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ROLE OF HER2/NEU OVEREXPRESSION IN DIAGNOSIS AND ITS PROGNOSTIC SIGNIFICANCE IN GASTRIC ADENOCARCINOMA

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Abstract:

Introduction: Gastric cancer is one of the most common tumor and remains the second most common cause of cancer mortality in the world. The mainstay of treatment is the surgical resection and can cure patients at an early stage. The survival rate of patient's with advance gastric cancers remains poor despite of new treatment strategies such as peri-operative chemotherapy or adjuvant chemoradiation. There are evidence of the role of HER2neu overexpression in gastric adenocarcinoma patient's and it has correlated with more aggressive course of disease and poor outcomes. HER2neu overexpression in gastric adenocarcinomas is also becoming important due to availability of targeted treatment with trastuzumab chemotherapy have proven effective.
Material and Methods: This was a retrospective study in cases of Gastric Adenocarcinoma presenting to a tertiary care hospital over a period of 02 years. A total of 30 cases were included and were subjected for ER, PR & HER2neu receptor study.
Results: The correlation studies were performed between Her2neu positivity and type of specimen, tumor histological subtype, tumor location, tumor differentiation, tumor grading, tumor staging, lymphovascular invasion, lymph node metastasis. In our study Her2 overexpression was observed in 13 % cases of gastric carcinoma. Higher HER2neu expression was seen in older age and in males. A positive association between HER2neu positivity and intestinal type cancers was identified along with significant association of HER2neu positivity with proximal tumors. More cases of HER2/neu positivity was seen with poorly differentiated carcinomas. No statistically significant correlation was found between HER2neu overexpression and tumor stage, lymphovascular and perineural invasion in the present study.
Conclusion: Her2neu IHC study is become very essential due to availability of trastuzumab drug which increases the significant overall survival in gastric adenocarcinoma. The overall reliability of HER2neu evaluation by IHC can be affected by diverse preanalytical, analytical and post-analytical variables. FISH should be done in all equivocal cases (2+) for increased diagnostic accuracy.
Keywords: Immunohistochemistry**,** Herceptin, Gastric adenocarcinoma**,** Trastuzumab

INTRODUCTION

Gastric cancer is one of the most common tumor and remains the second most common cause of cancer mortality in the world (1). The mainstay of treatment is the surgical resection and can cure patients at an early stage. Upper gastrointestinal cancers, including gastric cancer are among the most common cancers (4-8). Reports from different parts of the world shows very different results but a higher incidence found in males as compared to females in gastric cancer (9). A report from Iran [2012] showed that 11.4% of all cancer cases were gastric cancer it was considered as the second most prevalent cancer in the country. It was studied that it is the cancer with poor prognosis in Iran (GLOBOCAN, 2012), accounting for 15.5% of all mortalities caused by cancer (10). One investigations showed that more than 750,000 people die annually from gastric cancer, and men are at risk for gastric cancer 2 times more than women (11,12). This high mortality is due to the fact that gastric cancer is mainly diagnosed in advanced stages because of absence of symptoms in early gastric cancers so use of highly sensitive and specific diagnostic methods is very essential for identification of patient at risk. However the cases of gastric carcinoma are decreasing in recent decades. It has been shown that gastric cancer related to 39% of death due to cancer (5). Gastric adenocarcinoma is the most common type and it is originating from the glandular epithelium. Other types are lymphoma and sarcoma which originates in lymphoid and connective tissues, respectively (11,12). Gastric cancer is associated with high mortality and morbidity worldwide. It is the fourth most commonly diagnosed cancer and the second most common cause of cancer-related deaths globally (13-15). In Intestinal type, tumor cells shows adhesion and are arranged in tubular or glandular pattern

along with intestinal metaplasia(20).This is associated with lymphovascular invasion and metastasis. Intestinal gastric cancer most commonly occurs in elderly age, male patients commonly involve the gastric antrum and exhibit a longer course and better prognosis (21,22). In Diffuse type, tumor cells lack adhesion and infiltrate in the stroma as single cells or small cluster and appeared as scattered tumor cells(6). Intracellular mucus may push the nucleus of the cells aside to form signet ring cell. This type associated with younger patient and more common in female (20). Peritoneal metastasis of diffuse type is more common in the absence of recognized precursor lesion. This type of cancer usually affects the body of stomach, present shorter duration and worse prognosis in comparison to intestinal type (21,23).The pathogenesis of gastric carcinoma involves Methylation of DNA, chromosome recombination and histone modifications (27,28). The two subtypes have common dietary and environmental risk factors. Environmental factors are more important in intestinal type whereas diffuse type usually presents with genetic etiology (29).

ETIOLOGY OF INTESTINAL GASTRIC CANCER- Helicobacter pylori infection along with diet and some environmental factors is associated with the development of intestinal gastric cancer (29). This carcinogenic process involves multiple steps, including atrophic gastritis, dysplasia, intestinal metaplasia and finally gastric carcinogenesis (30). Helicobacter pylori is considered promoter of intestinal gastric cancer, however, the hypothesis that helicobacter pylori eradication would prevent gastric cancer remains controversial. Previous studies have suggested that the process preceding high-level neoplasia is potentially reversible and the probability of gastric atrophy and intestinal metaplasia decreases with eradication of helicobacter pylori infection thus leading to the prevention of gastric cancer (31,32). However, some studies revealed that the risk of developing gastric cancer is still there even after the cure of helicobacter pylori infection (33,34). A previous meta-analysis shows that curing helicobacter pylori infection may reduce the incidence of chronic atrophic gastritis, however, this may not prevent the development of intestinal metaplasia(35). Helicobacter pylori eradication does not decrease the incidence of chance of gastric carcinoma (36).

ETIOLOGY OF DIFFUSE GASTRIC CANCER - Diffuse gastric carcinoma arises from the gastric mucosa and is associated with gastritis (37). Thus it is less affected by environmental factors than the intestinal type, although helicobacter pylori infection may be also involved in the development of diffuse gastric carcinoma (38). However, in comparison of intestinal gastric cancer, the diffuse type develops as a direct result of chronic active inflammation even in absence of atrophic gastritis and intestinal metaplasia (39). The major risk factor for diffuse gastric cancer is active gastritis. A previous study reported that the level of DNA methylation in gastric mucosa is closely associated with helicobacter pylori related gastritis and that there may be a molecular mechanism underlying the development of diffuse gastric cancer (40).

The epidemiology of gastric cancer shows variation across geographical areas (13,14). While the incidence of gastric cancer showing decreasing trends in countries such as Japan where screening programs identify gastric cancer at early stages while in western countries till now late diagnosis is common (16). The most of the patients with gastric cancer present with advanced, metastatic or inoperable disease and have a poor prognosis. Although screening in Japan has led to a decrease in death rates for gastric cancer (two fold decrease in screened vs unscreened subjects), improved 5-year survival rates and a significant reduction in the overall incidence of advanced gastric cancer (16,17). The median 5-year survival rates reported for metastatic gastric cancer are in the range of 5–20% in the Western populations (14,18,19). The survival rate of patient's with advance gastric cancers remains poor despite of new treatment strategies such as peri-operative chemotherapy or adjuvant chemoradiation (1). There are evidence of the role of HER2/neu overexpression in gastric adenocarcinoma patient's and it has correlated with more aggressive course of disease and poor outcomes (1). HER2/neu overexpression is well known in breast malignancies, however the evidence of HER2/neu overexpression in gastric adenocarcinomas is also becoming important due to targeted treatment (1). Targeted therapy with trastuzumab along with chemotherapy have proven more beneficial in patients with HER2/neu expression in cases of gastric adenocarcinoma. However, patient individualised treatment aims to avoid unnecessary medication in patients who are unlikely to respond to therapy. Accurate assessment of the HER2 status of patients before the treatment with trastuzumab is very important. The results of these studies will contribute to a better knowledge of the efficacy and tolerance of trastuzumab based therapy in HER2/neu positive gastric adenocarcinoma's (1). HER2/neu encoded by a gene located on chromosome 17q21 (1). HER2 gene is located adjacent to the topoisomerase IIa gene and is related to the erb B of the erythroblastosis virus. HER2 protein is a 185 kDa transmembrane tyrosine kinase receptor and a member of epidermal growth factor receptor (EGFR) family. This family is composed of four members : HER1 also known as EGFR, HER2, HER3 also known as erb-B3 and HER4 also known as erb-B4. These receptors share the same molecular structure with a extracellular ligand-binding domain, a short transmembrane domain and an intracellular domain with tyrosine kinase activity (1). The binding of different ligands to the extracellular domain initiates a signal transduction cascade that can initiate tumor cell biology, cell proliferation, apoptosis, adhesion, migration and differentiation. Analysis of HER2/neu scoring by IHC, suggests that HER2 is overexpressed in 7-34% of gastric tumors (2). The rate of HER2 positivity lower in early gastric adenocarcinomas ranging from 10.4% to 13.6% while high in advance gastric adenocarcinoma (2). Despite the variability in overexpression rates, most studies support a strong association between HER2/neu positive status and Lauren's intestinal type adenocarcinomas (3).

OBJECTIVE

1. To perform immunohistochemical stain and examination of Her2/neu positivity on sections from blocks of known cases of Gastric adenocarcinoma.
2. To analyze its relationship with various histological types, grade, localization and staging of the disease.

RESEARCH METHODOLOGY

STUDY DESIGN
This is retrospective study on paraffin blocks of gastric cancer specimens.

STUDY POPULATIONS
Gatrectomy specimen and Endoscopic biopsy sample from gastric cancer received in the department of pathology at INHS ASVINI Mumbai.

STUDY PERIOD
The study was carried out for 02 years between Jun 2016 to Jun 2018.

SAMPLE SIZE
All the samples received during the study period were included in the study. From the histopathology records, total 36 gastric adenocarcinoma were diagnosed in our tertiary care hospital. Out of which we took 30 cases after excluding all the exclusion criteria for this study.

DATA COLLECTION TOOLS
Age ,sex site, histological type, grade, and stage of the disease were obtained from the medical records. Processed paraffin sections of 5 micron thickness from tumor areas were taken and stained with H and E stain and mounted on glass slides. After the diagnosis made on it 3 micron thickness were cut and immunohistochemical staining of HER2/neu was observed.

Inclusion criteria:

1.Cases of histologically proven Gastric Adenocarcinoma that are presenting to our hospital over a period of 2 years (June 2016- June 2018).
2. All cases of Gastric Adenocarcinoma irrespective of age and sex.

Exclusion Criteria:

1. All cases other than gastric adenocarcinoma will be excluded from the study.
2. All patients with any other upper gastro intestinal disease.
3. Cases of pre-operative chemoradiotherapy treated patient are also excluded.

ETHICAL APPROVAL AND INFORMED CONSENT
Permission of the institutional ethics committee was obtained prior to commencing the study.

MATERIAL & METHODS

Table 1: HUMAN EPIDERML GROWTH FACTOR RECEPTOR 2 IHC PROTOCOL TESTING ALGORITHM

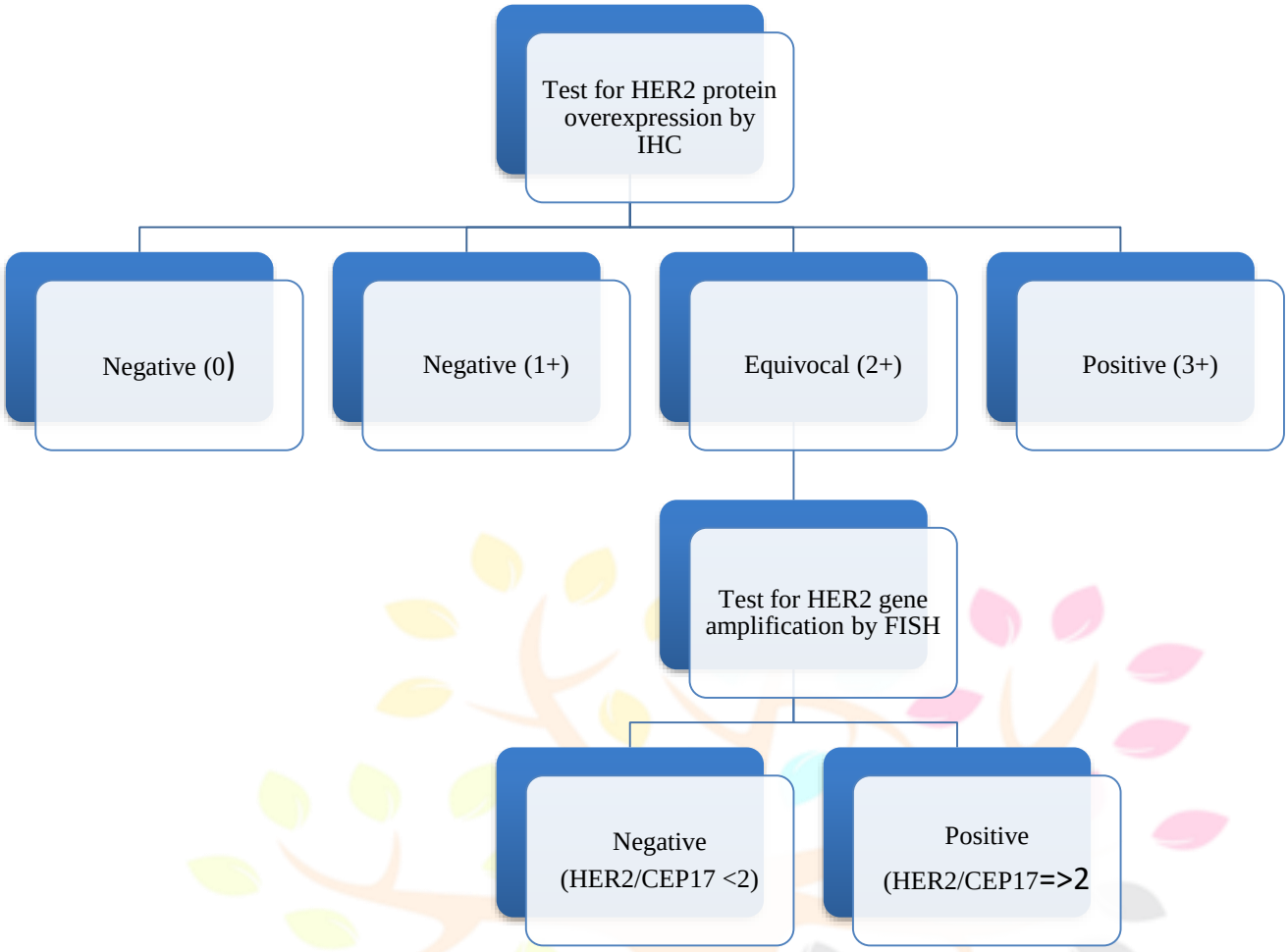

Table 2: DIFFERENCE IN HER2 EXPRESSION IN BIOPSY AND SURGICAL SPECIMEN-

SCORE	SURGICAL SPECIMEN – STAINING PATTERN	BIOPSY SPECIMEN – STAINING PATTERN	HER2 OVEREXPRESSION
0	No membranous staining or staining of <10% of the tumor cells.	No membranous staining or staining only in rare cells (< 5 cohesive cells).	Negative
1 +	Staining is weak or detected in only one part of the membrane in => 10% of the cells.	Staining is weak or detected in only one part of the membrane of atleast 05 cohesive cells.	Negative
2+	Moderate/weak complete or basolateral membranous staining in =>10% of the cells.	Moderate/weak complete or basolateral membranous staining of atleast 05 cohesive cells.	Equivocal
3+	Strong complete or basolateral membranous staining in =>10% of the neoplastic cells.	Strong complete or basolateral membranous staining of atleast 05 cohesive cells.	Positive

