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P16INK4a Expression of Uterine Cervical Biopsy Specimen in SBMCH

Tongbram Soni Devi[1] and Mary Lilly[1*]

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

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Introduction: The cervix is the lower portion of the uterus which connects this organ to the vagina through the endocervical canal. It is divided into the vagina (portiovaginalis) and one that lies above the vaginal vault (supravaginal portion). The outer surface of the portiovaginalis is known as the ectocervix or exocervix and the portion related to the endocervical canal corresponds to the endocervix. Carcinoma cervix accounts for the most common form of cancer by 13% of all the women affected cancers. The P16INK 4a is a tumour suppressor protein which is a CDKN2A gene product, an inhibitor of cyclin dependent kinase (CDK) 4 and 6 which is encoded by tumour suppressor gene INK 4a.
Objective: The present study is to evaluate the P16INK 4a expression in cervical biopsy specimens and to correlate it with the histopatholgical findings.
Materials and Methods: Cervical biopsy specimens were processed using rotary microtome and tissue blocks cut in sections of 3-5 µm was taken.

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

Results: P16INK4a expression on cervical biopsy specimen showed that those with inflammatory cervical histology, benign lesions of the cervix along with premalignant lesions of cervix are negative for P 16INK4a and all malignant lesions are P 16INK4a positive.
Conclusion: Hence P16INK4a immunoreactivity may be used for the diagnosis of neoplastic lesions of the cervix. But in our study, only a small size sample was positive. So, P16INK4a is used as one of the valuable markers for diagnosing neoplastic lesions of the cervix.

Keywords: Portiovaginalis; P16INK 4a; CDKN2A; carcinoma cervix; cyclin dependent kinase.

1. INTRODUCTION

Carcinoma cervix accounts for the most common form of cancer by 13% of all the women affected cancers [1]. In India, 1 in 53 Indian women are usually affected in the developed countries, 1 in 100 women are approximately affected [2]. Cervical Intraepithelial neoplasia (CIN), which is described by abnormal growth/dysplasia of squamous cells in the surface epithelium of cervix is a premalignant condition [3]. Human papilloma virus is the most important causative organism in the development of cervical cancer. They are transmitting sexually especially the high risk HPV types – 16,18,33; 16 and 18 mainly and then chronic infection causing CIN [4,5]. HPV 16 and 18 are the main sub types of papilloma virus causing cervical cancer and the other types are 45, 31,33,45,51,52,58 and 35.

HPV 31,33,35,45,52 and 58 can be taken into account after 16 and 18 for global cervical carcinogenesis of 20 % [6]. There are also other various subtypes around 200 and if left untreated, few cases developing to cervical cancer only after a long standing period usually within 5 to 10 years which persistent infection may be causing it. HPV being detected in all cases of cervical dysplasia and neoplasia cases, is a crucial factor [7] High risk HPV encodes twice known viral oncogenes, E6 and E7 in the association of cervical cancer?

E6- E6 inhibits the function of p53 indicated DNA damage and apoptosis pathway. It does not directly associate with p53 but work by forming a complex cellular E6-AP protein which is necessary 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 is a protein which controls the response to cellular stress including DNA damage and viral infection [7,8].

E7- E7 inhibits the function of tumour suppress 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 causing 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 [7,8,9].

1.1 P16INK 4a

The P16INK 4a is a tumour suppressor protein which is a CDKN2A gene product , an inhibitor of cyclin dependent kinase (CDK) 4 and 6 which is encoded by tumour suppressor gene INK 4a which normal function is to prevent cells from dividing in the absence of an in appropriate signal.

HPV transformed cervical cancer cells are dependent on the individual HR-HPV type and indicative of the carcinogenesis process. P16INK inhibits the cyclin dependent kinase by 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 [10,11,12,13].

Immunostaining of p16 INK 4a overexpression allowed specific and accurate negligible cases of CIN or cervical dysplasia in biopsy which allow improvement of diagnosis of cervical cancer [10].

2. MATERIALS AND METHODS

Hospital based cross sectional study was followed. The study was carried out in the Department of Pathology in collaboration with the Department of Obstetrics and Gynecology, Sree Balaji Medical College and Hospital (SBMCH), Chromepet.

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 consent for the study.

