Original Article

Immunohistochemical study of p16^{INK4a} expression in cervical carcinoma and dysplasia in correlation with histopathology

Kory Swetha[1*], Shantala P R[2], Ramdas Naik[3], Chanabasappa Chavadi[4], AijazMuzamil Dar[5]

[1,5]Resident, [2]Associate professor, [3]Professor and HOD, Department of Pathology, Yenepoya Medical College, Yenepoya University, Mangalore, Karnataka, INDIA.
[2]Assistant professor, Department of radiology, Kasturba medical college4, Mangalore, Manipaluniversity, Karnataka, INDIA.
Email: kory.swetha@gmail.com, bhatshanthala@yahoo.co.in, ramadas.nayak@gmail.com, chavadidoc@gmail.com, docmuzamil@gmail.com

Abstract

The association of high risk types Human papilloma virus(HR-HPV) with cervical intraepithelial neoplasia (CIN) and carcinoma are well known.P16^{INK4a}, a tumour suppressor protein suggested to be a surrogate marker forHR-HPV induced cervical neoplastic changes. To study was done to assess the expression status of p16^{INK4a} marker in CIN and cervical carcinoma. A study of 40 cases included cervical biopsies of cervical intraepithelial neoplasia and carcinoma. Immunohistochemical study of these cases were done usingp16^{INK4a} marker.The intensity of staining and percentage of p16^{INK4a} positive cells were observed. The study of 40 cases included 28 cases of squamous cell carcinoma (70%), one case of adenocarcinoma (2.5%), five cases of CIN (12.5%) and other three cases were non-neoplastic lesions (7.5%). One case each of CIN2 and CIN 3 were p16^{INK4a}marker positive (2+). Among the 28 cases of squamous cell carcinoma majority showed 3+ positivity (>75% positive cells). One case of adenocarcinoma in the study showed positivity. The strong association between p16^{INK4a} and cervical carcinoma (squamous cell carcinoma) supports the predictive role of p16^{INK4a} study in supplement to the biopsy study for cases of CIN.

Key words: Human papilloma virus, p16^{INK4a}, Cervical intraepithelial neoplasia, Cervical carcinoma.

[*]**Address for Correspondence:**
Dr. Kory Swetha, Flat 1205 'A' Block, Siliconia Apartment, Near Kuthar Junction, Mangalore- 575017, Karnataka, INDIA.
Email: kory.swetha@gmail.com

Received Date: 13/02/2016 Revised Date: 16/03/2016 Accepted Date: 10/04/2016

Access this article online

Quick Response Code:

Website: www.statperson.com

DOI: 12 April 2016

INTRODUCTION

Cervical carcinoma is the 2nd most common cancer in women, worldwide. Most (83%) of the cases are seen in developing countries.[1] India accounts for nearly third of the global cervical cancer deaths.[2] It is a well-established fact that infection with human papilloma virus high risk(HR-HPV)types especially HPV-16 is the key etiological factor associated with cervical intra epithelial neoplasia(CIN) and carcinomas.[3] The risk of developing precancerous lesion with HR-HPV 16 and 18 is found to be 10% and 15% respectively.[3] The incidence of association of the HR-HPV with carcinomas is nearly 99% in squamous cell carcinoma.[4] In India, cervical cancer screening programs are yet to be streamlined. The Bethesda system for reporting of pap smears has brought in some uniformity in reporting cervical cytology. However, the possibility of inter-observer variations in interpretation still exist.[5] Recently, amore specific approach to delineate the clinically significant infection or progressive neoplastic change by detection of viral gene expression through direct detection of viral mRNA transcripts or study of expression of tumour suppressor protein p16^{INK4a} have been done in some studies.[6] The p16^{INK4a} acts as a surrogate marker of HPV E7-mediated pRB catabolism and provides an evidence of transforming cervical mucosa.[3] The present immunohistochemical(IHC) study was to evaluate the expression status of p16^{INK4a} marker to gain an insight into the frequency of HPV related cervical carcinomas in this part of India and also to observe whether they support the histopathological findings of CIN.

