Original Research Paper

Pattern of expression of HER2/neu and p53 in premalignant and malignant lesions of uterine cervix

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ABSTRACT:
Methods: It was a descriptive study conducted in histopathologically diagnosed cervical biopsy specimens of carcinoma cervix and cervical intraepithelial neoplasia received in the Department of Pathology, Government Medical College Thiruvananthapuram from 2015 to 2017. Sample size was 50 and immunohistochemical analysis for HER-2/neu and p53 was performed and graded with histopathology as gold standard and analysed for expression of HER-2/neu and p53 and compared with type, grade and stage of tumour. The association was tested using Chi-square test. **Results** : Out of 50 cases, 24 (48%) were negative, 16 (32%) with 1+ positivity, 9 (18%) with 2+ positivity and 1 (2%) were showing 3+ HER-2/neu expression and it had correlation with higher clinical stage but a statistical correlation was not established between grade of SCC and intensity of HER-2/neu staining .Out of 50 cases, 17 (34%) were negative, 15(30%) were having 1+ positivity p53 expression, 10(20%) were showing 2+ positivity and 8 (16%) were showing 3+ positivity. Statistical correlation was established between grade of SCC and intensity of p53 staining but was not established between p53 expression and stage of tumor. **Conclusion**: The present study indicates that the intensity of HER-2 expression did not increase progressively as grade of lesion increased but showed correlation with higher clinical stage and higher expression among malignant cases as compared to premalignant lesion. There is no evidence to suggest that the overexpression of p53 may be useful as a prognostic indicator.

Keywords: Cervical lesions; cervix; HER-2/neu expression; immunostaining

INTRODUCTION:
Worldwide, cervical carcinoma is second only to breast carcinoma in its incidence and mortality. In India carcinoma cervix is the most common malignancy in women with an incidence of 9 to 44 per 100, 000 women[i]. In most of the cases, high-risk subtypes of the human papilloma virus (HPV) are the cause of the disease. Since the introduction of formal screening programmes in high-income countries, cervical cancer incidence and mortality have halved over the past 30 years. Human papillomavirus infection is recognized as the stronger etiological factor for the development of this tumor; however, overexpression of the epidermal growth factor receptor family members is also common and seems to play an important oncogenic role[ii]. The epidermal growth factor receptor (EGFR/HER) family of receptors has been associated with aggressive biological behavior and metastatic potential. Tumor response to

trastuzumab strongly correlate with HER2/neu expression. So by this study we can improve targeted therapy and survival in women with Cervical Intraepithelial Neoplasia and Carcinoma cervix.

Two possible explanations for the transition of HER2 expression are, first; a minor tumor clone originally expressing HER2 has a selective advantage with serial passages. One study indicates HER2-positive cells may have a selective advantage over HER2-negative cells both in vitro and in vivo[iii] Second, tumor cells previously not expressing HER2 began expressing HER2 after serial passaging. Some colon cancer cases exhibit cytoplasmic HER2 overexpression as was found in a patient tumor sample in one study[iv]. The accumulated HER2 protein in the cytoplasm can migrate to the membrane under the influence of the unfolded protein response[v]. The expression of HER-2 is believed to be associated with aggressive biological behavior and metastatic potential[vi] [vii]The best step to follow would be

the development of targeted therapies so as to improve the prognosis of cervical carcinoma patients. An association between p53 protein accumulation and aggressive behavior of cervical carcinoma including a genetic propensity toward metastasis, and recurrence has been observed. Chemotherapeutic drugs may also be more effective against high-stage, p53- mutated cancers than earlier stage cancers.

MATERIALS AND METHODS:

A Descriptive study was done on all histopathologically diagnosed cervical biopsy specimens of carcinoma cervix and cervical intraepithelial neoplasia received in the Department of Pathology from 2015 to 2017. Sample size was 50. Routine Hematoxylin and eosin slides were evaluated. The immunohistochemical analysis was performed on serial sections, using an immunoenzymatic soluble complex method.

The IHC staining procedure for HER-2/neu followed the avidin-biotin-peroxidase complex method after heating so as to maximize antigen retrieval. The HER-2/neu stained slides was evaluated on light microscopy.

The 2014 ASCO/CAP HER-2 Reporting Guideline (for Assessment of Her 2):-
Intensity of HER-2 expression:

0: No staining is observed or shows membrane staining that is incomplete and is faint/barely perceptible and within <10% of tumor cells.

1+: Membrane staining that is incomplete and is faint/barely perceptible and within >10% of tumor cells.

2+: Circumferential staining that is incomplete and/or weak/moderate within > 10% of tumor cells. or Complete intense circumferential membrane staining within < 10% of tumor cells.

3+: Complete intense circumferential membrane staining within > 10% of tumor cells.