2.2 Exclusion Criteria

Patient already treated and Patients not consenting for the study

Samples were processed as per the guidelines of inclusion and exclusion criteria. The preparation of the tissues preserved in formal saline for light microscopy involved the following steps. The specimens received were fixed in 10% formal saline. The tissue pieces were wrapped in filter paper and put inside the tissue basket and was dehydrated with descending grades of ethyl alcohol and cleared in xyelene in automated tissue processor. After clearing, the tissue was impregnated with molten paraffin and leuckhart's L-block.

With the help of rotary microtome, the tissue blocks were cut in sections of 3-5 µm. The sections were put into flotation bath and subsequently transferred to albuminised glass slide and placed in hot oven. Sections were treated in xylene to deparaffinised. Xylene was washed off with graded with graded alcohol for rehydration and was washed in water.

3. RESULTS

The present study on the histomorphology of cervical specimens and their relationship with the immunohistochemical expression of p16INK4a was conducted in the department of Pathology in collaboration with Obstretics and Gynaecology department, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu. A total of 50 cases were studied and the diagnosed. 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%).

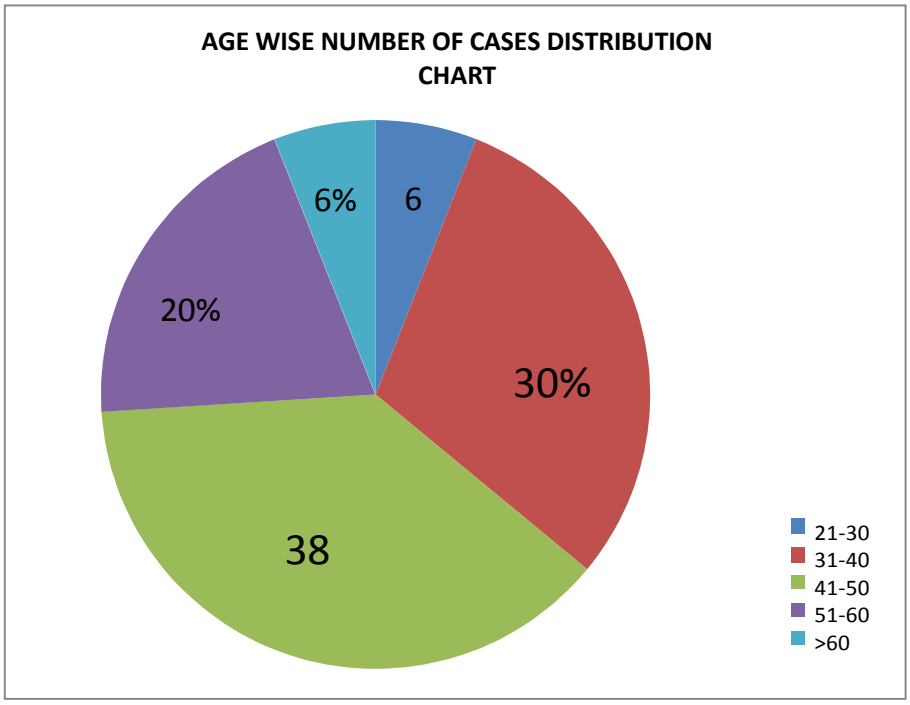

Fig. 1. Age (in years) wisenumber of cases distribution chart

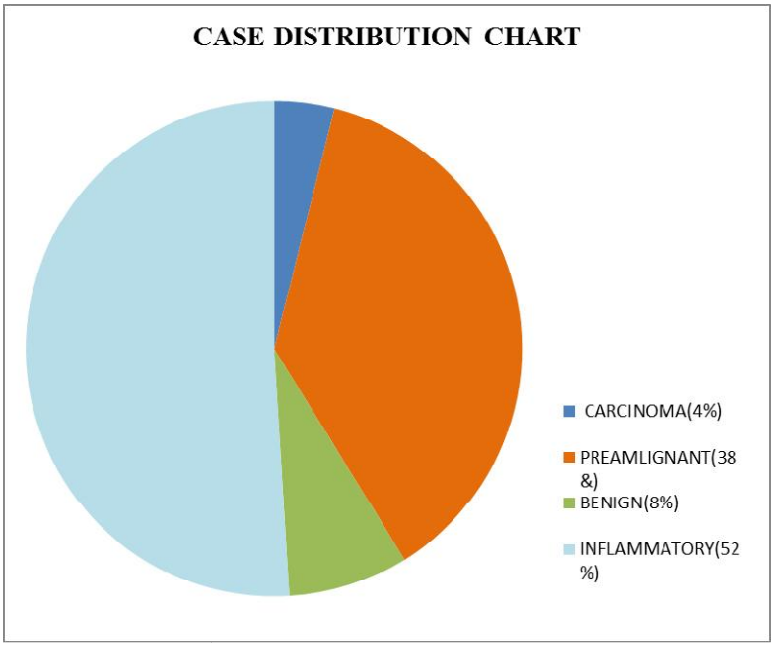

Fig. 2. Case distribution chart

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.

