Original Research Article

Immunohistochemical study of p53 expression as proliferative index in neoplasia of the uterine cervix

Monil Thakrar[1*], Kusum Mathur[2]

[1]3rd Year Resident, [2]Senior Professor
Department of Pathology, SMS Medical College, Jaipur, Rajasthan, India
[*]Corresponding author email: **monil.p.thakrar@gmail.com**

International Archives of Integrated Medicine, Vol. 4, Issue 12, December, 2017.

Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 12-11-2017 **Accepted on:** 18-11-2017
Source of support: Nil **Conflict of interest:** None declared.
How to cite this article: Monil Thakrar, Kusum Mathur. Immunohistochemical study of p53 expression as proliferative index in neoplasia of the uterine cervix. IAIM, 2017; 4(12): 16-25.

Abstract

Introduction: Cervical cancer is the third most common cancer in India. In women, it is the second most common after breast carcinoma. Human cancers arise from a protracted sequence of multiple genetic and epigenetic alterations which involve the growth regulatory genes like p53.
Aim: This study aimed at correlation of immunopositivity of p53 with the histopathological type and grade of carcinoma cervix.
Materials and Methods: A total of 100 cases of cervical biopsy/ hysterectomy specimens of carcinoma cervix received at the Department of Pathology, SMSMC Jaipur from January 2016 to September 2017 were examined for gross and microscopic features. Immunohistochemistry was used to study the p53 expression as proliferative index in cervical intraepithelial neoplasia, invasive squamous cell carcinoma as well as adenocarcinoma of the uterine cervix.
Results: The demographic profile of SCC and CIN in this study was in concordance with that described by epidemiological studies in India with larger group of patients presenting with advanced stage tumors. Increased expression of p53 was found in increasing grades of CIN and SCC. The p53 positivity showed a statistically significant association with squamous cell carcinoma (SCC) histologic type (P = 0.005).
Conclusion: The expression of p53 is greater in the malignant cervical neoplasms than pre-malignant cervical lesions, suggesting that p53 overexpression can be used as an early diagnostic tool and also as a prognostic indicator.

Key words

Cervical neoplasia, Immunohistochemistry, p53.

Introduction

Cervical cancer is the third most common cancer in India. In women, it is the second most common after breast carcinoma. Over 80% of the cases of the cervical cancer present at a fairly advanced stage and around 80,000 deaths are reported due to cervical cancer in India [1]. There are many biomarkers of cervical cancer, some of which have a differential expression in different types of cervical cancers. Human cancers arise from a protracted sequence of multiple genetic and epigenetic alterations which involve the growth regulatory genes. Evasion of apoptosis is one of the fundamental changes in the cell physiology required for malignant transformation. Wild-type p53 is believed to be continuously monitoring the integrity of the DNA molecule. Evasion of this protective mechanism occurs when p53 is mutated and the cells with DNA damage are allowed to accumulate mutations and progress to malignancy [2].Detection of these mutations, especially p53 has practical therapeutic implication in invasive cancer. There are primary and secondary biomarkers in cervical cancers. The primary marker being HPV DNA and secondary markers like p53, c-fos, p50, fra 1, p16, notch 1, rb and telomerase. Out of these markers the primary marker has been widely studied in India and not many studies have been done in India on secondary markers. The secondary marker p53 has been studied at various centres in the world and has been found to be disregulated in cervical cancers. The suppressor gene p53 plays a pivotal role in protecting against various cancers [3, 4].This study aims at correlation of immunopositivity of p53 with the histopathological type and grade of carcinoma cervix.

Materials and Methods

This was a descriptive and observational study on cases diagnosed as CIN (Cervical Intraepithelial Neoplasia), Squamous Cell Carcinoma (SCC) and Adeno Carcinoma (AC) conducted in the department of pathology, SMS Medical College, Jaipur (Rajasthan) from January 2016 to September 2017. A total of 100 histopathological specimens comprising of cervical biopsies, hysterectomy specimens and conization specimens positive for cervical neoplasia, received in the department of pathology were included in the study. Patients with history of HIV infection or any other malignancy, any benign conditions or any other inflammatory conditions were excluded from the study. Paraffin embedded section of tissues were used. Sections of 5-6 µ thickness were stained with hematoxylin and eosin. The cases were evaluated for the presence of malignancy and the histologic subtype of the tumor as per the WHO classification. The tumors were graded using modified Broder's grading, which is based on differentiation, nuclear pleomorphism, and mitotic figures. All the 100 cases were subjected to IHC study for p53 using polymer based ready to use IHC kit of Biogenex.

