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Histopathological Study of Cervical Intraepithelial Neoplasia and Carcinoma Cervix with Expression of P53 as IHC Marker

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***Abstract*: *Introduction:* Cervical malignancies are the third leading cause of mortality in women in developing countries. Various clinical, histopathological, and molecular-based screening methods have been developed for detection of cervical carcinoma. Aim: The aim of this study was to determine the frequency and pattern of p53 expression in cervical intraepithelial neoplasia (CIN), and invasive carcinoma with histo-pathological correlation.**

***Methodology:* A total of 100 cases of cervical biopsy /hysterectomy specimens of carcinoma cervix and cervical intraepithelial neoplasia (CIN) received at the Department of Pathology, from July 2013 to June 2018 were examined for gross and microscopic features. Immunohistochemistry was used to study the p53 expression in cervical intraepithelial neoplasia and carcinoma cervix. The p53 expression was correlated with various Histopathological and clinico-pathological prognostic parameters.**

***Results:* Of the 50 cases of cervical neoplasia, 84% of the cases showed p53 positivity. The single case of CIN III showed p53 positive cells in all the layers of squamous epithelium. Unlike normal cervical epithelium where p53 positivity was observed in the basal layer, in CIN 1, 2 and 3 the p53 positivity was present in one-third, two-third and all the layers of squamous epithelium respectively. Maximum positivity is observed in moderately differentiated squamous cell carcinoma. The p53 positivity increased with the age, parity, stage, and grade of the disease with no statistical significance.**

***Conclusion:* Our study indicates that p53 may be a used as an adjunct in differentiating Cervical Intraepithelial Neoplasia from carcinoma cervix. The p53 may be used as a prognostic marker as its expression increased with prognostic parameters such as histological type, grade, and stage of carcinoma cervix.**

***Keywords*: Cervical carcinoma, Cervical intraepithelial neoplasia, Clinico-pathological prognostic parameters, Immunohistochemistry.**

1. Introduction

Cervical cancer is third most common cancer in developing countries. In women, it is the most common after breast carcinoma. The incidence has increased from 0.12 million in the year 2000 to 0.17 million cases in 2014. Over 81% of the cases of the cervical cancer present at a fairly advanced stage and around 80,000 deaths are reported due to carcinoma cervix in developing countries. [1] Apoptosis, a programmed cell death, is governed by a balance between the pro and anti-apoptotic genes. It requires inactivation of pro-apoptotic genes, mainly the p53 gene, and activation of anti-apoptotic genes, mainly the bcl-2 gene, for the cell survival and proliferation to occur which results in neoplasm. [2] When p53 is mutated and the cells with DNA damage are allowed to accumulate mutations and progress to malignancy. [3] Radiotherapy and chemotherapy, the two main modalities of therapy of carcinoma cervix, mediate their effect by inducing DNA damage and there by apoptosis in the neoplastic cells. Therefore, a mutation in apoptotic genes, mainly the p53gene, may confer chemo-resistance or radio-resistance to tumors. [4] Knowledge of the status of these genes may help in determining the response to therapy or recurrence of these tumors. Some studies have shown that the weak expression of p53 in carcinoma cells before radiotherapy is regarded as a predictive factor for better prognosis [5] and positive staining for p53 was associated with survival disadvantage. [6] This study aims at correlation of immune positivity of p53 with the histopathological type and grade of carcinoma cervix.

2. Aims and objectives

- To assess the pattern of expression of IHC marker, p53 in cervical intraepithelial neoplasia, and carcinoma cervix.
- Comparison of the expression of p53 as IHC marker among various histologic types of cervical carcinomas.

3. Materials and methods

In this retrospective study, histopathological slides of 100 cases of carcinoma cervix and cervical intraepithelial neoplasia during July 2013 to June 2018 retrieved from archive of pathology department. The histological type was confirmed by reviewing H & E stained slides.

The most representative section for immunohistochemistry were selected. 3-4 micrometer thick sections from each tumor blocks were obtained. IHC is done on Leica Bond Max machine by automated method with positive and negative control. All the immunostained sections were scanned randomly at 100x

magnification for the most densely labelled areas. The nuclear counts were taken at400x magnification. A total of 1000 nuclei were counted in most densely labelled microscopic fields.
The grading system used is as follows [15]:
Staining intensity; absent (-), mild (+), moderate (++), severe (+++)
Percentage of positive cells (semi quantitative method) in 10 HP

Grade 1 - 1 to 5% of positive cells.
Grade 2 - 6 to 25% of positive cells.
Grade 3 - 26 to 50% of positive cells.
Grade 4 - 51 to 75% of positive cells.
Grade 5 - >75% of positive cells.

