Acta Scientific CANCER BIOLOGY (ISSN: 2582-4473)

Volume 8 Issue 1 January 2024

Research Article

Study of p16INK4a Immunostaining as Specific Biomarker in the diagnosis of Cervical Intraepithelial Neoplasia and Invasive Cancer: It is High Time to Prevent Cervical Cancer than to Cure

Sharada Patil[1], Kusuma Venkatesh[2]*, CP Manjula[3] and T Jayanthy[4]

[1]Former Postgraduate and Tutor at Kempe Gowda Institute of Medical Sciences, Bengaluru, Karnataka, India

[2]Former Professor and Head, Department of Pathology, Kempegowda Institute of Medical Sciences Hospital and Research Centre, Bengaluru, India

[3]Professor, Department of Pathology, Kempegowda Institute of Medical Sciences Hospital and Research Centre, Bengaluru, India

[4]Professor, Department of Obstetrics and Gynecology, Kempegowda Institute of Medical Sciences and Research Centre, Bengaluru, India

***Corresponding Author:** Kusuma Venkatesh, Former Professor and Head, Department of Pathology, Kempegowda Institute of Medical Sciences Hospital and Research Centre, Bengaluru, India.

DOI: 10.31080/ASCB.2024.08.0462

Received: October 25, 2023

Published: December 08, 2023

Abstract

Introduction: Cancer of the uterine cervix is the fourth most common cancer among women worldwide, with a higher burden in the low- and middle-income countries. Human Papilloma Virus (HPV) is the foremost aetiological factor which integrates into the genome of the epithelial cells at the cervical transformation zone leading to neoplasia.

Aim: This study was undertaken to demonstrate overexpression of p16INK4a as a biomarker for HPV infection in cervical biopsies; to know its frequency of occurrence in cases of cervicitis, low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL) and invasive cancer; to establish marker's specificity and the ability to differentiate between cervical dysplasia from benign morphological mimickers.

Materials and Methods: Totally 75 cervical biopsies were studied between 2014 to 2016, and categorized as inflammatory, precancerous and cancerous lesions. Routine histopathological examination was combined with Immunohistochemistry (IHC) using p16INK4a antibody. Grading of immuno-positivity was made depending on the number of cells exhibiting strong nuclear and/or cytoplasmic positivity.

Results: Age of the patients ranged between 24 to 80years and the commonest clinical complaint was vaginal discharge. Of the 75 biopsies studied, 32% were diagnosed as cervicitis, 12% as LSIL and 16% as HSIL. Totally 30(40%) cases of invasive cancers were studied which included 26 cases procured from a cancer hospital to conduct a pilot study. p16INK4a was positive in all invasive cancers (100%) and negative in all cases of cervicitis. Statistically significant results were noted between histopathological diagnoses and the grades of immunopositivity with p16INK4a.

Conclusion: This study reiterates the usefulness of p16INK4a as a reliable biomarker for HPV induced cervical lesions and unnecessary follow-up can be avoided in immune-negative cases. The fact that all cervical cancers were positive for HPV is alarming, signifying that developing nations have to give utmost importance to the primary prevention of cervical cancer with vaccination for HPV.

Keywords: Cervical Carcinoma; Human Papilloma Virus; Immunohistochemistry; p16INK4a Biomarker

Introduction

Cancer of the uterine cervix is the fourth most common cancer among women worldwide, with a global incidence of 13.3 per 100000 in 2020 [1]. The burden of cervical cancer is higher in low- and middle-income countries as 8 out of 10 cases are found in these countries due to deficiency of prevention and treatment [2,3]. In India, cervical cancer is the second most common cancer with 123,907 new cases in 2020 associated with 77348 deaths and the majority of them are squamous cell carcinoma followed by adenocarcinoma [4].

Human Papilloma Virus (HPV) is the main aetiological factor for carcinoma of cervix and the chances HPV infection increases with genetic predisposition, hormonal influence, decreased immunity and cigarette smoking. The high-risk HPV (hrHPV) integrates into the genome of the epithelial cells at the cervical transformation zone [5], causing inactivation of the tumour suppressor genes p53 and Rb and leads to cell proliferation and mutations [6]. As the persistent HPV infection causes cervical carcinoma, so early detection of pre-cancerous lesions such as low grade and high grade squamous intraepithelial lesions, or adenocarcinoma in situ (ACIS) is essential [7]. Hence prevention of cervical cancer relies on HPV vaccination (primary prevention) and detecting precancerous lesions before they progress to invasive cancer (secondary prevention).

The imunohistochemical (IHC) expression of hrHPV biomarker p16INK4a has been used as a marker for cervical dysplasia. Screening by cervical cytology aids in early diagnosis and intervention thereby reducing the mortality rate of cervical cancer [8]. The objectives of this study were to know: 1) the frequency of p16INK4a expression in cervical biopsies diagnosed as cervicitis, low and high grade squamous epithelial lesions and cancer cervix in the study group, 2) the specificity of p16INK4a as a biomarker for cervical Intraepithelial neoplasia (CIN), 3) the ability of this marker to differentiate dysplasia from benign mimics such as immature squamous metaplasia, atrophy and reparative epithelial changes and 4) to categorize cervical intraepithelial lesions using p16INK4a expression.

