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Original Research Paper

Pathology

STUDY OF P53 EXPRESSION IN CARCINOMA CERVIX AND NORMAL CERVICAL EPITHELIUM WITH CLINICAL CORRELATION

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KEYWORDS :

INTRODUCTION

Cervical cancer is a world- wide public health problem with an incidence of 530,232 new cases and 275,008 deaths every year. Across the world, carcinoma of the uterine cervix is the second most common malignancy in women, and is a major cause of morbidity and mortality. Cervical carcinoma is the leading cancer in India, common in the females between 15 and 44 years of age group [1]. India accounts for one-fifth of the world's burden of cervical cancer and the incidence of cervical carcinoma has increased from 0.11 million in the year 2000 to 0.16 million cases in 2012 [2].

Cervical carcinoma is unique among human cancers by being the first found to be virtually solely attributable to the effects of an infectious agent [3]. Infection with HPV is the greatest risk factor in the development of cervical carcinoma and its role in the progression of the precursor lesions to invasive cervical carcinoma is well established [4].

Histopathology

The development of cancer is a multifactorial process which includes the sequential activation of oncogenes and other genetic derangement. Screening has been shown to be an effective method for identifying pre-neoplastic stage early, thereby reducing the mortality [5].

There are primary and secondary biomarkers in cervical carcinoma. The primary marker being HPV DNA and secondary markers like p53, bcl2,ki67,mdm2,cyclin D1,cytokeratin,cyclinE,c-fos, p50, fra 1, p16, notch1, rband 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 [6]. P53 monitors the integrity of the genome [7]. DNA repair process is initiated by the activation of the p53 gene through DNA protein kinase during DNA damage. Mechanisms of inactivation or loss of the wild type of p53 gene can be by either of the following processes-mutation within the genome, virally encoded p53 binding E6 oncoprotein. Mutant p53 forms an oligomeric complex with wild type of p53 which turns out to be functionless and mutant p53 gains a new oncogenic function that overcomes the negative regulation by small quantities of the wild type p53 [8].

This study aimed at comparison of expression of p53 in carcinoma cervix and normal cervical epithelium. The study also aims at correlation of immune positivity of p53 with the histopathological type, grade of carcinoma cervix and clinico-pathological prognostic parameters.

The p53 is a 53-kDa phosphoprotein produced by p53 gene. This 20-kb human gene consisting of 11 exons is located on the chromosome 17p13.1. Normally it is expressed in small amounts with short half-life. The primary functions are growth arrest, apoptosis, senescence, differentiation and anti-angiogenesis. The vast majority of tumor associated p53 missense mutations occur within the core domain. More than 95% of alterations in p53 are point mutation that produces the mutant p53 proteins which loses its transactivational activity. Thus, mutated p53 has a changed conformation, a longer half-life (increased stability) and disordered function. Nuclear staining of the majority of tumor cells accompanied by absence of reactivity in surrounding uninvolved tissue or stroma is the most commonly observed pattern characteristic of the presence of a missense p53 mutation. The inability to delay cell division processes increases the probability that DNA damage will remain uncorrected during DNA replication, leading to neoplastic progression. Between 30 and 70% of malignant tumours of almost every organ and histologic subtype have a point mutation in one of the two p53 gene copies and loss of the other allele. In addition, loss of p53 function by other mechanisms may be important in some of the cancers that do not have p53 allele loss or mutation. Assessment of p53 function can be done by gene sequencing, IHC and functional tests. IHC is a rapid preliminary indication of p53 status in tumors and can be performed by immuno histochemical detection of nuclear p53 accumulation. The p53 protein may be biochemically altered by a different mechanism than gene mutation, possibly involving other p53-binding inhibitory proteins. Inactivation of p53 represents a key step in cervical carcinogenesis, similarly to other human cancers, in which the p53 gene is frequently mutated. It was recently proposed that the two p53variants at codon 72 might contribute differently to the development of invasive cervical cancer.

AIM AND OBJECTIVES

- Study of expression of p53 in cervical carcinomas of various histological types and normal cervical epithelium.
- Correlation of p53 expression with the histopathological grade of cervical carcinomas.

