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Study on p53 Expression in Carcinoma Cervix and it's Correlation with Clinical Parameters, Grading and Staging: A Cross-Sectional Study

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Abstract

Carcinoma cervix is the fourth most common cancer in women globally and its incidence and mortality are much higher in developing countries. The persistent high risk Human papilloma virus infection is the main etiologic agent and inactivation of p53 by HPV oncoprotein E6 represents the key event in cervical carcinogenesis. To evaluate the frequency and pattern of P53 expression in carcinoma cervix and correlate with age, parity, hormonal status, histologic type, grading and clinical staging. This cross-sectional study included a total of 100 biopsy/hysterectomy proven cases of cervical carcinoma during the two and half years study period. All relevant clinical parameters were noted and each case examined in detail for gross and microscopic findings. Immunohistochemical marker p53 was applied in all cases and its intensity and grading was assessed and correlated with clinicopathologic parameters. Data analysed by SPSS version 27 and correlation was measured by chi-square and Fischer exact test. The mean age of cervical carcinoma patients was 52. 5 years and 74% were postmenopausal. A total of 85% cases were squamous cell carcinoma, 9% were adenocarcinoma and 3% were premalignant (SIL) cases. There was no association between P53 positivity and score with age, parity, hormonal status, Broder's grading and FIGO clinical staging. The p53 expression was significantly associated with histologic variant as it is higher in SCC. The P53 expression was negative in two cases of LSIL whereas positive in HSIL. The p53 expression progressively increased from intra epithelial lesion to malignant lesion. This observation can be useful to differentiate SIL from invasive lesions in difficult situation. Thus it can be used as diagnostic biomarker.

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Key Words
P53, carcinoma, cervix, histopathology

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INTRODUCTION

At present, cancer is the most serious threat to mankind. Worldwide, cervical cancer is the fourth most frequent cancer in women with an estimated 660000 new cases and around 350000 deaths in 2022[1] and the second most common malignancy in women in India[2].

The carcinoma cervix progress from precursor lesions (Squamous intraepithelial lesion -SIL) to well differentiated tumor in sequential manner and HPV infection is strongly linked with it as main etiologic factor[3]. Biopsy cervix is definitive in establishing a diagnosis in cervical lesions. There are primary and secondary biomarkers in cervical carcinoma. The primary marker being HPV DNA and secondary markers like p53, c-fos, p50, fra1, p16, notch-1, rb and telomerase. Role of HPV DNA in cervical carcinogenesis is widely studied globally and also in India[3,4].

Secondary marker p53 suppresses outgrowth of genetically damaged, hence potentially neoplastic, cells in two distinct ways: by causing a pause in the cell cycle, and by apoptosis and acts as "guardian of the genome"[5]. Inactivation of p53 represents a key step in cervical carcinogenesis, similarly to other human cancers. The HPV viral oncoprotein E6 has the ability to associate with and neutralize the function of p53. E6 interacts with E6 associated protein (E6AP., also called ubiquitin-protein ligase E3A or UBE3A), which functions as an ubiquitin protein ligase. The dimeric complex then binds p53 and E6AP catalyzes multi-ubiquitination and degradation of p53. The ability to promote p53 degradation is an exclusive property of E6 from the high-risk HPV types[5]. Assessment of p53 function can be done by gene sequencing, IHC and functional tests. IHC is a rapid preliminary indication of p53 status in tumours and can be performed by immunohistochemical detection of nuclear p53 accumulation[6].

Thus, present study was conducted to study the expression patterns of immunohistochemical markers p53 in cases of cervical carcinomas. The study also aimed to identify the association of p53 with the different histopathological types, grades and stages of cervical carcinomas and with age, menopausal status and parity of patient.

MATERIALS AND METHODS

This two and half year, cross-sectional study was conducted in the Department of Surgical Pathology, Sri Aurobindo Medical College and PG Institute (SAMC and PGI), Indore from November 2016 to April 2019.

