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Pathology

IMMUNOHISTOCHEMICAL PROFILE OF HIGH RISK HUMAN PAPILLOMA VIRUS IN PRECANCEROUS LESIONS OF CERVIX

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ABSTRACT

INTRODUCTION: Carcinoma of the cervix is the second most common neoplasm in women world wide. Cervical cancer is related to the human papilloma virus. Viral E6 and E7 proteins are responsible for oncogenic effects of HPV. They promote DNA synthesis in viral affected cells and increase expression of P16. E6 oncogene inhibits apoptosis, inhibits P53 and increases the expression of Ki67 in injured cells.

AIM: To assess the potential use of immunohistochemical markers such as HPV (Panmarker), P16, and Ki67 in grading of intraepithelial lesions and to detect the risk of development of cervical cancer.

Materials and methods: 30 cervix biopsies with intraepithelial lesion were selected and submitted to immunohistochemical study for biomarker HPV (Panmarker), P16 and Ki67.

Results: 33% of CIN I lesions, 22% of CIN II lesions and 11% of CIN III lesions show positivity for HPV panmarker. 50% of CIN I cases and 66.6% of CIN II cases expressed P16. 90% of CIN III cases expressed P16. There was higher expression of Ki 67 in CIN III (66%) lesions than CIN II(44%) and CIN I (16%). P16 marker score was higher in CIN III lesions.

CONCLUSIONS: P16 is a potential biomarker used for the diagnosis of dysplasia, tumor progression and associated with high risk HPV oncogenes. Coexpression of p16 and Ki67 could help in grading of intraepithelial lesions and to detect the risk of developing cervical carcinoma.

KEYWORDS : HPV panmarker, p16, Ki67, cervical intraepithelial neoplasia, HPV

INTRODUCTION:

HPV is a small non enveloped DNA virus and it is a member of Papillomaviridae family. Human papilloma virus infection leads to broad spectrum of benign and malignant neoplastic epithelial changes in human beings. Warts were the earliest known lesions from the ancient Greeks and Romans from which the infectious nature of HPV was recognized.

Human papilloma viral infection plays an important role in the carcinoma cervix. Similarly carcinomas of the oral cavity, esophageal mucosa and larynx, are also caused by this papilloma virus. [1]

MOLECULAR AND ONCOLOGICAL ASPECTS

Human papilloma virus is classified as following categories based on oncogenic potential

a. High risk virus (HRHPV) - 16, 18, 33, 35, 51, 52, 59, 45, 68 and 73. These viruses typically induce malignant lesions and associated with more than 95% of cervical carcinomas. Among these HPV 16 is found in 50 to 70% of malignant lesions and HPV18 is present in 7-20% of malignancies.
b. Low risk virus (LR-HPV) - 6, 11, 13, 32, 42, 54 and 70. LR-HPV were associated with benign lesion such as wart and condyloma of genital tract.[2,3]

MOLECULAR BIOLOGY OF HUMAN PAPILLOMA VIRUS

The human papilloma virus consists of 8 early genes (E1 to E8) and 2 late genes (L1&L2). Early genes regulate viral DNA replication, gene transcription and interact with oncoproteins. Among these E6 and E7 proteins are considered to be important because the expression of these proteins leads to malignant phenotype. In the host cell E6 and E7 interact with tumor suppressor gene products pRb and p53 respectively. This results in cellular immortalization and transformation through interference with cell cycle and control of apoptosis. Above mentioned HPV oncoproteins activate oncogene and inactivate tumor suppressor genes. Viral E6 proteins interfere with p53 and inhibit apoptosis. E7 protein binds with pRb and inhibits complex formation with transcription factor E2F. E7 also binds with CDK inhibitors p21 and p27. Thus E7 protein initiates DNA synthesis and allows the continuous cell growth thereby enhancing the malignant transformation. [2,4]

P16

P16INK4A currently known as p16, is one of the most important biomarkers for human papilloma virus infections. Expressed p16 is considered as potential biomarker for the oncogenic potential of human papilloma virus. Human papilloma virions replicate in viable cells of cervical epithelium leading to overexpression of P16. So it is well established in high grade intraepithelial lesions and invasive squamous cell carcinomas. Increased P16 expression is due to feed back inhibition on pRb protein by HPV E7 protein. [5]

HPV PAN MARKER:

HPV 16 was the most frequent virus detected and HPV 18 was the second most common type. HPV L1 gene is predominantly expressed in nuclei of koilocytes. The higher positivity in intraepithelial lesions could be explained by the fact L1 gene is predominantly expressed in nuclei of koilocytes. There must be correlation of HPV with precancerous and cancerous cervical lesions along with cofactors.[6]

Ki67

Ki67 is a non histone protein that is expressed in the nuclei of cells in all phases of cell cycle except G0 and G1 phase. It has been associated with an increased degree of cervical intraepithelial lesions. Ki67 is also reported as an additional prognostic marker of cervical cancer.[7]

MATERIALS AND METHODS

The present study "Immunohistochemical profile of high risk human papilloma virus in precancerous lesions of cervix" was carried out in the Department of Pathology, KAPV Govt Medical College from march 2014 to February 2015.