MATERIALS AND METHODS

40 cases of cervical biopsy/hysterectomy submitted to the department of Pathology, CAIMS, Karimnagar for histopathological evaluation during the study period of January 2014 to October 2015 were studied. Of these, 40 cases were cervical biopsy/hysterectomy done for the clinical diagnosis of cervical cancer and 20 were hysterectomies done for other gynecologic causes like dysfunctional uterine bleeding, uterine fibroids etc. with normal cervical epithelium.

Clinical data was obtained from the patient's outpatient and inpatient records and Requisition forms accompanying the specimens to the department. On arrival to the department, the specimens were adequately fixed in 10% neutral buffer formalin following which the evaluation of gross features was done.

The gross details of specimens submitted for evaluation of malignancy were observed and recorded based on the protocol for evaluation of cervical malignancy.

The hysterectomies done for other gynecologic causes were grossed based on the routine protocol for hysterectomy specimen. Representative tissue is subjected for processing.

INCLUSION CRITERIA

- Cervix biopsies and hysterectomy specimens, of females above of 20yrs.
- Benign, premalignant and malignant epithelial lesions of cervix were included.
- Representative lesion areas are included for IHC.

EXCLUSION CRITERIA

- Unusual tumor types were excluded.
- Tumor tissue with necrotic areas was excluded for IHC.

Cervical biopsy specimen was subjected to routine processing for paraffin embedding four to five micron thick sections were taken from paraffin embedded blocks, stained with hematoxylin and eosin (H & E) stain and studied as per the proforma.

The tumors were typed according to the WHO classification system. The modified Broder's grading was used to grade the tumors. The important microscopic subtypes of squamous cell carcinoma, presence of additional in-situ component and pattern of infiltrative margins of invasive component were studied.

P53 Immunostaining using p53 antibody (Dako)

All the 60 cases were subjected to IHC study for p53. The 20 cases of hysterectomies done for other gynecologic causes were used to study the expression of p53 in normal cervical epithelium. Sections underwent histological evaluation to select blocks without necrotic and hemorrhagic areas. The polymer based IHC kit of DAB was used. Positive controls are serous adenocarcinoma of ovary, colorectal adenocarcinoma and negative controls are normal cervical epithelium.

Assessment of expression of p53

Nuclear staining either as coarse or fine granular dots was considered positive. The intensity of staining and the number or percentage of positive cells was assessed (**Table – 1, 2**).

Table - 1: Grading of intensity of p53 staining pattern [10]

Staining pattern	Grading of intensity
Absent	0
Mild	1+
Moderate	2+
Severe	3+

Table - 2: Percentage of positivity of p53 staining [10]

Percentage of cells showing positivity (in 10 HPF)	Grade
1-5% of the tumor cells	1
6-25% of the tumor cells	2
26-50% of the tumor cells	3
51-75% of the tumor cells	4
>75% of the tumor cells	5

Dataanalysis

Thecollecteddatawasenteredinexcelsheetandanalyzedusing Epiinfo softwareandthedescriptivestatistics,Chi-squaretest, Student'st-test,andother applicable statistic altests were appliedforthedataasapplicable.Thep value of< 0.05was considered statisticallysignificant.

RESULTS

In the present study, the age of patients of carcinoma cervix ranged from 31 to 77 years. Mean age of presentation 56.6 years. The peak incidence of carcinoma of Cervix was seen in the sixth decade. 13 subjects are premenopausal (32.5%) and 27 (67.5%) post-menopausal women, all the patients were parous women. Of the 40 cases, 17 patients (42.5%) had 2 child birth, 21 patients (52.5%) had 3 to 5child birth and 2 patients (5) more than 5 child birth. No nulliparous women present in this study.

The gross and microscopic features with special emphasison various Clinico-pathologicalprognosticparameters were studied. These cases were subjected to IHC for p53, the results ofwhichwere correlatedwithvariousclinic pathologic alprognostic parametersofcarcinoma cervix.

