Original Research Article

Histomorphological spectrum of small cervical biopsies with p16 staining in pre invasive lesions: A seven year retrospective analysis

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ABSTRACT

Introduction: Uterine cervix is a common site for many neoplastic and non-neoplastic lesions. Non-neoplastic lesions include acute and chronic cervicitis, hyperplasia, polyps and cysts. Neoplastic lesions include pre invasive lesions and cervical cancer. Cervical cancer is the second most common cancer in Indian women after breast cancer. Histo pathological studies of cervical lesions helps in early diagnosis and proper management of patients. Various biomarkers are available for diagnosis of HPV in pre invasive lesions of cervix.

Aims : To analyse the histo pathological spectrum of cervical lesions and their relation with demographics and clinical presentation in small cervical biopsies. Our aim was also to study the role of p16 immunohistochemistry on a small subset of pre invasive lesions of cervix.

Materials and Methods : This is a hospital based seven year retrospective study of 279 cervical punch biopsies performed on patients from January 2013 to December 2019. Clinical history, demographic details, histopathology (H&E) slides and reports of cervical punch biopsies of the patients were retrieved from past records and analyzed. Six cases of pre invasive lesions of cervix were studied using p16 immunohistochemistry.

Results : The age range of various cervical lesions was 21 to 70 years with a mean age of 45.5 years. The most common age group affected in non- neoplastic lesions was 30-40 years and among neoplastic lesions it was found to be in 40 to 50 years.

White discharge per vaginum was the most common complaint seen in122 (43.7%) cases, followed by backache in 50 (17.9%), abdominal pain in 37 (13.2%). Bleeding per vaginum was noted in 60 (21.5%) cases and post coital bleeding was reported in 10 cases (3.5%)

On histopathological examination, 163 cases (58.4%) were non-neoplastic, 68 (24.37%) were pre-invasive and 48 (17.2%) cases were malignant.

The most common non-neoplastic lesion was endocervical polyp reported in 90 biopsies. Among neoplastic cases, LSIL was seen in 30 (12.1%) HSIL in 37 (13.5%) and condyloma in 1 case (0.35%). Squamous cell carcinoma was diagnosed in 44 cases (15.2%). Adenocarcinoma was seen only in 4 cases (1.4%).

The percentage positivity of p16 staining on six biopsies, was 33.3% in LSIL and 66.6% in cases of HSIL. Invasive squamous cell carcinoma was taken as a positive control.

Conclusion : Histopathological diagnosis of cervical punch biopsy is a valuable tool in early diagnosis of neoplastic, pre-invasive and non-neoplastic lesions of cervix.Immunostaining with p16 can be a useful adjunct to routine histopathology in diagnosis of preinvasive lesions of cervix.

1. Introduction

Cervix uteri is a Latin word meaning neck of the uterus. Cervix consists of ecto cervix, endo cervix and

a transformation zone or the squamocoloumnar junction. This zone is susceptible to numerous microrganisms, and is a common site of squamous metaplasia, dysplasia, intraepithelial lesions and cervical cancer.[1]

Nonneoplastic cervical lesions are mainly acute and chronic cervicitis, cervical erosions, polyps, hyperplasia and cysts. The infective etiology is commonly due to staphylococcus, N. gonocooci, Trichomonas vaginalis, Clamydia, Candidia to Herpes simplex (HSV), human papilloma virus (HPV) and even HIV.[1]

Among the neoplastic lesions, in India, Cervical cancer contributes to approximately 6-29%of all cancers in women.[2] It is the second most common cancer seen after breast cancer Epidemiological and molecular studies have shown that HPV is the causative organism for condylomas, CIN-I-III and eventually cervical cancer. PCR, hybrid capture assay and p16 IHC are now commonly used to diagnose HPV infections.[3]

Immunostaining with p16 plays an important cooperative role with histopathology and helps in reducing the inter and intraobserver variability in diagnosis of preinvasive lesions of cervix.[4]

Cervical punch biopsies are used widely in gynaecology practice. They are less expensive and average time taken is 2 to 3 minutes.[5] Histopathology provides a final diagnosis and forms the basis of treatment plan. It not only serves as a gold standard for quality control of cytology and colposcopy, but is also a valuable source of diagnostic data for cancer registry. It is very helpful in evaluation of screening programs.