Method of Assessment of p53 Expression:

Nuclear staining either as coarse or fine granular brown dots will be considered positive. The intensity of staining and p53 grade will be assessed by semi-quantitative method.

Data was entered into Excel sheet and analysed using SPSS software .The qualitative variables was expressed in percentage. The association was tested using Chi-square test.

Institutional Ethics Committee clearance was obtained prior to commencement of study

RESULT:
Age:

Age range of patients ranged from 48 to 91 years. The average age at diagnosis was 66 year.

Percentage distribution of sample according to her 2

Out of the 50 cases, 24 cases (48%) were negative for Her 2, and 26 cases (52%) were positive for Her 2.

Percentage distribution of sample according to p53

Out of the 50 cases, 17 cases (34%) were negative for p53, and 26 cases (66%) were positive for p53.

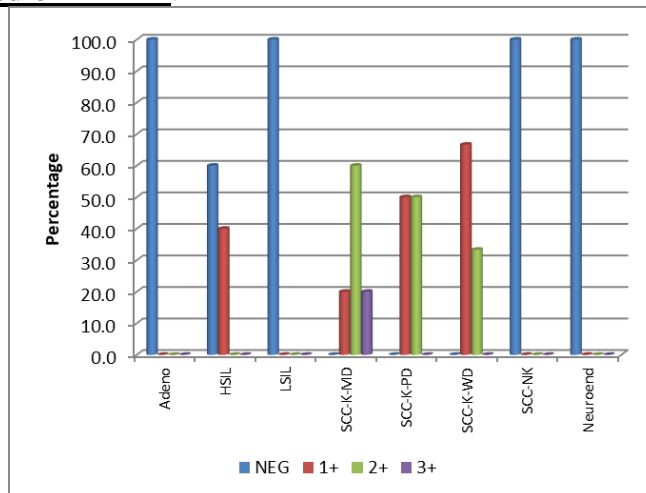## Distribution of HPR Dx based on HER-2:

Fig 1 : Distribution of HPR Dx based on HER-2
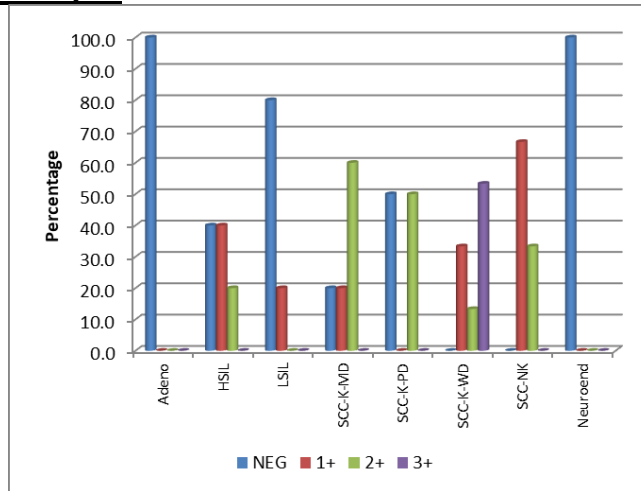
Distribution of HPR Dx based on p53:

Fig 2: Distribution of HPR Dx based on p53

COMPARISON OF GRADES OF SCC BASED ON HER 2:

SCC grade	HER2						Total	
	1+		2+		3+			
	N	%	N	%	N	%	N	%
Well differentiated	10	66.7	5	33.3	0	0	15	100
Moderately differentiated	1	20	3	60	1	20	5	100
Poorly differentiated	1	50	1	50	0	0	2	100
Total	12	54.5	9	40.9	1	20	22	100

Table 1 .Comparison of grades of SCC based on Her 2

COMPARISON OF GRADES OF SCC BASED ON p53:

SCC grade	p53								Total	
	Negative		1+		2+		3+			
	N	%	N	%	N	%	N	%	N	%
Well differentiated	0	0	5	33.3	2	13.3	8	53.3	15	100
Moderately differentiated	1	20	1	20	3	60	0	0	5	100
Poorly differentiated	1	50	0	0	1	50	0	0	2	100
Total	2	9.1	6	27.3	6	27.3	8	36.4	22	100

Table 2: Comparison of grades of SCC based on p53

Statistical correlation was established between grade of SCC and intensity of p53 staining (p=0.033)
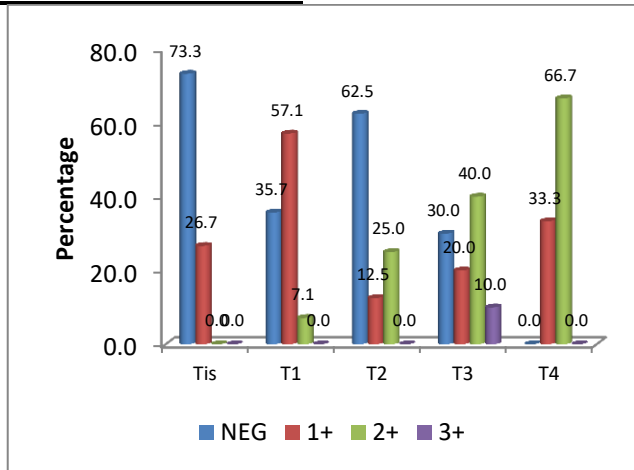
COMPARISON OF T STAGE BASED ON HER-2:

Fig 3: comparison of t stage based on her-2

Significant correlation between stage of cervical carcinoma and intensity of HER-2/neu staining was established (p=0.003) .
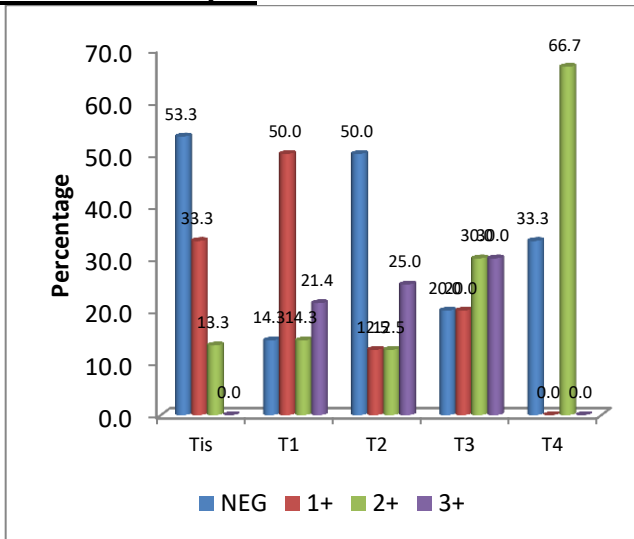
COMPARISON OF T STAGE BASED ON p53:

Fig 4 : comparison of t stage based on p53

Photomicrographs:

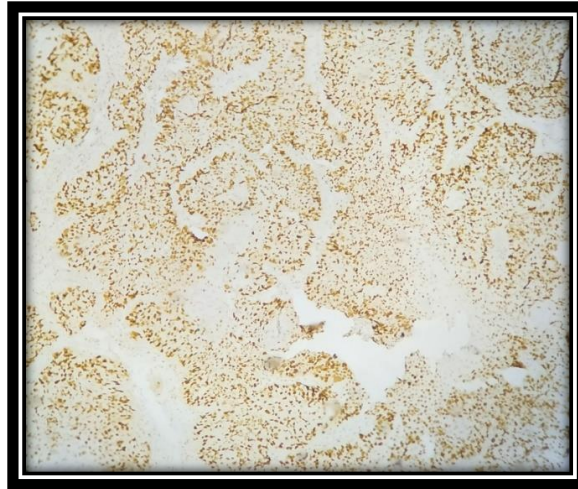**Figure 1. SCC POORLY DIFFERENTIATED- HER 2 (2+) (IHC 40X)**

Figure 2. SCC KERATINISING -p53 (3+) (IHC 20X)

DISCUSSION:
According to histopathological type:

HER2/neu positivity was seen in 40 % of high grade intraepithelial lesions and all the low grade intraepithelial lesions were negative for Her2. Premalignant lesions showed only of 1+type. In comparison to the present study HER2 expression was noted only in 37.5% of CIN cases by Li et al,(2013)[viii] while 60% of CIN cases showed HER2 positivity in a study by Gupta et al (2009).[ix] Lakshmi et al (1997)[x] noticed HER-2 expression in 86.25% of CIN cases and this was found to be statistically significant. Due to the fact that less number of cases was analyzed in the present study, discordance between the studies is there.

In the present study expression of HER 2/neu in cervical carcinoma was seen to be 52 %. Costa et al (1995)[xi] reported HER-2/neu expression in 77% of cervical carcinomas while Lakshmi et al (1997) reported HER-2/neu expression in 96.8% of squamous cell carcinomas of cervix.

All LSIL cases, adeno carcinomas, neuroendocrine carcinoma and squamous cell carcinoma non keratinising cases were negative for HER-2/neu immunostain .

Kihana et al (1994)[xii] studied 44 cases of adenocarcinoma and found 1+ and 2+ positivity in 34 cases. In the study conducted by Hale et al (1992) 5 out of 6 adenocarcinoma (83%) showed definite membranous positivity.

Pattern of expression of Her 2/neu:

All LSIL cases, adeno carcinomas, neuroendocrine carcinoma and squamous cell carcinoma non keratinising cases were negative for HER-2/neu immunostain .