Difficulty in reporting due to size of sample-

Because of intratumoral heterogeneity, the size of the tissue sample might interfere in HER2/neu analysis. Although Hofmann's HER2 scoring system was formulated for evaluating HER2/neu status in biopsy and surgical specimens, discordant HER2 results in paired specimens were observed in a small percentage of tumors. Intratumoral heterogeneity appears likewise to be the subject of conflicting results of HER2neu expression in primary and metastatic tumor samples. There is significant difference in sensibility when analysing HER2/neu expression in whole-tissue sections and in tissue microarrays. Evaluation of more than one sample, metastatic foci and all available specimens is required to exclude the chance of discrepancies (Image A). When only biopsies are available, it is recommended to have at least four fragments containing tumor cells. It is also recommended that all surgical specimens from patients that previously obtained HER2neu negative results in biopsies should also be tested to increase the chance of finding HER2neu positive tumors.

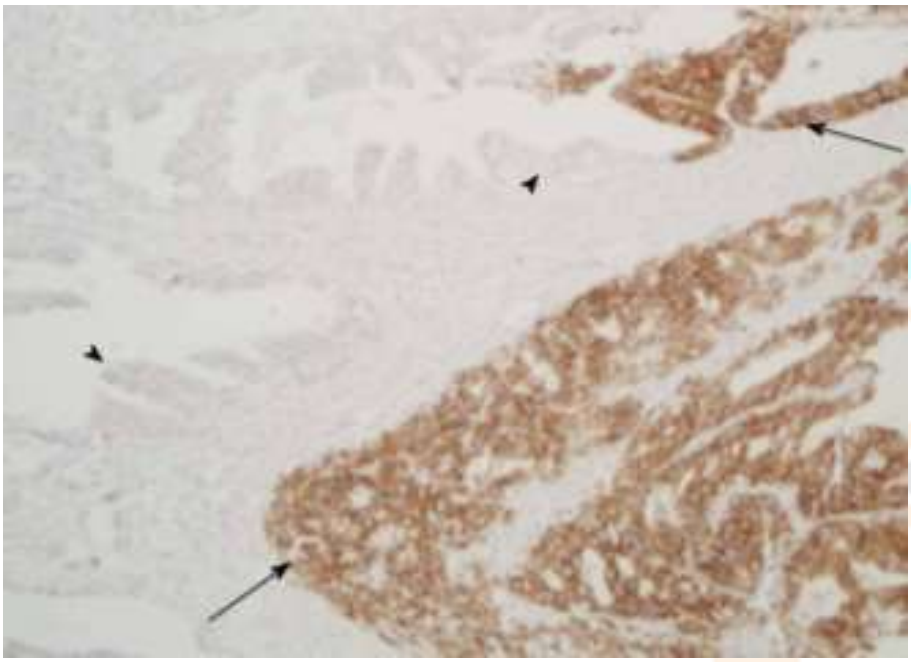

Image A - Intratumoral heterogeneity of HER2 expression. Arrow indicate areas with strong continuous membranous staining (score 3+). Arrow heads indicate negative areas - score 0 (IHC, X400)

IHC SCORE SYSTEM

The system proposed by Hofmann et al that has been assimilated by FDA, besides being specific for gastric tumors, also distinguishes biopsies from surgical specimens(82,61). Table 2 shows the IHC score system for HER2/neu in gastric cancer and image B, C, D, E illustrates it.

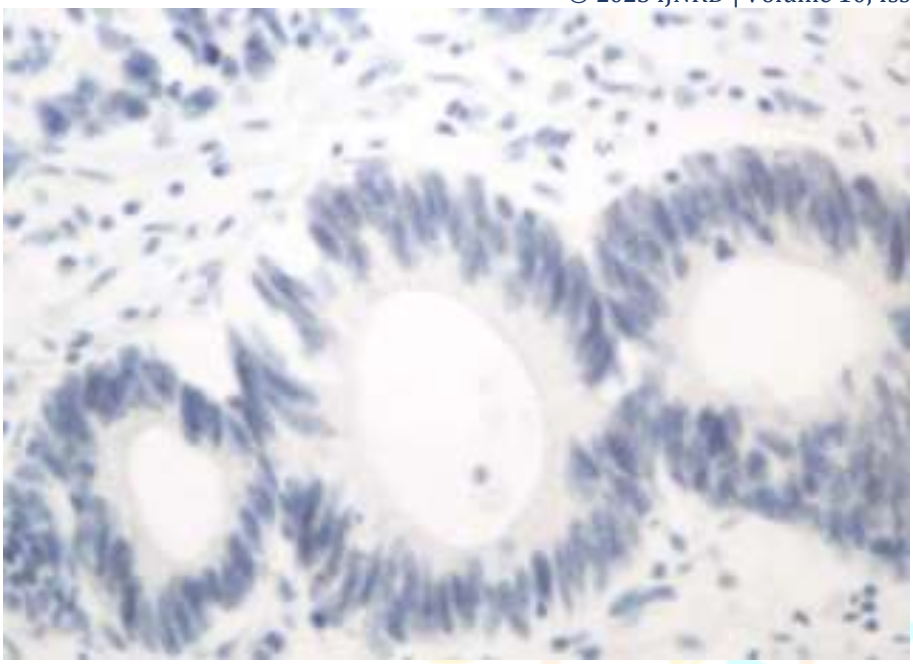

Image B - A HER2/neu negative (0) case (IHC, X400)

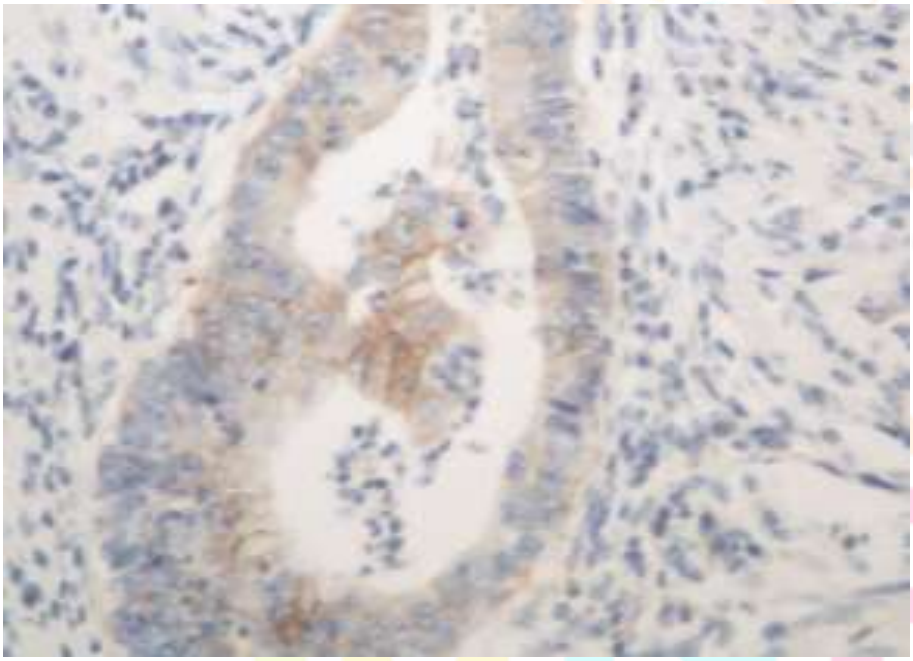

Image C - A HER2/neu negative (+1) case(IHC, X400

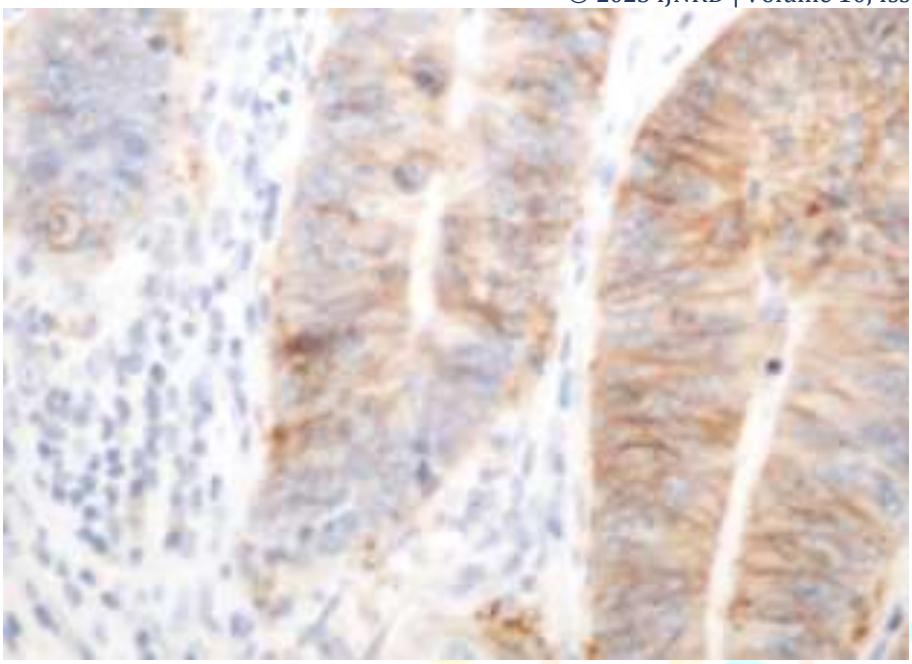

Image D - A HER2/neu equivocal (2+) case (IHC, X400)

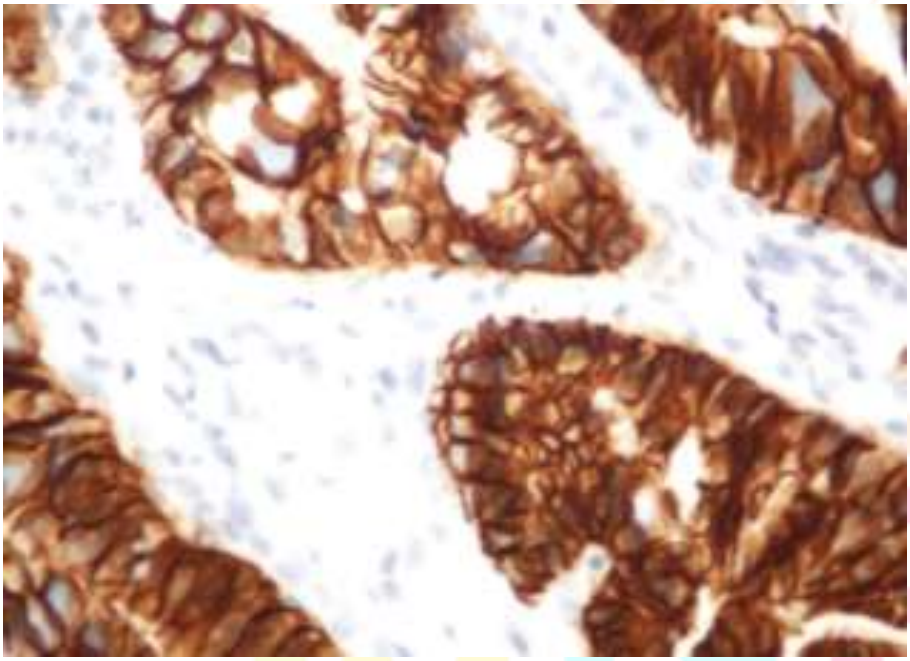

Image E - A HER2/neu positive (3+) case (IHC, X400)

IHC INTERPRETATION:

- The staining was scored as negative (0) when no membrane was stained or when membrane was stained in less than 10% of tumor cells and its taken as weakly positive (1+) if focal membrane was stained in more than 10% of tumor cells, intermediately positive (2+) if complete membrane was weak to moderately stained in more than 10% of tumor cells and strong positive (3+) if complete membrane was intensely stained in more than 10% of tumor cells. Scores 0 and 1 were considered negative, scores 2 as equivocal and 3 were considered positive.
- On IHC in this study out of 30 cases, Her2neu was positive in 4 cases (13%). In which 3 were resection specimen (75%) and 01 were gastric biopsies (25%)..

