Journal of Pharmaceutical Research International

32(13): 47-51, 2020; Article no.JPRI.54180
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Evaluation of the P16INK4a Expression in Cervical Biopsy Specimen SBMCH

Amish Aggarwal[1*], I. Venkatraman[1] and M. Prabakaran[1]

[1]Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i1330582
Editor(s):
(1) Dr. Giuseppe Murdaca, University of Genoa, Italy.
Reviewers:
(1) S. Danish Kadir, University of Rajshahi, Bangladesh.
(2) Zhen Sun Cell, Memorial Sloan Kettering Cancer Center, USA.
Complete Peer review History: http://www.sdiarticle4.com/review-history/54180

Original Research Article

Received 15 May 2020
Accepted 22 July 2020
Published 03 August 2020

ABSTRACT

In early 1980 human papillomavirus (HPV) were the risk factor and most commonly affects younger women. Many test have developed since then and among that a biomarker test system have developed and clinically evaluated. P16INK4a is used as an important marker for indicating neoplastic transformation for cervical dyplasia. This study was done to evaluate the P16INK4a expression in cervical biopsy in 50 cases. Two cases were identified a P16INK4a positive and remaining 48 didn't show P16INK4a expression proving the hypothesis that p16INK4a is capable of showing the dysplasia positive cases.

Keywords: Human papilloma virus; p16INK4a; dysplasia; biopsy.

1. INTRODUCTION

Carcinoma cervix the most common form of cancer by 13% of all the women affected cancers. In India, 1 in 53 Indian women are usually affected in the developed countries, 1 in 100 women are approximately affected. HPV being detected in all cases of cervical dysplasia and neoplasia cases, is a crucial factor [1]. High risk HPV encodes twice known viral oncogenes, E6 and E7 in the association of cervical cancer. E6- E6 act by inhibiting the function of p53 indicated DNA damage and apoptosis pathway. It acts by forming a complex cellular E6-AP

**Corresponding author: E-mail: deanpublications@bharathuniv.ac.in;*

protein which is useful for p53 interaction. E6 retarget E6- AP to induce ubiquitination and rapid proteosomal degradation of p53 HPV E6 can activate telomerase n TERT transcription also. P53, a protein, controls the response to cellular stress including DNA damage and viral infection [2].

E7- E7 which inhibits the function of tumour suppressor pRB mediated cell cycle regulation pathway. Inactivation of the pRB and p53 tumour suppressor pathways and expression of the catalytic telomerase subunit hTERT constitute the process and thus leading to overexpression of the CDK inhibitor P16INK 4a through negative feedback control to check the cell proliferation through regulation of CDK4 and 6. Hence P16INK 4a is overexpressed in HPV mediated cervical cancer [3].

The P16INK 4a which is a tumour suppressor protein and a CDKN2A gene product, an inhibitor of cyclin dependent kinase (CDK) 4 and 6 which is encoded by tumour suppressor gene INK 4a its normal function is to prevent cells from dividing in the absence of an inappropriate signal. P16INK inhibits the cyclin dependent kinase preventing the phosphorylation of RB (hypophosphorylated form); pRB binds to transcription factors, it loses the G1/S check – point controller. RB gene in cervical dysplasia is inactivated as a HPV E7 protein expression [4]. Since HPV infections supersede cell cycle controls, the immune detection of cell proteins that are differentially expressed in infected cells is currently being considered for use as tumour and prognostic marker. p16INK4a is a tumor-suppressor protein and cyclin-dependent kinase (cdk) inhibitor that blocks cdk4- and cdk6-mediated pRb phosphorylation to inhibit E2F-dependent transcription and cell-cycle progression [5]. Hence in this study the level of expression of the p16INK4a in the cervical biopsy has been evaluated.

2. MATERIALS AND METHODS

- Hospital based cross sectional study design was followed. The study was carried out in the Department of Pathology in collaboration with the Department of Obstretics and Gynaecology, SreeBalaji Medical College and Hospital (SBMCH), Chromepet. The study was constructed for a period of two years starting from November 2016 to October 2018. The study material included 50 cervical biopsy samples specimens received by the Department of Pathology.
- Samples were processed as per the guidelines of inclusion and exclusion criteria:

2.1 Study Population and Inclusion Criteria

- Both cone and punch biopsy are included.
- All the cervical biopsy specimens received in the department of Pathology within 2 years of the study period were included in the study.
- Patient irrespective of any age group undergoing cervical biopsy were included in the study.
- Patient who have consented for the study.

2.2 Exclusion Criteria

- Patient already treated.
- Patients not consenting for the study.
- The preparation of the tissues is preserved in formal saline then sent for after H&E stain and Immunohistochemistry stain P16INK4a and after that for light microscopy examination [6,7].