Majorityof thepatients of carcinoma cervixwerein their 6th decade (35%). Majorityof thepatients of carcinoma cervixwere of parity3 to 5 (52.5%).

Majority of the patients of carcinoma cervix presented with ulcero prolifer ative growth (70%). Majorityof thepatients of carcinoma cervixwere of clinical stageIIB (31.4%). Majority of the cases of carcinomacervixwereofsquamouscellCarcinoma (82.5%),followed by7.5% of end ocervical adenocarcinoma, 5% of papillary squamous cell carcinoma and5%of CINIII. Mostofthecasesofcarcinoma cervix were moderately differentiated(69.7%) followed by27.3% of well differentiated and 3%of poorly differentiated carcinoma. Mostofthe SCC were large cellnonkeratinizingtype (48.5%)followedby 45.5% largecell keratinizingtype and 6% of small cell non keratinizing type (**Figure – 1, 2**).

Figure - 1: Clinical stage of the tumor.

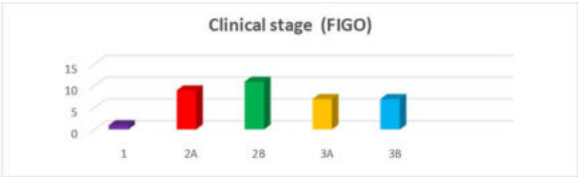


Figure - 2: Distribution of various histological types of Carcinoma cervix.

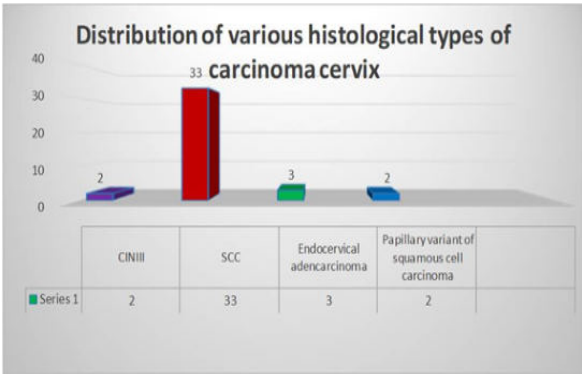
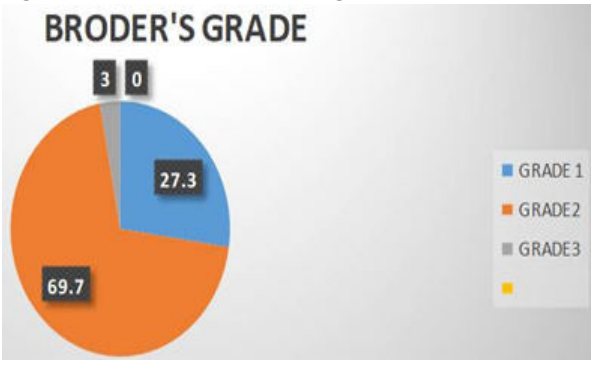


Figure - 3: Distribution of Broder's grade of tumor.



Gross examination: 38 cervical biopsies and 2 Wertheim's hysterectomy of carcinoma cervix were received.

Microscopic examination: The tumors were typed according to the WHO classification system. The modified Broder'sgrading was used to grade the tumors. In the present study, 33 were squamous cell carcinomas (82.5%),2 were papillary squamous cell carcinomas (5%), 3cases of endocervical adenocarcinomas (7.5%) and 2 cases (5%) of Cervical intra epithelial neoplasia (CINIII) **(Figure – 3).**

Table - 3: Correlation of p53 positivity with clinical parameters (parity, age, menopausal status).

Parity	TotalNo.	P53 positive(%)	P53 negative(%)
0-2	17	12 (70.6%)	5 (29.4%)
3-5	21	18 (85.5%)	3 (14.5%)
>5	02	02 (100%)	0
Age group (years)			
31`-40	06	04 (66,6%)	02(33.4%)
41-50	07	06 (85.4%)	01(14.6%)
51-60	10	08 (80%)	02 (20%)
61-70	14	12 (84%)	02 (16%)
71-80	03	02 (6%)	01(33.4%)
Menopausal status			
Pre-menopausal	13	10 (77.7%)	3 (22.3%)
Post-menopausal	27	22 (80.1%)	5 (19.9%)

Table - 4: Correlation of p53 positivity with FIGO staging.