Immunohistochemical grading will be done on the basis of presence of staining, number of cells taking up the stain, distribution and intensity of staining.

The grading system used will be as follows [5]:
Staining intensity: absent (-), mild (+), moderate (++), severe (+++).
Percentage of positive cells (semi quantitative method) in 10 HPF:

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. Though several studies have used different cut off values [6, 7], a score of >3 for p53 was taken as a cut off in this study to detect those tumors with at least a weak staining in up to 25% of cells.

Data Management and Statistical Analysis
The difference in the degree of p53 staining between CIN (Pre Malignant) and Carcinoma

(Malignant) to be assessed using the Pearson Chi-Square Test. Any p value < 0.05% to be considered statistically significant.

Results

During the period of two years from January 2016 to September 2017, a total of 30,659 specimens were received in the department of Pathology, SMS Medical College, Jaipur (Rajasthan) for histopathological examination of which 100 specimens of cervical neoplasia were included according to the inclusion criteria. Out of our 100 cases 12 were Wertheim's hysterectomy specimens and the remaining 88 were biopsy specimens.

In our study, maximum number of pre malignant cases were found in 36- 45 years of age (40%) while the maximum number of malignant cases were found in the later age group i.e. 46-55 years of age (31.1%). However, this age wise distribution was found to be statistically insignificant (p=0.429) (**Table – 1**). We noticed that the most common complaint in both, pre malignant and malignant cases, was bleeding per vaginum.

Table - 1: Age distribution of Pre malignant and malignant cases.

Age group	CIN (pre malignant)		Malignant (SCC, AC)		Grand Total	
	N	%	N	%	N	%
≤35 years	2	20	8	8.9	10	10
36-45 years	4	40	24	26.7	28	28
46-55 years	3	30	28	31.1	31	31
56-65 years	0	0	19	21.1	19	19
>65 years	1	10	11	12.2	12	12
Grand Total	**10**	**100**	**90**	**100**	**100**	**100**

Chi-square = 3.834 with 4 degrees of freedom; P = 0.429 (NS)

Table - 2: p53 expression in relation to type of lesion.

Type of lesion	p53 Positive		p53 Negative		P value
	N	%	N	%	
CIN 1	1	100	0	0	0.679
CIN 2	1	50	1	50	
CIN 3	4	57.1	3	42.9	
Moderately diff Adeno CA	2	33.3	4	66.7	
Well differentiated SCC	2	66.7	1	33.3	0.711
Moderately differentiated SCC	64	84.2	12	15.8	
Poorly differentiated SCC	4	80	1	20	
Grand Total	**78**	**100**	**22**	**100**	

In our study, there was a single case of CIN 1 which was found positive for p53. Out of 2 cases of CIN 2, one case was found positive for p53. In case of Adenocarcinoma, out of 6 cases positivity was observed in only 2 cases. Maximum positivity was observed in Squamous Cell Carcinoma cases. However, this difference of p53 expression in relation to type of lesion was found statistically insignificant (**Table – 2**). p53 positivity of grades 2, 3 and 4 was observed as 20% each in pre malignant cases whereas in malignant cases, grade 3 positivity was found to be maximum (33.3%) of cases. However, this difference was found to be statistically insignificant (p=0.682) (**Table – 3**).

