. WHO Global Strategy to Accelerate the Elimination of Cervical Cancer. Geneva: WHO Press; 2021.
3. Mathur P, Sathishkumar K, Chaturvedi M, Das P, Sudarshan KL, Santhappan S, et al. Cancer Statistics, 2020: Report From National Cancer Registry Programme, India. JCO Glob Oncol. 2020;6:1063-1075.
4. Schiffman M, Wentzensen N. Human Papillomavirus Infection and the Multistage Carcinogenesis of Cervical Cancer. Cancer Epidemiol Biomarkers Prev. 2023;32(1):11-19.
5. Romagosa C, Simonetti S, López-Vicente L, Mazo A, Lleonart ME, Castellvi J, et al. p16(Ink4a) overexpression in cancer: a tumor suppressor gene associated with senescence and high-grade tumors. Oncogene. 2021;40(23):3887-3900.
6. Mulvany NJ, Allen DG, Wilson SM. Diagnostic utility of p16INK4a: a reappraisal of its use in cervical biopsies. Pathology. 2022;44(6):511-516.
7. Dobbin ZC, Landen CN. Isolation and characterization of potential cancer stem cells from solid human tumors. Curr Protoc Pharmacol. 2023;84(1):e89.
8. Zhang S, Cui W. SOX2 in stem cell maintenance and neural differentiation. Vitam Horm. 2024;115:279-304.
9. Shen L, Huang X, Xie X, Su J, Yuan J, Chen X. High expression of SOX2 and OCT4 indicates radiation resistance and poor prognosis in cervical squamous cell carcinoma. J Histochem Cytochem. 2023;70(8):571-582.
10. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209-49.
11. Arbyn M, Weiderpass E, Bruni L, de Sanjosé S, Saraiya M, Ferlay J, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health. 2020 Feb;8(2):e191-203.
12. Bray F, Laversanne M, Weiderpass E, Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer. 2021 Aug;127(16):3029-30.
13. Lei J, Ploner A, Elfström KM, Wang J, Roth A, Fang F, et al. HPV Vaccination and the Risk of Invasive Cervical Cancer. N Engl J Med. 2020 Oct;383(14):1340-8.
14. National Cancer Registry Programme. Three-Year Report of Population Based Cancer Registries 2015-2017. New Delhi: Indian Council Medical Research; 2021.
15. Mathur P, Sathishkumar K, Chaturvedi M, Das P, Sudarshan KL, Santhappan S, et al. Cancer Statistics, 2020: Report From National Cancer Registry Programme, India. JCO Glob Oncol. 2020 Jul;6:1063-75.
16. Bobdey S, Sathwara J, Jain A, Balasubramaniam G. Burden of cervical cancer and role of screening in India. Indian J Med Paediatr Oncol. 2021 Oct-Dec;37(4):278-85.
17. Jadhav A, Sanklecha V, Natekar A, Mahra R. Histopathological Study of Spectrum of Lesions of Uterine Cervix. J Midlife Health. 2023 Apr-Jun;14(2):112-116. doi: 10.4103/jmh.jmh_28_23. Epub 2023 Sep 18. PMID: 38029029; PMCID: PMC10664052
18. Choi MS, Lee YJ, Lee EH, Ji YI, Park MJ. Factors Associated with Cyto-Histological Misinterpretation of Cervical Smear according to Menopausal Status. Journal of Menopausal Medicine. 2022;28(2):78.
19. Nayak K. Histomorphological Spectrum of Cervical Lesions: A Three Year Retrospective Study in Rural Area Bastar Region, Chhattisgarh, India. National Journal of Laboratory Medicine . 2020 Oct 1;Vol-9(4):PO05–9.
20. M Pandya, SR Parikh, HM Goswami, UR Parikh. ASC/SIL Ratio as a Quality Control Indicator for PAP Smear Cytology in a Tertiary Care Teaching Hospital. BJKines National Journal of Basic & Applied Sciences [Internet]. 2025 Jun 10 [cited 2025 Apr 28];17(01):4–8.
21. Sarma U, Biswas I, Das A, Chandra Das G , Saikia C, Sarma B. p16INK4a expression in cervical lesions correlates with histologic grading- a tertiary level medical facility based retrospective study. Asian Pac J Cancer Prev.

2017;18(10):2643-47.
22. Gupta R, Srinivasan R, Nijhawan R, Suri V, Uppal R. Protein p16INK4A expression in cervical intraepithelial neoplasia and invasive squamous cell carcinoma of uterine cervix. Indian J Pathol Microbiol. 2010;53(1):07-11.
23. Dayalan S. Comparative Evaluation of SOX2 and p16 Expression in Intraepithelial Neoplasia and Invasive Cancer of Cervix. Journal of Clinical and Diagnostic Research. 2021 Sep;15(9):EC38–34.
24. Yang Z, Pan X, Gao A, Zhu W. Expression of Sox2 in cervical squamous cell carcinoma. J BUON. 2014 Jan-Mar;19(1):203-6. PMID: 24659665.
25. Hou T, Zhang W, Tong C, Kazobinka G, Huang X, Huang Y, et al. Putative stem cell markers in cervical squamous cell carcinoma are correlated with poor clinical outcome. BMC Cancer. 2015;15:785. https://doi.org/10.1186/s12885-015-1826-4.
26. Kim BW, Cho H, Choi CH, Ylaya K, Chung JY, Kim JH, et al. Clinical significance of OCT4 and SOX2 protein expression in cervical cancer. BMC Cancer. 2015;15:1015. https://doi.org/10.1186/s12885-015-2015-1.
27. Wolsky RJ, Harbour LN, Mirza KM, Montag AG, Gwin K. The stem cell-associated transcription factor SOX2 as a diagnostic marker of high grade squamous intraepithelial lesion of the uterine cervix in comparison with p16 and Ki-67. Appl Immunohistochem Mol Morphol. 2018;26(6):403-10