3. RESULTS

The present study of immune histo-chemical expression of p16INK4a on cervical specimens was conducted in the department of Pathology in collaboration with Obstetrics and Gynecology department, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu. A total of 50 cases were studied and the diagnoses are shown in Table. Out of the 50 cases, maximum are inflammatory conditions i.e. 26(52%), mainly chronic cervicitis which comprises 15(30%) cases and malignancies/carcinoma accounted for 2(4%) cases. Premalignant conditions consisting Low grade Squamous Intraepithelial Lesion and Cervical Intraepithelial Neoplasia accounted for 18(36%) cases. Benign conditions accounted for 4(8%). The detailed analysis was presented in Table 1 and Fig. 1.

Out of the 50 cases, there were 2(4%) malignant cases in the study and both are infiltrating non keratinizing squamous cell carcinoma (Fig. 2).

Table 1. Distribution of cases and their percentages

Sl. no.	Diagnosis	Number of cases	Percentage
	A) Carcinoma/Malignant conditions:		
1.	Infiltrating non keratinized squamous cell carcinoma	2	4%
	B) Premalignant conditions:		
1.	Low grade squamous cell carcinoma	14	24%
2.	Cervical intraepithelial neoplasia	4	8%
	C) Benign conditions:		
1.	Dysplastic squamous epithelial cells	2	4%
2.	Endometrial hyperplasia with Nabothian cyst	2	4%
	D) Inflammatory conditions:		
1.	Chronic endocervicitis with Non Specific Reactive Changes in squamous epithelium	1	2%
2.	Chronic non specific cervicitis	2	4%
3.	Chronic cervicitis	15	30%
4.	Chronic papillary cervicitis	3	6%
5.	Chronic papillary endocervicitis with LSIL	3	6%
6.	Non specificendocervicitis	1	2%
7.	Chronic papillary cervicitis with non reactive cervicitis	1	2%
Total		**50**	**100%**