Table - 3: p53 expression in pre malignant and malignant lesion.

p53 Grade (% expression)	CIN (pre malignant)		Malignant (SCC, AC)		Grand Total	
	N	%	N	%	N	%
0 (Negative)	4	40	18	20	22	22
1 (1-5%)	0	0	2	2.2	2	2
2 (6-25%)	2	20	24	26.7	26	26
3 (26-50%)	2	20	30	33.3	32	32
4 (51-75%)	2	20	10	11.1	12	12
5 (75%)	0	0	6	6.7	6	6
Grand Total	**10**	**100%**	**90**	**100%**	**100**	**100%**

Chi-square = 3.772 with 5 degrees of freedom; P = 0.682 (NS)

Table - 4: p53 intensity in pre malignant and malignant lesion.

P53 intensity	CIN (pre malignant)		Malignant (SCC, AC)		Grand Total	
	N	%	N	%	N	%
Negative	4	40	15	16.7	22	22
Mild	3	30	18	20	21	21
Moderate	3	30	42	46.7	45	45
Intense	0	0	12	13.3	12	12
Grand Total	**10**	**100%**	**90**	**100%**	**100**	**100%**

Chi-square = 4.756 with 3 degrees of freedom; P = 0.254 (NS)

Table - 5: p53 expression in relation to differentiation of SCC.

p53 Grade (%expression)	Well differentiated		Moderately differentiated		Poorly differentiated		Grand Total	
	N	%	N	%			N	%
0 (Negative)	1	33.3	12	15.8	1	20	14	16.7
1 (1-5%)	0	0	2	2.6	0	0	2	2.4
2 (6-25%)	1	33.3	23	30.3	0	0	24	28.6
3 (26-50%)	0	0	29	36.8	1	20	30	35.7
4 (51-75%)	0	0	7	9.2	1	20	8	9.5
5 (75%)	1	33.3	3	3.9	2	40	6	7.1
Grand Total	**3**	**100%**	**76**	**100%**	**5**	**100%**	**84**	**100%**

Chi-square = 16.246 with 10 degrees of freedom; P = 0.093 (NS)

Table - 6: p53 intensity in relation to differentiation of SCC.

p53 intensity	Well differentiated		Moderately differentiated		Poorly differentiated		Grand Total	
	N	%	N	%	N	%	N	%
Negative	1	33.3	12	15.8	1	20	14	16.7
Mild	1	33.3	17	22.4	0	0	18	21.4
Moderate	0	0	42	55.3	0	0	42	50
Intense	1	33.3	5	6.6	4	80	10	11.9
Grand Total	**3**	**100%**	**76**	**100%**	**5**	**100%**	**84**	**100%**

Chi-square = 28.734 with 6 degrees of freedom; P < 0.001 (S)

Table - 7: p53 final score in pre malignant and malignant lesion.

p53 final score	CIN (pre malignant)		Malignant (SCC, AC)		Grand Total	
	N	%	N	%	N	%
0-2	4	40	19	21.1	23	23
3-4	3	30	32	35.6	35	35
5-6	3	30	28	31.1	31	31
7-8	0	0	11	12.2	11	11
Grand Total	**10**	**100%**	**90**	**100%**	**100**	**100%**

Chi-square = 2.701 with 3 degrees of freedom; P = 0.599 (NS)

Table - 8: p53 final score in relation to differentiation of SCC.

p53 final score	Well differentiated		Moderately differentiated		Poorly differentiated		Grand Total	
	N	%	N	%	N	%	N	%
0-2	1	33.3	13	17.1	1	20	15	17.9
3-4	1	33.3	31	40.8	0	0	32	38.1
5-6	0	0	27	35.5	1	20	28	33.3
7-8	1	33.3	5	6.6	3	60	9	10.7
Grand Total	**3**	**100%**	**76**	**100%**	**5**	**100%**	**84**	**100%**

Chi-square = 18.386 with 6 degrees of freedom; P = 0.005 (S)

The pre malignant cases with mild and moderate intensity were both found to have 30% cases each. Whereas, in malignant cases maximum number of cases (46.7%) showed moderate intensity staining. However, this difference in p53 intensity was found to be statistically insignificant (p=0.254) (**Table – 4**).

