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P53, EGFR AND HER2 EXPRESSION IN GASTRIC ADENOCARCINOMA AND ITS RELATIONSHIP WITH THE CLINICOPATHOLOGICAL PARAMETERS

Histopathology

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ABSTRACT

Gastric cancer is the fourth most common cancer worldwide. The intestinal and diffuse type gastric carcinoma evolve through different molecular pathways.Various diagnostic and prognostic markers are present of which p53,EGFR and HER2 are important.Few studies related to their expression in gastric cancer in Indian population is available.Therefore,this study was conducted to evaluate the expression of p53,EGFR and HER2 in gastric carcinoma by immunohistochemistry and correlate the results with clinicopathological parameters. A cross sectional , non interventional study was done in the department of pathology of a tertiary care hospital in East India using fifty two samples diagnosed as gastric adenocarcinoma.Immunohistochemical examination was done using monoclonal antibodies against p53,EGFR and HER2 and data was interpreted by light microscopy using semiquantitative method.Statistical analysis was done by Chi-square test and Fisher Exact Test. Out of fifty two cases,diffuse type gastric adenocarcinoma occurred mostly in younger age-group of 35-54years(83.3%)(p value 0.02256) with female preponderance(71.4%)(p value 0.026479).Most of the p53 expression(76.5%) occurred in the age-group of 35-54 years(p value <0.05).Most of the HER2 positive tumours were gastric adenocarcinoma diffuse type(65.6%) (p value 0.000367)and poorly differentiated (87.5%)(p value 0.000054).Also 55.6% EGFR expression and 58.8% p53 expression occurred in poorly differentiated gastric carcinoma.Significant correlation between EGFR expression,nodal involvement and lymphovascular space invasion was found(p value <0.05). HER2 expression is associated with histological type and grade of tumour.EGFR expression is significantly associated with nodal involvement and lymphovascular space invasion. p53 expression is associated with younger age-group while HER2 and EGFR expression is not associated with age and gender.

KEYWORDS

Gastric Adenocarcinoma, Intestinal type, Diffuse type, Immunohistochemistry

INTRODUCTION

Gastric cancer is the fourth most common cancer worldwide. It is also second most common cause of cancer related deaths not only in the world but also in India (Almasi et al., 2015)[1]. In India, overall cancer incidence is 100-121/ 100000 male population and 59-70/100000 in females. As such, they account for 3.1% to 12.9% of all cancer deaths in different parts of India, the highest of this being in southern India (ICMR bulletin 2015)[2].

Gastric cancer is the end result of multifactorial, multi genetic and multistep process. The intestinal type and diffuse type gastric carcinoma appear to evolve through different molecular pathways, involving different oncogenes and tumour suppressor genes. Development of the former follows a multistep progression, from chronic gastritis to intestinal metaplasia, dysplasia and carcinoma. Gene expression profiling studies show that diffuse type carcinoma exhibits altered expression of genes related to cell matrix interaction and extracellular matrix components, whereas intestinal type carcinoma exhibits enhancement of cell growth[3]. Histologically, Lauren's intestinal type of gastric cancer includes papillary,well differentiated adenocarcinoma, moderately differentiated adenocarcinoma or mucinous adenocarcinoma without signet ring cell and diffuse type gastric cancer includes poorly differentiated adenocarcinoma, signet ring cell carcinoma and undifferentiated adenocarcinoma of WHO classification [4].

A better understanding of the molecular basis of cancer has contributed to the development of rationally designed molecular targeted therapies, which interfere with the signalling cascades involved in cell differentiation, proliferation, and survival. Biological prognostic factors which represent a crucial step to gastric cancer includes Human epidermal receptor 2 (HER2), Epidermal growth factor receptor (EGFR), Vascular Endothelial Growth Factor Receptor (VEGF), microsatellite instability, E-cadherin, DNA copy number changes, and changes in the expression of several factors like thymidilate synthase, matrix metalloproteinases, beta-catenin, mucin antigen, p53, COX-2 [5]. Several studies have shown that HER2-positive expression was associated with male gender, intestinal type and also p53 expression was associated with younger age group in intestinal type carcinoma. And some of them are also molecular targets either to chemotherapeutics or targeted therapies, like transtuzumab in HER2 positive tumours.

