DOI: 10.7860/NJLM/2022/55890.2668

Original Article

Pathology Section

Immunohistochemical Expression of p16 Lesions of the Cervix: A Cross-sectional Study

RADHA PRIYADHARSINI[1], BINDHUJA JOSEPH[2], MEGA SAMLY[3], SANTHA SADASIVAN[4]

ABSTRACT

Introduction: Cervical cancer is the second most malignant cancer that affects women globally. The most common cause for cervical cancer is persistent infection of high risk Human Papillomavirus (HPV) subtypes 16,18. Over-expression of p16 may serve as a surrogate biomarker to confirm HPV infection.

Aim: The present study aimed to study high risk HPV in cervical biopsies using p16 immunostaining for detecting high grade lesions.

Material and method: This cross-sectional study was conducted in the Department of Pathology, Sree Mookambika Institute of Medical Sciences, Tamil Nadu from January 2019 to February 2021 involving all women with cervical biopsies and excluding HPV vaccinated women. Samples were collected in 10% formalin. Specimens were processed and paraffin embedded. The section were stained with Haematoxylin and Eosin (H&E) and immunohistochemical staining p16 and analysed using Statistical Package for the Social Sciences (SPSS) software trial version 20.0 by qualitative analysis test i.e Chi-square test.

Results: The study included 53 cases. Age distribution was between 32-78 years, with majority in 4th to 5th decades. In the study 26 (49.1%) cases were non neoplastic lesions, 16 (30.19%) were premaliganant lesions and the remaining 11 (20.75%) were malignant lesions. The difference in the proportion of HPV diagnosis between age group was statistically significant (p-value 0.003). All the non-neoplastic lesions of cervix showed negative p16 staining. Out of 16 (30.19%) premalignant lesions, 15 (93.75%) had Low grade Squamous Intraepithelial Lesion (LSIL) and only 1 (6.25%) had High grade Squamous Intraepithelial Lesion (HSIL). Few low grade lesions 7 (100%) had negative staining and all the cervical carcinoma patients showed p16 positivity.

Conclusions: p16 expression was progressively increased with increasing grades of cervical neoplasm. So p16 may be useful as an adjunct in histological sections to detect HPV in those lesions which can help us to predict the progression of disease.

Keywords: Cervical lesions, Immunostaining, Squamous cell carcinoma, Squamous intraepithelial lesion

INTRODUCTION

In developing countries like India, cervical cancer is the most common cancer in women and may constitute up to 25% of all female cancers [1]. The most common cause for cervical cancer is persistent infection of high risk human papillomavirus (HPV 16,18) [2]. It is estimated that the prevalance rate of cervical cancer cases in united states is said to be 20 million high. The link between cervical cancer and genital infections was established by Harold Zur Hausen, a german virologist in the year 1980 [3]. The correlation between biological and clinical relevance of dividing cervical carcinoma into HPV associated and independent was identified in the last World Health Organisation (WHO) classification. Diffuse and strong p16 positivity is highly noted in all HPV associated tumors [4,5]. Due to its strong association p16 negative lesions behaves more aggressive than p16 positive lesions.

In HPV related cervical lesions, HPV derived oncoprotein E7 interacts with pRb and inactivates it leading to the disruption of the regulatory pathway Cdk-Rb-E2F. Inactivated pRb passes the cell cycle to the checkpoint G1/S without any inhibition. As a response to this event, an overexpression of p16INK4a occurs [6]. Over-expression of p16 may serve as a surrogate biomarker of HPV infection which makes it useful in evaluating HPV associated preneoplastic and neoplastic lesions of cervix [7]. Studies have shown that expression of p16INK4a is upregulated with the increase in dysplastic cells from LSIL to HSIL and to cervical carcinoma which indirectly indicates degree of malignancy [8]. The objective of the study was to investigate histomorphological changes and to study high risk HPV in various cervical lesions of cervix using p16 immunostaining. The Novelty of the study depends on the presence or absence of p16. Absence of p16 is a diagnostic and independent prognostic marker in carcinoma of cervix.