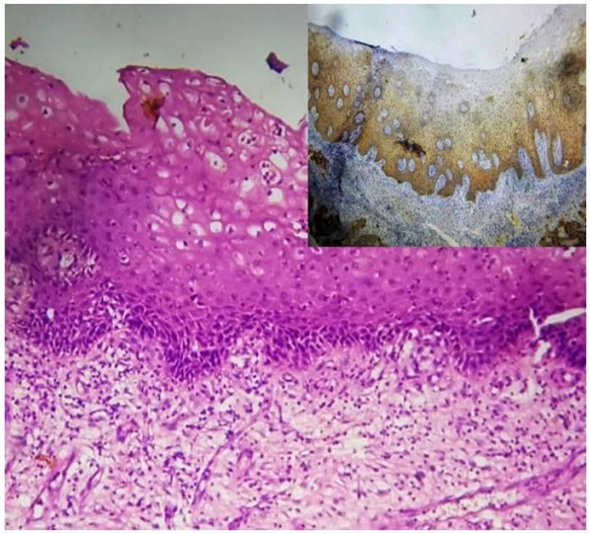
In Squamous Cell Carcinoma, well differentiated cases showed p53 grade of 2 and 5 positivity each being 33.3%. In moderately differentiated cases, maximum number of cases (36.8%) showed p53 grade 3 positivity. In poorly differentiated SCC maximum number of cases (40%) showed grade 5 positivity. This difference is however statistically insignificant (p=0.093) (**Table – 5**).

Out of 3 cases of well differentiated SCC, 1 case each showed mild and intense staining. While in moderately differentiated SCC majority of cases (55.3%) showed moderate p53 intensity. In poorly differentiated SCC majority number of cases (80%) showed intense p53 staining. This suggests that as the grade of differentiation progresses from well to poor the intensity of p53 staining increases and this difference was found to be statistically significant (p<0.001) (**Table – 6**).

On calculating the total score (p53 grade+ p53 intensity) majority of the pre malignant cases had score 0-2 while in malignant cases, majority of cases (35.6%) had scores 3 and 4. However this difference in pre malignant and malignant lesions was found to be statistically insignificant (p=0.599) (**Table – 7**).

While correlating the p53 final score in relation to grade of differentiation of SCC a high score was observed in poorly differentiated cases, a relatively lower score was observed in moderately differentiated cases. This difference in p53 final score in relation to differentiation of SCC was found to be statistically significant (p=0.005). This suggests a potential role of p53 as proliferative index in uterine cervical neoplasia (**Table – 8**).

Figure - 1: CIN 2 (10X). Loss of differentiation with basaloid cells in the lower and middle third of the epithelium is noted. Inset: (10X). p53 stained nuclei seen in the lower and middle third of the epithelium in CIN 2. p53 score 3.



Discussion

Demographic data

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. This finding was similar to studies done by Rajaram, et al. [8](52.1 years), Tjalma, et al. [9] (52 years) and Tan, et al. [10] (50.3 years). The peak age for cervical cancer incidence in India is 45-54 years, which is similar to the rest of South Asia (WHO/ICO Information Centre on HPV and Cervical Cancer) [7]. 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%). The average age of CIN patients in this study was a decade younger than the invasive cancer group concurrent with previous studies [12, 13]. In our study, maximum number of patients (49%) presented with abnormal bleeding similar to the study done by Rajaram, et al. [8].

Histopathological features

SCC was the most common histologic type of cervical cancer encountered in our study, with most tumors being moderately differentiated. These findings were in concordance with study done by Win, et al. (72.5%) [14].

Figure - 2: CIN 3 (10X). Loss of differentiation seen with basaloid cells replacing the entire thickness of epithelium. Inset: (10X). p53 stained nuclei seen in the entire epithelial thickness in CIN 3. p53 score 5.