The cervical biopsy specimens were collected from 721 clinically suspected cervical dysplasia patients in the age group of 30 to 80 years. The samples were fixed in 10% formalin, histopathological slides were prepared and stained with haematoxylin and eosin stain. All cervical biopsies were screened and 68 cases diagnosed as intraepithelial lesions. These lesions are classified into CIN 1, CIN II, CIN III. Among 68 cases 30 were selected and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemistry for HPV panmarker, P16 and Ki67 expression. The results were recorded and photographed.

IMMUNOHISTOCHEMICAL EVALUATION:

Immunohistochemical analysis of HPV panmarker, P16 and Ki 67 markers were done in paraffin embedded tissue samples for all cases which were diagnosed as cervical intraepithelial neoplasia as CIN I, CIN II, and CIN III. 3 sections were stained with HPV cocktail broad spectrum (HPV -1, 6, 11, 16-16, 18, 31) mouse monoclonal antibody, Anti -P16(INK4) (G175-405) mouse monoclonal antibody Biogenex and one Ki 67 proliferative marker.

The presence of even a single positive nucleus which appears brown colour was considered as positive for HPV panmarker. P16 Positivity

was seen as a chestnut brown reaction product staining the nucleus or cytoplasm or both. At least 10cells stained for P16 considered as positive.

Ki67 immunoreactivity was evaluated taking into the account of positivity of tumor cells and cell layer level of expression. The positivity was seen as dark brown reaction product staining the nuclei.

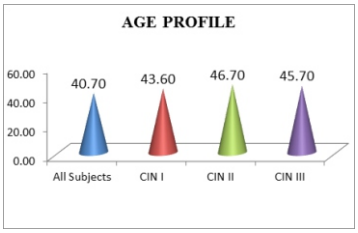
OBSERVATION AND RESULTS

In the study period from March 2014 to February 2015, 721 biopsy specimens were received in the department of pathology, KAPV Government Medical College and Hospital. Among them 68 cases were positive for cervical intraepithelial lesion which constitutes about 9.43%. Among 68 cervical intraepithelial lesions CIN I constitute about 37 cases (54.41%), CIN II 17cases (25%), CIN III 14 cases (20.58%). Among 68 cervical intraepithelial lesions 30 cases were selected for immunohistochemical analysis based on classical histological features, material adequacy. In 30 cases CIN I, II, III were 12, 9, 9 respectively.

AGE PROFILE

The mean age of women undergoing cervical biopsy in KAPV Government Medical College Hospital was 40.7 years. The mean age of patient diagnosed with CIN I was 43.6years, CINII was 46.7 years, and CIN III was 45.7years

CHART NO. 1



HPV PAN MARKER, P16 & KI 67IMMUNO HISTO CHEMICAL STAINING:

4cases of CIN I, 2 cases of CIN II, 1 case of CIN III were positive for immunostaining with HPV pan marker. 50% of CIN I cases, 66.6% of CIN II cases, 88.8% of CIN III cases showed positive immunostaining with P16. 16.6% of CIN I cases, 44.4% of CIN II cases and 66.6% of CIN III cases showed positive results for Ki 67.

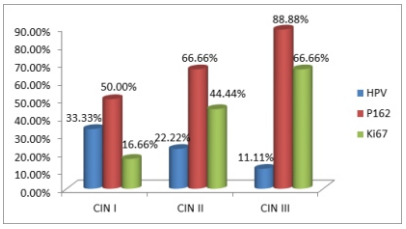
EXPRESSIONS OF HPV PAN MARKER, P16 AND KI67 IN RELATION TO THE GRADE OF CERVICAL INTRAEPITHELIAL NEOPLASIA

Table no 1

Histopathological diagnosis	HPV pan marker	P16	Ki67
CIN I	33.33%	50%	16.6%
CIN II	22.22%	66.66%	44.44%
CIN III	11.11%	88.88%	66.66%

HPV pan marker expression decrease as the lesion progress to high grade and there is significantly increase in expression of P16 and Ki67 in low grade to high grade lesion.

Chart no 2



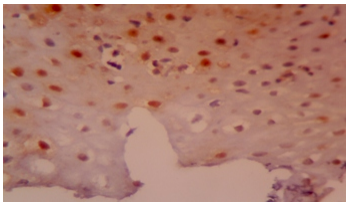


Figure no: 1 Cervical Intraepithelial Neoplasia III (HPV PAN MARKER 400X)

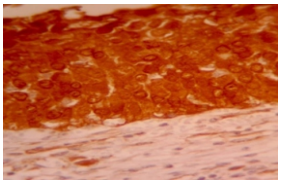


Figure no:2 Cervical Intraepithelial Neoplasia III(p16 100x)