Inclusion Criteria: All histopathological proven cases of carcinoma cervix with age more than 18 years, whose biopsies and/or hysterectomy specimens were received during study period.

Exclusion Criteria: Very small biopsies with inadequate tissue for further processing to apply immunohistochemistry.

Methods of Evaluation:

- Clinical parameters: Clinical details pertaining to age, gender, site of tumor, clinical signs and symptoms were noted
- Histological study of all cervix cancer cases was done. Tumor size was noted based on macroscopic examination
- Tumor grading was done on hematoxylin and eosin stained slides using modified Broder's grading system
- Staging of tumor was done based on TNM pathological staging system
- Immunohistochemical study of p53 was done in all cases of cervix carcinoma

IHC Staining Decision Criteria: Then slides were examined for presence of staining as well as for percentage and intensity of stained cells. The pathological diagnosis was considered definite if p53 were located in the nucleus of tumor cells indicated by tan or brown particles. At high magnification 10 different views were selected., for each view, 100 tumor cells were counted. Accounting to the percentage of positive cells p53 expression was considered positive or negative.

Scoring of biomarker staining was done as per the standard guidelines and expression patterns of various IHC markers were assessed in relation to different histological types of cervix carcinomas.

The score of p53 is the sum of intensity and percentage of positive cells. The correlation of this score was done with clinicopathological parameters.

Data Analysis: Data collected was tabulated using Microsoft Excel and presented with the help of descriptive statistics using SPSS software version 27. For qualitative variables, data was analysed by frequency and percentage and association was measured by chi-square test and fisher exact test.

RESULTS AND DISCUSSIONS

During study period, a total of 100 specimens of carcinoma cervix, were received and enrolled. The age of patients of carcinoma cervix ranged from 11-90

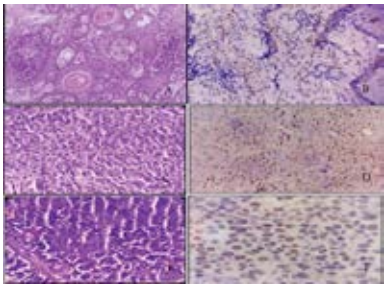

Fig. 1: Photomicrograph showing Well differentiated SCC (A) and P53 expression (400x) (B)., Moderately Differentiated SCC (C) and P53 expression (400x) (D)., Poorly differentiated SCC (E) and P53 expression (400x) (F).

Table 1: Intensity and grading of p53 staining[7,8]

Staining Pattern	Grading of Intensity
Absent	0
Mild	1+
Moderate	2+
Severe	3+
Percentage of Cells Showing Positivity (In 10hpf)	**Grade**
less than 5% of cells positive	1
5-24% of cells positive	2
25-49% of cells positive	3
50-75% of cells positive	4
>75% of cells positive	5

Table 2: Correlation of P53 positivity with age, parity and menopausal state.

Parameter	Total cases	P53 positive (%) N = 93	P53 negative (%) N = 7	p-value
Age (Yrs)*				
<30	02	02 (100)	00	0.990
31-40	19	18 (94.7)	01(5.3)	
41-50	29	26 (89.7)	03(10.3)	
51-60	26	25 (96.2)	01(3.84)	
>60	24	22 (91.7)	02 (8.3)	
Parity*				
0-2	35	33(94.3)	02 (5.7)	0.889
3-5	62	57 (91.9)	05 (8.1)	
>5	03	03 (100)	00	
Menopausal state#				
Premenopausal	26	24 (92.3)	2 (7.7)	0.872
Postmenopausal	74	69 (93.2)	5 (6.8)	

association measured by *Fisher exact test, # by Pearson chi-square test

Table 3: p53 protein expression in various histologic types of cervical carcinoma