Images of HER2/neu IHC slides in this study-

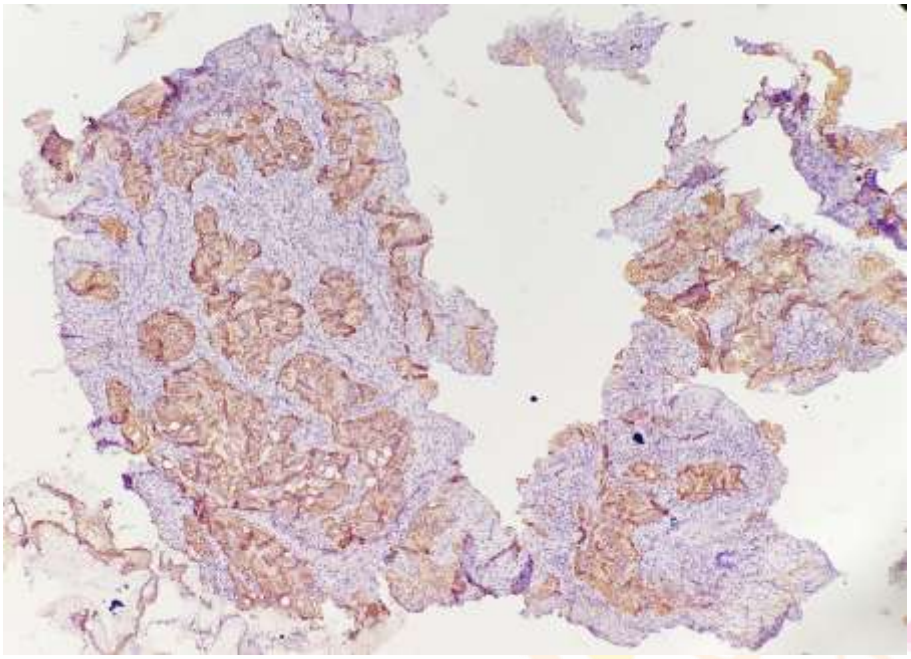

Image F - A HER2/neu positive (Score 3+) case

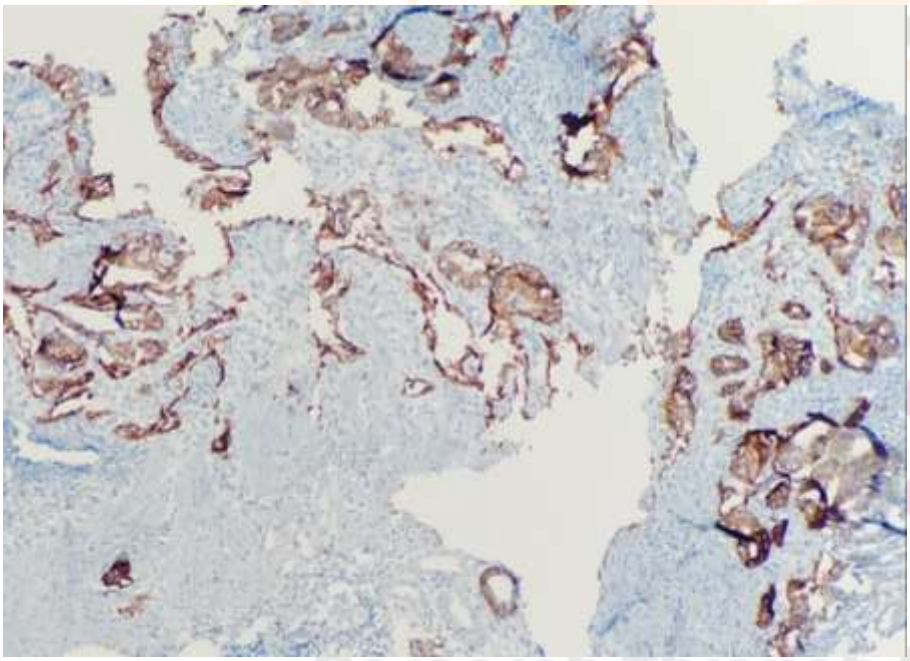

Image G – A HER2/neu positive (Score 3+) case

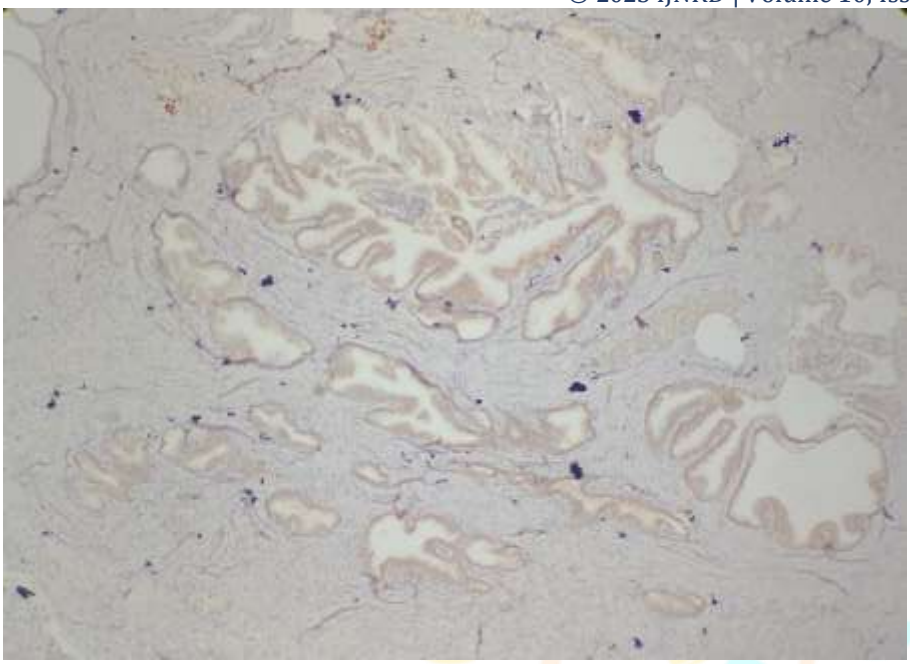

Image H – A HER2/neu equivocal (Score- 2+) case

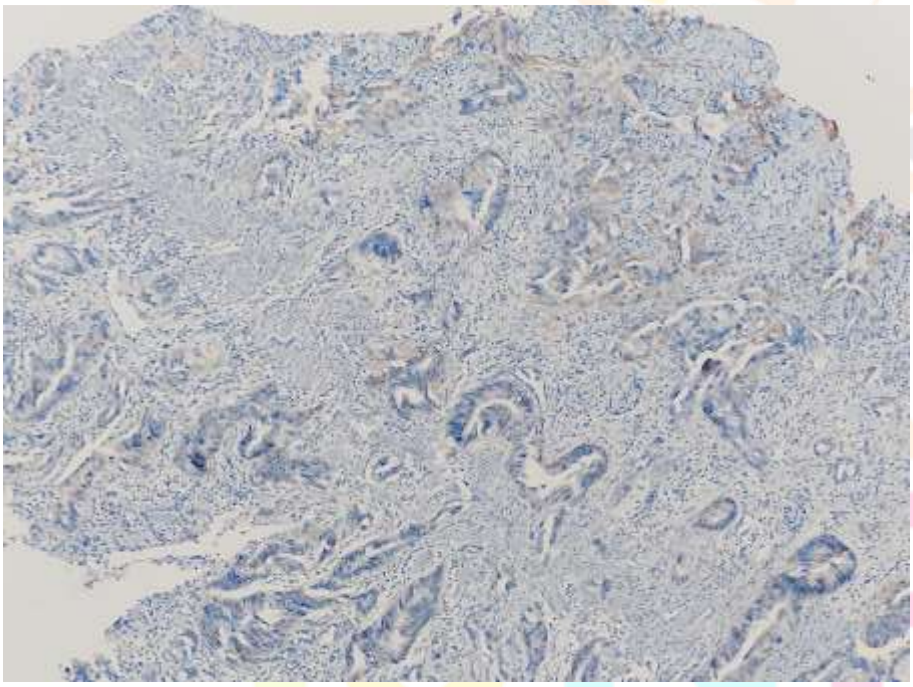

Image I – A HER2/neu negative (Score - 1+) case

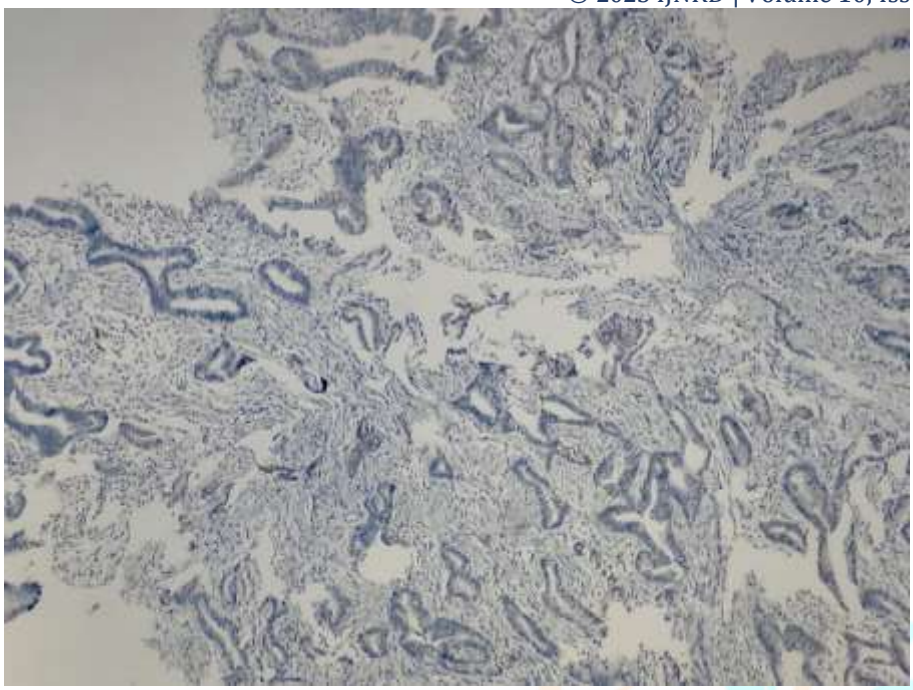

Image J - A HER2/neu negative (Score - 0) case

STATISTICAL ANALYSIS

Quantitative data was presented with the help of Mean and Standard deviation. Comparison among the study groups was done with the help of unpaired t test as per results of normality test. Qualitative data was presented with the help of frequency and percentage table. Association among the study groups was assessed with the help of Fisher test, Student 't' test and Chi-Square test. 'p' value less than 0.05 is taken as significant**.**

Pearson's chi-squared test

$$X^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Where X2 = Pearson's cumulative test statistic.
Oi = an observed frequency;
Ei = an expected frequency, asserted by the null hypothesis;
n = the number of cells in the table.
Results were graphically represented where deemed necessary. Appropriate statistical software, including but not restricted to MS Excel, SPSS ver. 20 was used for statistical analysis. Graphical representation was done in MS Excel 2010.

RESULT: A total of 30 cases were included out of which 22 were gastric biopsies and 08 were resection specimens (total/partial gastrectomy).

- **Gastric adenocarcinoma according to age -** The age range of patient varied from 28 to 69 yrs. More number of cases found in <= 60 year of age in comparison to >60 year of age as in fig-1. Peak age of incidence was between 40-60 yrs. The mean age of presentation was 50 years (fig -1).

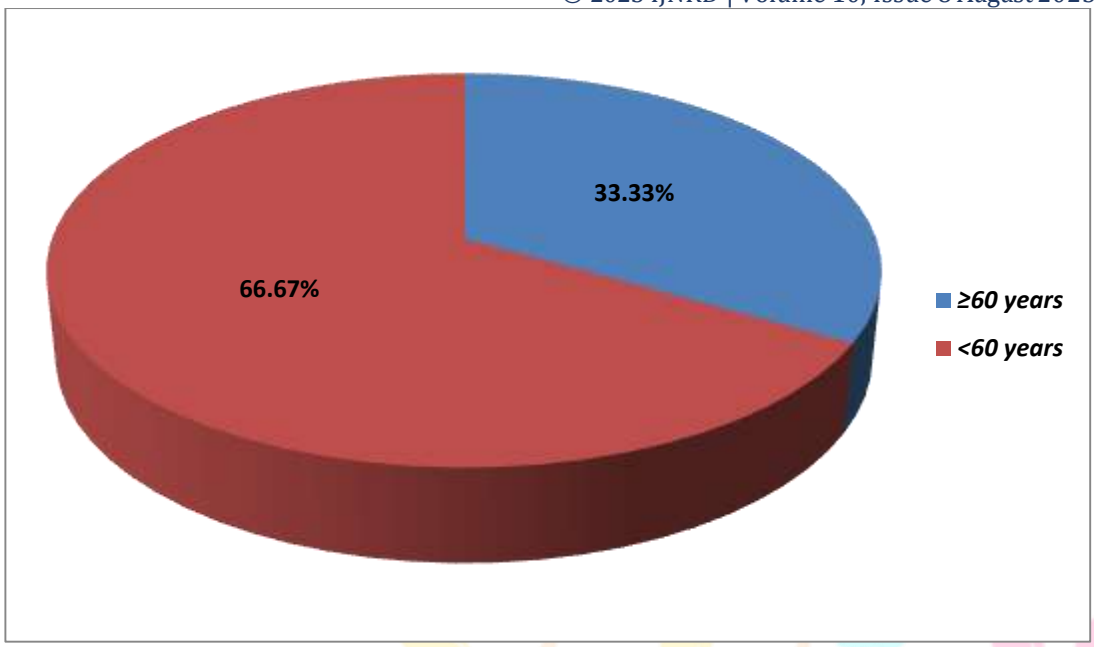

Figure 1: Descriptive statistics of tumor with age (N=30)

- **HER2/neu overexpression according to age -** Patient in older age group (> 60 years) showed higher HER2neu overexpression 20% in comparison to < 60 year of population in which HER2/neu overexpression is 10% as in fig-2. The correlation of HER2neu positivity with age was found not significant (p= 0.946) (fig-2).