4. DISCUSSION

Cervical cancer is the one of the most common forms of cancer in women worldwide. Many studies have shown that human papilloma virus (HPV) infection plays an important role in cervical carcinogenesis. In fact, HPV infection has been detected in almost all preneoplastic and neoplastic lesions of the cervix and is a critical factor in nearly all cases of cervical cancer [15,16,17].

The p16/cyclin D1/cdk4/pRb cell cycle regulatory cascade is central to regulation of the G1 to S phase transition and to understanding human cancers. HPV infection is critically involved in cervical carcinogenesis and plays an important role in the p16/cyclin D1/cdk4/pRb cell cycle regulatory cascade by binding of its oncoproteins E6 and E7 to tumour suppressor genes p53 and pRb respectively. In cervical cancers, loss of p53 function is the most frequently observed and best characterized epigenectic alteration caused by p53 protein degradation as a result of HPV E6 protein binding. These p53 alterations have been shown to influence the p16/cyclin D1/cdk4/pRb cell cycle regulatory cascade indirectly by altering p21 function. In the mentioned regulatory cascade, the correlation between pRb and p16 is obvious in various cancers. Various cancers with mutation or deletion of the Rb gene show overexpression of p16 and a reciprocal correlation between pRb and p16 has been established [18,19].

This study is to find out the correlation of p16INK4a immunohistochemical expression on various histological lesions received in our department. It has been observed that most of the cases received were those of inflammatory lesions mainly chronic cervicitis with or withoutpapillary hyperplasia and/ squamous metaplasia. These lesions had inflammatory cell infiltration with few having Nabothian cyst. Some of the lesions had papillary hyperplasia of the endocervix and few had squamous metaplasia. When p16INK4a immunostaining of the lesions were 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 [19].