Genetic alterations are known to play pivotal role in pathogenesis of cancers including carcinoma of the stomach. Among these, the most frequent change is observed in the p53 gene[6]. Normally, p53 gene located on chromosome 17p encodes wild-type p53 protein, which has a very short half-life, and controls the cell cycle (G1 phase arrest) and apoptosis of cells with damaged DNA[7].There has recently been an increase in the number of studies on p53 mutations and abnormal expression of p53 in gastric carcinomas, with variable expression rates of 22-61%[5]. Although TNM staging remains the most important prognostic factor for gastric cancer, there is a need for new prognostic and predictive factors, as prognosis varies among the patients of the same stage. The prognostic value of p53 nuclear expression in gastric carcinoma is still unclear . The objective of the study is to determine the value of p53 accumulation and correlate with the clinicopathological variables in gastric carcinoma.

Epidermal Growth Factor Receptor (EGFR) is a transmembrane tyrosine kinase receptor, and is one of the members of EGFR family of receptors. This receptor activation causes stimulation of the downstream signaling pathway that regulates cell proliferation, migration, adhesion, differentiation and survival.The frequency of EGFR over expression and⁄ or amplification in gastric carcinoma has been variously reported as ranging from 18 to 44%[8].

Few studies related to p53,EGFR and HER2 expression in gastric cancer in Indian population is available. Therefore, we have conducted this study to evaluate the percentage positivity of p53,EGFR and HER2 in gastric carcinoma in Indian population and to correlate the results with the clinicopathological parameters. As they are the molecular targets either to chemotherapeutics or targeted therapies, these are not only prognostic factors, but could also be predictive of therapy like transtuzumab in HER2 positive tumours.

METHODOLOGY :-

A cross sectional, non interventional study was done in Department of Pathology, R.G. Kar Medical College and Hospital, Kolkata over a period of eighteen months. Fifty two samples diagnosed as gastric adenocarcinoma were taken by systematic random sampling irrespective of age,gender.

Non malignant and benign gastric tumours**,** malignancies other than adenocarcinoma like lymphoma, leiomyosarcoma, Lauren's mixed type gastric adenocarcinoma , gastrectomy specimens of the patients who have been treated with chemotherapy or neoadjuvant chemotherapy and gastrectomies done due to causes other than gastric tumour were excluded from the study.

Ultra thin sections (3-4microns) were obtained by microtome from formalin fixed paraffin embedded blocks. After floatation they were picked on poly-L-lysine coated slides ,dried, deparaffinized and rehydrated in descending grades of alcohol.

Heat induced epitope retrieval (HIER) was done by microwave method using Tris Hydroxymethyl Aminomethane(TRIS) buffer, EMPARTA,pH 9.0. TRIS Buffer(EMPARTA ,pH 7.2) was used for washing. Endogenous peroxidase activity was blocked with PolyExcel Peroxidase Block (PATHNSITU) . Incubation with primary antibodies (Monoclonal antibody against p53-p53-BP-53-12 PATHNSITU, Monoclonal antibody against EGFR-EGFR-EP22 PATHNSITU & Monoclonal antibody against HER2- HER2/erb2-EP3 PATHNSITU) were done at 37°C for 60 minutes. For visualisation of result, serial incubation for 30 minutes each was done with PolyExcel Target Binder, PATHNSITU, Poly Horse Radish Peroxidase (HRP) (PolyExcel Stunn DAB Detection System,PATHNSITU) and chromogen (PolyExcel Stunn Dia DAB Buffer and PolyExcel Stunn Diaminobenzidine(DAB) Chromogen, PATHNSITU).

The sections were then counterstained with Harris Hematoxylin and mounted. For positive control - EGFR-Skin tissue, p53- High grade serous carcinoma of ovary, HER2 - Invasive breast carcinoma , were taken.

Expression of HER2, EGFR and p53 were evaluated by semiquantitative method using light microscopy.