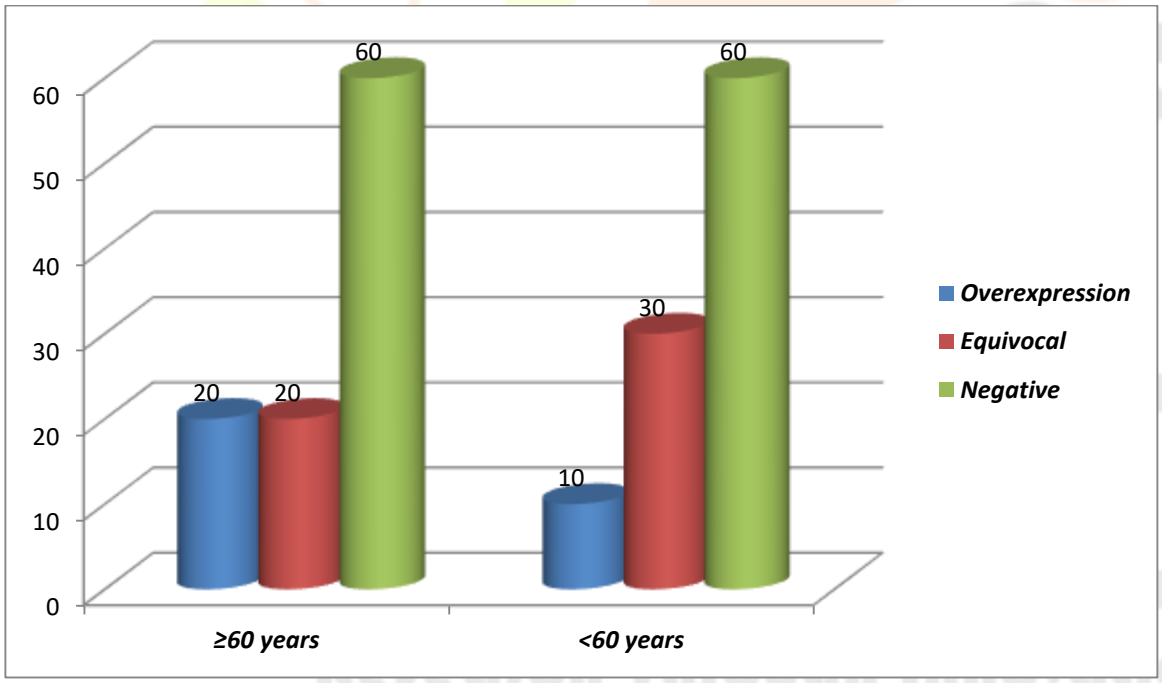

Figure 2: Comparison of the HER2 expression with age by using chi square test

- **Gastric adenocarcinoma according to gender -** Of the total 30 cases of gastric adenocarcinoma 19 patients were male and 11 were female as (fig-3). The male to female ratio was 1.7:1 (fig- 3).

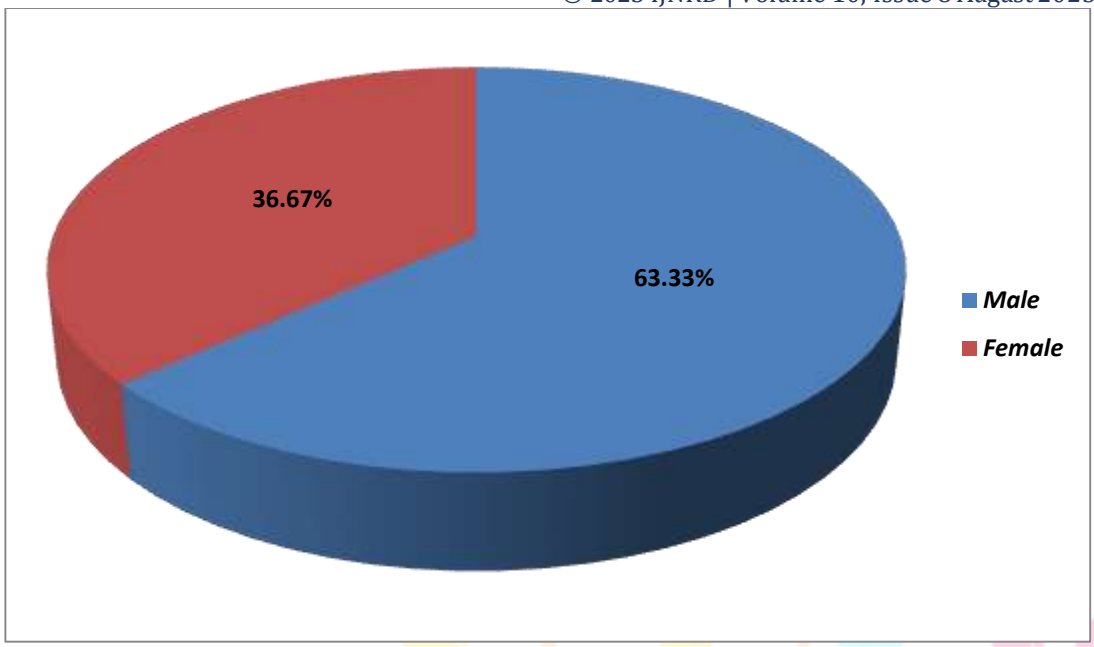

Figure 3: Descriptive statistics of tumor with gender (N=30)

- **HER2/neu overexpression according to gender -** Her2 was positive in 02 out of 19 male patients (14%) and in 02 of 11 female patients (11%) however tumors had more predilection for male patient in comparison to female. So the Her2 expression in male and female patient is found to be not statistically significant (p= 0.792) and chi square value: 0.464 (fig-4)

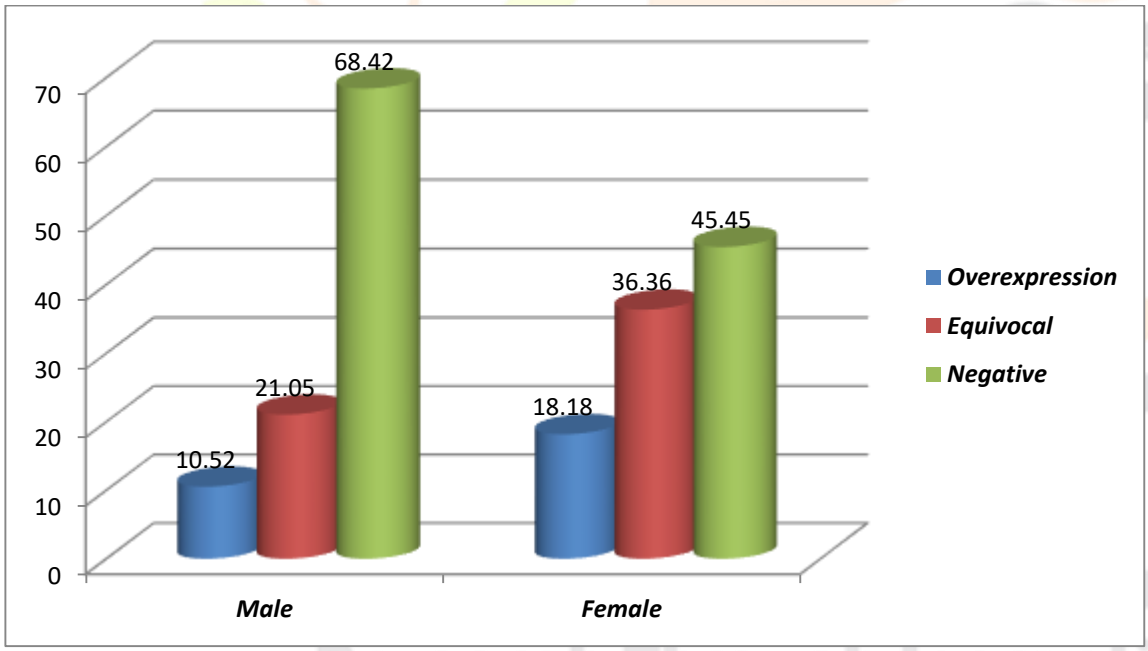

Figure 4: Comparison of the HER2 expression with gender using chi square test.

- **Gastric adenocarcinoma according to tumor subtype-**On histological examination, there were 17 intestinal, 11 diffuse and 2 mixed tumors subtypes using lauren's classification for gastric tumors as in (fig-5).

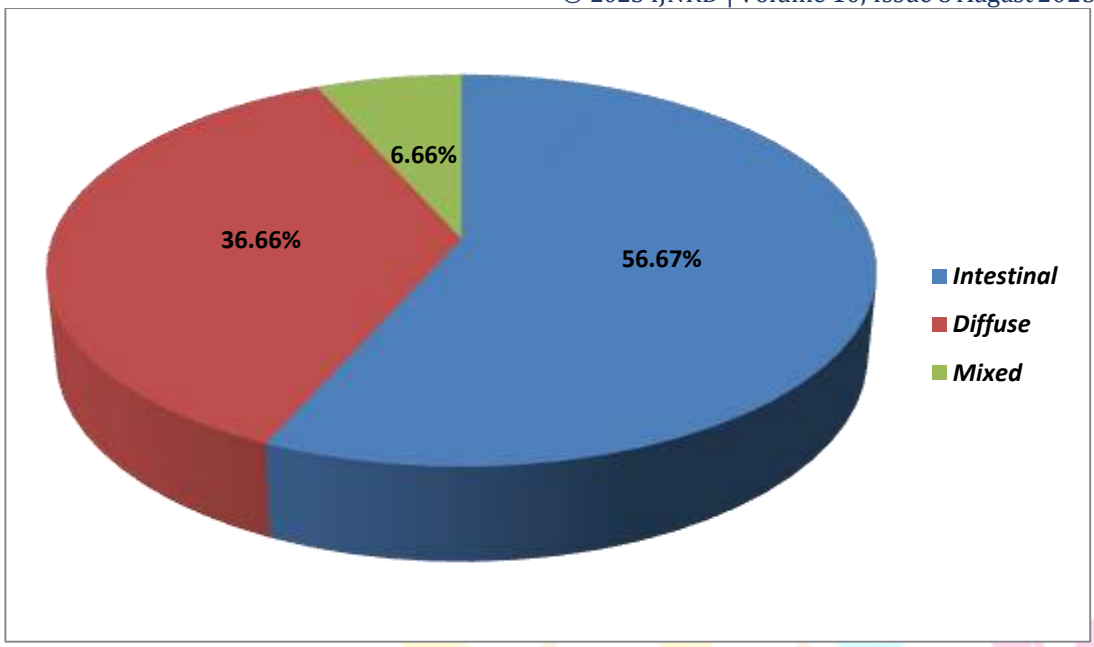

Figure 5: Descriptive statistics Tumor Subtype (N=30)

- **HER2/neu overexpression according to tumor subtype -** 19 % of Intestinal type of tumor showed Her2neu overexpression, whereas 09% of diffuse type of tumor showed Her2neu overexpression. The correlation of Her2neu positivity with tumor subtype was not statistically significant (p= 0.980)(fig- 6).

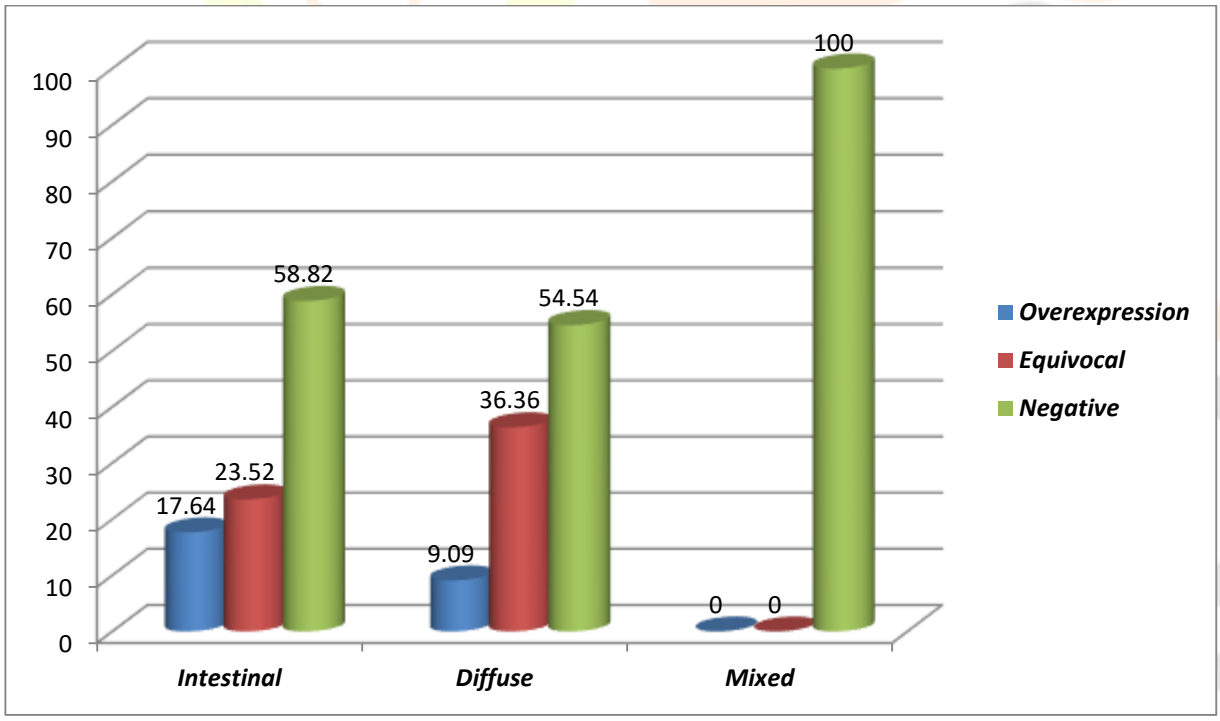

Figure 6: Comparison of the HER2 expression with tumor subtypes by using chi square test.