How to site this article: Kory Swetha, Shantala P R, Ramdas Naik, Chanabasappa Chavadi, AijazMuzamil Dar. Immunohistochemical study of p16^{INK4a} expression in cervical carcinoma and dysplasia in correlation with histopathology. *International Journal of Recent Trends in Science and Technology*. April 2016; 18(3): 493-497. http://www.statperson.com (accessed 14 April 2016).

MATERIAL AND METHODS

The retrospective study included a total of 40 cases of cervical biopsies of histopathologically diagnosed cases of CIN, carcinomas and also non neoplastic cervical lesions, received in our Medical college and Hospital. The HandE stained slides of the cases were reviewed. One paraffin-embedded block was selected and standard 4µm sections were subjected to immunohistochemical study. $P16^{INK4a}$ marker(a mouse monoclonal antibody of G175-405 clone, Pathinsitucompany) was used. The poly excel HRP(non-biotin, micro-polymer based)/diaminobenzidine(DAB) Detection system procedure was followed. The results were interpreted as positive if both nuclei and cytoplasm of cell stained with formation of brown product. The scoring of percentage of $p16^{INK4a}$ positive cells were done as 0 (negative), 1+ (<25% cells), 2+ (25-75%), 3+ (>75% positive cells). The intensity of staining was recorded as weak, moderate, and strong positivity.

RESULTS

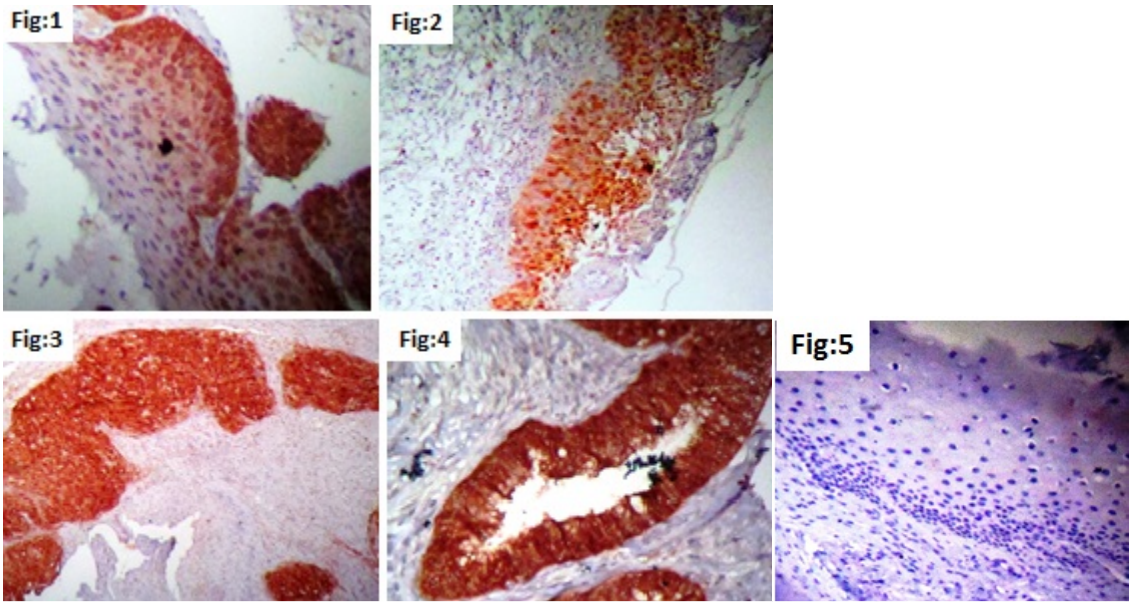
Out of 40 cases included, 28 cases of squamous cell carcinoma (70%), 1 case of adenocarcinoma (2.5%) and 5 cases were CIN (12.5%).Among 5 cases of CIN there was 1 case of CIN-1, 2 cases of CIN-2 and 2 cases of CIN-3. The non-neoplastic lesions included 3 cases (7.5%)(2with squamous metaplasia and 1 case of micro glandular hyperplasia). Three cases (7.5%) were normal cervix. Inimmunohistochemical (IHC) study,$p16^{INK4a}$ expression was negative in three cases, which included one case each of CIN-1, CIN-2 and CIN-3. One case each of CIN-2 and CIN-3 showed 2+ positivity with moderate intensity of staining as shown(Fig:1,2). Among the 28 cases of squamous cell carcinoma, majority (89%) showed 3+ (>75% positive cells) as shown in fig 3, and rest of the cases (10.8%) showed 2+(50-75%positive cells)(Table 1). The intensity of staining was strong and diffuse in 20 cases (71.4%), moderate in 7 cases (25%). One case(3.7%) showed weak staining. There was only one case of adenocarcinoma with 3+ (>75% cells) and strong intensity of staining (fig 4).Three cases of normal cervix(fig 5), 2 cases of squamous metaplasia and one case of microglandular hyperplasia were negative (Table 2). Cases which showed both nuclear and cytoplasmic staining were considered positive and in this study all the cases showed both nuclear and cytoplasmic positivity except for one case of CIN 1 which showed only weak cytoplasmic positivity which was considered as negative.