A final immunoscore was calculated by adding scores of % and intensity

4. Results and observations

100 specimens of cervical neoplasia were included according to the inclusion criteria.

Table 1
Age distribution of study subjects

Age group	Number of subjects	Percentage
≤35 years	8	8
36-45 years	30	30
46-55 years	35	35
56-65 years	16	16
>65 years	11	11
Total	100	100

In our study from age wise distribution, it is noted that maximum number of subjects belonged to 46-55 years of age (35%) group closely followed by 36-45 years of age (30%).

Table 2
p53 expression in relation to Age group

Age group	p53 Negative		p53 Positive	
	N	%	N	%
≤35 years	1	25	3	75
36-45 years	3	23	10	76.9
46-55 years	2	14.2	12	85.7
56-65 years	1	12.5	7	87.5
>65 years	1	9	10	90
Grand Total	8	16	42	84

In our study, in all the age groups the number of cases having positive p53 expression was found to be greater (84%), than the negative cases (16%).

Table 3
p53 expression in relation to type of lesion

Type of lesion	p53 Positive		p53 Negative	
	N	%	N	%
CIN 1	3	50	3	50
CIN 2	1	100	0	0
CIN 3	1	100	0	0
Well differentiated SCC	5	83.3	1	16.6
Moderately differentiated SCC	28	90.3	3	9.6
Poorly differentiated SCC	4	80	1	20
Grand Total	42	100	8	100

In our study, there was single case of CIN2 and CIN3 which was found positive for p53.Maximum positivity was observed in moderately differentiated squamous cell carcinoma.

Table 4
p53 expression in pre malignant and malignant lesion

p53 Grade (0% expression)	CIN (pre Malignant		Malignant (SCC, AC)	
	N	%	N	%
0 (Negative)	3	37.5	5	11.9
1 (1-5%)	0	0	2	4.7
2 (6-25%)	2	25	12	28.57
3 (26-50%)	2	25	15	35.71
4 (51-75%)	1	12.5	5	11.9
5 (75%)	0	0	3	7.1
Grand Total	8	100%	42	100%

In our study, P53 positivity of grades 2, 3 was observe as 25% each in pre malignant cases. Where as in malignant cases, grade 3 positivity was found to be maximum (35.71%).

Table 5
p53 Intensity in pre malignant and malignant lesion

p53 intensity	CIN (pre Malignant)		Malignant (SCC, AC)	
	N	%	N	%
Negative	3	37.5	5	11.9
Mild	3	37.5	9	21.42
Moderate	2	25	21	50
Intense	0	0	7	16.6
Grand Total	8	100%	42	100%

In our study, pre malignant cases with mild intensity was found to have 37.5% cases. Whereas, in malignant cases maximum number of cases (50%) showed moderate intensity staining.

Table 6
p53 final score in pre malignant and malignant lesion

p53 final score	CIN (pre Malignant		Malignant (SCC, AC)	
	N	%	N	%
0-2	3	37.5	10	23.8
3-4	3	37.5	18	42.85
5-6	2	25	10	23.80
7-8	0	0	4	4.5
Grand Total	8	100%	42	100%

On calculating the total score (p53 grade+ p53 intensity) majority of the pre malignant cases had score 0-2 and 3-4. While in malignant cases, majority of cases (42.85%) had scores 3- 4.

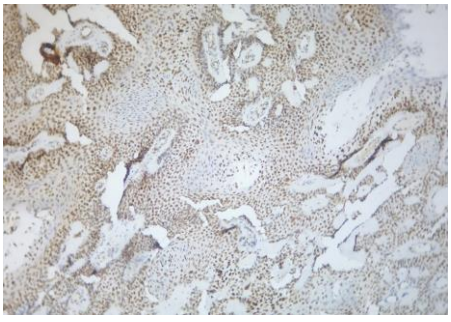

Fig. 1. Well differentiated keratinizing squamous cell carcinoma (P53, X10)