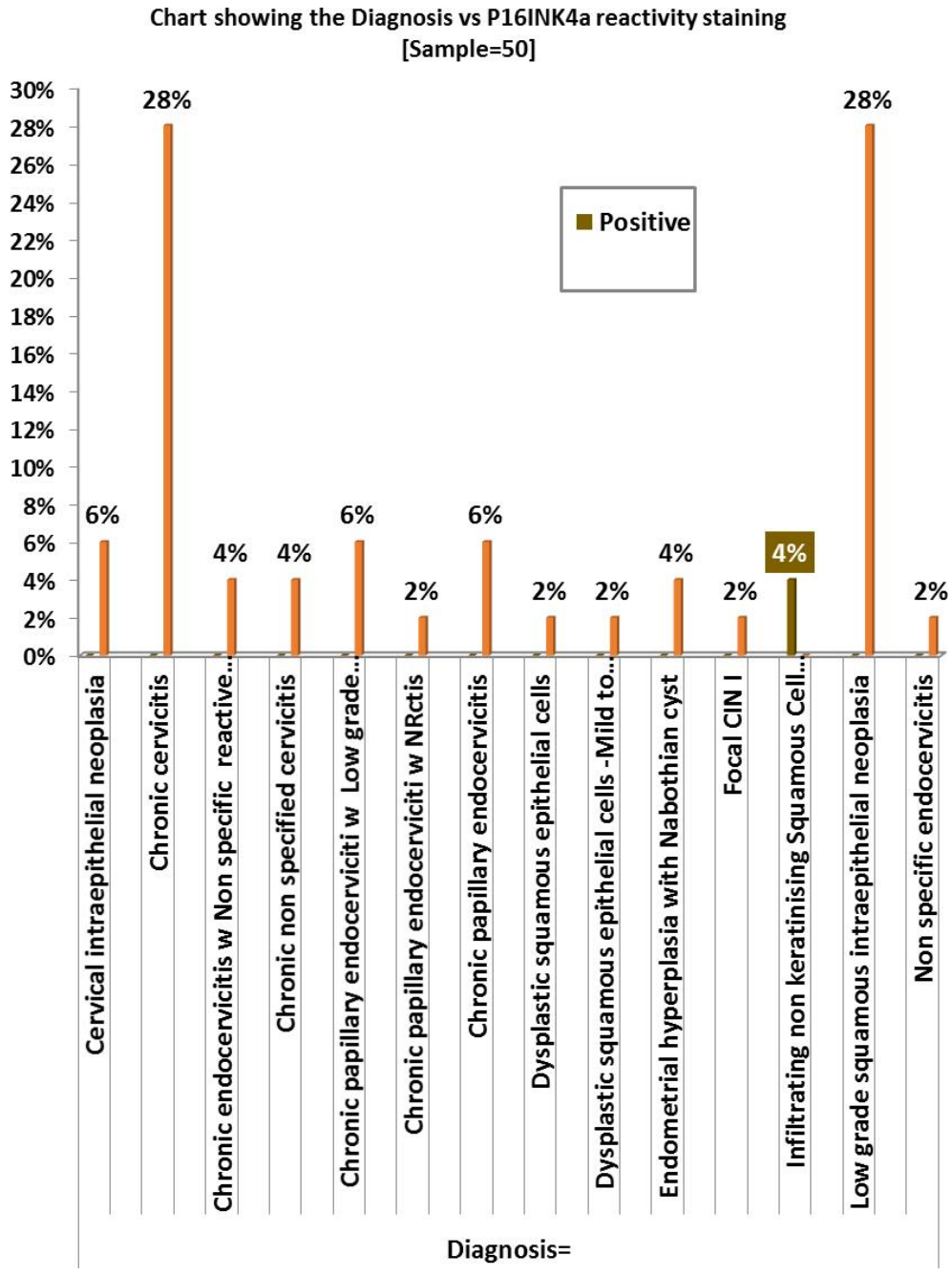

Fig. 3. Chart showing the diagnosis vs p16INK4a reactivity staining [14]

Sano et al conducted a study on the expression status of the p16INK4a protein associated with HPV oncogenic potential in cervical and genital lesions. To clarify the relationship between p16 overexpression and HPV infection in cervical carcinogenesis, immunohistochemical analysis of p16INK4a and detection of HPV by in situ hybridization and polymerase chain reaction were performed on 139 formalin fixed & paraffin embedded samples of cervical & genital condylomatous and neoplastic lesions [20].