Materials and Methods

This cross-sectional and observational study was undertaken for a period of two years between 2014 to 2016, in the department of Pathology of a tertiary health care centre. The clinical details of the patients were procured from the case files and from the treating clinicians. Totally 75 cervical biopsies were selected and analysed.

- **Inclusion criteria:** All the cervical biopsies selected for study were sent in 10% neutral buffered formalin and adequate for giving a diagnosis. All the necessary clinical data for each case was procured.
- **Exclusion criteria:** Cervical biopsies which were not adequate in quantity and those not fixed properly in formalin were excluded.

All the biopsy specimens were fixed in formalin & then subjected to routine tissue processing and paraffin embedding. From each block a minimum of two sections were cut, one section of 4-5µ thick was stained with Haematoxylin and Eosin (H&E) stain. The second section of 3µ thickness was taken on Poly-L-Lysine coated slide and stained with p16INK4a antibody by following the standard method for IHC. A pilot study was done using 26 biopsies of cancer cervix, procured on request from a cancer centre were also included in this study. Therefore, the total number of cervical cancers in this study does not indicate the actual frequency or incidence.

The H&E sections were studied for features of inflammation, epithelial dysplasia, metaplasia and neoplasia. Those having inflammation without epithelial dysplasia were diagnosed as 1) Chronic cervicitis, those with dysplasia were classified as 1) Low-grade Squamous Intraepithelial Lesions (LSIL) or CIN I, 2) High-grade Squamous Intraepithelial Lesions (HSIL) (including CIN II with CIN III) and 3) invasive Carcinoma.

Irrespective of the routine histopathological diagnosis, all the selected cervical biopsies, were stained for p16INK4a using 60 Biogenex Lifesystems Histology Kit (Clone G175-405) in accordance with the manufacturer's instructions. Positive and negative controls were run with each batch.

Interpretation of p16INKa Staining: The sections showing either strong nuclear and/or cytoplasmic immunostaining were considered positive p16INK4a. Depending on the number of epithelial cells showing IHC positivity, grades were given according to an arbitrary scale: Grade 0 with no positive staining, Grade 1 in case of <10% cells showing positive staining, Grade 2 in which >10% but <50% cells show positive staining and Grade 3 when >50% cells are positive.

Statistical analysis

The statistical calculations were done using SPSS software for windows version 24. The p value of < 0.05 was considered statistically significant. The collected data was analysed using frequency distribution charts, descriptive statistics and contingency coefficient test.

Results

In the study period of two years 75 cervical biopsies were subjected to histopathological evaluation with H and E stain and IHC for P16INK4a. The youngest patient was 24 years old and the oldest was 80 years old with a mean age of 49.25 years. Most of them were in the age group of 41 to 50 years comprising 40% (30/75) followed by 51 to 60 years age group comprising 20% (15/75) [Table-1]. All the patients including the younger ones between 20-to-30-year age had not taken vaccine for HPV.

Table 1: Age distribution amongst patients of the study group.

Age group (years)	No. of cases
20-30	3
31-40	13
41-50	30
51-60	15
61-70	10
71-80	4
Total	75

These patients presented with varied clinical complaints, the commonest being foul smelling vaginal discharge in 51 cases, followed by white discharge in 49, vaginal bleeding in 34, weight loss in 15 and abdominal pain in 11 patients.

The number of cervical biopsies taken are lesser compared to the previous years, as in most of the cases biopsy is taken only when indicated by Pap smear results precancerous lesions or features suggestive of malignancy.

Histopathological examination of 75 biopsies stained with H&E, revealed the following diagnoses. 24 (32%) cases of chronic cervicitis, 9 (12%) cases of LSIL and 12 (16%) were HSIL.

Out of the 30 (40%) cases of invasive carcinoma of cervix 26 belonged to the biopsies used in the pilot study. Therefore, the number of cancers in this study appear to be more and do not represent the actual frequency or incidence of the disease [Table-2].

Table 2: Types of lesions of uterine cervix included in this study.

Cervical Lesions	Frequency	Percentage
Chronic Cervicitis	24	32%
LSIL (CIN I)	9	12%
HSIL (CIN II & III)	12	16%
Invasive Carcinoma*	30	40%
Total	75	100%

*26 out of 30 cases of cancers belonged to the pilot study for p16INK4a.

Among the 30 cases of invasive carcinomas majority of them were SCC (76.67%) followed by adenocarcinoma (16.67%), Adenosquamous Carcinoma (3.33%) and biopsies with features suspicious of Malignancy (3.33%) [Table 3].

Correlation of Histopathological diagnosis with IHC for p16INK4a grading: Out of 75 cases, 24 (32%) cases were chronic cervicitis on routine histopathology and all were negative for p16INK4a. Of the 9 (12%) cases of LSIL, grade 1 positive staining with p16INK4a was noted in one (11.11%) case, grade 2 staining in 3 cases (33.33%) and grade 3 staining in 3 cases (33.33%). Among the 12 (16%) cases of HSIL, 1 (8.33%) case showed grade 2 staining and 9 (75%) cases showed grade 3 staining. However, 2 cases each of LSIL and HSIL were negative p16INK4a.