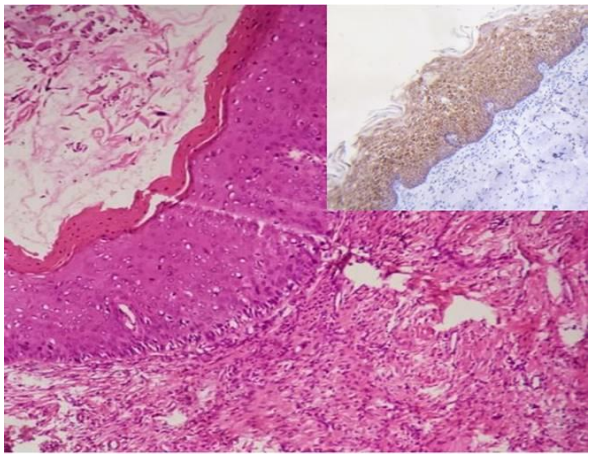
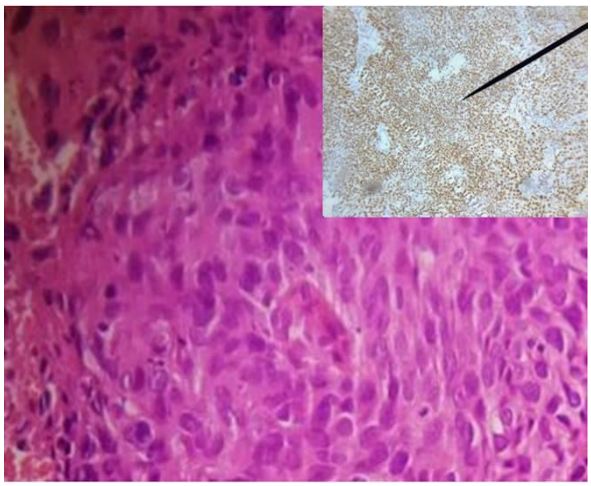


Figure - 3: Moderately differentiated squamous cell carcinoma of the cervix (40X). Broad trabeculae and sheets of pleomorphic cells are noted. Inset: High immunoexpression of p53 seen in squamous cell carcinoma of the cervix. p53 score 8. This case was diagnosed as moderately differentiated SCC on H&E but was upgraded to poor differentiation after IHC with p53.

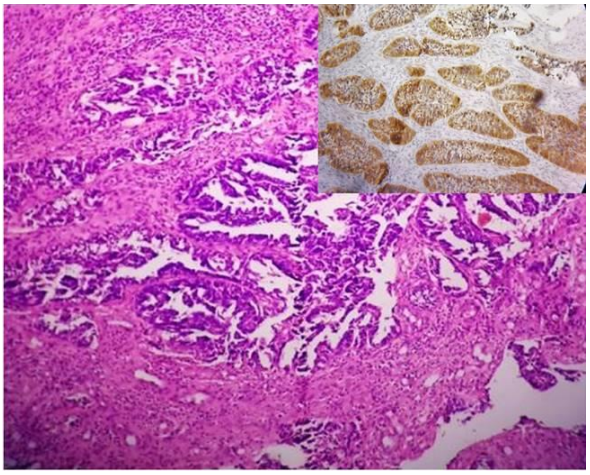


Immunoexpression of p53

The pathogenesis of cervical cancer is thought to occur through a multistep process involving HPV infection in more than 95% of the cases [8]. The

viral proteins E6/E7 of HPV functionally interfere with cell cycle control by inactivating tumor suppressor gene p53 and the retinoblastoma protein. Positive staining for p53 protein by IHC is considered to be abnormal and felt to be a poor prognostic predictor in many types of malignancies, although conflicting results are available in literature [15]. Wild-type p53 protein usually resides in normal cell nuclei. This protein is unstable and has a half-life of only 20–30 min, while the mutant p53 protein is more stable and has a prolonged half-life, resulting in detectable immunohistochemical staining [16].

Figure - 4: (10X) Well differentiated Adenocarcinoma of the cervix shows glandular proliferation with nuclear atypia. Inset: (10X) A case of well differentiated Adenocarcinoma showing strong nuclear positivity for p53 (score 7).



In the present study, the incidence of p53 positivity in neoplastic lesions was 80%. In various studies, the range of nuclear p53 positivity in cervical carcinoma was observed to be 25.2-85.7%. Few studies [10, 14, 17, 18] have showed high p53 positivity similar to our study, whereas others [19, 20] have showed a lower positivity of p53 in cervical cancer (**Table - 9**).

The varying range in different studies could be attributed to the composition of the study population, different specimen fixation techniques, and antigen retrieval methods.