- **Gastric adenocarcinoma according to tumor location -** Distribution of tumor according to location, 8 tumors were in the proximal stomach which included GEJ tumors, cardiac and fundus of the stomach. 22 tumor found in distal tumors included those in body and antrum of the stomach (fig-7).

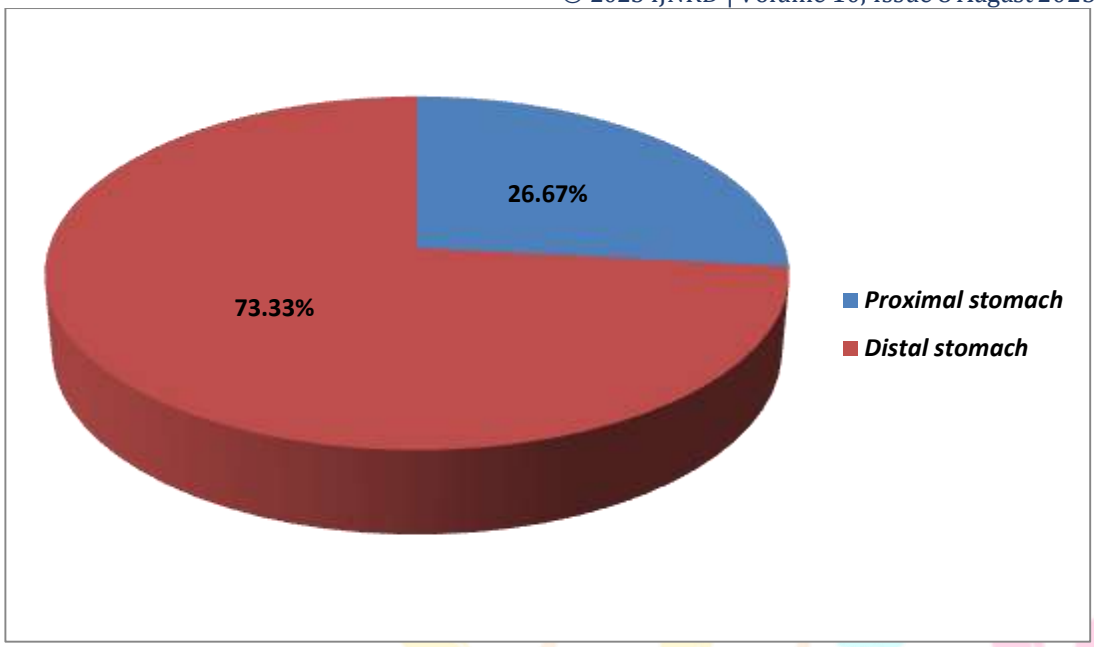

Figure 7: Descriptive statistics (N=30).

- **HER2/neu expression according to location -** Her2 positivity of tumors with respect to localization in proximal or distal stomach was analyzed. Number of cases showing Her2neu overexpression are same in both proximal and distal but in comparison to number of cases the percentage of proximal tumor HER2/neu overexpression (25%) is more than distal location tumors (9%). The correlation of Her2 positivity with tumor localization was found not significant(p= 0.838) (fig-8).

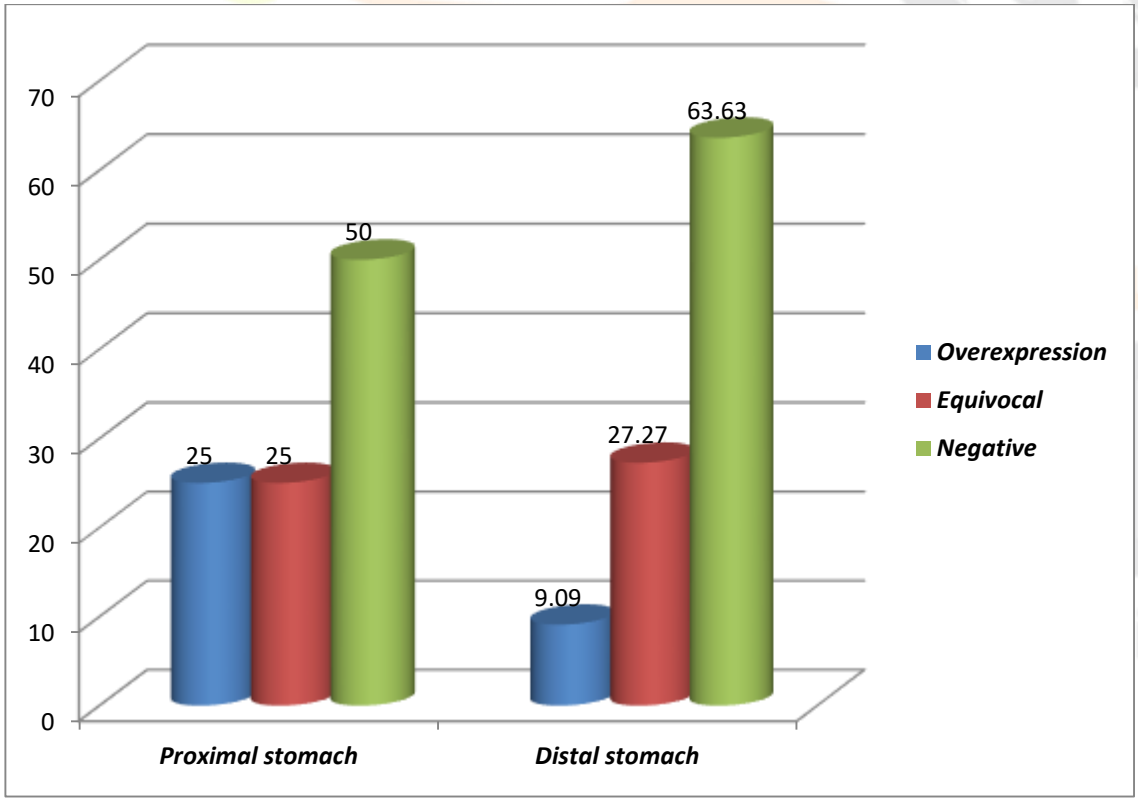

Figure 8: Comparison of the HER2 expression with location by using chi square test

- **Gastric adenocarcinoma according to Tumor Differentiation -** Out of all 10 cases were showing moderate differentiation whereas 20 cases were showing poorly differentiated tumor (fig- 9).

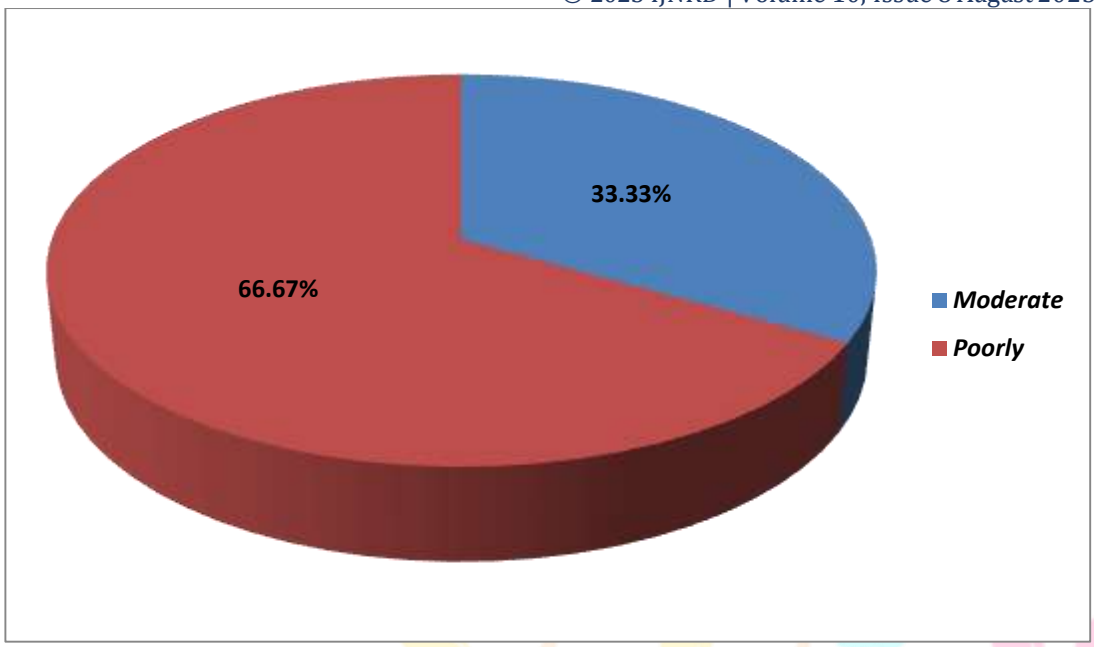

Figure 9: Descriptive statistics of Tumor differentiation (N=30)

- **HER2/neu expression according to Differentiation-** Poorly differentiated tumor shows 15% of HER2/neu positivity in comparison to 10% of HER2neu positivity in moderately differentiated tumor. Despite this trend of Her2 positivity in poorly differentiated intestinal tumors, the association was not statistically significant (p=0.946) (fig-10).

TUMOR DIFFERENTIATION-

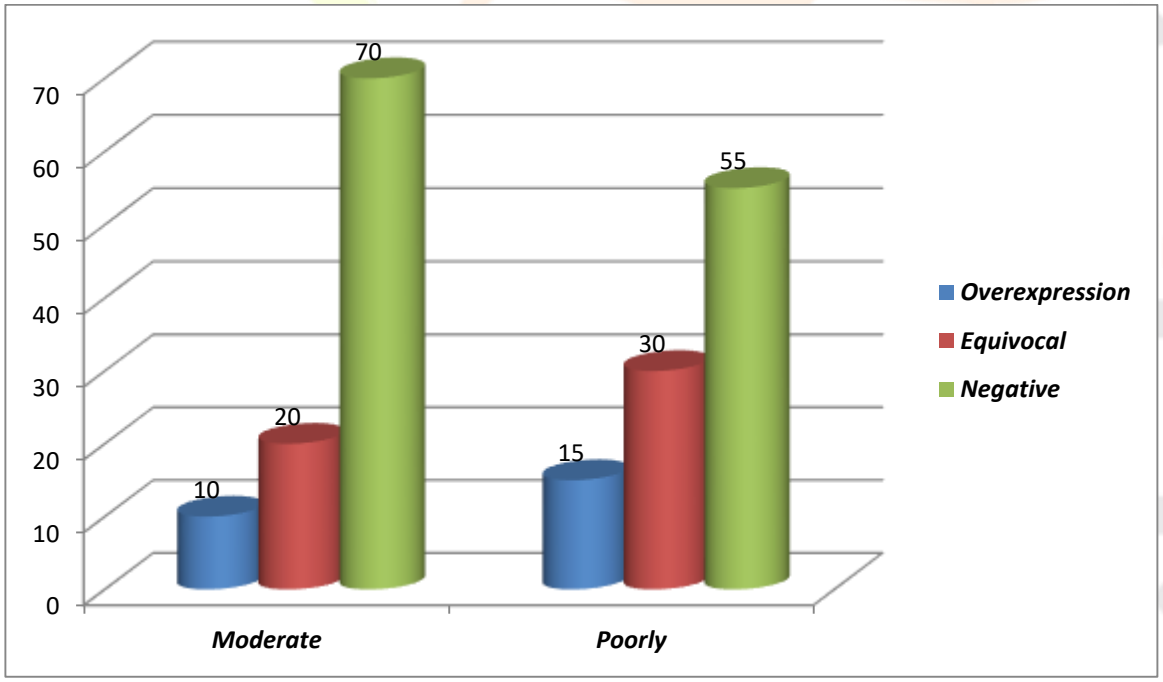

Figure 10: Comparison of the HER2neu expression with tumor differentiation by using chi square test.

- Of the 17 intestinal subtype tumors, 06 were moderately differentiated and 11 were poorly differentiated (Her2neu positivity was higher in poorly differentiated intestinal tumors as compared to those of moderate differentiation (27% vs 0%). None of the moderately differentiated tumors showed Her2neu positivity. While out of 11 diffuse subtype tumors, 03 were moderately differentiated and 08 were poorly differentiated (27% vs 72%).

In Gastrectomy Specimen-

- **Gastric adenocarcinoma according to lymphovascular involvement -** Among all 8 gastrectomy specimen 5 of them shows lymphovascular involvement.

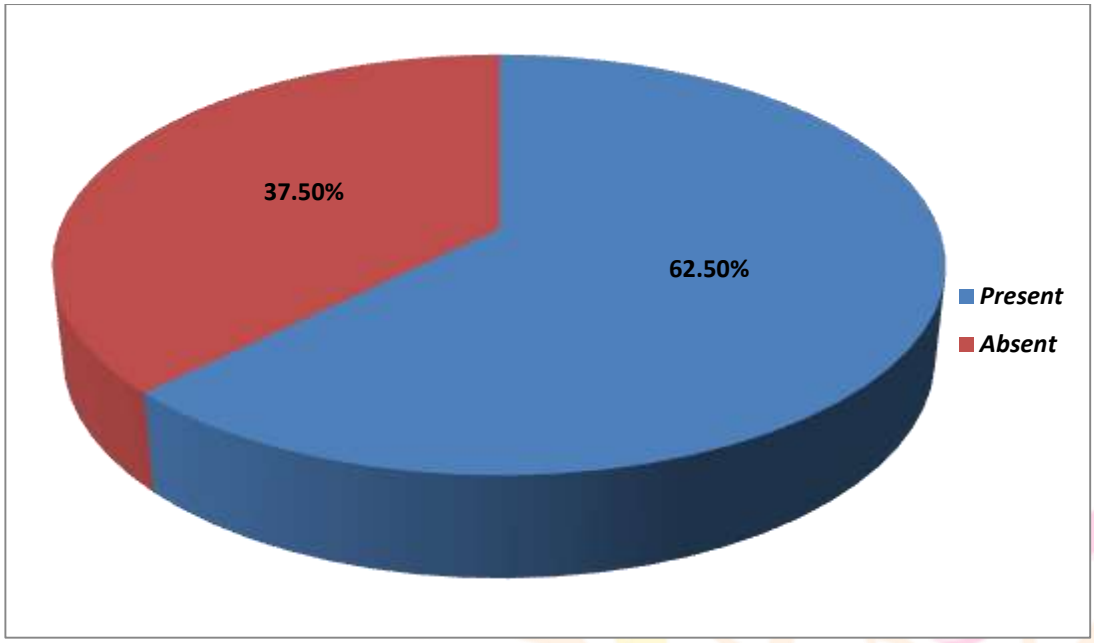

Figure 11: Descriptive statistics of Lymphovascular involvement (N=8)

- **Gastric adenocarcinoma according to perineural involvement-**

In all 8 gastrectomy specimen only 1 showing perineural spread (fig -12).