MATERIALS AND METHODS

This cross sectional study was conducted in the Department of Pathology, Sree Mookambika Institute of Medical Sciences, Tamil Nadu over a period of two years from January 2019-February 2021. A total of 53 cases was studied.

Sample size calculation:

$N = 4pq/d^2$

P = Prevalence

q = 100-p

d = Allowable error (20%)

Prevalence: 65.4% [9]

4 x 65.4 x 34.6=9051.36

20% of 65.4 =13.08

9051.36/ (13.08) 2 = 52.9

Rounded off to 53 samples, these were included in this study involving all women with cervical biopsies from Department of Obstetrics and Gynaecology in this study.

Inclusion criteria: All cases of cervical biopsies received from Department of Obstetrics and Gynaecology for histopathological examination were included in the study.

Exclusion criteria: The HPV vaccinated patients and those on chemoradiotherapy were excluded from the study.

After getting approval from the Institutional Human Ethical Committee (IHEC) NO- 26/2019, clinical history and results of relevant investigations done were collected from the patient case files. Punch biopsy from both lips of cervix were received in the pathology department in adequate 10% formalin.After a detailed specimen description, sampling was done. Entire cervical biopsies

were processed and paraffin embedded. Tissue sections of 4 µm thickness were cut and stained by H&E staining for histopathological study.

Study Procedure

Immunohistochemistry (IHC) procedure: Sections were taken on glass slide coated with adhesive (Poly-L-Lysine) for IHC p16. The positive control used was well differentiated squamous cell carcinoma with known p16 positivity and the negative control was phosphate buffer solution.The primary antibody used was-p16–INK4(MX007)-monoclonal antibody, and the secondary antibody was Horse Raddish Peroxidase (HRP conjugated antibody).Chest brown colour in nucleus and or cytoplasm positivity is considered positive.

The slide was incubated for 20 minutes at 60ºC. Deparaffinise, dehydrate and rehydrate tissues.Subject tissues to heat epitope retrieval using immune Deoxyribonucleic acid (DNA) retriever citrate buffer, pH 6 by keeping in pressure cooker for one whistle, after cooling keep in tap water, followed by distilledwater wash. It was then washed in IHC wash buffer for five minutes. The slide was placed in polydetector peroxidaseblocker for 15 minutes. It was washed in IHC wash buffer for three minutes. The sections were incubated with the primary antibody for 30 minutes and washed in IHC wash buffer twice for three minutes. The tissues were covered with polydetector HRP label for 30 minutes and washed in IHC wash buffer thrice for three minutes each.

Deaminobenzidine (DAB) was prepared by adding one drop of polydetector DAB chromogen per mL of polydetector DAB buffer and mix. The tissue was covered with prepared DAB substrate chromogen solution and incubated for 10 minutes. It was then rinsed with deionised water and counterstained with haematoxylin (4-8 dips), then dehydrated, cleared and the slide was mounted with DPX. Positive control (well differntiated squamous cell carcinoma of cervix) and negative controls was run with each batch of slides.

Two parameters were evaluated in p16 expression:

1. Percentage of p16 positive cells and
2. Reaction intensity of p16 immunostaining.

IHC scoring: Score was obtained by semiquantitative IHC score(0-3 points) depending on intensity: [10]

0 points-negative staining

1 point- weak staining

2 points- moderate staining

3 points- strong staining

Grading of IHC: [10]