Table 1: Histopathology and percentage of$p16^{INK4a}$expression.

Histopathology	Total number	Negative	Positive		
			1+	2+	3+
Normal Cervix	3	3			
Squamous metaplasia	2	2			
Microglandular hyperplasia	1	1			
CIN-1	1	1			
CIN-2	2	1		1	
CIN-3	2	1		1	
Squamous cell carcinoma	28			3	25
Adenocarcinoma	1				1

Table 2: Histopathology and intensity of expression of $p16^{INK4a}$ marker

Histopathology	Total Number	Negative	Positive		
			Weak	Moderate	Strong
Normal Cervix	3	3			
Squamous metaplasia	2	2			
Microglandular hyperplasia	1	1			
CIN-1	1	1			
CIN-2	2	1		1	
CIN-3	2	1		1	
Squamous cell carcinoma	28		1	7	20
Adenocarcinoma	1				1



Legend

Figure 1: Moderate intensity of staining and 2+ positivity of $p16^{INK4a}$ in CIN-2.
Figure 2: Moderate intensity of staining and 2+ positivity of $p16^{INK4a}$ in CIN-3.
Figure 3: Strong and diffuse (3+) expression of p16INK4a in Squamous cell carcinoma of cervix.
Figure 4: Strong and diffuse (3+) expression of p16INK4a in adenocarcinoma of cervix.
Figure 5: Negative expression of p16INK4a in normal cervical epithelium.

DISCUSSION

One of the commonest types of cancer that affects female reproductive organs is cervical cancer. Of the several risk factors causing cervical carcinoma (smoking, age, oral contraceptives, low age at 1st sexual intercourse, deficient diet and family history) human papilloma virus infection is the most common risk factor. Cervical cancer has a morphologically well recognized precancerous state called cervical intraepithelial neoplasia. Cellular protein alterations can be analysed at different progressive levels of CIN1 to CIN3 and cervical carcinoma. P16 encoded by CDKN2A gene, located on chromosome 9 p16 is a cyclin-dependent kinase (CDK) inhibitor that slows down the cell cycle by prohibiting progression from G1 phase to S phase. P16 acts as a tumour suppressor gene by binding to CDK4/6 and preventing its interaction with cyclin D.[7] This interaction ultimately inhibits the downstream activities of transcription factors, such as E2F1 and arrests cell proliferation. The hyper methylation, mutation, or deletion of p16 leads to down regulation of the gene and can lead to cancer through the dysregulation of cell cycle progression. Thus, p16 has been studied as a biomarker for detecting and determining prognosis of cancer. Persistence of HPV infection by viral genomic integration into host cell DNA leads to the upregulation of $p16^{INK4a}$ expression which has been well documented in various studies.[3] Reciprocal relationship has been observed between $p16^{INK4a}$ and RB expression. Functional inactivation of RB by the HPV E7 protein results in the over expression of $p16^{INK4a}$ in cervicalcarcinomas.[8] However non-HPV mediated cervical carcinomas have also been documented[9] and $p16^{INK4a}$ negative CINs and carcinomas do exist.[8,10] $P16^{INK4a}$ overexpression is also noted in other tumours like colon carcinoma, gall bladder carcinoma, uterine leiomyosarcoma, astrocytoma, breast carcinomas, lung carcinoma and undifferentiated high grade pleomorphic sarcomas.[11]