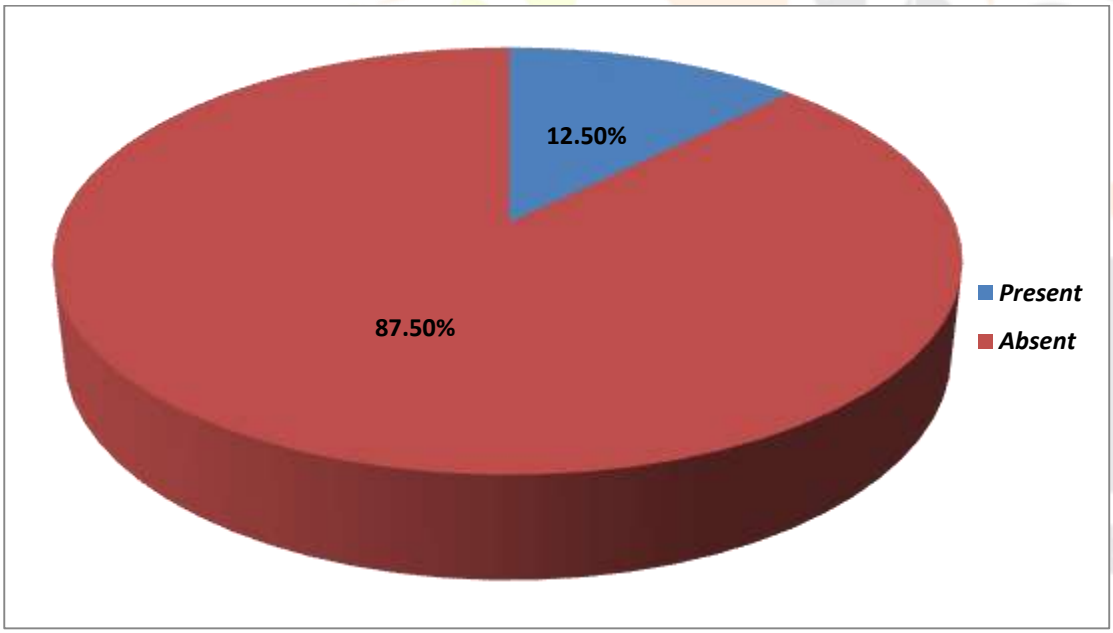

Figure 12: Descriptive statistics of Perineural spread (N=8).

- **Gastric adenocarcinoma according to Tumor stage (pT) -** Out of all no cases were showing T1 stage, 4 cases showing T2 stage, 4 cases were showing T3 stage, no cases showing T4 stage, however HER2neu overexpression were higher in stage pT2 stage in comparison to stage pT3 (75% vs 25%) (fig-13).

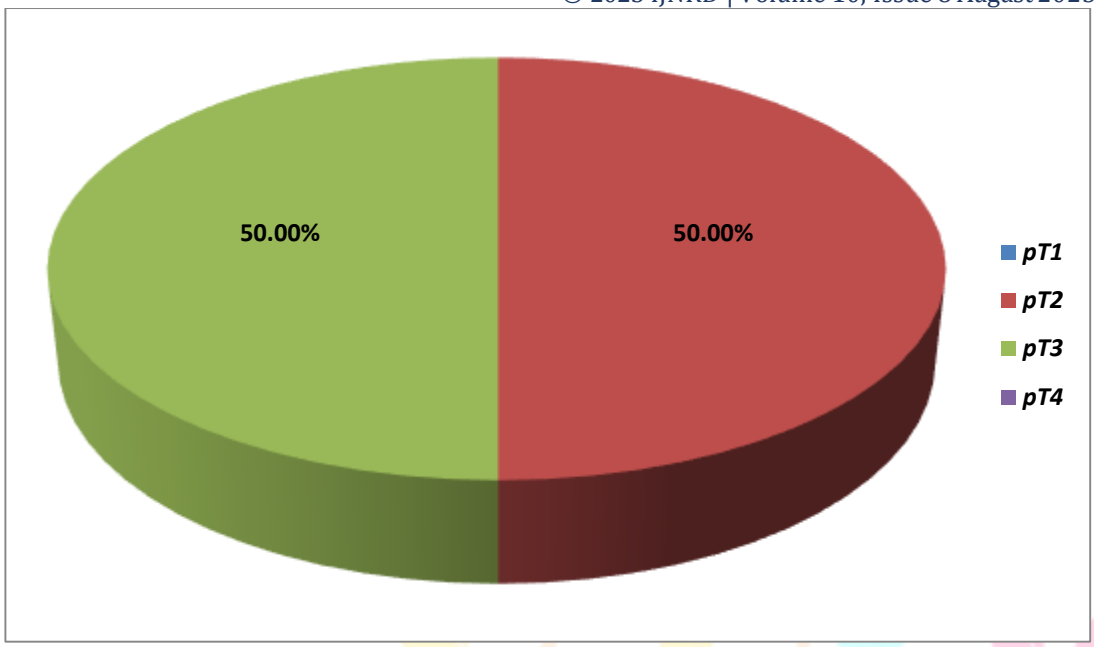

Figure 13: Descriptive statistics of pT stage of tumor (N=8)

- **HER2/neu expression according to (pT)stage of tumor -** Despite this trend of high Her2neu positivity in T2 stage tumors, the association was not statistically significant (p=0.974) (fig-14).

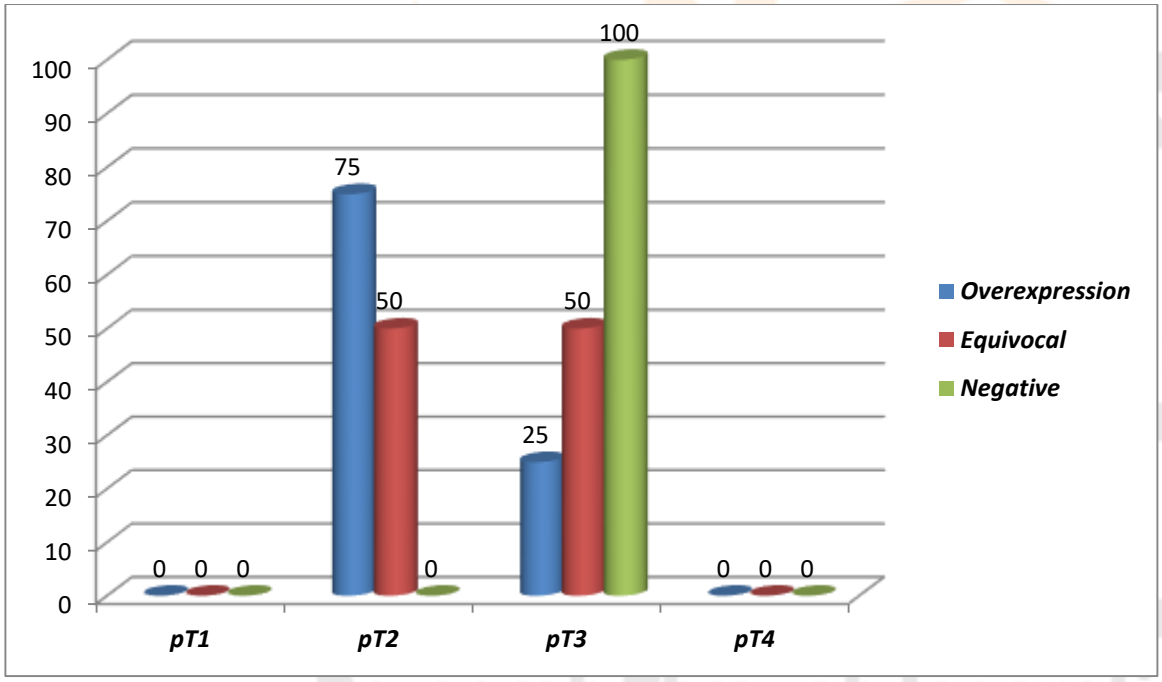

Figure 14: Comparison of the HER2 expression with pT status by using chi square test

- **Gastric adenocarcinoma according to Lymph Node Positivity -** Among all 8 gastrectomy specimen 7 specimen having positive lymph nodes (fig- 15).

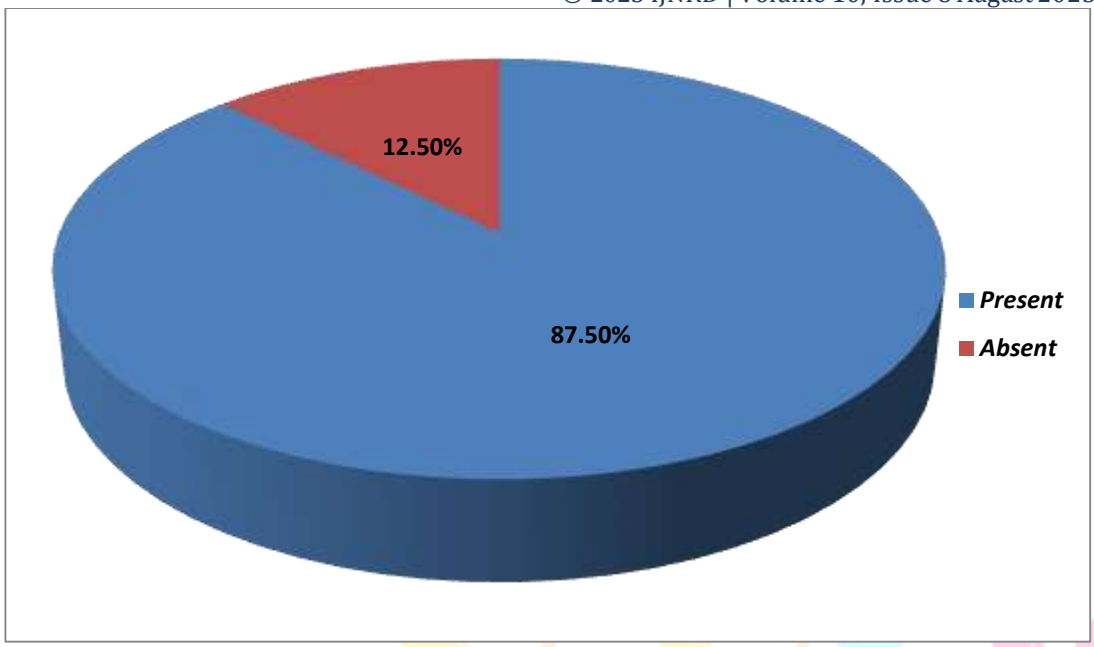

Figure 15: Descriptive statistics of lymph node positivity (N=30)

- Out of 8 gastrectomy specimen ,3 specimen were N1 stage(1-2 regional lymph node), 1 specimen was N2 stage (3-6 regional lymph node) and 3 specimen were showing N3 stage (>= 7 lymph node) (fig-16).

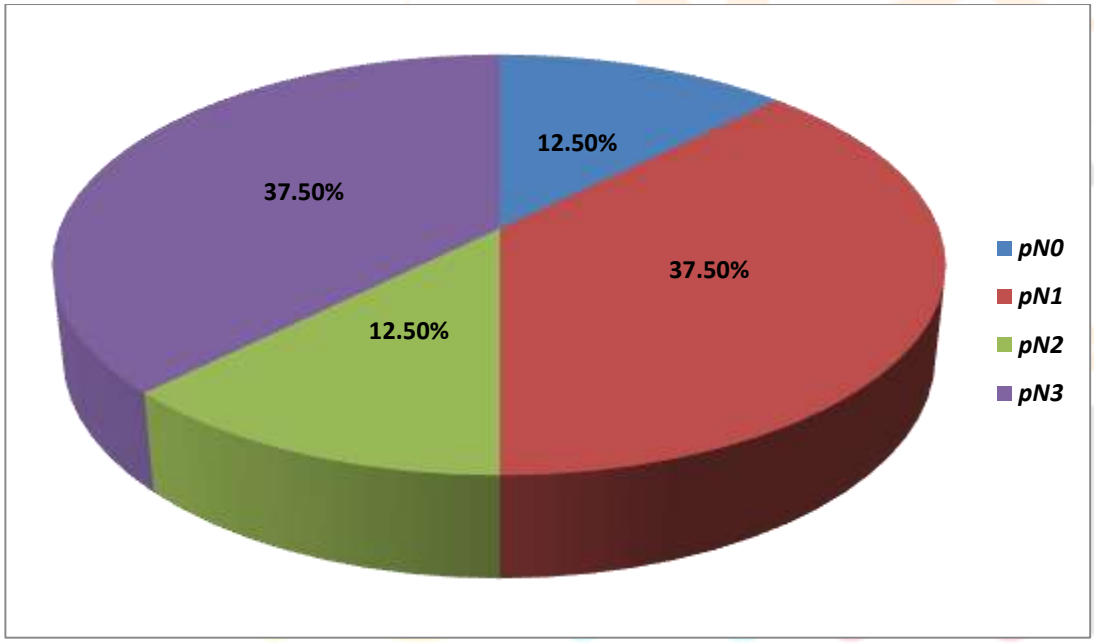

Figure 16: Descriptive statistics of pN stage(N=30)

- **HER2/neu expression in respect to Node status (pN) -** Her2 positivity was equal in pN1 and pN3 (50% vs 50%), rest all shows no overexpression. When Lymph node positivity compared with Her2 positivity, the association is not statistically significant (p=0.969) (fig – 17).