Amongst the 30 cases of invasive carcinoma, 1 (3.33%) case showed grade1 positive staining with p16INK4a, 2 (6.67%) cases showed grade 2 and 27 (90%) cases showed grade 3 positivity.

Table 3: Types and frequency of invasive cervical carcinomas studied.

Lesions	Frequency	Percentage
Squamous cell carcinoma (SCC)	23	76.67%
Adenocarcinoma	5	16.67%
Adenosquamous carcinoma	1	3.33%
Suspicious of malignancy	1	3.33%
Total	30	100%

Analysis of the routine histopathological diagnosis with H&E stain and comparison with IHC for p16INK4a grade [Table 4], showed concordant results as the contingency co-efficient was 0.635 which was highly statistically significant (p = 0. 000). Chronic cervicitis was significantly associated with IHC grade 0, whereas HSIL and invasive carcinoma were significantly associated with IHC grade 3.

Histomorphology of the various types of cervical lesions on H & E in comparison with IHC are shown in the microphotographs [Figures – 1,2,3 and 4]. There was no epithelial abnormality noted in cases of cervicitis and chronic inflammatory cell infiltration of variable degree was present in the sub-epithelial stroma. The Squamocolumnar junction exhibited features of dysplasia in one third of the epithelium in LSIL which corresponds to CIN I. Dysplasia involving more than one third of the epithelium was noted in HSIL (includes CIN II and III). The dysplastic epithelial cells exhibited loss of polarity with enlarged, hyperchromatic nuclei and increased number of abnormal mitotic figures.

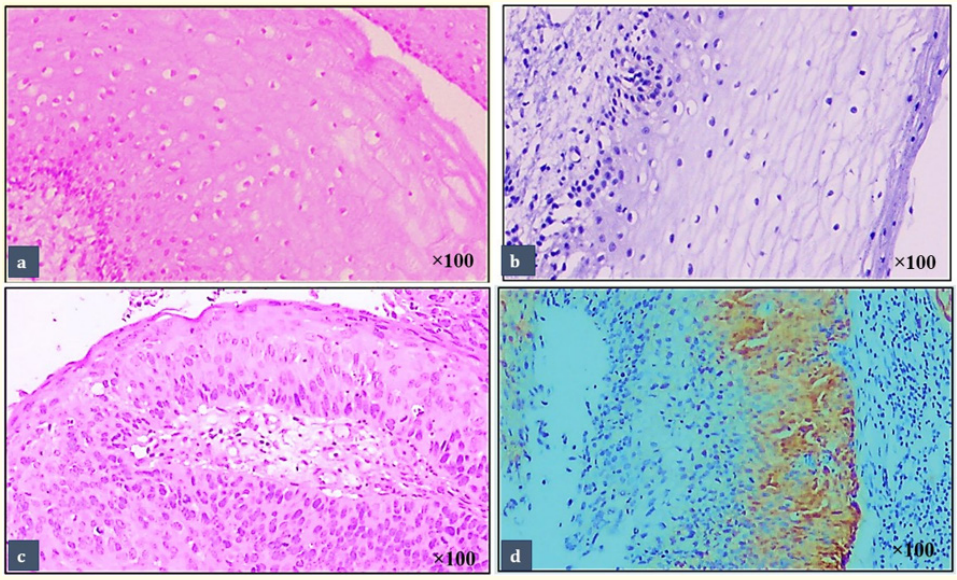

Figure 1: (a) Chronic cervicitis (H&E, ×100); (b) Squamous epithelium is negative for p16INK4a in chronic cervicitis (×100); (c) LSIL exhibiting dysplasia in the lower 3rd of epithelium (H & E, ×100); (d) LSIL exhibiting positivity for p16INK4a in lower 3rd of epithelium (×100).

Table 4: Correlation of Histopathological diagnosis with IHC for p16INK4a grade.

H & E diagnosis	IHC for p16INK4a grading*				Total
	Grade 0	Grade 1	Grade 2	Grade 3	
Chronic cervicitis	24 (100%)	0	0	0	24
LSIL	2 (22.22%)	1 (11.11%)	3 (33.33%)	3 (33.33%)	09
HSIL	2 (16.67%)	0	1 (8.33%)	9 (75.00%)	12
Invasive carcinoma	0	1 (3.33%)	2 (6.67%)	27 (90.00%)	30
Total	28	2	6	39	75

*Statistically significant.