The present study was undertaken to analyse the histopathological spectrum of various cervical lesions across various age groups and their clinical correlation in past seven years. It also aimed at studying the role of p16 immunohistochemistry in subset of pre invasive lesions of cervix.

2. Materials and Methods

This seven year Hospital based retrospective study was conducted in Department of Pathology from January 2013 to December 2019. Approval from the Ethical committee was taken. Clinical history, demographic details, histopathology slides and reports of cervical punch biopsies of the patients of 279 patients were retrieved from past records and analyzed and wherever necessary new sections were prepared and stained with Haematoxylin and Eosin stain. The cervical lesions were classified according to WHO classification of tumours of cervix.[6]

Six paraffin blocks, 3 each reported as LSIL and HSIL were analysed for p16 immuno histochemical staining.The standard guidelines of IHC staining of the institute were followed. p16 positive squamous cell carcinoma of cervix served as the positive control. The staining pattern (focal or diffuse), percentage positivity and intensity of the staining

were observed.

A Scoring system proposed by Vinyuvat S et al[7] was followed:

A. Percentage of positive cells: 0: 80% positive cells

B. Intensity of reaction: 0: No reaction, 1: weak reaction, 2: variable (both weak and strong) reaction, 3: Strong reaction

C. Cellular reaction pattern: 0: No reaction, 1: Focal reaction, 2: Diffuse reaction.

A diffuse reaction was defined as a positive reaction greater than 1 low power field (100x magnification).

A total score was then given on the basis of all three parameters with a range of 0 to 8.

3. Results

The most common age group involved in non- neoplastic lesions was 30-40 years and among neoplastic lesions it was found to be 40-50 years (Tables 1 and 2).

Table 3 White discharge per vaginum was the most common presentation seen in122 (43.7%) cases, followed by backache in 50patients (17.9%). Abdominal pain was reported in37 (13.2%) cases. Bleeding per vaginum was noted in 60 (21.5%) cases and lastly post coital bleeding was observed in 10 cases (3.5%).

On histo pathological examination, 163 cases (58.42%) were non-neoplastic, 68 (24.37%) were found to be pre-invasive and 48 (17.20%) cases were malignant.

Among non-neoplastic lesions which includes both inflammatory and tumor like lesions, endo cervical polyps were seen in 90 cases (32.2 %), followed by 34cases (12.1%) of chronic nonspecific cervicitis, 24 cases of chronic cervicitis with koliocytosis (8.6%). Only 10 (3.5%) were chronic nonspecific cervicitis with squamous metaplasia and only 3 cases of chronic cervicitis with micro glandular hyperplasia (1.07%) were reported.

We divided the neoplastic into pre invasive intraepithelial lesions and invasive lesions. LSIL was seen in 30 (10.7%) HSIL (CINII &CIN III) in 37cases (13.2%) and condyloma in 1 case (0.35%). Squamous cell carcinoma was diagnosed in 44 cases (15.2%). Adenocarcinoma was seen only in 4 cases (1.4%) (Tables 4 and 5).

Squamous cell carcinoma was most common neoplastic lesion.

The frequency of Cervical Intraepithelial neoplasia (CIN) in this study was 24.3%with majority of cases falling in the age group of 31-40 years, followed by age group of 41-50 years. All the intraepithelial lesions found were squamous in origin. Squamous cell carcinoma was the commonest malignancy

4. Discussion

In the present study, age of patients ranged from 21 years to 70 years with a mean age of 45.5 years, Sriknath S,Omoniyi

Table 1: Age wise distribution ofnon-neoplastic cervical lesions

Age (Years) Lesion	21-30	31-40	41-50	51-60	61-70	Total
Chronic nonspecific cervicitis	11	13	08	01	01	34
Chronic cervicitis with microglandular hyperplasia	00	02	01	00	00	03
Cervicitis with squamous metaplasia	06	04	02	0	0	12
Endocervical polyp	34	35	14	05	02	90
Chronic cervicitis with koiliocytosis	02	14	07	01	00	24
Total	53	68	32	07	03	163

Table 2: Age wise distribution of pre invasive and neoplastic Cervical Lesions

Lesion	21-30	31-40	41-50	51-60	61-70	Total
CIN I (LSIL)	04	12	10	02	02	30
CIN II (HSIL)	04	10	04	01	01	20
CIN III (HSIL)	00	05	07	04	01	17
Squamous cell carcinoma	00	04	21	18	01	44
Adenocarcinoma				3	1	04
Condyloma		01				01
TOTAL	08	32	42	28	5	116