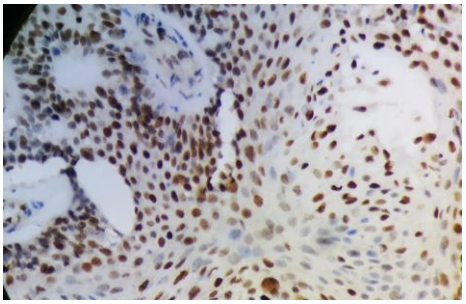

Fig. 2. Well differentiated keratinizing squamous cell carcinoma (p53, x40)

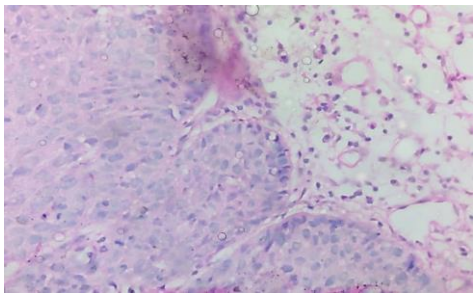

Fig. 3. Cin-2 showing moderate dysplastc changes (h&e, x40)

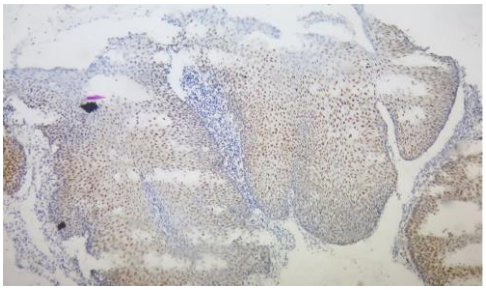

Fig. 4. Cin -2 showing moderate dysplastc changes (p53, x10)

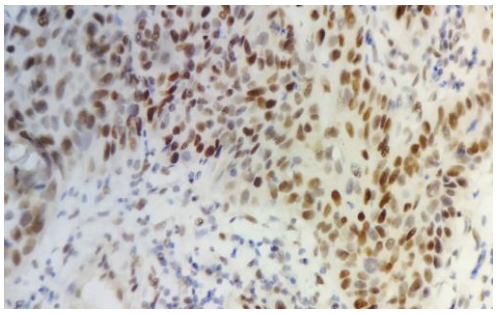

Fig. 5. Papillary squamous cell carcinoma (p53, x40)

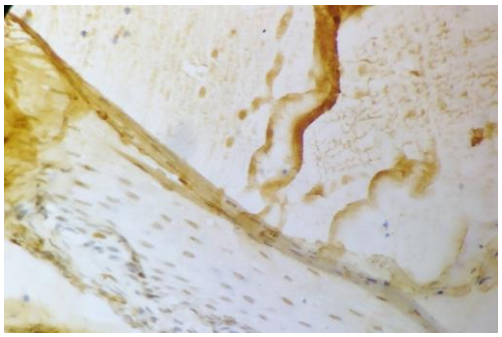

Fig. 6. Cin-1 showing mild dysplastic changes (p53, x40)

5. Discussion

In our study of 100 cases of cervical neoplasia, patient's age ranged from 28 to 76 years and most cases were observed in elderly women with a mean age of 50.45 years). Maximum number of pre malignant cases were found in age range 36-45 years (40 %) while that of malignant cases were found in the later age group i.e. 46-55 Years (31.1%). In our study, maximum number of patients (49%) presented with abnormal bleeding. In the present study, the incidence of p53 positivity in neoplastic lesions was 84 %.

Table 13
p53 incidence in various studies

Study	p53 incidence (%)
Oka et al.[9]	52.1
Haenrgen at al.[7]	85.7
Ngan at al.[8]	25.2
Tjalma ar al.[9]	42.0
Win at al.[10]	80.0
Madhumati at al.[11]	45.5
Baskaran at al.[12]	83.0
Sandhu at al.[13]	86.7
Tan et al.[14]	85.2
Present Study	84.0

Our study had 8 cases of CIN of which 6 case was of CIN 1,1 case was of CIN 2 while the remaining 1 was of CIN3, unlike normal cervical epithelium where p53 positivity was observed in the basal layer, in CIN 1, 2 and 3 the p53 positivity was present in one-third, two-third and all the layers of squamous epithelium respectively.

6. Conclusion

The role of p53 as IHC marker in carcinoma cervix and cervical intraepithelial neoplasia that has been previously investigated for prognostic information in cervical cancer. The pattern of positivity was different in cervical intraepithelial neoplasia and carcinoma cervix with increase in p53 grade and score. This observation could be used to advocate the use of p53 as a screening and prognostic biomarker in cervical carcinoma.

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