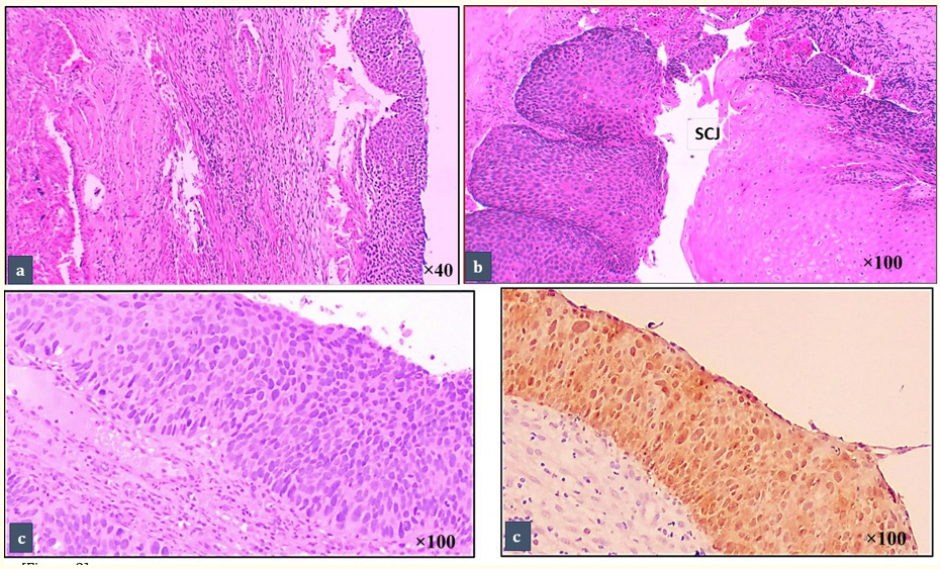

Figure 2: (a) High Grade Squamous Intraepithelial Lesion (HSIL) with full thickness epithelial dysplasia (H&E, ×40); (b) HSIL at the Squamocolumnar junction (SCJ) with adjacent benign epithelium (H&E, ×100); (c) HSIL exhibiting nuclear pleomorphism and hyperchromatic nuclei (H&E, ×100); (d) Squamous epithelium of HSIL shows diffuse positivity for p16INK4a (×100).

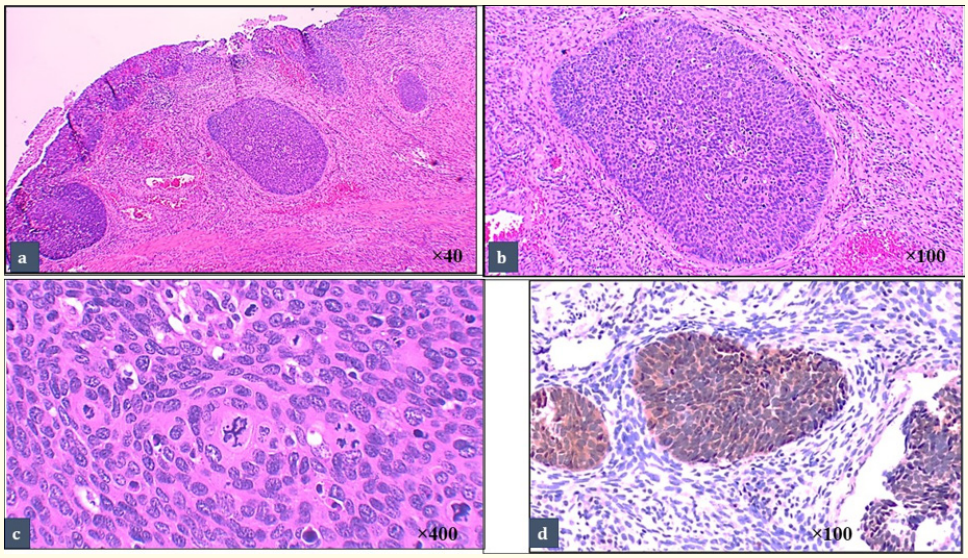

Figure 3: (a) Cervical carcinoma with infiltrating nests of neoplastic cells (H&E, ×40); (b) One island of malignant cells in the stroma (H&E, ×100); (c) Malignant cells exhibit many abnormal mitotic figures (H&E, ×400); (d) Nests of cancer tissue invading the subepithelial stroma are positive for p16INK4a (×100).

