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Original Research Article

Association of Cervical Non-Neoplastic, Precancerous and Neoplastic Lesion with Protein p16^{ink4a}: A Prospective Observational Study

Arpita Singh[1], Swaraj Sharma[2], Anju Chandra[3], Mohd. Fahimuddin[4], Navin Kumar[5]

[1]Assistant Professor, Department of Pathology, Rani Durgavati Medical College, Banda, Uttar Pradesh, India

[2]Assistant Professor, Department of Pathology, Maharshi Vashishtha Autonomous State Medical College, Basti, Uttar Pradesh, India

[3]Assistant Professor, Department of Pathology, Rajkiya Medical College, Jalaun, Uttar Pradesh, India

[4]Associate Professor, Department of Pathology, Rani Durgavati Medical College, Banda, Uttar Pradesh, India

[5]Statistician cum Assistant Professor, Department of Community Medicine, Rani Durgavati Medical College, Banda, Uttar Pradesh, India

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Corresponding author: Dr. Mohd. Fahimuddin

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Abstract:

Background: p16^{INK4a} (tumor-suppressor protein), used as biomarker of human papillomavirus (HPV) oncogenic activity have shown higher positivity rate in high grade cervical cancer lesions.

Aims: The study aimed to assess association of cervical non-neoplastic, precancerous and neoplastic lesion with protein p16^{INK4a}.

Setting and Design: This study was hospital based prospective study.

Methods and Material: The study was done among 110 patients in the department of Pathology, Vydehi institute of medical Sciences and Research centre, Bangaluru, Karnataka. The duration of the study was of one year five month from January 2015 to May 2016. All specimens were subjected to histopathological examination whereas immunohistochemistry staining for p16^{INK4a} was done in all cancerous, pre-cancerous and non-neoplastic cervical glandular lesions.

Statistical analysis: The number and percentage were calculated for all the variables and chi-square test was done to find association. P value <0.05 was considered significant.

Results: Most common histopathological type of lesion encountered were inflammatory lesions (57/110-51.8%). Cervical intraepithelial neoplasm-3(CIN III) was the most common pre-cancerous lesion accounting for 56.6% of the cases (13/23). Among neoplastic lesions, squamous cell carcinoma was the most common invasive carcinoma (17/21- 80.9%). The association of non-neoplastic, pre-cancerous and neoplastic lesion with protein p16^{INK4a} was found to be statistically significant.

Conclusion: p16^{INK4a} expression was significantly higher in neoplastic lesions. p16^{INK4a} staining intensity increases from sporadic to diffuse as disease severity progress from cervical intraepithelial neoplasm-1(CIN I) to neoplastic lesions.

Keywords: p16^{INK4a}, cervical cancer, cervical intraepithelial neoplasm (CIN), cervical dysplasia, histopathology.

Introduction

Cervical cancer ranks fourth among female cancers in worldwide, with maximum contribution (85% cases) from developing countries while in India it ranks second among female cancers with mortality rate of 20.7% and age standardized rate of 22 per 100,000 females.[1]

The pre-cancerous lesions of the cervix viz. low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL) transformation rate to neoplastic lesions varies. Estimates showed that 1%–2% of women have cervical intraepithelial neoplasm 2+ (CIN2+)/ HSIL whose progression rate to cervical intraepithelial neoplasm 3+ (CIN3+) and neoplastic lesions is approximately 20% and 5%, respectively.[2,3]

Different studies have shown conflicting results on evaluation of p16^{INK4a} protein expression prognostic value in cervical cancer while some reported that no influence on survival for p16^{INK4a} expression[4] others reported that improved survival outcome

prediction by $p16^{INK4a}$ expression for cervical carcinoma.[5,6]$p16^{INK4a}$ (p16), a tumour suppressor protein regulates cell cycle by decelerating cell progression from G1 to S phase.[7] Other risk factors which includes sexual and reproductive factors, socioeconomic status can also show association with cervical cancer that may vary geographically.[8]

$p16^{INK4a}$immunohistochemical staining has been reported to improve the inter-observer reproducibility of cervical intraepithelial neoplasia histologic diagnoses.[9] $p16^{INK4a}$overexpression acts as a marker of the oncogenic activity of high-risk human papilloma virus infection in cancer cervix.[10] Therefore, $p16^{INK4a}$ immunohistochemical staining can significantly improve the accuracy of cervical pre-cancer histologic diagnoses by reducing both false-negative and false-positive biopsy results.[9,11] The burden of follow up in $p16^{INK4a}$ negative cervical intraepithelial neoplasia grade 1 patients will decrease as the chance of progression to high grade cervical intraepithelial neoplasia grade 2 or 3 is rare.[12]The present study was therefore conducted to assess association of cervical non-neoplastic, precancerous and neoplastic lesion with protein $p16^{INK4a}$.