	Total No	P53 +VE	P53 -VE
	40	32	08
FIGO Stage	35	29	06
1	1	1(100%)	0
2A	9	7 (77%)	2 (23%)
2B	11	9 (81.8%)	2 (18.2%)
3A	7	6 (85.5%)	1 (14.5%)
3B	7	6 (85.5%)	1 (14.5 %)

Table - 5: Correlation between p53 grade and FIGO staging.

Grade of p53 expression (% of cells positive/10 HPF)	1	2A	2B	3A	3B
1-5 (Grade1)	0	2(28.6%)	4(44.4%)	1(16.7%)	1(16.7%)
6-25(Grade2)	0	3(42.8%)	4(44.4%)	2(33.3%)	2(33.3%)
26-50(Grade3)	1(100%)	0	1(11.1%)	1(16.7%)	1(16.7%)
50-75(Grade4)	0	1(14.3%)	0	2(33.3%)	1(16.7%)
>75(Grade5)	0	1(14.3%)	0	0	1(16.7%)
Total	1	7	9	6	6

In the 33 cases of various invasive squamous cell carcinomas of cervix studied,9cases(27.3%) were well differentiated (Grade I), 23 cases (69.7%) were moderately differentiated (Grade II) and 1case (3%) was poorly differentiated (Grade III) carcinoma. The cases of SCC were further sub typed into large cell non keratinizing type (LCNK), Large cell keratinizing type (LCK) and small cell non keratinizing type (SCNK). Among the 33 cases of squamous cell carcinoma subtypes16 (48.5%) were large cell non-Keratinizing type and 15 (45.5%) were large cell keratinizing type. 2 (6%) cases of smallcell non-Keratinizing SCC types were found.

The grade and intensity of p53 expression was studied in all the 40 cases of Carcinoma cervix in the present study in a semi-quantitative manner. Among the 40 cases, p53 was expressed in 32 cases (80%) of carcinoma cervix. It was negative in the rest of cases (20%).The expression of p53 studied in 20 cases of hysterectomies done for Other gynecologic causes showing normal cervical epithelium (ecto-and endo-cervical epithelium) to assess p53expression in normal cervical epithelium. All cases were negative of p53 expression **(Table – 3).**

Figure - 4: Distribution p53 positivity in FIGO Stages.

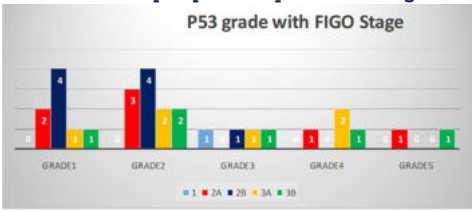


Figure - 5: Gross picture of carcinoma cervix hysterectomy specimen and cut section is showing irregular growth in the cervix.

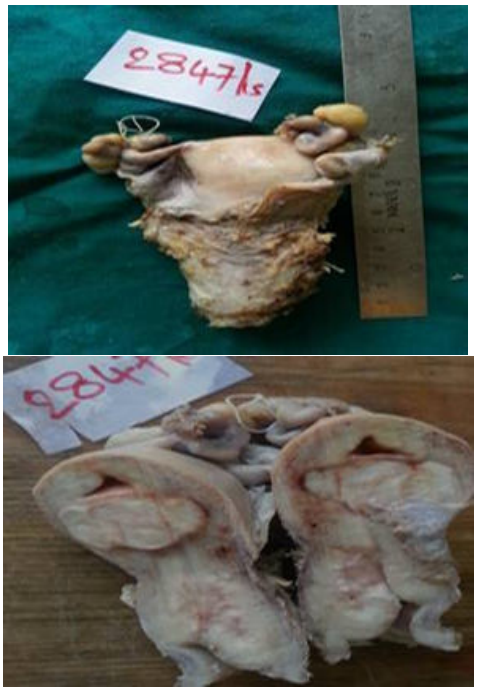
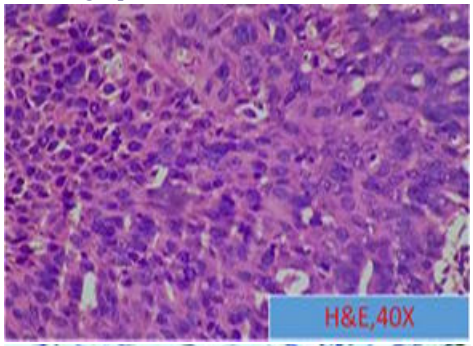