Figure - 5: (10X) This photomicrograph depicts a case which was histologically diagnosed as CIN 3 and after applying p53 a foci of microinvasive carcinoma was noted.

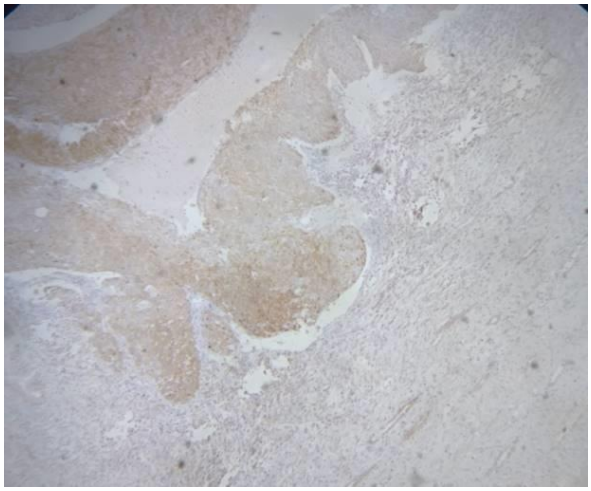


Table - 9: p53 incidence in various studies.

Study	p53 incidence (%)
Oka, et al. [19]	52.1
Haenrgen, et al. [17]	85.7
Ngan, et al. [20]	25.2
Tjalma, et al. [9]	42.0
Win, et al. [14]	80.0
Madhumati, et al. [21]	45.5
Baskaran, et al. [18]	83.0
Sandhu, et al. [22]	86.7
Tan, et al. [10]	85.2
Present Study	80.0

Bahnassy, et al. [23] in their study of 110 cases of SCC and CIN concluded that aberrations of p27, cyclin E, CDK4, and p16INK4A are early events in HPV 16 and 18 associated cervical carcinoma, whereas cyclin D1 and p53 pathway abnormalities are considered as late events. In contrast, Tjalma, et al. [9] observed higher p53 positivity in Stage 1A, 1B, and 2B, and Ikuta, et al. [26] observed that p53 expression was an indicator of unfavorable prognosis in Stage 1B of SCC. Vasilescu, et al. in their study concluded that p53 was a prognostic factor for the aggressiveness of tumor when more that 30% positivity was seen in tumor nuclei [25]. These findings were similar to our study.

Our study had 10 cases of CIN of which 1 case was of CIN1, 2 cases were of CIN 2 while the remaining 7 were 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 1/3rd, 2/3rd and all the layers of squamous epithelium respectively (**Figure – 1, 2**). Jeffers, et al. [4] and Hunt, et al. [3] observed similar patterns in their studies. Tan, et al. have observed similar findings [10]. In the studies done by Baskaran, et al. [18] and Bahnassyet al.[23] a gradual increase in p53 positivity was observed as the lesion progressed from CIN to ISCC. This finding was utilized by Singh, et al. [7] in cytology smears where they found that abnormal expression of p53 was noted in cervical dysplasia, and it increased with higher cytological grades. In our study there was one case which was histologically diagnosed as CIN 3 and after applying IHC with p53 a foci of microinvasive carcinoma was noted and the lesion was upstaged (**Figure - 5**). Similar to our study, Madhumati, et al. [21] concluded in their study that p53 could be used as an important marker for low-grade CIN lesions showing high proliferative index and that p53 overexpression can be utilized as a marker to differentiate difficult cases of CIN 3 from microinvasive SCC. While correlating histologic type with the p53 score (**Table 8**) and p53 intensity (**Table - 6**), a statistically significant association was observed in our study indicating that p53 positivity was predominantly seen in SCC (**Figure - 3**). A similar pattern was observed in other studies [8, 9, 14]. In our study, p53 positivity increased in grade and intensity with the increase in pleomorphism of the nuclei, similar to the study done by Carrilho, et al. [24], which was statistically significant. In a study done by Jiko, et al., p53 mutations were seen in 32% ADC and the incidence of these mutations was higher in cases at advanced clinical stages and with high grades of nuclear and structural atypia [11]. We had six cases of Adenocarcinoma cervix, out of which 2 cases were p53 positive (**Figure - 4**) and the remaining 4 were negative. Similar results are found in various studies. In our study, p53 positivity increased as the Broder's grade worsened (**Tables - 5, 6, 8**), similar to study done by Tjalma, et al. [9]. This difference of final score of p53 in relation to Broder's Grade was statistically significant (P = 0.005) (**Table - 8**).