IHC Interpretation Of P53 Immunostaining[9]

Intensity of stain	Interpretation
No nuclear staining of tumour cells (complete absence)	Negative
Weak, scattered, or patchy positivity	Weakly Positive
Strong nuclear staining in more than 10% of the tumour cells	Strongly Positive

IHC Interpretation Of HER2 Immunostaining[10,11]

Score	Surgical specimen staining pattern	HER2 overexpression assessment
0	No reactivity or membranous reactivity in<10% of tumour cells	Negative
1+	Faint/barely perceptible membranous reactivity in≥10% of tumour cells; cells are reactive only in part of their membrane	Negative
2+	Weak to moderate complete,basolateral or lateral membranous reactivity in≥10% of tumour cells	Equivocal
3+	Strong complete, basolateral or lateral membranous reactivity in≥10% of tumour cells	Positive

IHC Interpretation Of EGFR Immunostaining[12,13]

EGFR Negative Tumour- Absence of membrane staining above background in all tumour cells.

EGFR Positive Tumour- EGFR positive staining as any IHC staining of tumour cell membranes above background level, whether it is complete or incomplete circumferential staining.

Staining intensity	Percent of Tumour Cells Staining
1+,2+,3+.	>10%

For statistical analysis, the data collected were analysed by Chi-square test and Fisher Exact Test using Statistical Package for the Social Science (SPSS) version 25.0

RESULT AND ANALYSIS

In our study population, out of 52 cases, 15.4% were in the age group of 35-44 years of which 50% were males and 50% were females.53.8% of the study population had gastric adenocarcinoma intestinal type and 46.2% had gastric adenocarcinoma diffuse type. Most of the cases in our study had poorly differentiated gastric cancer (46.2%).67.3% of study population had lymph node metastasis out of which 80.8% had lymphovascular space invasion.

In our study, we found that *diffuse type gastric adenocarcinoma occurs mostly in the younger age-group of 35-54years (83.3%) and in female patients (71.4%), while intestinal type gastric carcinoma occurs in the higher age-group of 55-74years(76.5%) and in male patients (85.7%). The result was statistically significant and p value was 0.02256 and 0.026479 respectively(vide table no. 1).*

Table 1: Association between Age(years) and Gender with Histological type of tumour

Histological type	Age(Years)		Gender		Total
	35-54	55-74	Male	Female	
Intestinal	15	13	24	4	56
Row percentage	26.8	23.2	42.9	7.1	100
Column percentage	42.9	76.5	63.2	28.6	53.8
Diffuse	20	4	14	10	48
Row percentage	41.7	8.3	29.2	20.8	100
Column percentage	57.1	23.5	36.8	71.4	46.2
Total	35	17	38	14	104
Row percentage	33.7	16.3	36.5	13.5	100
Column percentage	100	100	100	100	100
P value	0.02256		0.026479		

61.5% of the study population showed positive HER2 expression and most of the patients were in the age-group of 45-54 years(63%) of which 78.6% were female. However, *no association between HER2 expression and the age and gender of the patients (p=0.743518 and 0.125419 respectively) was found.* EGFR expression was more in the age-group of 45-54 years (55.6%) whereas, in the younger age-group of 35-44 years there was no EGFR expression. However, we did not find any correlation between age and gender of patients with the EGFR expression (p=0.188678 and 0.578169 respectively). *Most of the p53 expression (76.5%) occurred in the age-group of 45-54 years. There was no p53 expression in the age groups of 55-64 and 65-74 respectively. This result was statistically significant with a p value of <0.05 (vide table no.4).* We did not find any association between p53 expression and gender of the patients.

We also found that most of the *HER2 positive tumours were gastric adenocarcinoma diffuse type (65.6%) and poorly differentiated (87.5%).* Only 1 case of well differentiated carcinoma out of 11 cases showed a positive HER2 expression. *These results were statistically significant (vide table no.2).*

Table 2: Association between Histological type and Grade of tumour with HER2 expression

HER2 status	Histological type		Grade			Total
	Intestinal	Diffuse	Well	Moderate	Poor	
HER2 positive	11	21	1	10	21	64
Row percentage	17.2	32.8	1.6	15.6	32.8	100
Column percentage	39.3	87.5	9.1	58.8	87.5	61.5
HER2 negative	17	3	10	7	3	40
Row percentage	42.5	7.5	25	17.5	7.5	100
Column percentage	60.7	12.5	90.9	41.2	12.5	38.5
Total	28	24	11	17	24	104
Row percentage	26.9	23.1	10.6	16.3	23.1	100
Column percentage	100	100	100	100	100	100
P value	0.000367		0.000054			

Most of the EGFR expression was seen in the diffuse type gastric adenocarcinoma (55.6%) and only 28.6% cases of intestinal type showed a positive EGFR expression. Also *55.6% EGFR expression occurred in poorly differentiated gastric carcinoma. 41.2% of moderately differentiated carcinoma showed positive EGFR expression* and only 1 case of well differentiated gastric carcinoma showed positive EGFR expression. However, these results were not statistically significant with a p value 0.322409 and 0.134194 respectively.