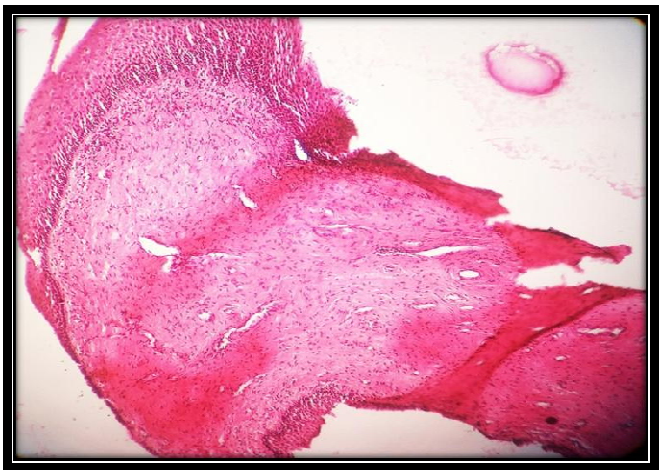

Fig. 4. Hyperplastic epithelium H&E 10x magnification

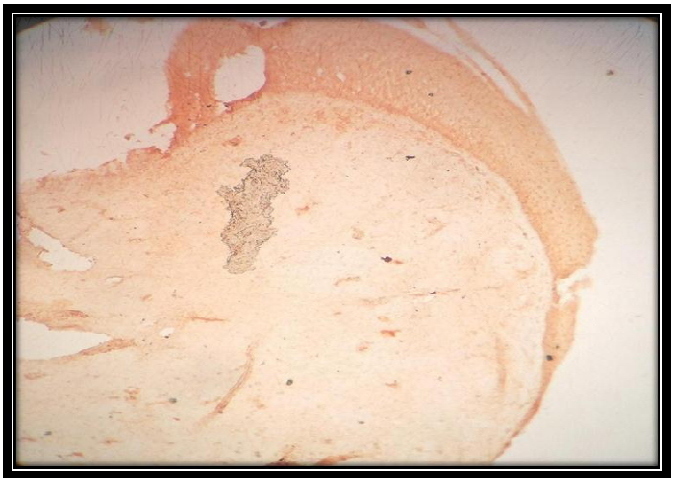

Fig. 5. Hyperplastic epithelium IHC stain P16INK4a negative 10xmagnification

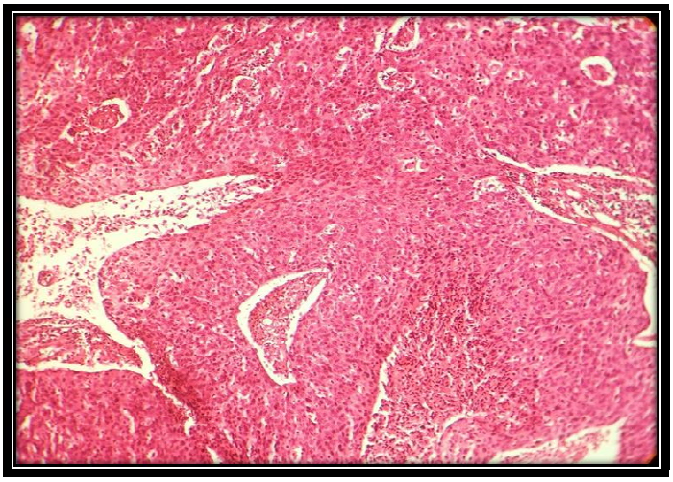

Fig. 6. Infiltrating non keratinizing squamous cell carcinoma H&E 40xmagnification

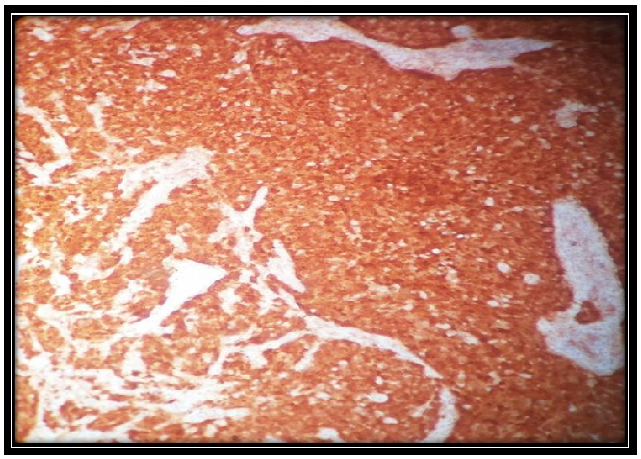

Fig. 7. Infiltrating non keratinizing squamous cell carcinoma showing Cytoplasmic &nucleoli both positive for P16INK4a 40xmagnification