The aim of this study was to evaluate the role of p53 as proliferative index in neoplasia of uterine cervix that has been previously investigated for prognostic information in cervical cancer, and it covers a variety of major functions in cervical carcinogenesis. Few studies have showed that p53 accumulation in the tumor was associated with shorter overall patient survival [9].In our study, p53 positivity increased with SCC histologic type and higher tumor grade. The pattern of positivity was different in normal cervical epithelium, CIN, and invasive SCC 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. In future, studies with a larger sample size representative of all histologic categories could be performed to evaluate the correlation of p53 positivity with clinico-pathological parameters, prognosis and response to therapy in Indian patients with CIN and invasive cervical carcinomas.

Conclusion

Squamous neoplasia of the uterine cervix which is the most common cancer of women in India has a distinct demographic profile with larger number of patients presenting with higher stage tumors. Higher immunoexpression of p53 in higher grades of CIN and SCC seen in this study throws light on the pathogenetic mechanisms of development of squamous neoplasia in the uterine cervix. It highlights the role played by these proteins in early carcinogenesis itself and the possible association with high risk HPV types. The expression of p53 is greater in the malignant cervical neoplasms than pre-malignant cervical lesions, suggesting that p53 overexpression can be used as a poor prognostic indicator. Also the percentage of positive cells and staining intensity increases with higher

histological grade. p53 could also be used to differentiate CIN III from SCC in difficult situations. It can also be used to categorize CIN lesions. Furthermore, an infectious etiology with high risk HPV is suggested leading to overexpression of p53 and further HPV studies are indicated.

References

1. Aswathy S, Reshma J, Avani D. Epidemiology of cervical cancer with special focus on India. Int J Womens Health, 2015; 7: 404-14.
2. Pietsch EC, Sykes SM, Mc Mahon SB, Murphy ME. The P53 family and programmed cell death. Oncogene, 2008; 27(50): 6507-521.
3. Hunt CR, Hale RJ, Buckley CH, Hunt J. p53 expression in carcinoma of the cervix. Journal of Clinical Pathology, 1996; 49: 971.
4. Jeffers MD, Richmond J, Farquharson M, McNicol AM. p53immunoreactivity in cervical intraepithelial neoplasia and non-neoplastic cervical squamous epithelium. Journal of Clinical Pathology, 1994; 47(12): 1073.
5. Thomas MD, McIntosh GG, Anderson JJ, Mcenna DM, Parr AH, Stone JR, et al. A novel quantitative immunoassay system for p53 using antibodies selected for optimum designation for p53 status. Journal of Clinical Pathology, 1997; 50: 143.
6. Cheah.P, Looi L. P53 immunohistochemical expression: messages in cervical carcinogenesis. Pathology, 2002; 34: 326–31.
7. Singh M, Srivastava S, Singh U, Mathur N, Shukla Y. Coexpression of P53 and Bcl2 proteins in Human Papilloma virus induced premalignant lesions of the uterine cervix: Correlation with progression to malignancy. Tumor Biol., 2009; 30: 276-85.
8. Rajaram S, Gupta G, Agarwal S, Goel N, Singh KC. High-risk human papillomavirus, tumour suppressor protein p53 and mitomycin-c in invasive squamous cell carcinoma cervix. Indian Journal of Cancer, 2006; 43: 156.
9. Tjalma WA, Weyler JJ, Bogers JJ, Pollefliet C, Baay M, Goovaerts GC, et al. The importance of biological factors (bcl-2, bax, p53, PCNA, MI, HPV and angiogenesis) in invasive cervical cancer. Eur J ObstetGynecolReprod Biol., 2001; 97: 223-30.
10. Tan GC, Sharifah NA, Salwati S, Shiran MS, Hattaa AZ, Ng HO. Immunohistochemical study of p53 expression in premalignant and malignant cervical neoplasms. Med Health, 2007; 2: 125-32.
11. Satija A. Cervical cancer in India. Neoplasma, 2000; 47: 143-7.
12. Shanta V, Krishnamurthi S, Gajalakshmi CK, Swaminathan R, Ravichandran K. Epidemiology of cancer of the cervix: global and national perspective. Journal of the Indian Medical association, 2000; 98: 49-52.
13. Kurman RJ, Blaustein A. Blausteins Pathology of the female genital tract. 5th edition, Springer, 2002.
14. Ne Win, TheinMyint Thu, AaingNaingTun, ThiThi Aye, SoeSoe, KyawNyunt, et al. p53 expression in carcinoma cervix. Myanmar Medical Journal, 2004; 48: 1-4.
15. Khunamornpong S, Siriaunkgul S, Manusirivithaya S, Settakorn J, Srisomboon J, Ponjaroen J, et al. Prognostic value of p53 expression in early stage cervical carcinoma treated by surgery. Asian Pac J Cancer Prev., 2008; 9: 48-52.
16. Levine EL, Davidson SE, Roberts SA, et al. Apoptosis as a predictor of response to radiotherapy in cervical carcinoma. Lancet, 1994; 344: 472.
17. Gabriela Haenrgen, Ulf Krause, Axel Becker, Peter Stadler, Christine