Figure - 6: Grade 2 Positivity of P53 expression in large cell non keratinizing squamous cell carcinoma.



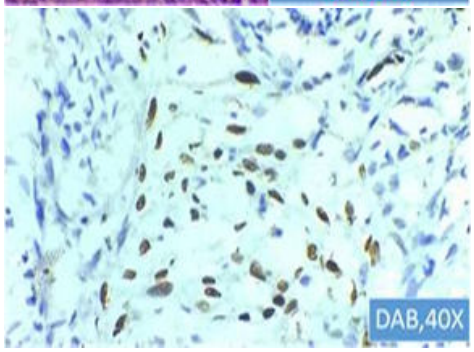
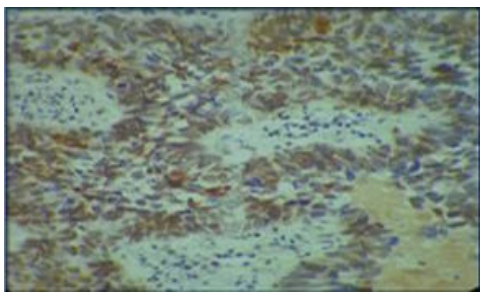


Figure - 7: Grade 3 positivity of p53 expression in papillary adeno carcinoma.



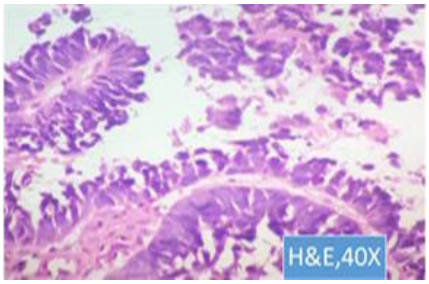


Figure - 8: Grade 2 positivity of p53 expression in CIN III.

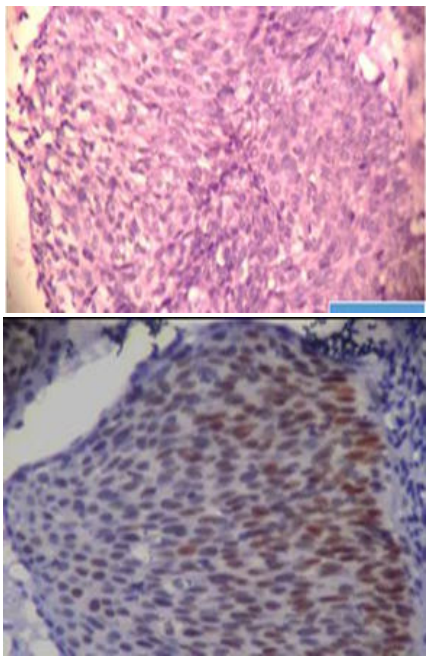
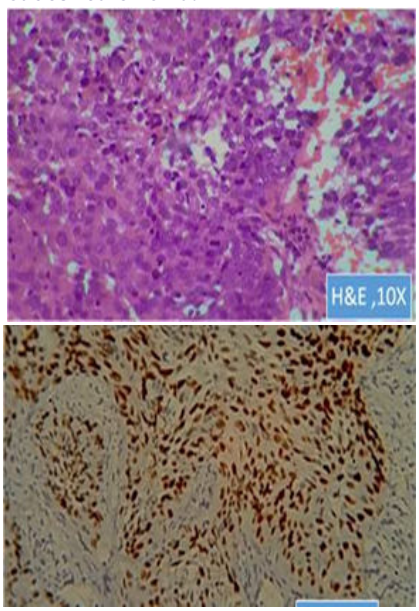


Figure - 9: Grade 5 positivity of p53 expression in moderately differentiated large cell non keratinizing squamous cell carcinoma.