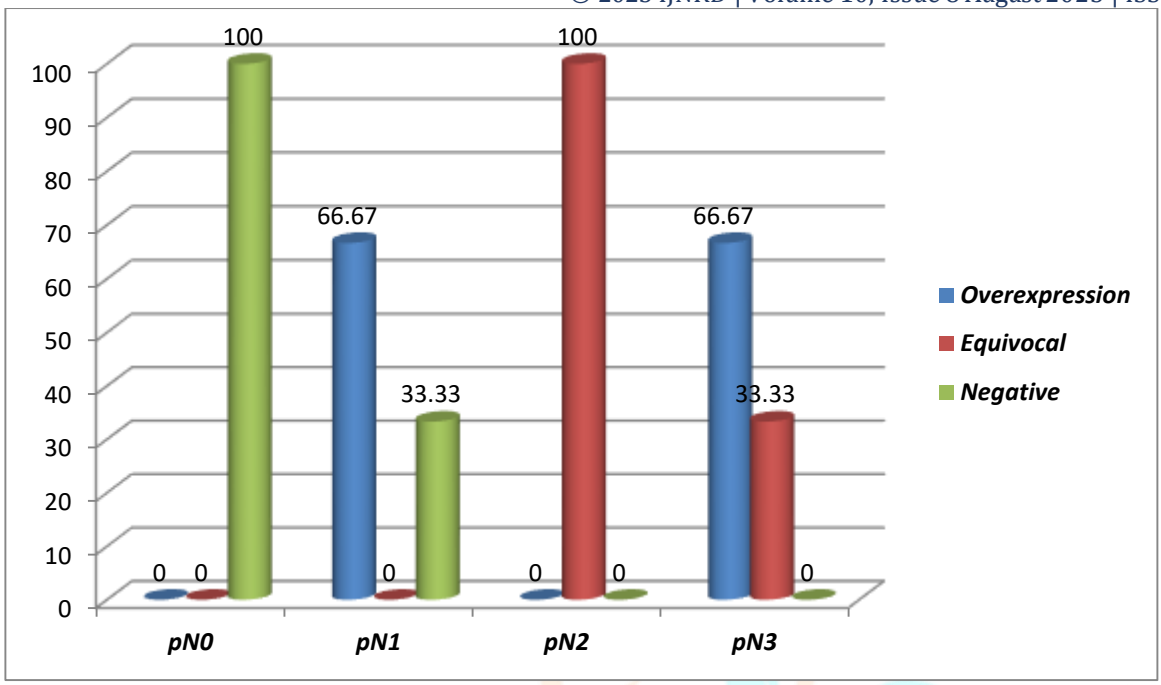

Figure 17: Comparison of the HER2 expression with pN status by using chi square test

- **Gastric adenocarcinoma according to metastasis (Mx) -** No metastasis noted among all 8 gastrectomy specimen (fig-19).

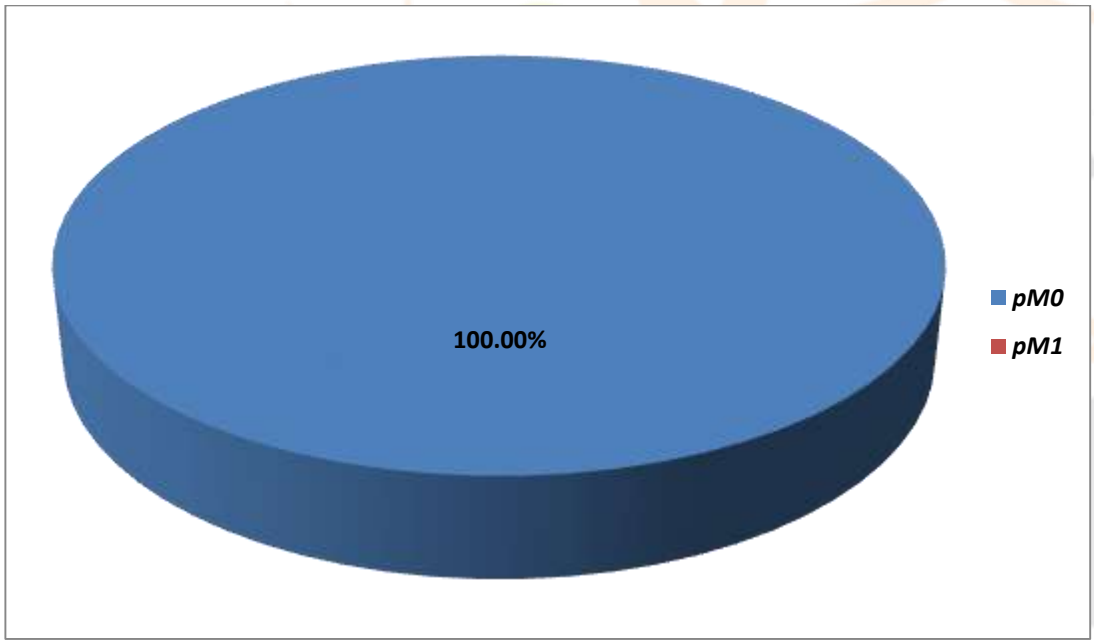

Figure 19: Descriptive statistics of number of tumor showing metastasis (N=30)

All tumor shows no metastasis and the association are not statistically significant (p=0.855).

- **Gastric adenocarcinoma with tumor stage (pTNM stage) of tumor -** Among all 8 gastrectomy specimen TNM stage II A and IIIB were equally noted by respectively 3, 3 specimen. While stage IIB and IIIA were noted respectively by 1,1 specimen (fig - 20).

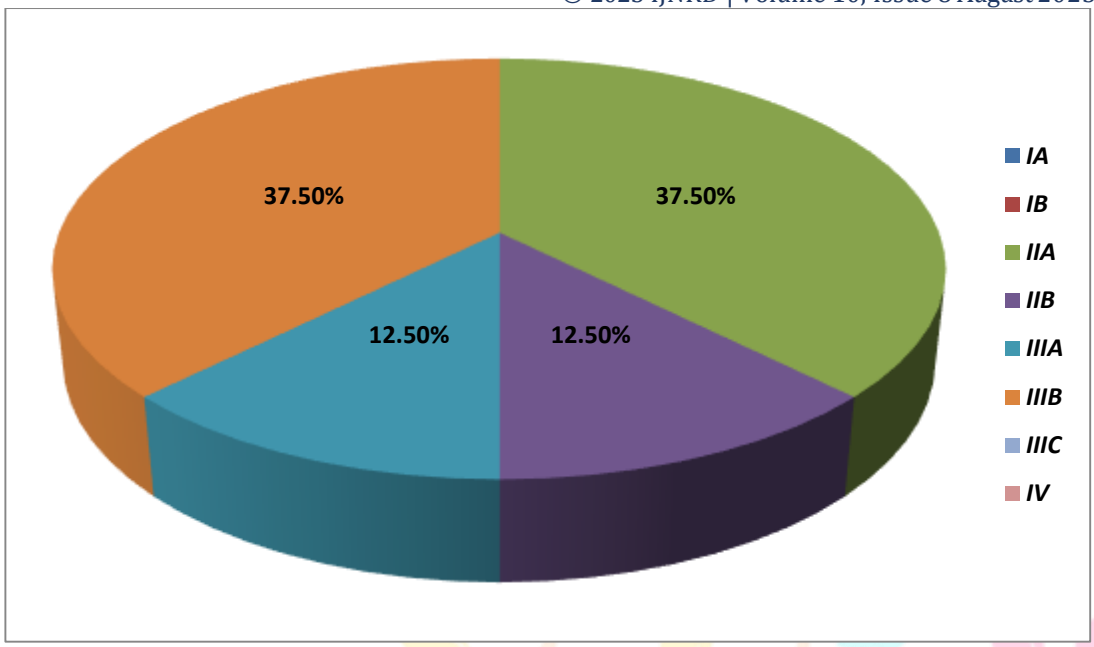

Figure 20: Descriptive statistics of TNM stage (N=30)

- **HER2/neu expression according to tumor stage (pTNM stage) of tumor** - Stage IIA and IIIB shows equal percentage of HER2 overexpression, rest all shows no HER2neu overexpression. This trend of Her2 positivity shows not statistically significant association (p=0.999) (fig-18).

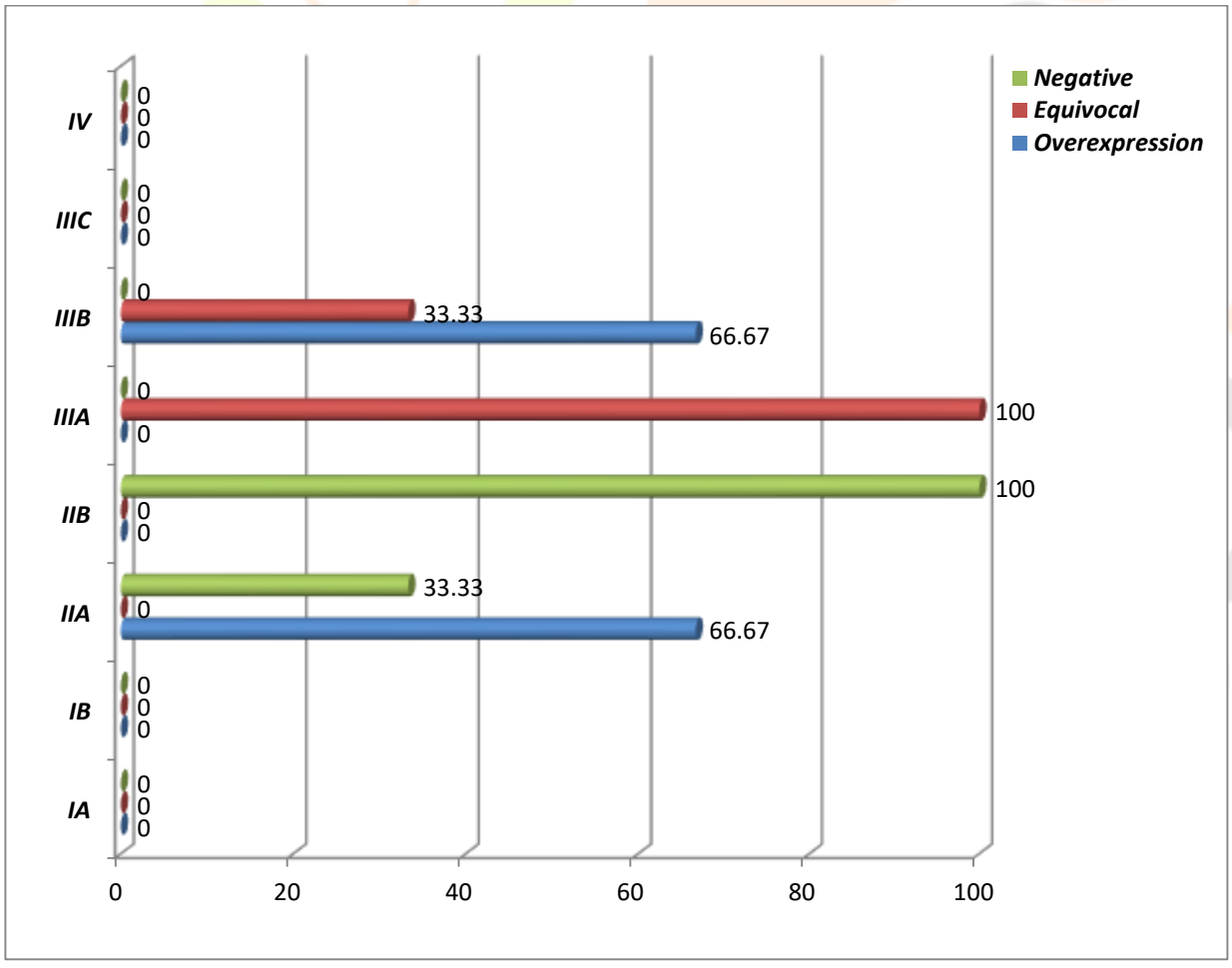

Figure 18: Comparison of the HER2 expression with TNM stage of tumor by using chi square test.

- **Comparison of various clinicopathological features with Her2 expression mentioned in table-**

Table 1: Descriptive statistics (N=30)

Variables	*Sub-groups*	*Number (n)*	*%*
Gender	**Male**	19	63.33
	Female	11	36.67
Age at diagnosis	**≥60 years**	10	33.33
	<60 years	20	66.67
Tumor location	**Proximal stomach**	8	26.67
	Distal stomach	22	73.33
Tumor subtype	**Intestinal**	17	56.67
	Diffuse	11	36.66
	Mixed	2	6.66
Tumor differentiation	**Moderate**	10	33.33
	Poorly	20	66.67

Table 2: Descriptive statistics (Gastrectomy specimen) (N=8)

Variables	*Sub-groups*	*Number (n)*	*%*
pT status	**pT1**	0	0.0
	pT2	4	50.0
	pT3	4	50.0
	pT4	0	0.0

pN status	**pN0**	1	12.5
	pN1	3	37.5
	pN2	1	12.5
	pN3	3	37.5
pM status	**pM0**	8	100.0
	pM1	0	0.0
PTNM stages	**IA**	0	0.0
	IB	0	0.0
	IIA	3	37.5
	IIB	1	12.5
	IIIA	1	12.5
	IIIB	3	37.5
	IIIC	0	0.0
	IV	0	0.0
Lymphovascular involvement	**Present**	5	62.5
	Absent	3	37.5
Perineural spread	**Present**	1	12.5
	Absent	7	87.5
Lymph node positivity	**Present**	7	87.5
	Absent	1	12.5

Table 3: Comparison of the HER2 expression with different variables using chi square test.