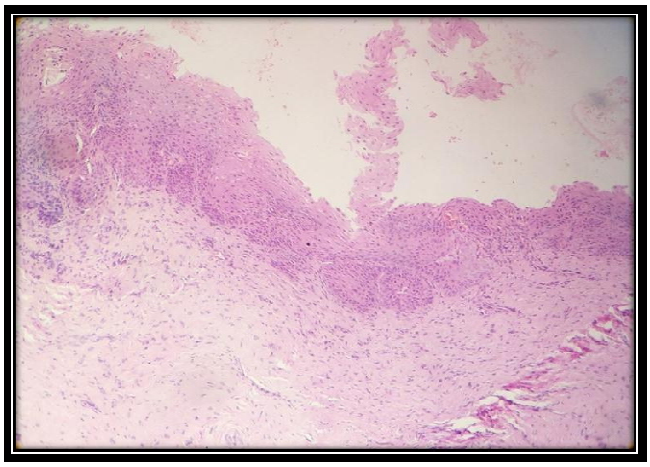

Fig. 8. Dysplastic epithelium CIN I H&E 10x magnification

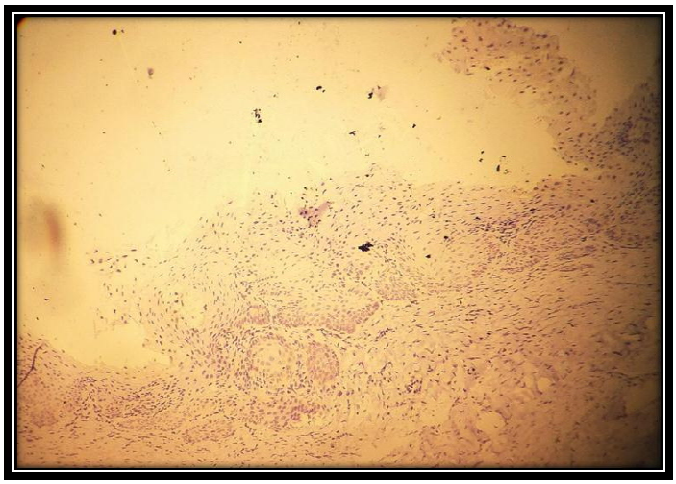

Fig. 9. Dysplastic epithelium CIN I IHC (P16INK4A) negative 10x magnification

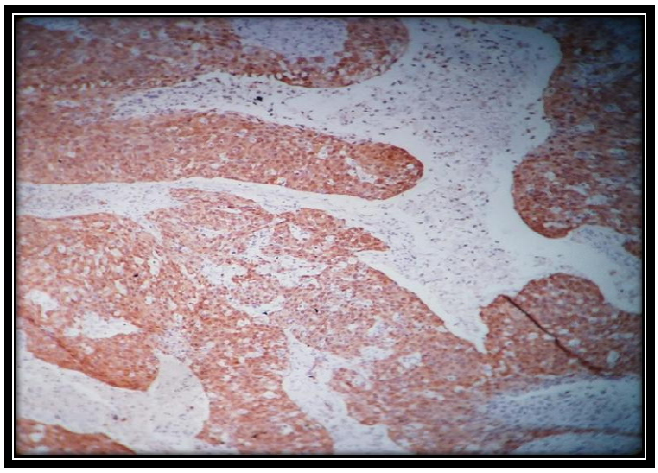

Fig. 10. P16INK4a Positive in low power 10xmagnification

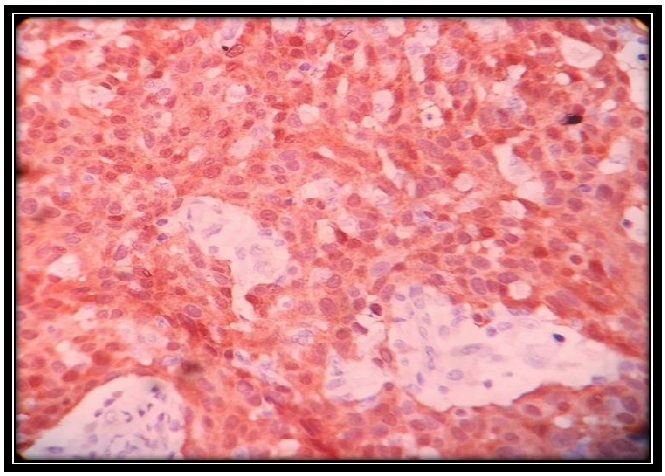

Fig. 11. P16INK4a Positive in high power 40 x magnification

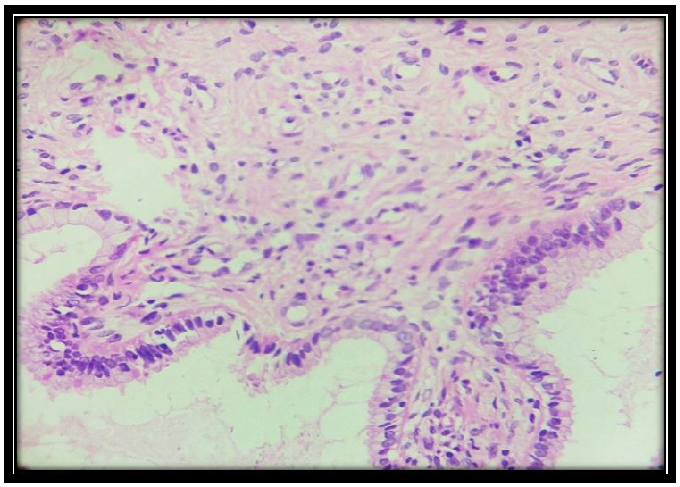

Fig. 12. Chronic papillary endocervicitis H&E 40xmagnification

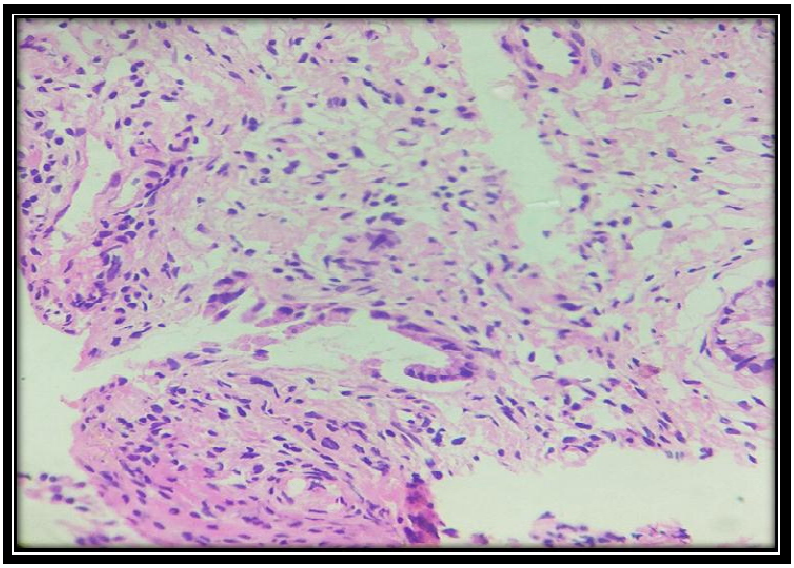

Fig. 13. Chronic papillary endocervicitis H&E 40xmagnification

Marked overexpression of p16INK4a protein, i.e. diffuse and strong immunostaining was observed in all cervical cancers and preneoplastic lesions with infection by high and intermediate risk HPVs i.e. subtypes 16,18,31,33,52 and 58.

Volgareva G et al. in their study—Protein p16INK4a as a marker of dysplastic and neoplastic alterations in cervical epithelial cells‖ found that in control samples which consisted of vaginal smears of healthy women and biopsy samples of cervical ectopia were negative for p16 INK4a. Overexpression of p16INK4a was detected in samples of cervical dysplasia (CINs) & carcinomas [21].

Jedpiyawongse A et al conducted a study 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. The specimens were taken from the cervical biopsy, cervical conization & hysterectomy in the year 2000 at National Cancer Institute. Strong immunoreactivity for the p16INK4a protein was observed in only the nuclei and cytoplasm of all cervical neoplastic cells [22].

Horn yet al conducted a study on 247 punch biopsies and 249 cone biopsies whose initial assessment was based on H&E slides only. Inclusion of a p16INK4a stained slide in the evaluation after a washout phase led to a significant increase in inter observer agreement for both punch and cone biopsies (from 0.49 to 0.63 to 0.70 respectively).