In the present study, one case in clinical stage I showed p53 Positivity (100%). The p53 expression was positive in 7 of 9 cases (77%) of clinical stage 2a, 9 of 11cases (81.8%) of clinical stage 2b, 6 of 7 cases (85.5%) of 3a and of 7 cases (85.5%) of clinical stage 3b. No statistically significant association was noted between clinical stage and P53 positivity (p value =0.97) **(Table – 4, 5, Figure – 4 to 9).**

DISCUSSION

Uterine cervical cancer accounts for 15% of all cancers in females. Of these, 80% of cervical cancer is from developing countries while only 20% are from developed world. The p53 gene is one of the most important targets of the HPV E6 gene. It was found that E6 protein has the ability to stimulate p53 degradation, and inhibits several functions of the wild type p53 including the suppression of malignant growth P53 abnormalities may be important in the pathology of cervical carcinoma. Studies have shown that point mutations of the p53 suppressor gene are correlated to the malignant transformation. It has also been suggested that complex binding between thep53 protein and the E6 protein from the human papilloma virus may result in the disturbance of the growth-inhibitory effect of wild-type p53 which in turn results in uncontrolled cell proliferation and malignant transformation.

Newin, et al. [10] suggested that the expression of p53 increased proportionally to the grade of CIN and cervical cancer. Therefore, p53 immunoreactivity can be helpful to decide a neoplastic lesion, but the absence of p53 does not exclude neoplasia. Thep53 protein did not show labeling in normal epithelium. In the invasive cervical cancer specimens we found that 80% (32/40) were P53positive, though showing expression in far nuclei of the carcinoma cells when compared to the expression pattern of other markers. The literature describes conflicting data for p53 expression in cervical cancer, with rates ranging from very low percentages to 62.0% of cancer cells. Although the basis of this marked disparity of results is unclear, it may relate to different Causes including different tissue fixation, as well as the antigen retrieval methods and adopted cutting points. It is also known that in many types of human cancer, the p53 is over expressed as a result of mutations which modify their transcriptional activity, that may, in turn, affect other regulatory proteins for example, MDM2 (also called HDM2 for the human protein).

Development and progression of cervical tumors are associated with alterations in apoptosis, disturbance in immune surveillance, increased cell growth and/or loss of growth suppression (uncontrolled proliferation).Apoptosis plays an important role in the balance between cell death and cell proliferation that is influenced by the expression of a specific set of genes. In the present study most of the patients of carcinoma cervix were of child birth 3to 5 with a mean parity of 3.50±1.93.This finding is in concordance with the study done by Rajaram, et al. [11] in Delhi (5.23±2.34).

While correlating p53 over expression with age of the cervical carcinoma patients, p53 positivity was high in the patients of 5thand 6thdecade and low in patients of 4thdecade. Highest p53 score was observed in 5thdecade. Similar finding of high p53positivity with increasing age was observed by Madhumati, et al. [12] where women less than 30 years of age showed 20% positivity as compared to women more than 30years of age showed 47.6% p53 positivity. Therefore the p53 expression increased with increasing age of the patients. While correlating p53 positivity with parity of patients with carcinoma cervix high p53 expression was seen in women with high parity (93.7% in parity 3 to 5, 100% in parity of more than 5) as compared to only 75% in patients with parity 2.This finding suggests that expression of p53 increased in women with high parity, however, no statistically significant association was observed between parity and p53score (p=0.542). In our study p53 over expression is was higher in post-menopausal women (68.75%) as compared to premenopausal women (31.25%) similar to study done by Madhumati, et al. [12] where the p53 over expression was higher in postmenopausal women (67.5%) as compared to premenopausal women (32.5%). No significant association was found between menopausal status and p53 over expression in our study.