Lantenschlarger, Wolfgang Wohlrab et al. Tumour hypoxia, p53 and prognosis in cervical carcinoma. International Journal of Radiation Oncology, Biology, Physics, 2001 July; 50(4): 865-72.

18. Baskaran K, Karunanithi S, Sivakamasundari I, Sundresh NJ, Thamaraiselvi B, Swaruparani S. Overexpression of p53 and its role as early biomarker in carcinoma of uterine cervix. Int J Res Pharm Sci., 2013; 4: 198-202.
19. Kuniyuki Oka, Yoshiyuki Suzuki, Takashi Nakano. Expression of p27 and p53 in cervical squamous cell carcinoma patients treated with radiotherapy alone. Cancer, 2000; 88: 2766-73.
20. Ngan HY, Cheung AN, Liu SS, Cheng DK, Ng TY, Wong LC. Abnormal expression of pan-ras, c-myc and tp53 in squamous cell carcinoma of cervix: Correlation with HPV and prognosis. Oncol Rep., 2001; 8: 557-61.
21. Madhumati G, Kavita S, Anju M, Uma S, Raj M. Immunohistochemical Expression of Cell Proliferating Nuclear Antigen (PCNA) and p53 Protein in Cervical Cancer. J ObstetGynaecol India, 2012; 62: 557-61.
22. Sandhu JK, Shivakumar S. Study of p53 in Cervical Intraepithelial Neoplasia and Carcinoma Cervix with Clinico-pathological Correlation. Int J Sci Stud., 2016; 4(1): 208-214.
23. Bahnassy AA, Zekri AR, Saleh M, Lotayef M, Moneir M, Shawki O. The possible role of cell cycle regulators in multistep process of HPV-associated cervical carcinoma. BMC ClinPathol., 2007; 7: 4.
24. Carrilho C, Gouveia P, Cantel M, Alberto M, Buane L, David L. Characterization of human papillomavirus infection, P53 and Ki-67 expression in cervix cancer of Mozambican women. Pathol Res Pract., 2003; 199: 303-11.
25. Vasilescu F, Ceausu M, Tanase C, Stanculescu R, Vladescu T, Ceausu Z. p53, p63 and Ki-67 assessment in HPV-induced cervical neoplasia. Rom J MorpholEmbryol., 2009; 50: 357-61.
26. Ikuta A, Saito J, Mizokami T, Nakamoto T, Yasuhara M, Nagata F, et al. Correlation p53 expression and human papilloma virus deoxyribonucleic acid with clinical outcome in early uterine cervical carcinoma. Cancer Detect Prev., 2005; 29: 528-36.