1. Negative-when no cells stained and weak cytoplasmic staining.
2. Positive

Grade 1- positive cells 0-5%

Grade 2-positive cells 6-25%

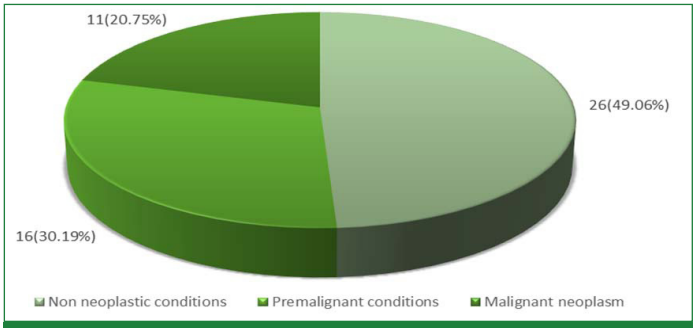
Grade 3-positive cells >25%

STATISTICAL ANALYSIS

Significance level was decided before starting of study which is found to be 5%. Qualitative analysis (Chi-sqaure test) as the statistical test used in the data analysis. The data was collected and entered in Microsoft excel and analysis was done using SPSS software trial version 20.0.

RESULTS

Among the study population, 11 (20.75%) of them were up to 40 years, 21 (39.62%) of them were aged between 41 to 50 years, 12 (22.64%) of them were aged between 51 to 60 and 9 (16.98%) of them were more than 60 years old. Majority of the cases 26(49.1%) were non neoplastic lesions, 16 (30.19%) were premalignant lesions and 11 (20.75%) were malignant lesions [Table/Fig-1].



[Table/Fig-1]: Histopathological diagnosis of cervical biopsies.

Comparison of histopathological diagnosis between age group is shown in [Table/fig-2].The difference in the proportion of histopathological diagnosis between age group was statistically significant (p-value 0.003).

In patients with non-neoplastic lesion, majority of 11 (42.31%) had squamous metaplasia, 93 (4.62%) cases had koilocytosis, 5 (19.23%) had koilocytosis and squamous metaplasia and 1 (3.85%) had tunnel cluster. Out of 16 cases with squamous intraepithelial lesion, 15 (93.75%) had LSIL and only 1 (6.25%) had HSIL. Out of 11 with carcinoma, 9 (81.82%)were nonkeratinizing squamous cell carcinoma and only one was keratinizing and verrucous squamous cell carcinoma for each respectively.

p16 immunostaining was done on all cervical biopsy specimens. Percentage of p16INK4a expression showed increase in score from non neoplastic to premalignant lesions to malignant lesions [Table/Fig-3]. In the present study it was noted that 7 out of 15 cases of LSIL showed negative staining pattern.

Intensity of p16INK4a expression showed increase in score from non-neoplastic to premalignant lesions to malignant lesions [Table/Fig-4]. LSIL/HSIL intensity and percentage positivity were shown separately in [Table/fig-5,6] In the present study it was noted that none of the premalignant showed strong reaction intensity for p16 immunostaining [Table/Fig-7]. All the malignant lesions showed strong intensity for p16 [Table/Fig-8,9,10].

Histopathological diagnosis	Age group		Chi-square	p-value
	Up to 50 (N=32)	>50 (N=21)		
Non-neoplastic conditions	17 (53.13%)	9 (42.86%)	11.373	0.003
Premalignant conditions	13 (40.63%)	3 (14.29%)		
Malignant neoplasm	2 (6.25%)	9 (42.86%)		

[Table/Fig-2]: Histopathological diagnosis vs age distribution.

HPV diagnosis	p16 Percentage positivity			
	Negative (N=33)	1+ (N=7)	2+ (N=3)	3+ (N=10)
Non-neoplastic conditions	26 (78.79%)	0	0	0
Premalignant conditions	7 (21.21%)	7 (100%)	2 (66.67%)	0
Malignant neoplasm	0	0	1 (33.33%)	10 (100%)

[Table/Fig-3]: Comparison of HP diagnosis across p16 percentage positivity.

HP diagnosis	p16 intensity staining			
	Negative (N=33)	1+ (N=6)	2+ (N=5)	3+ (N=9)
Non-neoplastic conditions	26 (78.79%)	0	0	0
Premalignant conditions	7 (21.21%)	6 (100%)	3 (60%)	0
Malignant Neoplasm	0	0	2 (40%)	9 (100%)

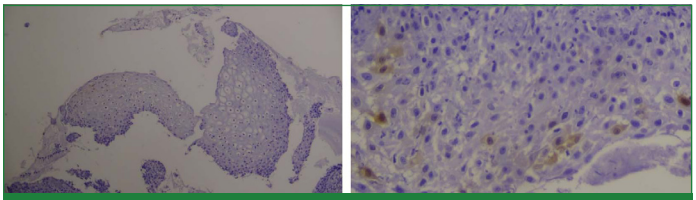
[Table/Fig-4]: Comparison of HP diagnosis across p16 intensity staining.