Materials and Methods

The study was done among 110 patients in the department of Pathology, Vydehi institute of Medical Sciences and Research centre, Bangaluru, Karnataka. The duration of the study was of one year five month from January 2015 to May 2016 among 110 patients. The specimens for the study were taken from punch biopsy, hysterectomy, endocervical curettage, and polypectomy specimens.

All the specimens were fixed in 10% neutral buffered formalin; tissues processed; paraffin embedded tissue blocks were prepared which were cut at 4-5 microns thickness. They were subsequently stained with Haematoxylin and Eosin. The histopathological classification of tumours was done according to recommendation by WHO.[13]

Immunohistochemistry staining for $P16^{INK4a}$ was done in all cancerous, pre-cancerous and non-neoplastic cervical glandular lesions.

Interpretation of $p16^{INK4a}$ staining

The distribution of $p16^{INK4a}$ nucleo-cytoplasmic positivity was done. As per Klaes and colleagues criteria.[14]

Negative: <1% of the cells were positive

Sporadic: isolated cells were positive (< 5%)

Focal: small cell clusters, but < 25% of the cells were positive

Diffuse: ≥25% of the cells were stained.

Statistical analysis

Data was entered in MS excel, then imported to trial version of SPSS version 20.0 for statistical analysis. The number and percentage were calculated for all the variables and chi square test was done to find association. P- value <0.05 was considered significant.

Results

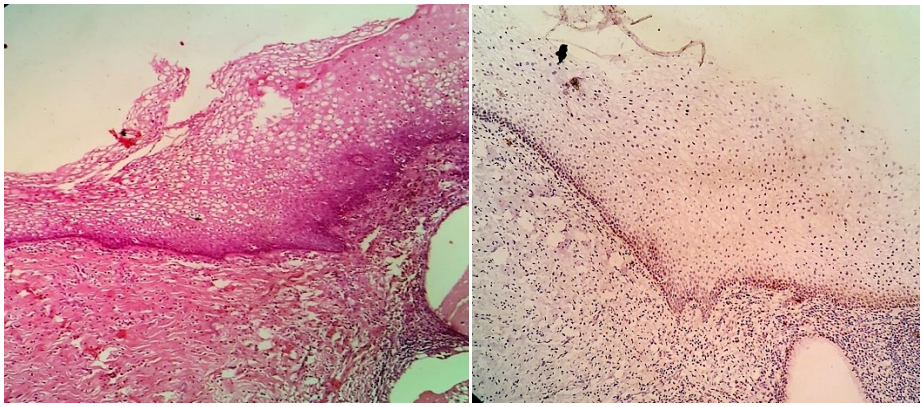
Punch biopsy (46.4%) was the most common type of specimen received for histopathological evaluation followed by hysterectomy (40%), endocervical curettage (8.2%) & Polypectomy (5.4%).

Table 1: Distribution of histopathological types of cervical lesions(n=110)

Cervical lesions	Number (n)	Percentage
Neoplastic	19	17.3
CIN (pre-cancerous)	23	20.9
Non- neoplastic		
Inflammatory	57	51.8
Non-neoplastic cervical glandular lesion	3	2.7
Benign	8	7.3
Non-neoplastic cervical glandularlesions(n=3)		
Microglandular hyperplasia	2	66.7
tunnel clusters	1	33.3
Benign cervical lesions (n=8)		
Leiomyoma	3	37.5
Endocervical polyp	5	62.5
Cervical Intraepithelial Neoplasia (n=23)		
CIN I	7	30.4
CIN II	3	13.0
CIN III	13	56.6
Cervical malignancy (n=21)		
Squamous cell carcinoma	17	80.9
Adenocarcinoma	1	4.8
Neuroendocrine carcinoma	3	14.3