Table 3: Distribution of cases in relation to symptoms

Chief Complaints	No. of cases	Percentage
White discharge per vaginum	122	43.72
Bleeding per vaginum	60	21.50
Backache/	50	17.92
Abdominal pain	37	13.26
Postcoital bleeding	10	03.58

Table 4: Spectrum of non-neoplastic lesions

Lesion	Number of cases	Percentage from total cases
Endo cervical polyp	90	3.2
Chronic nonspecific cervicitis	34	12.1
Chronic cervicitis with koiliocytosis	24	8.6
Cervicitis with squamous metaplasia	12	4.3
Chronic cervicitis with microglandular hyperplasia	03	1.07
Total	163	58.4

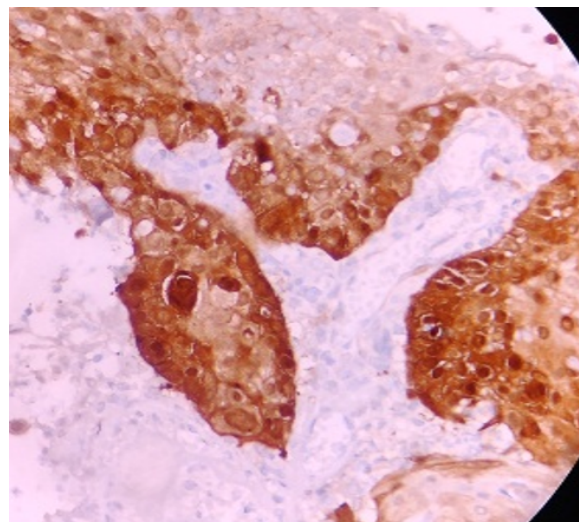**Table 5:** Spectrum of pre invasive and invasive neoplastic lesions

Lesion	Number of cases	Percentage from total cases
CIN I (LSIL)	30	12.18
CIN II (HSIL)	20	7.52
CIN III (HSIL)	17	6.09
Condyloma	01	0.358
Squamous cell carcinoma	44	15.77
Adenocarcinoma of cervix	04	01.43
Total	116	43.4

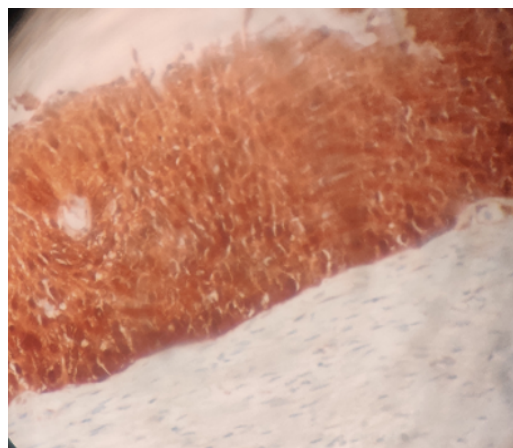**Table 6:** Showing % positivity in six cases.

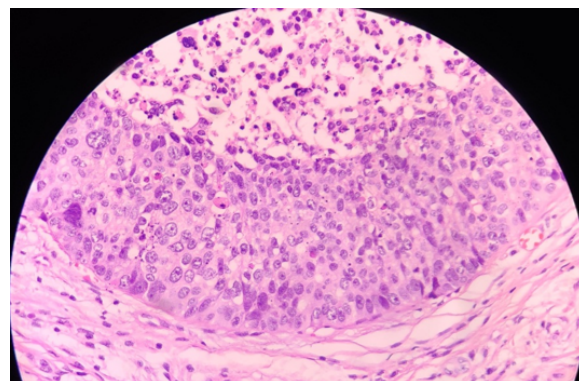| Preinvasive lesion	%positivity
LSIL	33%(1/3)
HSIL	66%(2/3)
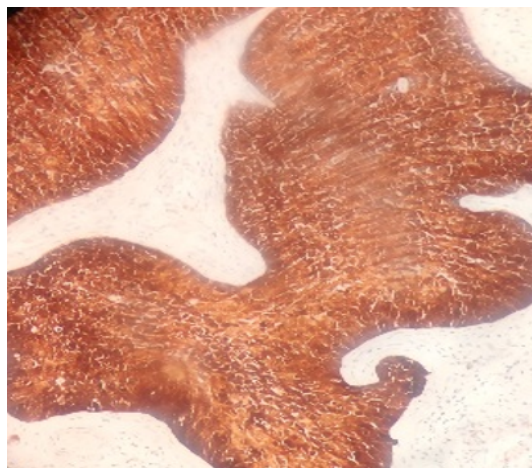
Fig. 1: p16 staining in Invasive carcinoma of cervix(positivecontrol)
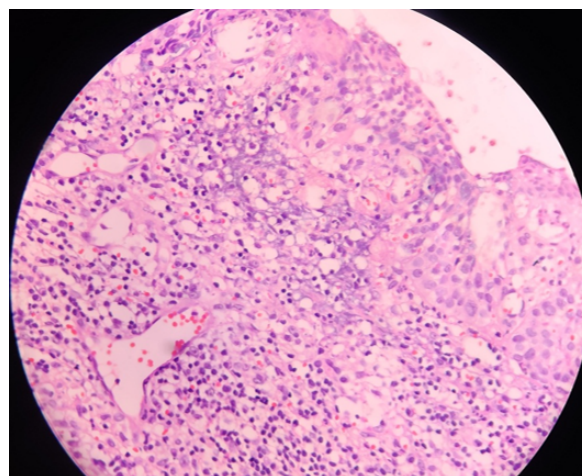