Table 3: Association between Lymph node status and Lymphovascular space invasion with EGFR expression

EGFR Status	Lymph node status		Lymphovascular space invasion		Total
	Positive	Negative	Positive	Negative	
EGFR positive	18	0	18	0	36
Row percentage	50	0	50	0	100
Column percentage	51.4	0	42.9	0	34.6

EGFR negative	17	17	24	10	68
Row percentage	25	25	35.3	14.7	100
Column percentage	48.6	100	57.1	100	65.4
Total	35	17	42	10	104
Row percentage	33.7	16.3	40.4	9.6	100
Column percentage	100	100	100	100	100
P value	<0.05		<0.05		

We also found that most of the tumours with positive p53 expression were diffuse type (58.8%).In Intestinal type of gastric carcinoma only 25% of the tumours showed a positive p53 expression. Also most of the p53 expression was found in poorly differentiated gastric carcinoma (58.8%). However, we did not find any statistical association between histological type and grade of tumour with p53 expression (p value 0.201513 and 0.259941 respectively).

There was no significant association between lymph node metastasis and lymphovascular space invasion with HER2 expression (p=0.779128 and p=0.540549 respectively).

51.4% of lymph node positive tumours showed EGFR expression while lymph node negative tumours did not show EGFR expression. 42.9% of lymphovascular space invasion positive tumours showed EGFR expression while lymphovascular space invasion negative tumours did not show EGFR expression.These results were statistically significant with a p value <0.05 (vide table no.3).

Table 4: Association between Age and Lymphovascular space invasion with p53 expression

p53 status	Age(Years)		Lymphovascular space invasion		Total
	35-54	55-74	Positive	Negative	
p53 Positive	17	0	17	0	34
Row percentage	50	0	50	0	100
Column percentage	48.6	0	40.5	0	32.7
p53 Negative	18	17	25	10	70
Row percentage	25.7	24.3	35.7	14.3	100
Column percentage	51.4	100	59.5	100	67.3
Total	35	17	42	10	104
Row percentage	33.7	16.3	40.4	9.6	100
Column percentage	100	100	100	100	100
P value	<0.05		<0.05		

No significant correlation was found between p53 expression and lymph node involvement, though correlation with lymphovascular space invasion was found (p <0.05) (vide table no.4).

DISCUSSION

In our study, we found that *diffuse type gastric adenocarcinoma occurs mostly in the younger age-group of 35-54years with a female preponderance.*

This result is in concordance with a study done by *Shoichi Nora, et al.* on 207 patients with gastric carcinoma, where intestinal type was found to be more common in the aged male patients while diffuse type was more common in younger female patients[15].

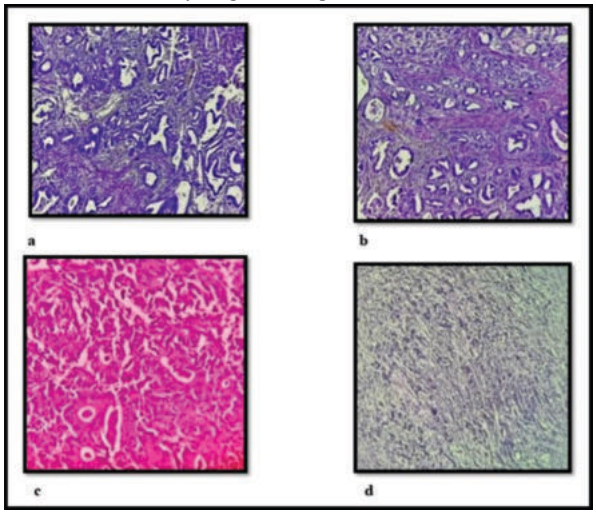

Fig. 1 a,b Photomicrograph showing Well differentiated Gastric Adenocarcinoma, Intestinal type,H&E,100x , c Photomicrograph showing Moderately differentiated Gastric Adenocarcinoma, Intestinal type,H&E,100x, d Photomicrograph showing Poorly differentiated Gastric Adenocarcinoma, Diffuse type,H&E,100x

No association was found between HER2 and EGFR expression with the age and gender of the patients . *However, most of the p53 expression (76.5%) occurred in the younger patients (<55years).* Though we did not find any association between p53 expression and gender of the patients.