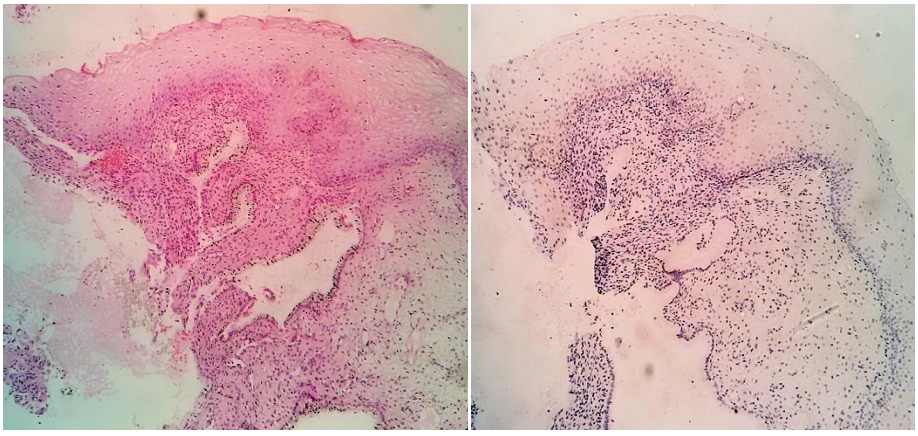
Most common histopathological type of lesion encountered were inflammatory lesions (51.8%) followed by cervical intraepithelial neoplasm (20.9%), neoplastic lesions (17.3%), benign lesions (7.3%) & non neoplastic cervical glandular lesions (2.7%) respectively. Among 57(51.8%) inflammatory lesions, chronic non-specific cervicitis (koilocytic change and squamous metaplasia) was seen in 8.8% (5 cases). (Table 1)

CIN III was the most common pre-cancerous lesion accounting for 56.6% of the cases followed by 30.4% cases of CIN I and 13.0% cases of CIN II. Among neoplastic lesions, squamous cell carcinoma was the most common invasive carcinoma comprising of 80.9% of the cases followed by 14.3% cases of neuroendocrine carcinoma and 4.8% cases of adenocarcinoma.



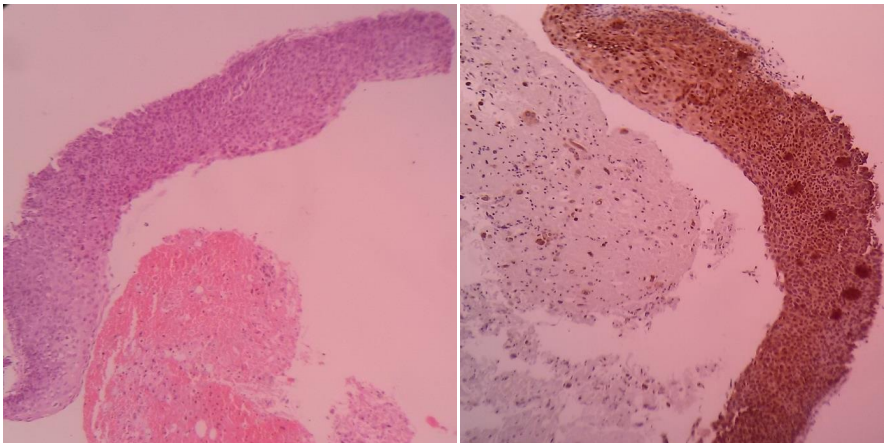
(A) H & E stain (40x) **(B) Negative for p16 stain (40x)**

Figure: 1 CNSC associated with koilocytic change.



(A) H & E stain (40x) **(B) Negative for p16 stain (40x)**

Figure 2:



(A) H & E stain (40x) **(B) p16 showing diffuse Positivity(40x)**

Figure 3:

Table 2: Association of non-neoplastic, precancerous and neoplastic lesion with protein $p16^{INK4a}$ (n=54)

Type of lesion	$p16^{INK4a}$ staining				Total
	Negative	Sporadic	Focal	Diffuse	
Non-neoplastic lesion (n=8)					
Chronic nonspecific cervicitis with koilocytic change and squamous metaplasia	5	0	0	0	5
3Non neoplastic glandular lesions	3	0	0	0	3
Precancerous lesion (n=23)					
CIN I	5	0	1	1	7
CIN II	1	1	1	0	3
CIN III	1	1	4	7	13
Neoplastic lesion (n=21)					
Squamous Cell Carcinoma (SCC)	0	1	4	12	17
Adenocarcinoma	0	0	0	1	1
Neuroendocrine carcinoma	0	0	1	2	3
	χ^2=28.28, df=2, p value= <0.0001				