Fig. 2: P16 staining in HSIL

Fig. 3: P16 staining in koiliocytosis

Fig. 4: P16 staining in LSIL
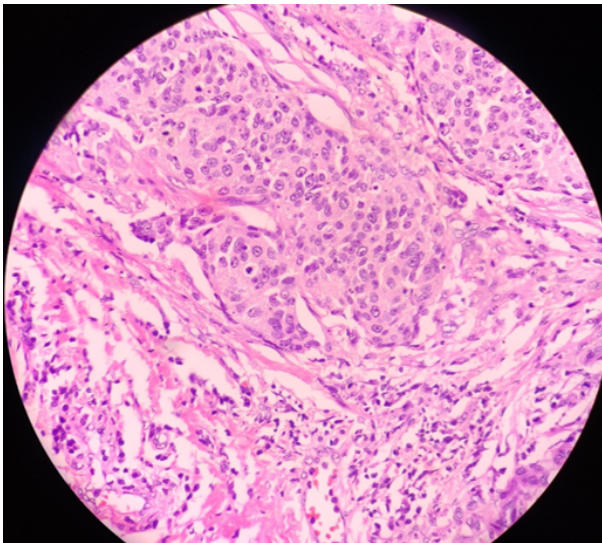

Fig. 5: H&E Section showing HSIL

Fig. 6: H&E Section showing LSILand koiliocytsis

Fig. 7: H&E section showing Squamous cell carcinoma of cervix

–Esan et al and Nwachokor F N, also found the peak age incidence of 40 to 49 years in their studies on non-neoplastic lesions of cervix. [8–10]

The commonest presenting symptom was white discharge per vagina 122 (43.7%). It is the second most common complaint after menstrual disorders. One out of ten women complain of vaginal discharge over a year. [11] Most common microbial causes of abnormal white discharge are bacterial vaginosis, trichomoniasis and candidiasis, mixed infections of candida and trichomonas.

Among the non-neoplastic lesions of cervix, endo cervical polyp was the most common lesion seen in 90(32.2%) of total cases. Endocervical polyps are common benign proliferations composed of fibro vascular core and endocervical glandular or metaplastic squamous epithelium. [1] Sixty nine (76.6%) cases were seen in the age group of 21 to 40 years with a mean age of 30.5 years. Tirlapur et al studied the clinicopathological spectrum of cervical polyps and found that there was no case of malignanacy, majority are seen in parous women and they can recur in 12.6% of women. [12]

Chronic non-specific cervicitis was next common lesion seen in 30(12.1%) cases. The incidence of chronic non-specific cervicitis in our study is quite low as compared to other studies. The exclusion of hysterectomy samples in present study could be the reason for the lower incidence of chronic non- specific cervicitis. The reason to exclude out hysterectomy samples was that in majority of the cases the indications of hysterectomy are not due to cervical pathology, but are chiefly endometrial or myometrial in nature and the majority of hysterectomy samples do not show morphologically changes of cervix. [13] Diagnosis of chronic non-specific cervicitis was made on the presence of lympho plasmacytic infiltrate, some cases showed loss of surface columnar epithelium and three cases showed microglandular hyperplasia. Infective causes of cervicitis include a wide spectrum of organisms ranging from bacterial, viral, protozoan and fungal. According to Paavonen et al [14] the etiology of chronic non-specific cervicitis is variable. If untreated it may lead to endometritis, salphingitis and chorioamnionitis and pelvic inflammatory disease through ascending intraluminal spread. It may also lead to the initiation or progression of cervical neoplasia.