Squamous intraepithelial lesion	p16 percentage positivity		
	Negative (N=7)	1+ (N=7)	2+ (N=2)
LSIL	7 (100%)	6 (85.71%)	2 (100%)
HSIL	0	1 (14.29%)	0

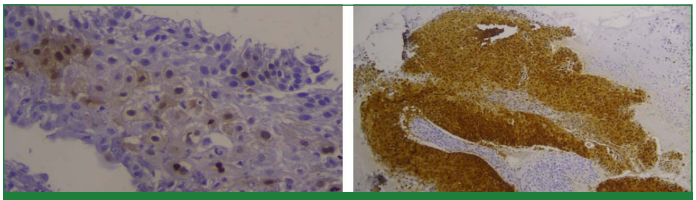
[Table/Fig-5]: Comparison of squamous intraepithelial lesion between p16 percentage positivity (N=16).

Squamous intraepithelial lesion	p16 intensity staining		
	Negative (N=7)	1+ (N=6)	2+ (N=3)
LSIL	7 (100%)	5 (83.33%)	3 (100%)
HSIL	0	1 (16.67%)	0

[Table/Fig-6]: Comparison of squamous intraepithelial lesion between p16 intensity staining (N=16).



[Table/Fig-7]: IHC p16- Negative staining in koilocytosis (10x).
[Table/Fig-8]: IHC p16 -Positivity in low grade squamous intraepithelial lesion (40x). (Images from left to right)



[Table/Fig-9]: IHC p16 -Positivity in SCC's (40x). **[Table/Fig-10]:** IHC p16- Positivity in SCC – large cell non keratinizing type (10x). (Images from left to right)

DISCUSSION

Among many effective immunomarkers used in diagnosis of cervical lesions, p16 is considered to be a cost effective and has a higher rate of sensitivity in the detection of cervical tumors. The p16 intensity staining reaction to be considered more superior than other parameters in diagnosing cervical lesions. Although numerous screening methods are available, Papanicolaou test (Pap smear) is the most widely used screening method [11].

The new WHO classification (2020) has included HPV along with histopathologic subtypes of cervical adenocarcinoma [12]:

1. Squamous cell carcinoma (HPV-associated, independent), Not Otherwise Specified (NOS)
2. Adenocarcinoma (HPV-associated, independent-NOS, gastric type, clear cell type, mesonephric type), NOS

Screening programmes like liquid based cytology and HPV genotyping are mandatory to detect High Risk (HR) HPV groups. The most common HR-HPV genotypes are HPV52, HPV16, HPV51 and the Low Risk (LR) HPV groups are HPV72, HPV62. International guidelines recommend HPV genotyping to be the effective screening method in case of high risk HPV infection. In earlier days, pap smear was the auxiliary method followed which is now replaced by Liquid Based Cytology (LBC) due to various interference factors. The Society of Cervical Pathology's (ASCCP) guidelines have included combination of all the three as a good diagnostic tool in screening high risk cervical cases [13].

The overall age range of the 53 patients was 32-78 years. This is similar to studies done by Kim TH et al., and Poste P et al., in which the age group commonly involved ranges from 24-80 years and 29 to 80 years respectively [14,15]. Majority of malignant lesions of cervix were seen above 50 years of age group, which is similar to study done by poste et al., [15]. The difference in the proportion of HP diagnosis between age group was statistically significant (p-value 0.003).