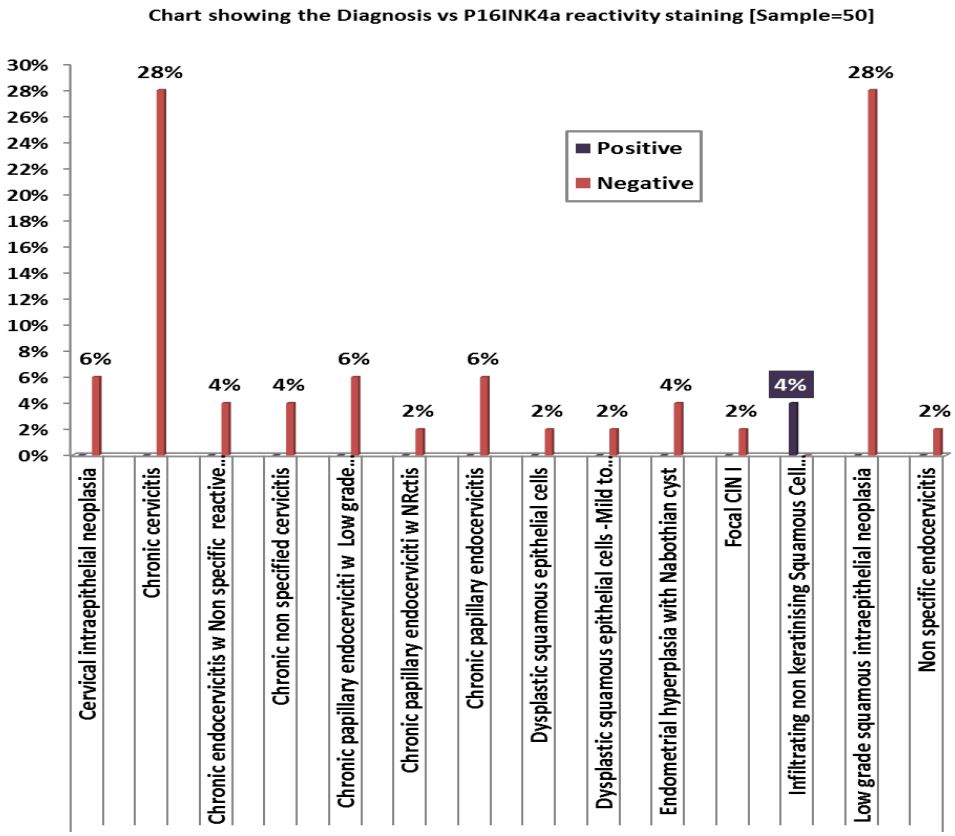

Fig. 1. Case distribution chart

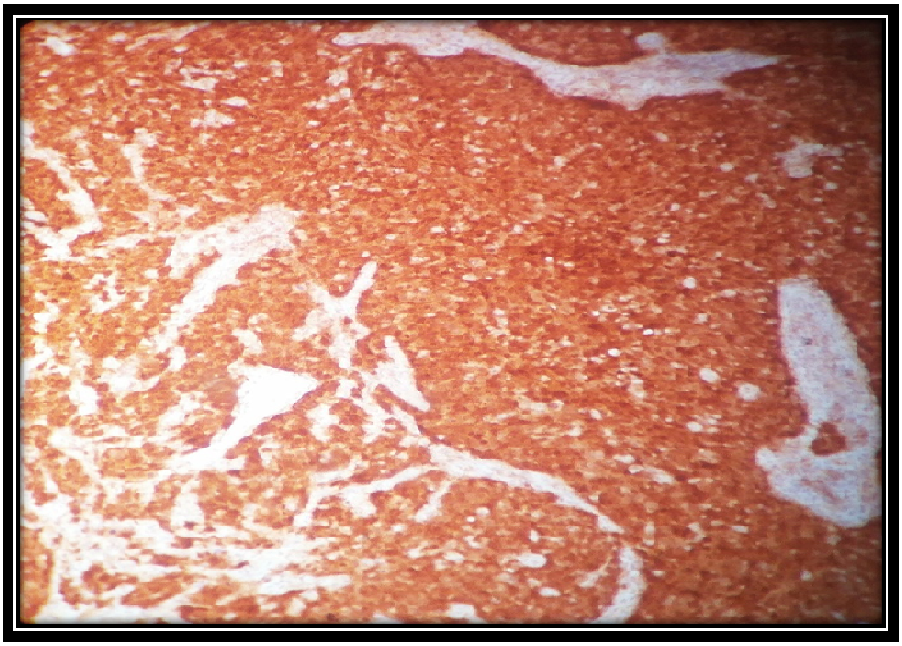

Fig. 2. Infiltrating non keratinizing squamous cell carcinoma showing Cytoplasmic & nucleoli both positive for P16INK4a 40xmagnification

4. DISCUSSION

Cervical cancer is the one of the most common forms of cancer in women worldwide. In my study out of the total number of 50 cases studied, inflammatory conditions comprised of the highest number with 26(52%) cases followed by premalignant conditions and benign conditions with 18(36%) cases and 4(8%) cases respectively. Carcinoma cases were the least having accounted for just 2(4%) cases only.

The earlier studies found that out of 26 inflammatory conditions (mainly chronic cervicitis and its variants).In this study done, non-specific chronic cervicitis showed no expression of p16INK4a WHICH is similar to our study [8].

The study was conducted on 53 formalin fixed and paraffin embedded samples of various stages of cervical neoplastic lesions. There were squamous cell carcinomas in situ of 8 cases, squamous cell carcinoma in situ with glandular involvement of 16 cases, microinvasive squamous cell carcinoma of 13 cases and invasive squamous cell carcinoma of 16 cases. Strong immunoreactivity for the p16INK4a protein was observed in only the nuclei and cytoplasm of all cervical neoplastic cells [9,10].

5. CONCLUSION

This study is to find out p16INK4a immune histochemical expression on cervical biopsy received in our department. It has been observed that most of the cases received were those of inflammatory lesions mainly chronic cervicitis with or without papillary hyperplasia and/or squamous metaplasia. When p16INK4a immune staining of the lesions was done, it showed that the benign lesions, inflammatory conditions did not take up the stain. It was expected that few premalignant lesions may take up the stains but it also showed negative IHC results. Hence, they were considered to be p16INK4a negative.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval was obtained from the ethical committee of Sree Balaji Medical College and Hospital.

ACKNOWLEDGEMENT

The authors thank Sree Balaji Medical College and Hospital for providing the facility for carrying out the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010; 127(12):2893-917.
DOI: 10.1002/ijc.25516
2. The challenge ahead - Progress and setbacks in breast and cervical cancer, Institute for health metrics and evaluation, University of Washington. IHME; 2011. ISBN: 978-0-9840910-3-4
3. Hawley-Nelson P1, Vousden KH, Hubbert NL, Lowy DR, Schiller JT. HPV16 E6 and E7 proteins cooperate to immortalize human foreskin keratinocytes. EMBO J. 1989;8(12):3905-10.
4. Agoff SN, Lin P, Morihara J, Mao C, Kiviat NB, Koutsky LA. p16(INK4a) 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.
5. Izadi-Mood N, Asadi K, Shojaei H, Sarmadi S, Ahmadi SA, Sani S, Chelavi LH. Potential diagnostic value of P16 expression in premalignant and malignant cervical lesions. J Res Med Sci. 2012; 17(5):428-33.
6. Koh J, Enders GH, Dynlacht BD, Harlow E. Tumour-derived p16 alleles encoding proteins defective in cell-cycle inhibition. Nature. 1995;375(6531):506-10.
7. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ, International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003; 348(6):518-27.
8. Jedpiyawongse A, Homcha-Em P, Karalak A, Srivatanakul P. Immunohisto-chemical overexpression of p16 protein associated with cervical cancer in Thailand. Asian Pac J Cancer Prev. 2008; 9(4):625-30.
9. Kanthiya K, Khunnarong J, Tangjitgamol S, Puripat N, Tanvanich S. Expression of p16 and Ki67 in cervical squamous intraepithelial lesions and cancer. Asian Pac J Cancer Prev. 2016;17:3201.
10. Bruni L, Barrionuevo-Rosas L, Albero G, et al. Human papillomavirus and related diseases in India. Summary report. ICO Information Centre on HPV and Cancer (HPV Information Centre). 2016; 3–20.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/54180