In India, cervicalcarcinoma is one of the most common cancers in females and there is paucity of Indian studies done on evaluation of $p16^{INK4a}$expression in cervical cancers.[12] The present study results are in concordance with many of previous studies(Table 3)[12,13,14,15,16,17] The expression of $p16^{INK4a}$ is heterogeneous with respect to different stages of CIN but the expression was diffuse in all the carcinomas. In the present study, the diffuse and strong expression of the $p^{16INK4a}$ gene product is seen in progressive stages of CIN2 to carcinomas, whereas normal cervical and non-neoplastic lesions were negative. There was only one case of CIN1 which was considered as negative even though there was weak cytoplasmic staining. Most of the Indian studies considered anynuclear or cytoplasmic staining as positive[12,18,19,20] but the studies done by others[8,4,5] consider both nuclear and cytoplasmic staining as

positive, similar to the present study. A study done by Tsoumpou *et al* reported that over expression of $p16^{INK4a}$ increased with the degree of cytological or histological abnormality, and showed that 38% of CIN1, 68% of CIN2 and 82% of CIN3cases.[21] $P16^{INK4a}$ is strongly over expressed in almost all cervical carcinomas and the intensity of positivity and percentage of cells showing positivity also increases as the grade of dysplasia. In various studies, $p16^{INK4a}$ expression was found to be in the range of 0-12% in non-dysplastic lesion, 25-91% in CIN-1, 63-100% in CIN-2, 91-100% in CIN3 and 89-100% in invasive carcinomas.[6] $P16^{INK4a}$isalso usually found to be positive in other histological types of cervical carcinoma like adenocarcinoma[16,22] and small cell carcinoma.[23] Some of the studies done have studied and compared HR-HPV types and$p16^{INK4a}$ expression status. In one such study$p16^{INK4a}$ expression was found to be increased significantly with HR-HPV 16 and 58types.[24] This over expression of $p16^{INK4a}$ in cervical carcinoma has been associated with increased overall and disease free survival rate and better prognosis. Interestingly it has been proposed that over expression is recognized by the immune system to initiate an anti tumour response in cervical carcinomas.[7] In the present study though only $p16^{INK4a}$ over expression was studied, this being a surrogate marker for (HR-HPV mediated) carcinomas indirectly shows that most of carcinomas are HR-HPV mediated carcinomas.