The result is in concordance with study done by *Padma K. Malini et al.* on 50 cases of gastric biopsies over a period of 4 years. The study showed that, p53 expression was associated with younger age group [2].

We also found that most of the *HER2 positive tumours were gastric adenocarcinoma diffuse type (65.6%) and poorly differentiated (87.5%).* This result is in discordance with other studies where HER2 expression was found to be associated with Intestinal type of gastric adenocarcinoma, well or moderately differentiated. However, in one study by *Sekaran et al.* HER2 overexpression rates were found to be similar for intestinal type as compared to diffuse type gastric adenocarcinoma[14]**.** A similar study by *Piyali Ghosh et al. also confirmed the same* [16].

We did not find any significant association between EGFR and p53 expression with the histological type and grade of tumours though most of the EGFR and p53 expressions were shown by diffuse type carcinoma and poorly differentiated.

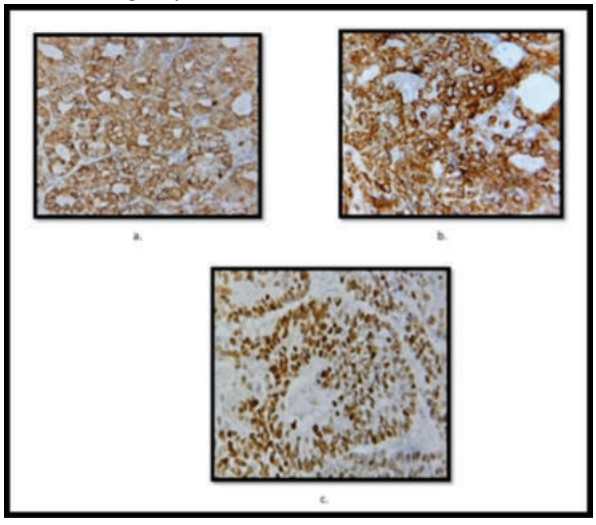

Fig. 2 a Photomicrograph showing Positive HER2 expression (Score 3), 400x, b Photomicrograph showing Positive EGFR expression (Score 3),400x, c Photomicrograph showing Positive p53 expression, 400x

There was no significant association between lymph node metastasis and lymphovascular space invasion with HER2 expression, though we found association between EGFR expression with nodal involvement and lymphovascular space invasion.

These results were in concordance with the study done by *YK Wang, et al.* where EGFR expression was found to be associated with lymph node metastasis[17] .

In case of p53, no significant correlation was found between p53 expression and lymph node involvement, though significant correlation with lymphovascular space invasion was found (p <0.05).

CONCLUSION

HER2 expression is associated with histological type and grade of tumour. HER2 expression is not associated with age , gender, lymph node involvement and lymphovascular space invasion.

EGFR expression occurs mostly in poorly differentiated gastric cancer and is significantly associated with nodal involvement and lymphovascular space invasion. EGFR expression is not associated with age , gender and histological type of tumours.

p53 expression is associated with a younger age-group. Though p53 expression was found mostly in diffuse type and in poorly differentiated tumours,no statistical correlation was found. Also p53 expression is associated with lymphovascular space invasion. No correlation between p53 expression with gender of patients and lymph node involvement was found.

Various studies have shown that HER2, EGFR and p53 expression correlate with histological type, grade, nodal involvement and lymphovascular space invasion and the present study has strengthened the results of various other studies on HER2, EGFR and p53 expression in gastric adenocarcinoma.

However, owing to the small sample size from a relatively small geographical area, the results are to be confirmed in a wider set up. Also we were unable to follow up the cases due to Covid-19 pandemic. Hence,further multicentric studies involving large number of cases over a larger geographic area and duration with proper follow up will be required to verify our findings and identify patients with reduced survival and those eligible for targeted therapy.

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