Tsoumpou et al. performed a meta-analysis of 61 published studies on the correlation of the p16INK4a immunostaining to the degree of cytological or histological abnormality. They reported that the proportion of cervical smears over expressing p16INK4a increased with the severity of cytological abnormality. In histology only 2% of normal biopsies and 38% for CIN I showed staining for p16INK4a compared to 68% of CIN II and 82% CIN III.

Munoz et al. 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.

Wang et al conducted a study on p16INK4a and p14ARF expression pattern by immunohistochemistry in human papilloma virus related cervical neoplasia. Serial consecutive biopsies representing normal cervical epithelium to cervical intraepithelial neoplastic &/or invasive cervical cancer were stained with immunohistochemistry for p16INK4a, p14ARF & proliferating cell nuclear antigen. The positive rates of these markers were significantly highest

in cervical intraepithelial neoplasia and in squamous cell carcinoma [23].

Klaes et al. in their study of p16INK4a in cervical biopsy specimens, reported no significant p16INK4a immunoreactivity in inflammatory lesions and reserve cell hyperplastic changes without concurrent evidence of dysplasia. It also helped in discriminating reactive inflammatory changes from the presence of dysplasia in the company of inflammation [24].

5. CONCLUSION

P16INK4a expression on cervical biopsy specimen showed that those with inflammatory cervical histology, benign lesions of the cervix along with premalignant lesions of cervix are negative for P 16INK4a and all malignant lesions are P 16INK4a positive. Hence P16INK4a immunoreactivity may be used for the diagnosis of neoplastic lesions of the cervix. But in our study, only a small size sample was positive. So, P16INK4a is used as one of the valuable markers for diagnosing neoplastic lsisons of the cervix.

CONSENT

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

ETHICAL APPROVAL

Study was approved from the Institutional Ethics Committee (IEC), SBMCH was taken before starting the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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