In the present study majority of the cervical lesions were non neoplastic (49.06%) followed by premalignant lesions (30.19%) and then malignant lesions (20.75%) This was in contrast to study conducted by Ismail AT et al., which showed majority of their cases were premalignant lesions [16]. In another study by Rawat N et al., analysed 80 biopsy samples, with benign lesions reported in 17.5%, Cervical Intraepithelial Neoplasia (CIN) 1 in 7.5%, CIN 2 in 7.5%, Squamous Cell Carcinoma (SCC) in 17.5% of the study population [17].

In the present study, among the nonneoplastic lesions of cervix 11 (42.31%) cases showed squamous metaplasia in correlation with Battacharya S et al., [18] in their study of 686 cases 344 cases (50.14%) showed squamous metaplasia. This is in contrast to study done by Pallipady A et al., where 78.12% of the cases showed squamous metaplasia [19].

Koilocytosis is considered as the histological hallmark of papilloma virus infection.Koilocytosis was diagnosed in nine cases and five cases showed koilocytosis along with squamous metaplasia among all cervical specimens during the study period. This is in correlation with studies done by Pallipady A et al., and Naik R et al., showed 18 out of 2053 cervical biopsies and 3 out of 50 cervical biopsy specimens respectively [19,20].

The incidence of squamous intraepithelial lesion (cervical dysplasia) was 16 (30.19%) of 53 cases. 15 out of 16 cases had LSIL (CIN 1) and one out of 16 cases had HSIL (CIN 3). This is similar to studies done by Nam EJ et al., where the incidence of low grade cervical lesion, CIN 1 (LSIL) is more when compared to CIN 2 and 3 (HSIL) [21].

Carcinoma cervix in the present study was 11 (20.75%) of the total 53 cases and found all the cases were SCC's. Low incidence of carcinoma cervix was reported by Solapurkar ML, with two cases in a total of 551 cases and high incidence was noted in the study done by Nega B et al., with 2318 (55.7%) of 4155 cases [22,23].

Immunohistochemistry

p16INK4a in cervical lesions: Out of 26 non neoplastic lesions including nonspecific cervicitis, this study showed no expression of p16INK4a. In the study done by Munhoz NG et al., nonspecific cervicitis showed no expression of p16INK4a and 100% of cases show 6.6% of cells positivity for ki67 [24].

In the present study, out of 15 LSIL slides, seven cases were negative for p16INK4a, six cases were positive for score 1 and two cases were positive for score 2. It was observed that p16 negativity in majority of the LSIL samples. Tan GC et al., in 2010 stated that low grade lesion with p16 negativity may be due to infection with low risk HPV or due to subclinical infection [25]. A study by Volgareva G et al., in 2004, observed that some of the preneoplastic and neoplastic lesions of cervix do not express p16 [26]. They concluded that absence of p16 expression may be due to p16 mutation, deletion or hypermethylation.

In the present study, one HSIL case showed positivity (100%) for p16INK4a with score 1. In the study done by Lorenzato M et al., [27] 100% of HSIL cases show 100% show nuclear and cytoplasmic positivity for p16INK4a marker. In the study done by Reuschenbach M et al., he demonstrated positivity in 86.95% of HSIL cases for p16INK4a and 85.45% of cases were positivite for ki67 [28].

In total, 100 % (11 out of 11) of the cervical carcinoma patients showed positivity for p16INK4a. All cases of SCC in this study showed an over-expression of p16INK4a. Similar evidence were obtained by the studies done by Volgareva G et al., [26]. This reflects the close association of overexpression of p16INK4a in HSIL and SCC with the degree of premalignancy and malignancy and its pivotal role in the progression of malignancy in cervix.

In present study, it was demonstrated that a graded increase was seen in the expression of the protein p16INK4a from nonspecific cervicitis to LSIL and further to HSIL and invasive squamous cell carcinoma. Thus, expression of p16INK4a protein in cervical biopsies can serve as a specific marker for diagnosing premalignant and malignant lesions.

Limitation(s)

The sample size was low in number however, the attempt for HPV DNA detection studies to validate the utility of p16 for detection of HPV in cervical neoplasm could not be made due to financial constraint.