CONCLUSION

In the view of nearly 100% expression of $p16^{INK4a}$ in cervical squamous cell carcinomas, studying the expression of status of $p16^{INK4a}$ in biopsy would support the histopathological features of CIN and also predict the risk of those progressing to carcinoma.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics 2002. CA Cancer J Clin2005;55:74-108.
2. Kaarthigeyan K. Cervical cancer in India and HPV vaccination.Indian J Med PaediatrOncol 2012;33(1):7-12.
3. Hwang S, Shroyer K. Biomarkers of Cervical Dysplasia and Carcinoma. J Oncol 2012;2012:1-9.
4. Simionescu C, Margaritescu C, Stepan A, Georgescu CV, Niculescu M, Muntean M. The utility of p16, E-cadherin and Ki67 in cervical squamous intraepithelial lesions diagnosis.Rom J MorpholEmbryol 2010;51(4):621-6.
5. Tan G, Norlatiffah S, Sharifah N, Razmin G, Shiran M, Hatta A *et al*. Immunohistochemical study of p16 INK4A and survivin expressions in cervical squamous neoplasm. Indian J PatholMicrobiol 2010;53(1):1.
6. Cuschieri K, Wentzensen N. Human Papillomavirus mRNA and p16 Detection as Biomarkers for the Improved Diagnosis of Cervical Neoplasia.CancerEpidemiol Biomarkers Prev 2008;17(10):2536-45.
7. Lin J, Albers A, Qin J, Kaufmann A.Prognostic significance of overexpressed p16INK4a in patients with cervical cancer: A meta-analysis.PLoS One2014;9(9):e106384.
8. Lesnikova I, Lidang M, Hamilton-Dutoit S, Koch J. p16 as a diagnostic marker of cervical neoplasia: a tissue microarray study of 796 archival specimens. DiagnPathol2009;4(1):22.
9. Zhao C, Yang H, Li Z. Evidence emerging for HPV-negative cervical cancer.Cytopathology and more.2014 Jan.
10. Klaes R, Friedrich T, Spitkovsky D, Ridder R, Rudy W, Petry U *et al*. Overexpression of p16INK4a as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. Int J Cancer 2001; 92:276-84.
11. Romagosa C, Simonetti S, López-Vicente L, Mazo A, Lleonart M, Castellvi J *et al*. p16Ink4a overexpression in cancer: a tumour suppressor gene associated with senescence and high-grade tumours. Oncogene 2011;30(18):2087-97.
12. Kumari K, Vadivelan A. P16INK4A expression in cervical intraepithelial neoplasia and cervical cancer. Brunei Int Med J 2013;9(3):165-71.
13. Gupta R, Srinivasan R, Nijhawan R, Suri V, Uppal R, Gupta R. Protein p 16INK4A expression in cervical intraepithelial neoplasia and invasive squamous cell carcinoma of uterine cervix. Indian J PatholMicrobiol 2010;53(1):7.
14. Ishikawa M, Fujii T, Saito M, Nindl I, Ono A, Kubushiro K *et al*. Overexpression of p16 INK4a as an indicator for human papillomavirus oncogenic activity in cervical squamous neoplasia.Int J Gynecol Cancer 2006 ;16(1):347-53.
15. Karcheva M, Popovska S, Nachev R. Immunohistochemical investigations ofp16 ink 4a expression in carcinomas and high grade cervical lesions. J of IMAB2007;13(1):24-6.
16. Volgareva G, Zavalishina L, Andreeva Y, Frank G, Krutikova E, Golovina D *et al*. Protein p16 as a marker of dysplastic and neoplastic alterations in cervical epithelial cells. BMC Cancer 2004;4:58.
17. Reuschenbach M, Seiz M, Doeberitz C, Vinokurova S, Duwe A, Ridder R *et al*. Evaluation of cervical cone biopsies for coexpression of p16INK4a and Ki-67 in epithelial cells. Int J Cancer 2011;130(2):388-94.
18. Lakshmi S, Rema P, Somanathan T. p16 ink4a Is a Surrogate Marker for High-Risk and Malignant Cervical Lesions inthe Presence of Human Papillomavirus. Pathobiology 2009;76:141–8.
19. Srivastava S. P16INK4A and MIB-1: An immunohistochemical expression in preneoplasia and neoplasia of the cervix. Indian J PatholMicrobiol 2010; 53(3):524.
20. Wang JL, Zheng BY, Li XD, Angstrom T, Lindstrom MS, Wallin KL. Predictive Significance of the Alterations of p16INK4A, p14ARF,p53, and Proliferating Cell Nuclear Antigen Expression in the

Progression of Cervical Cancer.ClinCancer Res2004; 10:2407.
21. Tsoumpou I, Arbyn M, Kyrgiou M, Wentzensen N, Koliopoulos G, Martin-Hirsch P. p16(INK4a) immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis. Cancer Treat Rev 2009;35(3):210-20.
22. Agoff S, Lin P, Morihara J, Mao C, Kiviat N, Koutsky L. p16INK4a Expression Correlates with Degree of Cervical Neoplasia: A Comparison with Ki-67 Expression and Detection of High-Risk HPV Types. Mod Pathol 2003; 16(7):665-73.
23. Wang H, Lu D. Detection of Human Papillomavirus DNA and Expression of p16, Rb, and p53 Proteins in Small Cell Carcinomas of the Uterine Cervix. Am J SurgPathol2004; 28(7):901-8.
24. Nam E, Kim J, Hong J, Jang H, Lee S, Jang S *et al*.Expression of the the p16INK4a and Ki-67 in relation to the grade of cervical intraepithelial neoplasia and high-risk human papillomavirus infection.JGynecolOncol2008;19(3):162-8.

Source of Support: None Declared
Conflict of Interest: None Declared