Histologic variant	Total cases	P53 positive (%)	P53 negative (%)	p-value
WD SCC	25	24 (96)	1 (04)	
MD SCC	55	54 (98.2)	1(1.8)	
PD SCC	5	4 (80)	1 (20)	
Adenocarcinoma	7	6 (85.7)	1 (14.3)	
Papillary Adenocarcinoma	2	1(50)	1(50)	
Clear Cell Carcinoma	2	2 (100)	0 (0)	0.0003
Transitional Cell Carcinoma	1	1 (100)	0 (0)	
SIL	3	1 (33.3)	2 (66.7)	

Table 5: Correlation of p53 score with age, parity, menopausal status

Parameter	P53 score (%)			
	0-2 (n = 25)	3-5 (n = 52)	6-8 (n = 23)	p-value
Age (years)				
<30	0	2 (100)	0	0.791
31-40	4 (21.1)	12 (63.2)	03(15.8)	
41-50	7 (24.1)	14 (48.3)	08 (27.6)	
51-60	9 (34.6)	10 (38.5)	07(26.9)	
>60	5 (20.8)	14 (58.3)	05 (20.8)	
Parity*				
0-2	10 (28.6)	15 (42.9)	10 (28.6)	0.491
3-5	14 (22.6)	36 (58.1)	12 (19.4)	
>5	1 (33.3)	1 (33.3)	1 (33.3)	
Menopausal state#				
Premenopausal	5 (19.2)	16 (61.5)	5 (19.2)	0.522
Postmenopausal	20 (27)	36 (48.7)	18 (24.3)	

Table 6: Correlation of p53 score with Broder's grading and clinical staging

Parameter	P53 score (%)			
	0-2 (n = 25)	3-5 (n = 52)	6-8 (n = 23)	p-value
Broder's grading				
Well differentiated	6 (16.7)	24 (66.7)	6 (16.7)	0.210
Moderately differentiated	17 (28.8)	26 (44.1)	16 (27.1)	
Poorly differentiated	2 (40)	2 (40)	1 (20)	
FIGO Clinical Staging				
Tis	2 (66.7)	1 (33.3)	0	0.270
IA	1 (50)	0 (0)	1 (50)	
IB	0 (0)	6 (100)	0 (0)	
IIA	1(14.3)	3 (42.9)	3 (42.9)	
IIB	5 (22.7)	12 (54.5)	5 (22.7)	
IIIA	1 (10)	7 (70)	2 (20)	
IIIB	15 (32.6)	20 (43.5)	11(23.9)	
IVA	0	3 (75)	1 (25)	

years with a mean age of 52.5 years. The peak incidence was seen in the fifth decade (29%) followed by sixth decade (26%). 74% of females were postmenopausal. Of these 100 cases, 35 patients were of parity 0-2, 62 patients were of parity 3-5 and 3 patients were of parity more than 5. The commonest presentation was postmenopausal bleeding (45%), followed by white discharge per vaginum (30%). The majority (46%) of the patients belonged to Stage III B and none of the patient was in stage IVB.

Histologically, 85% cases were squamous cell carcinoma (SCC), followed by 9% of adenocarcinoma and 3% SIL. Among SCC, moderately differentiated SCC (MD SCC) were 64.7%, well-differentiated (WD SCC) were 29.4% and poorly differentiated were 5.9%. (Fig. 1)

Among the 100 cases, p53 was expressed in 93 cases (93%) of carcinoma cervix. The correlation of P53 expression with age, parity and menopausal status was also studied but the association was statistically insignificant. (Table 2).