In the present study the HER2/neu expression was seen in 52 % cases while 48 % cases did not show any positivity for HER2/neu. Comparing the HER2/neu positivity with other studies, it was seen that about 63% of cases showed positivity for HER2/neu in Gupta et al (2009). The positivity rate in the present study is close to the study by Sharma et al (2016) and Sarwade et al (2016) who showed 52% and 44% positivity respectively.

In this study the premalignant lesion showed HER 2 expression score upto 1+only , while there were none of the premalignant cases showing score of 2+ and 3+ .Higher score of HER 2/neu was seen only in malignant cases .

Grade:

A statistical correlation was not established between grade of SCC and intensity of HER-2/neu staining (p=0.231)

Joseph et al (2015) in their study reported 100 % positivity rate for all histopathological grades; however ,with respect to IHC score ,they also reported an increasing trend of higher scores(3+) increasing from 0% well differentiated and 27% moderately differentiated to 72.7% in poorly differentiated histopathological grades respectively but did not find any relation between HER2/neu expression and grades of SCC.

In my study, the findings are not in agreement with the literature and does not show incremental trend of HER 2/neu IHC expression with increasing loss of differentiation.

Stage:

Significant correlation between stage of cervical carcinoma and intensity of HER-2/neu staining was established (p=0.003) .

In the present study it was found that higher intensity of HER2/neu expression among cases under stage III and stage IV . A statistically significant relationship was

established between HER 2/neu expression and stage of the tumour (p value- 0.003).

Similar to our study Ndubisi et al(1997) and Gupta et al (2009) found a significant correlation between HER-2/neu expression and higher stage of cervical carcinoma.

Kim *et al[xiii]* stated that HER-2 expression in cervical carcinoma was an independent prognostic factor.

P53:
Pattern of expression

Out of 50 cases, 17 (34%) were negative, 15(30%) were having 1+ positivity p53 expression, 10(20%) were showing 2+ positivity p53 expression and 8 (16%) were showing 3+ p53 expression.

All LSIL cases, adeno carcinomas, neuroendocrine carcinoma cases were negative p53 immunostain

According to histopathological type:
All cases of adenocarcinoma and neuroendocrine carcinoma were negative for p53. Among 10 cases of HSIL 4 cases(40%) were negative and rest showed 1 + and 2+ positivity. Among 5 cases of LSIL 4 cases(80%) were negative,rest showed 1+ positivity. Among 6 cases of squamous cell carcinoma non keratinising 4 cases(66.7%) showed 1 + positivity and remaining 2 showed 2 + positivity.

Grade:
Statistical correlation was established between grade of SCC and intensity of p53 staining (p=0.033)

Stage:
Significant correlation between stage of cervical carcinoma and intensity of HER-2/neu staining was not established (p=0.084)

Mandai *et al[xiv]* and Yong *et al[xv]* analyzed HER-2 expression in 39 and 74 cases of cervical adenocarcinoma respectively and stated that a significant correlation existed between HER-2 expression and lymph node metastasis.

CONCLUSION:
HER 2:
The average age at diagnosis of cervical carcinoma was 66 years. Mean age of patients with cervical intraepithelial neoplasia was 67 years, and mean age of diagnosis of cervical cancer was 69.5 years.

A statistical correlation was not established between grade of SCC and intensity of HER-2/neu staining (p=0.231) .HER 2/neu positivity rate did not show an incremental trend from well differentiated to poorly differentiated .

In the present study it was found that higher intensity of HER2/neu expression among cases under stage III and stage IV and on statistical analysis, HER 2/neu expression in cervical carcinoma showed correlation with higher clinical stage.(p=0.003).

Higher expression of HER 2 /neu among malignant cases as compared to premalignant lesion indicated the possibility of gradual progression in HER2/neu expression from premalignant to malignant cases although several previous studies do not endorse the same in terms of positivity alone. Thus, the findings of present study are in agreement with the literature and show that with increasing loss of differentiation, the HER 2/neu IHC expression rate shows an incremental trend.

Immunohistochemical verdict of HER2/neu expression do not distinguish subgroups of patients at higher risk of recurrence of disease. Hence HER2/neu oncogene may not represent a future target for monoclonal antibody directed therapy for cervical cancer. Therefore further studies on various subtypes and their prognostic significance are needed.

P53:
Statistical correlation was established between grade of SCC and intensity of p53 staining (p=0.033) Statistically significant correlation was not established between p53 expression and stage of tumor (p=0.084)It seems unlikely that p53 analysis will be of value in determining prognosis in carcinoma of the uterine cervix. The p53 protein does indeed seem to play a pivotal role in the pathogenesis of carcinoma of the cervix but, despite the relatively small numbers involved in this series, there is no evidence to suggest that its overexpression may be useful as a prognostic indicator. Traditional parameters such as tumour size and lymph node status still seem to be the most important determinants of prognosis in carcinoma of the uterine cervix.

Data Availability:
All relevant data are included in this manuscript.

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