CONCLUSION(S)

The p16INK4a was found to show graded increase in score from nonspecific cervicitis to LSIL and further to HSIL and invasive squamous cell carcinoma. So p16 may be useful as an adjunct in histological sections to detect HPV in those lesions which can help us to predict the progression of disease. If the association of HPV in SCC it can predict the prognosis of the patient.The detection of p16 can help in categorising high risk HPV cases,which can be further treated.

REFERENCES

[1] Jo H, Kim JW. Implications of HPV infection in uterine cervical cancer. Cancer Ther. 2005 Jul;3 (41):434.

[2] Zhang S, Xu H, Zhang L, Qiao Y. Cervical cancer: Epidemiology, risk factors and screening. Chinese Journal of Cancer Research. 2020 Dec 31;32(6):720–8.

[3] Zur Hausen H. Papillomavirus infections- a major cause of human cancers. Biochimica et biophysica acta (BBA)-reviews on cancer. 1996 Oct 9;1288(2):F55-78.

[4] Hohn AK, Brambs CE, Hiller GG, May D, Schmoeckel E et al. 2020 WHO classification of female genital tumors. Geburtshilfe und Frauenheilkunde. 2021 Oct;81(10):1145-53.

[5] Nwachukwu CR, Harris JP, Chin A, Von Eyben R, Giaretta S et al. Prognostic significance of P16 expression and P53 expression in primary vaginal cancer. International Journal of Gynecological Pathology. 2019 Nov 1;38(6):588-96.

[6] Paraskevaidis E, Arbyn M, Sotiriadis A, Diakomanolis E, Martin-Hirsch P, Koliopoulos G, Makrydimas G, Tofoski J, Roukos DH. The role of HPV DNA testing in the follow-up period after treatment for CIN: a systematic review of the literature. Cancer treatment reviews. 2004 Apr 1;30(2):205-11.

[7] Galgano MT, Castle PE, Atkins KA, Brix WK, Nassau SR et al. Using biomarkers as objective standards in the diagnosis of cervical biopsies. Am J SurgPathol. 2010 Aug;34(8):1077–87.

[8] Izadi-Mood N, Asadi K, Shojaei H, Sarmadi S, Ahmadi SA et al. Potential diagnostic value of P16 expression in premalignant and malignant cervical lesions. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2012 May;17(5):428.

[9] Bhatla N, Lal N, Bao YP, Ng T, Qiao YL. A meta-analysis of human papillomavirus type-distribution in women from South Asia: implications for vaccination. Vaccine. 2008 Jun 2;26(23):2811-7.

[10] Gupta R, Srinivasan R, Nijhawan R, Suri V, Uppal R. Protein p 16INK4A expression in cervical intraepithelial neoplasia and invasive squamous cell carcinoma of uterine cervix. Indian journal of Pathology and Microbiology. 2010 Jan 1;53(1):7.

[11] Mishra GA, Pimple SA, Shastri SS. An overview of prevention and early detection of cervical cancers. Indian Journal of Medical and Paediatric Oncology. 2011 Jul;32(03):125-32.

[12] International Agency for research on cancer (IARC).WHO Classification of female genital tumours.5th ed.Edited by the WHO classification of tumours editorial board,International agency for research on cancer (IARC);2020.

[13] Wright Jr TC. 2006 American Society for Colposcopy and Cervical Pathology-sponsored Consensus Conference. 2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ. J Low Genit Tract Dis. 2007;11:223-39.

[14] Kim TH, Han JH, Shin E, Noh JH, Kim HS et al. Clinical implication of p16, Ki-67, and proliferating cell nuclear antigen expression in cervical neoplasia: improvement of diagnostic accuracy for high-grade squamous intraepithelial lesion and prediction of resection margin involvement on conization specimen. Journal of cancer prevention. 2015 Mar;20(1):70.

[15] Poste P, Patil A, Andola SK. Incidence of neoplastic cervical pathologies recorded at a medical College. International journal of applied science-research and review. 2015 Mar 18;2(2):051-68.