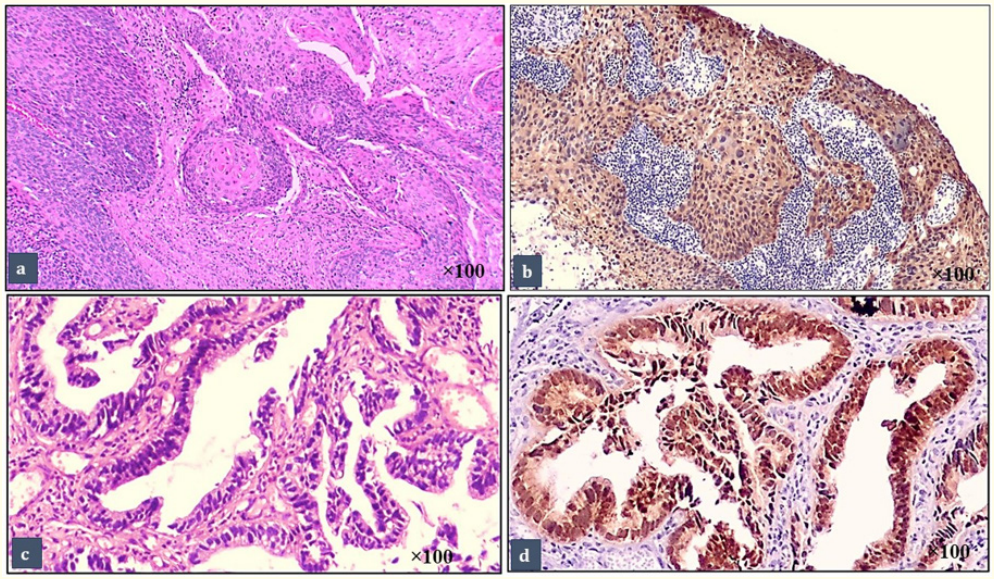

Figure 4: (a) Squamous cell carcinoma (SCC) with cells having eosinophilic cytoplasm, hyperchromatic nuclei and keratin pearls (H&E ×100); (b) SCC stained for p16INK4a shows diffuse and strong positivity (×100); (c) Adenocarcinoma showing glands lined by columnar cells having hyperchromatic nuclei (H&E ×100); (d) Adenocarcinoma with IHC for p16 INK4a shows strong positivity (×100).

Among the 30 cases of carcinomas, all 23 (76.66%) cases of SCC, 1 (3.33%) case of Adeno-squamous carcinoma, 1 (3.33%) case of suspicious for malignancy showed grade 3 immunostaining. Whereas, of the 5 (16.66%) cases of adenocarcinoma, 1(20%) case showed grade 1 positive staining, 2 (40%) cases each showed grade 2 and grade 3 positivity.

Discussion

Integration of hrHPV types 16 and18 in the cell genome is an essential step in the oncogenic pathway. In a similar study percentage of atypical squamous cells of undetermined significance (ASCUS) lesions progressing to LSIL was 9.6% and to HSIL was 1.6%. The progression of LSIL to HSIL was found in 9.0%. The observed progression of HSIL to invasive carcinoma is nearly constant with 21% developing carcinoma in 5 years, 28% in 10 years, 33% in 15 years and 38% in 20 years [9].

p16INK4a is a cellular protein and in HPV related cancers, its expression correlates with the increase in oncogenic E6/E7 mRNA. The continuous expression of E7 is necessary to maintain a malignant phenotype. The expression of p16INK4a seems to be independent of the HPV type causing the oncogenic change, obviating the need to detect different HPV types in DNA and RNA assays. In contrast to many classic tumour markers such as ki-67 or MYC, p16INK4a is not associated with cell proliferation, but associated with senescence and cell cycle arrest. p16INK4a is not expressed in normal basal cells of cervical epithelium or in other proliferative cells [10].

There is upregulation of p16INK4a in cases of CIN and cervical cancer. It is basically a nuclear protein hence IHC should show nuclear staining. However, in severe dysplasia and neoplasia both nuclear and cytoplasmic staining with p16INK4a is observed. This is possibly because of post transcriptional modification and overproduction of p16INK4a protein forcing its transfer into the cytoplasm [11].

Inflammatory lesions

The present study had 24 (32%) cases of chronic cervicitis and all were negative for p16INK4a immunostaining 0/24(0%) and results were similar with those found by of Klaes., *et al*. [8], Murphy., *et al*. [12], Redman., *et al*. [13], Srivatsava [11] and Han., *et al*. [14]. However, the study done by Kumari., *et al*. 4 of the 16 cases of chronic cervicitis showed p16INK4a immunopositivity. Review of these cases were made, which revealed H&E-stained sections of all these biopsies to be having either LSIL or HSIL. In cases of LSIL, the

histopathological findings were very subtle and were picked up by IHC [15]. Studies by Wang IL, Bolanca and Lee also reported immunopositivity for p16INK4a in cases of cervicitis [16-18] [Table 5].

Table 5: Comparison of the results of IHC for HPV in chronic cervicitis.

Results of the similar studies	p16INK4a immunopositivity in Chronic cervicitis
Klaes (2001)	0/48 (0%)
Murphy (2003)	0/21 (0%)
Liu Wang (2005)	1/8 (12.5%)
Bolanca (2007)	1/14 (7.1%)
Redman (2008)	0/110 (0%)
Srivatsava (2010)	0/15 (0%)
Han (2011)	0/21 (0%)
Lee (2012)	16/60 (26%)
Kumari (2013)	4/16 (25%)
Our study (2016)	0/24 (0%)

LSIL

In the present study, 9(12%) cases were diagnosed as LSIL, of these 2/9 (22%) were immuno-negative and 7/9 (78%) cases were immuno-positive for p16INK4a. These results are compared with that of Klaes., *et al*. [8] who observed 40/47 (85%) cases of LSIL and Ordi., *et al*. [19] found 56/86 (65.8%) cases to be immuno-positive. Hu et.al observed low positivity of 20/45 (44%) in LSIL and explained that in p16INK4a negative LSIL cases, HPV infection was transient and that viral DNA was not integrated into the host cells [20]. In the study by Nam *et al*, p16INK4a immunopositivity was very low (2/12 cases, 16.6%). The possible reason for this is certain percentage of LSIL are caused by low-risk HPV types and the affinity of the E7 protein of low-risk HPV is much lower than that of hrHPV types and there would not be overexpression of p16INK4a [21]. A study by Murphy., *et al*. yielded 38/38 (100%) rates of immunopositivity in LSIL [12].