In the present study, the most common clinical feature was White discharge per vagina (WDPV) followed by Post-menopausal bleeding (PMB) and Contact bleeding (CB). The most common clinical presentation in Pre-menopausal women was WDPV and CB and in postmenopausal women it was PMB and WDPV. In the present study most women presented with ulcero proliferative growth (70%) followed by ulcerative lesions (23.3%). This finding is similar to the study Conducted by Rajaram, et al. [11] where the most common clinical finding was exophytic growth (60%) followed by ulcerative growth (33.3%). In the present study most patients presented later in course of the disease. This finding matched with the findings of,Rajaram, et al. [11]. Of the 40 cases of carcinoma cervix majority were squamous cell carcinoma(86.7%) followed by 2cases papillary squamous cell carcinomas(5%),1case of Mucinous endo cervical adenocarcinoma (2.5%) and 2cases of endocervical adenocarcinoma (5%) and, and 2 cases of CINIII (5%).This finding is in concordance with, W.A. Tjalma, et al. [13], Geok Chin, et al. [14], RAF Crawford, et al. [15] and Tan GC, et al. [16]. Of the 33 cases of Squamous cell carcinomas, most of the cases were of large cell non keratinizing subtype(48.5%) followed by large cell keratinizing subtype (45.5%).This finding is similar to the studies done by AbeerA.Bahnassy, et al. LCNK-60.5%).

In the present study p53 expression was assessed in 20 cases of normal Cervical epithelium .The p53 protein did not show labeling in normal epithelium, similar to studies done by Abeer A.Bahnassy, et al. and Grace, et al. [18].In contrast to study done by Florina, et al. ,the p53 positive nuclei were restricted to the basal layers of squamous epithelium. This study is contrast to Wang, et al. P53 stained positively in the basal layer of normal epithelium and in CIN2, but was slightly more intense in CIS and SCC. In our study, the one case of CINIII was positive for p53.In comparison to the normal cervical epithelium the pattern of staining was different. In CINIII the p53positive cells were present throughout the layers of lining epithelium as compared to normal cervical epithelium where the p53 was negative. P53 positivity present in basal layer of squamous epithelium was observed by Jeffers MD, et al.].This marker in the tissue section can be used as an adjunct to definitelydiagnose pre neoplastic and neoplastic lesions in the cervix.

In our study it was noted that the positivity was present in the dysplastic nuclei and with the increase in pleomorphism of the nuclei the intensity of p53 expression increased. Similar finding was observed by Geok Chin, et al. [14].These findings suggest that the p53 marker may be useful to study the metastasis of the epithelial cell which may further help in identifying dysplastic lesions andprogression of disease in patients suffering cervical cancer. In the study done by AbeerA. Bahnassy, et al.gradual increase in p53 positivity was observed as the lesion progressed from CIN to invasive SCC. Madhumati, et al. [12] concluded in their study that p53 could be used as an important marker for low grade CIN lesions showing high proliferative index. The p53 over expression can be used as a marker to differentiate difficult cases of CIN III from micro invasive SCC. The p53 was expressed in 80% of the cases of carcinoma cervix in our study the p53 expression in various studies ranged from 25.2% to 87.5%. The varying range in different studies may be because of the fixation and AR methods.

While correlating grade of p53 positivity, a semi quantitative method was used. In our study 20% cases showed p53 positivity in more than 50% of tumor cells. Similarly studies done by Abeer A.Bahnassy, et al. [17] and Tan GC, et al. [16] 57.9%and 65.2% cases displayed p53 positivity in more than 50% of the tumor cells. Florina, et al. [19] in their study concluded that p53 was a prognostic factor for the aggressiveness of the tumor when more >30% positivity was seen in tumor nuclei.

CONCLUSION

Ourstudyevaluatedthep53expressionin20caseswithnormal cervicalepitheliumand 40 cases of various types of carcinoma cervix.

A comparison between thep 53 expressionin normal cervicale pithelium,CINand invasivecarcinomaof cervix wasdone.

The p53 expression wascorrelatedwiththevariousclinicaland histopathological parametersincasesofcarcinomacervix. (grade) and the intensityofp53 were also studied.

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