[16] Ismail AT, Ahmed NY, Hameed AA. Prevalence of HPV immunostaining in benign, preneoplastic and neoplastic cervical lesions of kurdish women in Erbil City/ Kurdistan of Iraq. Am J Res Commun. 2014;2:67-74.

[17] Rawat N, Shivapriya R. Immunohistochemical Expression of p16INK4A in The Lesions of Uterine Cervix. Ann Pathol Lab Med. 2020;7(10):01-07.

[18] Bhattacharya S, Banerjee U, Das S, Basu A, Sanyal S, Sanyal MK. Histopathology audit of cervical epithelium in non-neoplastic conditions of cervix. J Obstet Gynecol India. 1996;46:514-20.

[19] Pallipady A, Illanthody S, Vaidya R, Ahmed Z, Suvarna R et al. A Clinico-Morphological spectrum of the Non-neoplastic lesions of the uterine cervix at AJ Hospital Mangalore. Journal of clinical and diagnostic research. 2011;5(3):546-50.

[20] Naik R, Pai MR, Baliga P, Nayak KS, Dighe P. Mast cell profile in uterine cervix. Indian Journal of Pathology & Microbiology. 2004 Apr 1;47(2):178-80.

[21] Nam EJ, Kim JW, Hong JW, Jang HS, Lee SY 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. 2008 Sep 1;19(3):162-8.

[22] Solapurkar ML. Histopathology of uterine cervix in malignant and benign lesions. J Obstet Gynaecol India. 1985;35:933-938.

[23] Nega B, Gebrehiwot Y, Kiros K, Ergete W. A five-year analysis of histopathological results of cervical biopsies examined in a pathology department of a teaching hospital (2003-2007). Ethiopian Journal of Reproductive Health. 2010;4(1):52-7.

[24] Munhoz NG, Rodrigues DA, Takase Y, Pedregosa JF, Rodrigues JO et al. The use of molecular markers (p16, Ki-67 and E-Cadherin) in uterine cervical biopsies. The Open Pathology Journal. 2009 Mar 13;3(1): 10- 17.

[25] Tan GC, Norlatiffah S, Sharifah NA, Razmin G, Shiran MS et al. Immunohistochemical study of p16 INK4A and survivin expressions in cervical squamous neoplasm. Indian Journal of Pathology and Microbiology. 2010 Jan 1;53(1):1.

[26] Volgareva G, Zavalishina L, Andreeva Y, Frank G, Krutikova E et al. Protein p16 as a marker of dysplastic and neoplastic alterations in cervical epithelial cells. BMC cancer. 2004 Dec;4(1):1-0.

[27] Lorenzato M, Caudroy S, Bronner C, Evrard G, Simon M et al. Cell cycle and/or proliferation markers: what is the best method to discriminate cervical high-grade lesions?. Human pathology. 2005 Oct1;36(10):1101-7.

[28] Reuschenbach M, Seiz M, Doeberitz CV, Vinokurova S, Duwe A et al. Evaluation of cervical cone biopsies for coexpression of p16INK4a and Ki-67 in epithelial cells. International journal of cancer. 2012 Jan 15;130(2):388-94.

PARTICULARS OF CONTRIBUTORS:

1. Junior Resident, Department of Pathology, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Tamil Nadu, India.
2. Associate Professor, Department of Pathology, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Tamil Nadu, India.
3. Assistant Professor, Department of Pathology, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Tamil Nadu, India.
4. Professor, Department of Pathology, Sree Mookambika Institute of Medical Sciences, Kulasekharam, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Bindhuja Joseph,
119, Jose Bhavan, Puthukudiyerrupu, Nagercoil, Tamil Nadu, India.
E-mail: bindhuja85@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Mar 02, 2022
- Manual Googling: May 14, 2022
- iThenticate Software: Jun 20, 2020 (23%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

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

Date of Submission: **Feb 24, 2022**
Date of Peer Review: **Apr 04, 2022**
Date of Acceptance: **May 05, 2022**
Date of Publishing: **Oct 01, 2022**