HSIL

In the present study, 12/75 cases (16%) were diagnosed as HSIL, 2 of them (16.67%) were immuno-negative and 10 (83.33%) cases were positive for p16INK4a. Results are comparable with that of studies reported by Murphy., *et al*. 78/79 cases (98.7%) [12], Agoff., *et al*. 52/56 (92.8%) [22], Guimaraes., *et al*. 13/18 (86.6%) [23], Zhang., *et al*. 101/135 (74.8%), [24] Mood., *et al*. 10/11(90.9%) [25], Cheah., *et al*. 24/27(88.9%) [26] and Wei., *et al*. 26/36(72.2%) [27] of immunopositivity. One hundred percent positivity was noted in the study by Klaes., *et al*. [8] and Bolanca., *et al*. [17].

Guimaraes., *et al*. explain that not all hrHPV types possess the same potential for the cell cycle disruption or altered gene expression that leads to p16INK4a upregulation. Thus, these results highlight the possible potential of p16INK4a as a marker for type specific HPV-related HSIL and cervical cancer progression [23].

In the study by Zhang., *et al*. 74.8% cases of HSIL were p16INK4a immune-positive and it was stated that in all those classified as HSIL and p16INK4a negative cells had abundant eosinophilic cytoplasm, enlarged hyperchromatic nuclei with coarsely clumped chromatin, and showed frequent mitotic figures. In most of these, mitosis could be identified at approximately the midway point of the epithelium. In approximately one-third of the cases, mitosis was identified in the upper half of the epithelium. This histological appearance is reminiscent of reactive/reparative changes, but associated with greater nuclear atypia [24].

Invasive carcinoma

In the present study 30 of the 75 cases were diagnosed as invasive carcinomas and all of them showed (100%) p16INK4a immunopositivity. These results are consistent with that of Srivatsava (2010) [11] who observed 15/15 (100%), Kumari., *et al*. (2013) [15] whose study yielded 16/16 (100%) and Wei., *et al*. (2013) who showed 25/25 (100%) rates of immunopositivity [27]. On the contrary, Klaes., *et al*. (2001) showed 96.6% [8], Tan., *et al*. (2010) showed 98.6% [28], Cheah (2012)., *et al*. [26] showed 86.8% and Mood., *et al*. (2012) showed 90% rates of immunopositivity in SCC [25] [Table 6].

Srivatsava., *et al*. have reported 100% p16INK4a immunopositivity in cervical carcinoma and there was no detectable p16 expression in normal cervical epithelium. Therefore, p16 is a negative regulator of normal proliferation, works through negative feedback loop to down-regulate CDK4. This function is bypassed by HPV-7, causing p16 up-regulation and proliferation of cells. The detection of elevated levels of p16 is a clear indicator of abnormal proliferation [10].

There are studies showing that a proportion of the cervical cancer cases had neither HPV infection nor p16INK4a expression. The possible explanation for the absence of p16INK4a expression in these lesions could be methylation of the p16INK4a promoter resulting in silencing of the p16INK4a gene [27].

Table 6: Comparative results of p16INK4a immunopositivity in cervical carcinoma.

Authors reporting Results of p16INK4a immunopositivity	p16INK4a immunopositivity in Invasive Cervical Carcinoma
Klaes (2001)	58/60 (96.6%)
Srivatsava (2010)	15/15 (100%)
Tan (2010)	71/72 (98.6%)
Cheah (2012)	46/53 (86.8%)
Mood (2012)	18/20 (90%)
Kumari (2013)	16/16 (100%)
Wei (2013)	25/25 (100%)
Present study	30/30 (100%)

Comparison of grading system: The present study adopted the immunohistochemical grading system which was used by Murphy., *et al*. [12]. The study by Murphy., *et al*. showed that absence of p16INK4a over expression in all 21(100%) cases of normal cervical tissue (grade- 0). In 38 cases of LSIL, 3 (7.8%) cases showed grade 1 staining, 11(29%) cases showed grade 2 staining and 24 (63%) cases showed grade 3 staining. 79 cases of HSIL were studied in which 1 (1.2%) case showed grade 0 staining, 12(15%) cases showed grade 1 staining, 23 (29%) showed grade 2 staining and 43 (54.4%) cases showed grade 3 staining. All 10 cases of Invasive carcinomas (100%) (8 cases of SCC and 2 cases of adenocarcinoma) showed grade 3 staining. Their study showed that in the cervical biopsies all normal epithelia, metaplastic, endocervical, reactive and inflammatory regions were not stained with the monoclonal anti-p16INK4a antibody. In addition, all normal regions adjacent to SIL did not show detectable p16INK4a expression. The p16INK4a immunopositivity progressively increased from LSIL to HSIL to invasive carcinomas. All cases of invasive carcinoma exhibited strong overexpression of the p16INK4a. Although a small number of LSIL cases showed exclusive nuclear staining, interestingly, the remaining LSIL, HSIL, and invasive cancer cases showed a combination of nuclear and cytoplasmic staining. The presence of p16INK4a in the cytoplasm may result from a type of post-transcriptional modification or, more simply, overproduction of the protein may force its transfer into the cytoplasm.