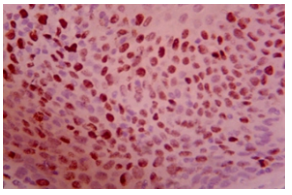


Figure no: 3 Cervical Intraepithelial Neoplasia III (ki67 400 x)

Discussion

Cervical carcinoma is the second most common cause of death among women in developing countries. There are many documented evidences for the association of human papilloma virus infection with cervical carcinoma. Among cervical carcinomas, the major type is squamous cell carcinoma preceded by intraepithelial precursor lesion. Only a fraction of intraepithelial lesions are progress to invasive carcinomas in which HPV is a strong factor for progression. So, identifying high risk HPV in cervical biopsies has implications in follow up protocols and for prophylactics in HPV vaccine trials. High grade cervical intraepithelial lesions are usually diagnosed among women of 25 to 35 years of age whereas invasive cancer is most commonly diagnosed after the age of 40yrs typically after 8 to 13 yrs after diagnosis of high grade lesions.[8]

In developing nations mean age for intraepithelial neoplasia was 37.6 yrs. In the current study total numbers of cases sampled were 721. The mean age of women undergoing cervical biopsy in our institute was 40.7 years. The mean age of patients diagnosed with CIN I was 43.6 years, for CIN II, 46.7 years and for CIN III, 45.7 years.[9]

HPV PAN MARKER IMMUNOHISTOCHEMISTRY IN CERVICAL INTRAEPITHELIAL LESION:-

The presence of viral infection was evidenced by strong nuclear expression of viral infection marker HPV 6, L1 capsid protein in all precancerous lesions. 30% of CIN I cases, 10% of CIN II cases, 13.3% of CIN III lesions showed nuclear positivity.[10]

HPV expression decreases as the lesion progress to high grade. This may be due to L1 capsid protein being highly expressed in nuclei of koilocytes and its expression limited more towards well differentiated cells. So, its expression is more in low grade lesions.[6]

P16 IMMUNOHISTOCHEMISTRY AND HIGH RISK HUMAN PAPILLOMA VIRUS:

P 16 expression underlies a negative feedback control through pRb. Reduction or loss of pRb function through HPV E7 results in increased expression of P16. Thus P16 represents specific and sensitive biomarker for cells with active expression of HPV oncogenes. P16 immunomarker is also useful to identify tumor progression because P16 overexpression correlates with the grade of the lesion. Thus P16 is used to detect the low grade lesion with increased risk for progression to high grade lesions and invasive carcinomas.

P16 can also be useful to differentiate precancerous lesions from reactive and hyperplastic lesions. They also concluded that staining pattern also correlate with the type of human papilloma virus. Begum et al concluded that strong and diffuse cytoplasmic and nuclear staining indicates the presence of high risk human papilloma virus in precancerous lesions of cervix.

Occasionally p 16 may show negative expression due to P16 gene mutation, deletion, or hypermethylation.

It may also due to following factors,

1. HPV inactivated by host immune surveillance

2. The infection could be very recent
3. Defective virus.

P16 gene inactivation is rare in cervical carcinogenesis supporting the hypothesis of P16 up regulation is a consequence of high risk HPV infection.[11]

HPV immunostaining has less sensitivity to detect viral infection when compared to P16 which is a highly sensitive marker for high risk HPV infection. In our study, 43.3% of cervical intraepithelial lesions that were negative for HPV showed positivity for P16 immunomarker.[12]

In our study P16 expression in CIN I, CIN II and CIN III were 33.33%, 44.4%, and 77.7% respectively. Among 30 cases, 9 cases showed strong positivity that may be associated with high risk HPV virus. . Significantly increased P16 expression in HSIL indicates integrated state of high risk HPV in the lesion leads to E7 over production and the subsequent P16 overexpression.[13]

KI67 EXPRESSION IN CERVICAL INTRAEPITHELIAL LESIONS:

Cell proliferation can be assessed by the expression of cell proteins such as PCNA, and Ki67 proliferation marker. 6% of CIN I cases, 44.4 % of CIN II cases, 66.6% of CIN III cases were positive for Ki67 proliferation index. These cases with ki67 expression may progress to subsequent high grade lesions. But Ki67 use as a potential marker is not conclusive because it is expressed in all proliferating cells. We can overcome this difficulty by correlation with P16 expression.[7,14]

Coexpression of P16 and Ki67 could help in the differential diagnosis between intraepithelial lesions and it may be a good marker to detect risk of developing cervical carcinoma in women infected by HPV.

CONCLUSION

HPV pan marker expression decreases as the lesion advances to high grade because of loss of differentiation. Similarly it does not correlate with P16 expression levels. Hence, HPV panmarker is not much useful in a study about high risk HPV types as per literature. Whereas P16 is a potential biomarker used for diagnosis of dysplasia, tumor progression and associated high risk HPV oncogenes. Thus P16 immunomarker can be used for planning of screening program to identify group of patients at risk in LSIL phase. However further studies have to be conducted in future to assess the progression of P16 positive lesions for a period of 5-10 years and identify the specific viral type using specific strain marker in Indian population.

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