Kiviat et al found that the loss of surface coloumnar epithelium seen in chlamydial, gonococcal and HSV infections may be responsible for possible association of risk of HIV in such cases. [15]

Microglandualr hypeplasia is a benign condition seen in women of reproductive age and can be confused with adenocarcinoma. [16,17]

Koiliocytic change and squamous intraepithelial lesions (LSIL&HSIL) are considered as morphological hallmarks of Human Papilloma Virus (HPV) infection.

Sometimes reactive squamous cells with vacoulated appearance also look like koiliocytes. The identification of koliocytosis should prompt further testing for HPV. [18] The American Society for Colposcopy and Cervical Pathology (2019) guidelines have aligned the management with natural history of HPV and cervical carcinogenesis It recommends more frequent surveillance in high risk cases and longer intervals for lower risk. [19]

In the present study 23.8% of the cases showed cervical squamous intraepithelial lesions which is comparable to the study done by Badge et al. [20] Majority of them present in the fourth decade which is comparable to the study done by Badge etal Kumari and Tamboli et al. [20–22] (Supplementary Information Table 7)

The problem of inter observer variability and reproducibility for diagnosis has made a paradigm shift from routine haematoxylin and eosin staining to immunohistochemistry and genotyping. [5]

P16 is cyclin dependant kinase inhibitor (CDKN2) and is up regulated in HPV related lesions. Lesnikova et al evaluated the usefulness of p16 in diagnosis of cervical neoplasia in 796 tissue micro array specimens and concluded that $p16^{INK4a}$ is useful diagnostic tool and found that its expression correlated with degree of dysplasia. [23] Agoff et al studied the possible utility of p16 IHC in differentiating LSIL from HSIL They found that use of p16 was statistically significant to differentiate between the two and suggested it as a tool to H&E histopathology. [24] Murphy et al studied 149 cases with different histopathological diagnosis, and found that p16 displayed good sensitivity and specificity to differentiate the various dysplastic lesions. p16 was also found to be better than proliferation markers MCM5and CDC6. [25] The presence of positive p16 IHC suggest increase risk of progression in these patients.

(Supplementary Information Table 8)

Among different histopathological types of cervical cancer, squamous cell carcinomas account for 75-80% of cervical cancers, adenocarcinoma 15-25%, and adenosquamous carcinomas 3-5%.[26] In the present study, among malignant lesions squamous cell carcinoma (SCC) was the commonest (91.6%) lesion.

Incidence of adenocarcinoma is increasing since the 1970s; especially in females younger than 35 years of age, partly attributed to increasing prevalence of human papilloma virus (HPV) infection.[27]

The limitations of the present study are, small sample size for p16 immuno staining. However, in future this can be taken up as a prospective study on a larger scale.

Table 7: Comparison of pre invasive and malignant lesion with various studies

STUDY	LSIL (%)	HSIL (%)	SCC (%)
Badge et al[20]	16	10	15.3
Kumari et al[21]	6.3	9	33
Tamboli et al[22]	33	12	17.7
Present study	10.7	13.2	15.7

Table 8: I.2: Various studies showing percentage positivity of p16

Study	CIN 1	CIN 2	CIN 3
Lesnikova et al[23]	72.3%	91%	98.3%
Agoff et al[24]	57%	75%	91%
Murphy et al[25]	100%	100%	98%
Hu L et al[28]	44%	93%	100%
Gupta et al[29]	50%	60%	70%
Srivastava etc al[30]	80%	100%	100%
Kumari et al[31]	62.5%	75%	81.25%
Kishore et al[26]	25%	50%	75%

5. Conclusion

Cervical lesions are the major source of morbidity and mortality among females, especially cervical cancer. Histopathological diagnosis of cervical biopsy is an important diagnostic test for the timely treatment and management of the patients. Larger scale prospective studies with use of HPV-DNA and p16 studies are required to give more comprehensive analysis of cervical lesions.

6. Acknowledgements

7. Source of Funding

8. Conflict of Interest

The authors declare they have no conflict of interest.

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