The present study establishes that p16INK4a over-expression was restricted to LSIL, HSIL and invasive carcinomas of cervix. No detectable p16INK4a over expression was observed in cases of chronic cervicitis. The rates of immunopositivity increased from LSIL to HSIL and associated with higher grade in carcinoma. These findings clearly support previous studies in confirming that p16INK4a is indeed over expressed in dysplastic and neoplastic cells of the uterine cervix.

Majority of lesions that progress into HSIL is HPV 16 positive. Progression into HSIL has not been found with low-risk HPV types 6 and 11. Interestingly a significant percentage of women with normal cervical epithelium but positive for HPV-DNA developed HSIL in 2 years without evolving through low grade lesions. Ten percent of cancers may arise without any detectable surface abnormality [9].

A study conducted in central India on 782 women which used cervical swabs for genomic DNA extraction and screening for HPV with MY09/11 and HPV-16 specific primers. An overall prevalence of 7.1% HPV infection was observed, and a significant incidence (95%) of HR-HPV 16 genotype was found. The prevalence decreased with age; young adults between 15 and 29 years (86.4%) followed by women aged between 30 and 54 years (13.2%) [29].

The first HPV vaccine was introduced into clinical practice in the year 2006 and all the vaccines available at present provide protection against hrHPV type 16 and 18, as approximately 70% of cervical cancers worldwide are attributable to these two types [30]. WHO has a target for 194 countries to adopt HPV vaccination by 2030 in its "Global Strategy to Accelerate the Elimination of Cervical Cancer as a Public Health Problem" [8]. By 2020, however, only 114 countries most of which were high income countries had introduced HPV and less than 25% of low-income countries have it in their national immunization programmes. Most of the countries of Africa and Asia are not having this immunization where the burden of cervical cancer is very high [31].

In this study all the patients, including the three who were under the age of 30 years, none of them were immunized with vaccine for HPV. As there is marked lack of awareness amongst the people about the cause of cervical cancer and its prevention, increased number of health campaigns about HPV related diseases and about

their primary prevention with HPV vaccination are needed. These are essential to reduce the mortality due to cervical cancer which requires governments coming together with non-governmental organizations, communities for public health and healthcare systems taking care of patients. Development of less expensive vaccine, which is indigenous, preferably prescribing single dose is necessary and including it in the regular vaccination programmes is essential to eliminate cervical cancer.

Limitation

This study did not involve follow up of the cases studied and therefore progression of lower grade lesions to higher grade could not be assessed.

Conclusions

Cervical cancer is an overbearing disease leading to high morbidity and mortality among women. In the present study, p16INK4a overexpression was noted in majority of preneoplastic lesions and in all (100%) the cases of invasive carcinoma of cervix. p16INK4a is an easily available, robust, stable and strong predictive biomarker for the diagnosis of squamous intraepithelial lesions and invasive cervical cancer. There was significant correlation with IHC grade and the severity of cervical lesions. These findings reiterate that p16INK4a is a valuable, specific marker for HPV induced cervical dysplasia and neoplasia and it also decreases the variation in the results reported by different pathologists. When the biopsy is negative for p16INK4a unnecessary follow up can be avoided and more importantly follow up and proper management of the patient can be planned in immune-positive subjects. Cervical cancer has been reduced to a large extent due to the efficient screening programmes, however primary prevention with HPV vaccination should be taken seriously in all the developing nations and this is the need of the present time.

Acknowledgement

We are grateful to the help and support from the department of pathology of Sri Shankara Cancer Hospital and Research Centre, Bengaluru.

Bibliography

1. Sung H., *et al*. "Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries". *CA: A Cancer Journal for Clinicians* 71 (2021): 209-249.

2. Oberlin AM., *et al*. "Making HPV vaccination available to girls everywhere". *International Journal of Gynecology and Obstetrics* 143 (2018): 267-276.

3. Alfaro K., *et al*. "Removing global barriers to cervical cancer prevention and moving towards elimination". *Nature Reviews Cancer* 21 (2021): 607-608.

4. Bruni L., *et al*. "ICO Information Centre on HPV and Cancer (HPV Information Centre)". Human Papillomavirus and Related Diseases in the India. Summary Report (2021).

5. Bhat D. "The 'Why and How' of Cervical Cancers and Genital HPV Infection". *Cytojournal* 19 (2022): 22.

6. Syrjänen SM and Syrjänen KJ. "New concepts on the role of human papillomavirus in cell cycle regulation". *Annals of Medicine* 31 (1999): 175-187.

7. Castellsagué X. "Natural history and epidemiology of HPV infection and cervical cancer". *Gynecologic Oncology* 110 (2008): S4-7.

8. Klaes R., *et al*. "Over expression of p16INK4A as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri". *International Journal of Cancer* 92 (2001): 276-284.