Among a total of 85 cases of SCC, 82 cases (96.5%) showed p53 positivity. Out of 3 cases of SIL, two cases were low grade SIL (LSIL) and were p53 negative and a single case of high grade SIL (HGSIL) showed p53 expression. Association of histologic variant and p53 positivity was statistically significant. (Table 3)

The present study also done correlation of p53 scoring with age, parity, menopausal status, Broder' grading and clinical stage but not find any statistically significant association. (Table 5,6)

The present study conducted on 100 cases of Cervical carcinoma. The mean age of the patients was 52.5 years with the peak incidence in the fifth decade. The similar findings were observed by Singh and Bannur[9] (49.8 yrs), Mucharla *et al.*[10] (56.6 yrs) Rajaram *et al*.[8] (52.1 yrs) and Tjalma *et al*.[11] (52 yrs). In the index study 62% of the patients of carcinoma cervix were of parity 3-5 and 74% were postmenopausal. This findings are similar with Singh and Bannur[9] who reported 64% cases in parity 3-5 and 60% cases in postmenopausal state. The commonest presentation was postmenopausal bleeding (45%) followed by white discharge (30%) in the index study. The findings are in concordance with study by Sandhu and Shivakumar[12]. The present study observed maximum patients of carcinoma cervix were of stage IIIB (46%), whereas in other studies[9,10,12] stage IIB was the most common clinical stage.

Among 100 cases, SCC was the most common histologic subtype of carcinoma cervix (85%) with MD SCC variant (64.7%) commonest in the present study, similar to other studies[9,12,13]. However, Vasilescu *et al*.[14] found PD SCC as the commonest histologic variant.

In the index study, p53 positivity was observed in 93% of cases. There is wide variation in p53 positivity among various studies ranging from 17.1-100%[7,15,16]. This variation could be because of difference in demographic profile of the study population, antigen retrieval method and different antibodies used.

The p53 positivity was more with higher age and parity but was not statistically significant, similar findings were observed in many studies[9,10,12]. However, Ikuta *et al*.[17] in their study on 49 cases, showed statistically significant rise in p53 expression with rise in age. The present study also did not find any association between hormonal status and p53 overexpression among the study population. However, Singh and Bannur[9], Ikuta *et al*.[17] and Madhumati *et al.*[18] found significantly more p53 positivity among postmenopausal cases in their studies.

The association between p53 positivity and histologic variant of carcinoma cervix was statistically significant in the present study as p53 positivity was more among SCC in contrast to other variants. This finding is in line with other studies[8,9,12,13].

In the present study, 33.3% cases of premalignant lesions (SIL) and 96.5% cases of SCC exhibited p53 positivity. The findings are concordant with Tan *et al*.[19] who studied differences in p53 expressions in pre-malignant and malignant cervical neoplasms. They found 66.7% premalignant cases and 87% SCC were positive for p53 protein and also observed more diffuse and intense staining in SCC than focal staining in premalignant lesions. They concluded that expression of p53 is greater in the malignant cervical neoplasms than the pre-malignant cervical lesions. Baskaran *et al.*[20] in their study also found the significantly increased expression from LSIL to HSIL and the highest expression in SCC with no difference among the stages.

The present study also did not find any association of p53 score with age, parity and menopausal status similar to Sandhu and Rajakumar[12]. However, few studies found significant association of p53 score with menopausal state as higher score was observed in postmenopausal women[9,17].

The present study revealed that only 20% cases of PD SCC (Broder's Grade III) showed the highest p53 score, whereas 27.1% of MD and 16.7% of WD SCC associated with 6-8 score. In contrast, Sandhu and Shivkumar[12] found 6-8 score in all cases (100%) of PD SCC. However, they also did not find any significant association between Broder's grading and P53 scoring similar to the index study.

CONCLUSION

The p53 positivity was more in multiparous and postmenopausal women. However, the association was not significant. The p53 expression was significantly higher in SCC, than in the premalignant lesions and adenocarcinomas. Similarly, the p53 score was lower with LSIL than HSIL and SCC. These observations could be useful in differentiating the low grade and high grade SILs and also SCC. However, grading and staging were not significantly associated with P53 positivity and scoring. Thus, a multicentric, large sample sized study which includes all histologic types of cervical carcinoma needed to evaluate the association of p53 with different grades and stages of cancer for assessment of prognosis and targeted therapy in patients of cervical carcinoma.

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