All the cases of CNSC associated with koilocytic change, squamous metaplasia and non- neoplastic glandular lesions were negative for $p16^{INK4a}$. Pre-cancerous lesion i.e., CIN accounts for 23 cases, p16 was positive in 16 (69.6%) cases, and negative for 7 (30.4%) cases. In the group of CIN I out of 7 cases 2 cases (28.6%) were positive for p16, and 5 cases (71.4%) were negative. Out of 2 positive cases; 1 case showed diffuse positivity whereas other case showed focal positivity. CIN II was observed in 3 cases; out of the 3 cases, 2 cases (66.66%) were positive for p16 & 1 case (33.33%) was negative. Out of 2 positive cases one case showed focal positivity while the other showed sporadic positivity. CIN III comprises maximum no of cases i.e., 13 cases. Out of 13 cases 12 cases (92.3%) were positive & only 1 case (7.7%) was negative. Most cases of CIN III showed diffuse positivity i.e., 7 cases. In 4 cases focal positivity was seen & one case showed sporadic positivity. A total of 21 neoplastic lesions were observed which include 17 cases of SCC, 3 cases of small cell carcinoma & 1 case of adenocarcinoma. All neoplastic lesions were positive for $p16^{INK4a}$. Adenocarcinoma showed diffuse positivity, small cell carcinoma showed diffuse & focal positivity (2:1), while 4 cases (23.5%) of SCC showed focal positivity, 1 case (5.9%) showed sporadic positivity & 12 cases (70.6%) showed diffuse positivity. The association of non-neoplastic, pre-cancerous and neoplastic lesion with protein $p16^{INK4a}$ was found to be statistically significant.

Discussion

The rate of positive staining for $p16^{INK4a}$ in normal/chronic cervicitis, LSIL and HSIL were 33.3, 75 and 96.3%, respectively. $p16^{INK4a}$ expression significantly increased with disease progression. 64.3% cases were low positive (+) in LSIL, and 92.6% cases were strong positive (3+) in HSIL. With increasing severity of the cervical lesion, $p16^{INK4a}$positive expression significantly increased.[15]

No expression of P16 in was observed in all cases with chronic cervicitis, while in the cases with CIN, it was expressed in 25 cases (86.2%) and in the cases with carcinoma it was expressed in 20 cases (95.2%). There was statistically significant difference in the $p16^{INK4a}$expression between chronic cervicitis, CIN, and carcinoma. 50% cases with CIN I showed grade II positivity; 62.5% cases with CIN II showed grade II positivity, 12.5% grade III positivity;76.9% cases with CINIII showed grade III positivity, 15.4% cases showed grade II positivity; 85.7% cases with carcinoma showed grade III positivity. There was statistically significant difference in the grades of $p16^{INK4a}$expression and histopathological grades of CIN.[16]

No expression of $p16^{INK4a}$ in was observed in all cases with chronic cervicitis, while P16 expression was seen in 58.3% cases of HSIL and 26.3% cases of LSIL.[17]

P16 expression was seen in 80%, 87.5%, 78.7% cases of HSIL and 50%, 24.4%, 10.4% cases of LSIL.[18,19,20]

P16 expression was significantly higher in cancerous tissues in comparison to normal & pre-cancerous tissues and it was also significantly higher in pre-cancerous tissues in comparison to normal tissues.[21]

Out of 47 cases of carcinoma cervix, 45 were squamous cell carcinoma and two were adenocarcinoma. No $p16^{INK4a}$ expression was seen in all cases of normal (non-dysplastic) cervical epithelium while it was 25% in CIN I, 50% in CIN II, 75% in CIN III and 100% in carcinoma cervix.[22]

$p16^{INK4a}$ staining was negative in chronic cervicitis; in LSIL group, no brown particles appeared in cells; in HSIL group, stained part extended to 1/3 to 2/3 layer of squamous epithelium of the cervix; in SCC group, the stained granules were diffused

throughout the whole cervical squamous epithelium. No expression of $p16^{INK4a}$ in was observed in all cases with chronic cervicitis, while $p16^{INK4a}$ expression rate was 9.09%, 65% and 95.65%, in LISL, HISL and SCC groups, respectively. Chronic cervicitis group had significant difference compared with HSIL and SCC groups, and LISL group had significant difference compared with HSIL and SCC groups, there was also a significant difference between HSIL and SCC groups.[23]

$p16^{INK4a}$ was positive in 96% of invasive cervical cancer, 66.6% in HSIL, and 37.5% in LSIL, while it was negative in all the non-neoplastic controls.[24]

Conclusion

Inflammatory lesions are the most frequent non-neoplastic cervical lesions. Cervical screening with $p16^{INK4a}$ will help in timely detection and differentiation of benign, inflammatory, premalignant and malignant cervical lesions and reducing burden of follow up of patients.

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