9. Prat J. "Female Reproductive System". In: Damjanov I, Lindu J, editors. Anderson's pathology. Tenth edition. Missouri: Elsevier 1 (2009): 2231-2309.

10. Cuschieri K and Wentzensen N. "HPV mRNA and p16 detection as biomarkers for the improved diagnosis of cervical neoplasia". *Cancer Epidemiology, Biomarkers and Prevention* 17.10 (2008): 2536-2545.

11. Srivastava S. "p16INK4A and MIB-1: An immunohistochemical expression in preneoplasia and neoplasia of the cervix". *Indian Journal of Pathology and Microbiology* 53.3 (2010): 518-524.

12. Murphy N., *et al*. "p16INK4A as a marker for cervical dyskaryosis: CIN and cGIN in cervical biopsies and Thin Prep smears". *Journal of Clinical Pathology* 56 (2003): 56-63.

13. Redman R., *et al*. "The Utility of p16Ink4a in Discriminating Between Cervical Intraepithelial Neoplasia 1 and Nonneoplastic Equivocal Lesions of the Cervix". *Archives of Pathology and Laboratory Medicine* 132 (2008): 795-799.

14. Han H., *et al*. "Decreased D2-40 and increased p16INK4A immunoreactivities correlate with higher grade of cervical intraepithelial neoplasia". *Diagnostic Pathology* 6 (2011): 59.

15. Kumari K and Arcot AV. "P16INK4a expression in cervical intraepithelial neoplasia and cervical cancer". *Brunei International Medical Journal* 9.3 (2013): 165-171.

16. Wang JL., *et al*. "p16INK4A and p14ARF expression pattern by immunohistochemistry in human papillomavirus-related cervical neoplasia". *Modern Pathology* 18 (2005): 629-637.

17. Bolanca IK and Ciglar S. "Evaluation of p16INK4a in Cervical Lesion of Premenopausal and Postmenopausal Women". *Collegium Antropologicum* 31 (2007): 107-111.

18. Lee S., *et al*. "The Utility of p16INK4a and Ki-67 as a Conjunctive Tool in Uterine Cervical Lesions". *The Korean Journal of Pathology* 46 (2012): 253-260.

19. Ordi J., *et al*. "p16INK4a Immunostaining Identifies Occult CIN Lesions in HPV-positive Women". *International Journal of Gynecological Pathology* 28 (2009): 90-97.

20. Hu L., *et al*. "Human papillomavirus genotyping and p16INK4a expression in cervical intraepithelial neoplasia of adolescents". *Modern Pathology* 18 (2005): 267-273.

21. Nam EJ., *et al*. "Expression of the p16INK4a and Ki-67 in relation to the grade of cervical intraepithelial neoplasia and high-risk human papillomavirus infection". *Journal of Gynecologic Oncology* 19.3 (2008): 162-168.

22. Agoff SN., *et al*. "p16INK4a Expression Correlates with Degree of Cervical Neoplasia: A Comparison with Ki67 Expression and Detection of High-Risk HPV Types". *Modern Pathology* 16.7 (2006): 665-673.

23. Guimaraes MCM., *et al*. "Immunohistochemical Expression of P16INK4a and bcl-2 According to HPV type and to the Progression of Cervical Squamous Intraepithelial Lesions". *Journal of Histochemistry and Cytochemistry* 53.4 (2005): 509-516.

24. Zhang Q., *et al*. "Impact of utilizing p16INK4A immunohistochemistry on estimated performance of three cervical cancer screening tests". *International Journal of Cancer* 120 (2006): 351-356.

25. Mood I N., *et al*. "Potential diagnostic value of P16 expression in premalignant and malignant cervical lesions". *Journal of Research in Medical Sciences* 17.5 (2012): 428-433.

26. Cheah P., *et al*. "P16INK4a is a useful marker of Human Papillomavirus integration allowing Risk Stratification for cervical malignancies". *Asian Pacific Journal of Cancer Prevention* 13 (2012): 469-472.

27. Wei Q., *et al*. "Combined detection of P16INK4a and IMP3 increase the concordance rate between cervical cytologic and histologic diagnosis". *International Journal of Clinical and Experimental Pathology* 6.8 (2013): 1549-1557.

28. Tan GC., *et al*. "Immunohistochemical study of P16INK4a and survivin expressions in cervical squamous neoplasm". *Indian Journal of Pathology and Microbiology* 53.1 (2010): 1-10.

29. Bharti Gupta., *et al*. "Prevalence of human papillomavirus 16 genotype in Anuppur district, Madhya Pradesh". *Molecular Biology Reports* 48.1 (2021): 503-511.

30. Markowitz LE., *et al*. "Quadrivalent human papillomavirus vaccine: recommendations of the advisory committee on immunization practices (ACIP)". *MMWR Recommendations and Reports* 56 (2007): 1-24.

31. World Health Organization. Global strategy to accelerate the elimination of cervical cancer as a public health problem (2020).

32. Doshi D., *et al*. "HPV, Cervical Cancer and Pap Test Related Knowledge Among a Sample of Female Dental Students in India". *Asian Pacific Journal of Cancer Prevention* 16.13 (2015): 5415-5420.