Age at diagnosis		***HER2/neu expression***		
		Overexpression	***Equivocal***	***Negative***
≥60 years	***Number***	2	2	6
	% within group	20.0	20.0	60.0
<60 years	***Number***	2	6	12
	% within group	10.0	30.0	60.0
Total	***Number***	4	8	18
	% within group	13.33	26.67	60.0
Chi square value: 0.687 P value: 0.946				

Gender		***HER2/neu expression***		
		Overexpression	***Equivocal***	***Negative***
Male	***Count***	2	4	13
	% within group	10.52	21.05	68.42
Female	***Count***	2	4	5
	% within group	18.18	36.36	45.45
Total	***Count***	4	8	18
	% within group	13.33	26.67	60.0

Chi square value: 0.464 P value: 0.792				

Tumor location		***HER2/neu expression***		
		Overexpression	***Equivocal***	***Negative***
Proximal stomach	***Count***	2	2	4
	% within group	25.0	25.0	50.0
Distal stomach	***Count***	2	6	14
	% within group	9.09	27.27	63.63
Total	***Count***	4	8	18
	% within group	13.33	26.67	60.0
Chi square value: 0.352 P value: 0.838				

Tumor subtype		***HER2/neu expression***		
		Overexpression	***Equivocal***	***Negative***
Intestinal	***Count***	3	4	10
	% within group	17.64	23.52	58.82
Diffuse	***Count***	1	4	6
	% within group	9.09	36.36	54.54
Mixed	***Count***	0	0	2
	% within group	0.0	0.0	100.0

Total	***Count***	4	8	18
	% within group	13.33	26.67	60.0
Chi square value: 0.426 P value: 0.980				

Tumor differentiation		***HER2/nu expression***		
		Overexpression	***Equivocal***	***Negative***
Moderate	***Count***	1	2	7
	% within group	10.0	20.0	70.0
Poorly	***Count***	3	6	11
	% within group	15.0	30.0	55.0
Total	***Count***	4	8	18
	% within group	13.33	26.67	60.0
Chi square value: 0.109 P value: 0.946				

Gastrectomy specimen (pT status)		***HER2/neu expression***		
		Overexpression	***Equivocal***	***Negative***
pT1	***Count***	0	0	0
	% within group	0.0	0.0	0.0
pT2	***Count***	3	1	0

	% within group	75.0	25.0	0.0
pT3	***Count***	1	1	2
	% within group	25.0	25.0	50.0
pT4	***Count***	0	0	0
	% within group	0.0	0.0	0.0
Total	***Count***	4	2	2
	% within group	50.0	25.0	25.0
Chi square value: 1.25 P value: 0.974				

Gastrectomy specimen (pN status)		***HER2/neu expression***		
		Overexpression	***Equivocal***	***Negative***
pN0	***Count***	0	0	1
	% within group	0.0	0.0	100.0
pN1	***Count***	2	0	1
	% within group	66.67	0.0	33.33
pN2	***Count***	0	1	0
	% within group	0.0	100.0	0.0
pN3	***Count***	2	1	0
	% within group	66.67	33.33	0.0
Total	***Count***	4	2	2

	% within group	50.0	25.0	25.0
Chi square value: 1.333 P value: 0.969				

Gastrectomy specimen (pM status)		***HER2/neu expression***		
		Overexpression	***Equivocal***	***Negative***
pM0	***Count***	4	2	2
	% within group	50.0	25.0	25.0
pM1	***Count***	0	0	0
	% within group	0.0	0.0	0.0
Total	***Count***	4	2	2
	% within group	50.0	25.0	25.0
Chi square value: 0.313 P value: 0.855				

Gastrectomy specimen (PTNM stages)		***HER2/neu expression***		
		Overexpression	***Equivocal***	***Negative***
IA	***Count***	0	0	0
	% within group	0.0	0.0	0.0

IB	***Count***	0	0	0
	% within group	0.0	0.0	0.0
IIA	***Count***	2	0	1
	% within group	66.67	0.0	33.33
IIB	***Count***	0	0	1
	% within group	0.0	0.0	100.0
IIIA	***Count***	0	1	0
	% within group	0.0	100.0	0.0
IIIB	***Count***	2	1	0
	% within group	66.67	33.33	0.0
IIIC	***Count***	0	0	0
	% within group	0.0	0.0	0.0
IV	***Count***	0	0	0
	% within group	0.0	0.0	0.0
Total	***Count***	4	2	2
	% within group	50.0	25.0	25.0
Chi square value: 1.333 P value: 0.999				

DISCUSSION AND SUMMARY

Gastric carcinoma is a leading cause of cancer mortality all over the world and nationally the second most common cause of cancer related deaths. 5 year survival rate of patients with gastric cancer ranges from 10% to 30% in the studies. Gastric carcinoma has been managed with different treatment strategies around the world and surgery is the mainstay of treatment for non-metastatic disease. Adjuvent chemotherapy is recommended to prevent recurrence after resection. In advanced or metastatic disease, chemotherapy is

considered as treatment of choice to provide palliation and to prolong survival, however prognosis remains poor. A great interest in targeted therapies has emerged in recent past, and several molecular targeting agents are being tested.

❖ In our study Her2 overexpression was observed in 13 % cases of gastric carcinoma. This results is near to the result of ToGA trial and falls close to range of Her2 positivity by IHC observed in most of the studies across the world.
❖ In the ToGA trial, the percentage of HER2/neu positive (IHC 3+ or IHC 2+/FISH positive) gastric or gastroesophageal cancer patients was 22.1% overall and around 10.4% of IHC 3+ in resected samples, similar to the present result (86). Recent studies also reported a range of 8.5% to 10.3% for HER2 overexpression in gastric carcinoma(87-89). Recently, three studies in Chinese Gastric cases applied the same FDA approved reagents and scoring criteria and reported HER2 IHC 3+ rates of 9.0% (77/860), 6.9% (10/145), and 5.8% (4/69), respectively, all slightly lower than the present result (90,91,92). This variation may be partly explained by different sample sets.
❖ In this study no endoscopic biopsy showed HER2/neu overexpression while in the ToGA study, both biopsies and surgically resected specimens were included and the HER2/neu positivity rate was higher in biopsies than in surgically resected specimens (23.1% vs. 19.9%; P=0.03) and biopsy samples were also more likely to be HER2-amplified than surgical samples when analyzed by FISH (P=0.01) than by IHC (23).
❖ Higher HER2/neu expression was seen in older age and in males while in above study more cases are found <60 years of age while the number of cases showing HER2/neu positivity was same in male and female, however the correlation did not completely fulfil the criteria of significant study. Her2 positivity was more common with intestinal type (18%) of tumors as compared to diffuse type (9%) and but did not reach statistically significant (p=0.980).
❖ A positive association between HER2/neu positivity and intestinal type cancers was identified in this study and also in other studies(93-97). In the ToGA trial, countries with higher ratios of intestinal / diffuse had increased HER2/neu positivity rates(86).
Her2 expression in intestinal type of tumors as opposed to diffuse type can be attributed to specific tumor characteristics, similar to breast carcinoma.
❖ Fan et al shows a significant association of HER2/neu positivity with proximal tumors. We also found high number of HER2/neu positive cases at proximal in comparison to distal tumors however the total number of cases are more seen at distal location but statistics did not reach statistically significant (p=0.980).
❖ More number of intestinal subtype found at proximal location along with more number of HER2 positivity also found at proximal location.
❖ More cases of HER2/neu positivity was seen with poorly differentiated carcinomas in the present study (P= 0.946) but did not reach statistically significance. However, other studies demonstrated both an association and no association between HER2 overexpression and tumor differentiation (90,97). These conflicting data may be due to different sample sizes and the low prevalence of HER2 in Gastric carcinoma.
❖ No statistically significant correlation was found between HER2neu overexpression and tumor stage, lymphovascular and perineural invasion in the present study and in other studies also(87-89). This can be due to small sample size. The reason of this discrepancy between resection and biopsy specimens is due to different scoring criteria applied for the two types of specimens. Resection specimens require 10 % cells showing strong positivity for 3+ score in contrast to biopsy specimens where any number of cells showing definite expression is considered positive.
❖ Lymph node positivity are more seen with HER2 positive tumors in this study however not statistically significant.
❖ The need for standardized processing method and a modified scoring system emerged due to discrepant results in various studies and dissimilarity of gastric cancer to breast cancer in the form of intratumoral heterogeneity and basolateral membrane staining by Her2 antibody. As the most suitable testing method for HER2/neu evaluation, IHC and FISH have emerged as most preferred techniques.
❖ The European medicines agency recommends initial evaluation of Her2 expression by IHC followed by confirmation of gene amplification of equivocal (2+) cases by FISH providing a therapeutic advantage to such patients.
❖ According to US Food and Drug administration (FDA) and ToGA trial all cases subjected to IHC and FISH simultaneously. This provide an added advantage of picking up those gene amplified cases (candidates for trastuzumab therapy) which showed no or 1+ Her 2 expression on IHC.
❖ In ToGA trial after IHC and FISH these cases was only 4% thus rendering the assumed advantage of this approach to be only theoretical.

SUMMARY

The present study was undertaken with 30 patients to observe the HER2/neu overexpression after use of IHC. The following observations were noted:

1. The age range of patient varied from 28 to 69 yrs. More number of cases found in <= 60 year of age in comparison to >60 year of age, while HER2 positivity more in >60 year of age.
2. Number of cases are more in male in comparison to female while HER2/neu positivity are equal in both male and female.
3. Distal location is more common for gastric adenocarcinoma although HER2/neu positive tumors are more in proximal site.
4. Intestinal subtype is more common than diffuse subtype and HER2/neu overexpression are also more common with intestinal subtype than diffuse subtype.
5. Intestinal subtype is seen more commonly at proximal location.
6. Her2 positivity more commonly seen with poorly differentiated tumor.
7. Lymph node positivity are found to be more associated with HER2 positivity.

CONCLUSION

❖ Trastuzumab increases the overall survival of the patients with gastric adenocarcinoma. This monoclonal antibody can be given in patients with Her 2 positivity. Therefore, Her2neu testing should be done in such patients.
❖ Gastric tumor tissue should undergo testing for HER2 at the time of initial diagnosis and scoring should be performed with recommended scoring criteria.
❖ The overall reliability of HER2 evaluation by IHC can be affected by diverse pre- analytical, analytical and post-analytical variables. FISH should be done in all equivocal cases (2+) for increased diagnostic accuracy.

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Appendix A - List of abbreviations used in the study

IHC- Immunohistochemistry

FISH- Fluorescent in situ hybridisation

FDA- Food & drug administration

GC- Gastric carcinoma

GEJ- Gastroesophageal carcinoma

MASTER CHART-

Biopsy no	Age	Sex	T.L	T.S	Specimen	Tumor Size	T.D	LVI	PNI	T. Stage	TNM Stg	Signet ring cell	Ass Feature	Lym Node	HER2
B/3592/18	69 yrs	M	2	Intestinal	End Bio	```	Moderate					Not Present	Ben gast glnd		1
B/5983-90/16	51 yrs	F	2	Diffuse	Gastrec Sp	5X2.5X1 cm	Moderate	Present	Not Pre	pT3N0M0	Stage IIA	Not Present	Chr gas	Negative	1
B/5525/16	62 yrs	F	2	Intestinal	End Bio	6.4x4x3.4 cm	Poorly					Present	Chr gas		1
B/1051/16	46 yrs	M	1	Intestinal	End Bio	5.7x4x3 cm	Moderate					Not Present	Chr gas		2
B/1347/16	52 yrs	M	2	Diffuse	End Bio	6.6x5.1x3 cm	Poorly					Present	Chr gas		1
B/1393-99/18	48 yrs	M	2	Intestinal	Gastrec Sp	12x6x2 cm	Poorly	Not Pre	Not Pre	pT2N1M0	Stage IIA	Present	Chr gas	Positive	3
B/1943-57/16	52 yrs	F	2	Diffuse	Gastrec Sp	6X5X2.8 cm	Moderate	Present	Not Pre	pT3N1M0	Stage IIB	Not Present	Mucin pool	Positive	1
B/3201/16	50 yrs	M	2	Intestinal	End Bio	9x6.3x5.5 cm	Poorly					Not Present	Necro inf infil		2
B/4008-22/16	68 yrs	M	2	Intestinal	Gastrec Sp	5x2.5x1 cm	Poorly	Present	Not Pre	pT3N3aM0	Stage IIIB	Present	Chr gas	Positive	3
B/1347-63/16	28 yrs	M	2	Diffuse	Gastrec Sp	2x1.5x1 cm	Poorly	Not Pre	Not Pre	pT3N2M0	Stage IIIA	Present	Chr gas	Positive	2
B/4012/16	44 yrs	F	2	Diffuse	End Bio	4.5x3x3 cm	Poorly					Not Present	Chr gas		2
B/3337-90/17	63 yrs	F	2	Intestinal	Gastrec Sp	5.5x2x1.5 cm	Poorly	Present	Present	pT2N3bM0	Stage IIIB	Present	Den hyaliniza	Positive	3
B/1769/17	56 yrs	M	2	Intestinal	End Bio	7.6x2x3.3 cm	Poorly					Not Present	Chr gas		2
B/1034/17	36 yrs	M	1	Intestinal	End Bio	4.4X2X3 cm	Poorly					Not Present	Chr gas		1
B/1907-39/17	67 yrs	F	2	Diffuse	Gastrec Sp	3.5X1X1 cm	Poorly	Not Pre	Not Pre	pT2N3bM0	Stage IIIB	Present	Non cas granu	Positive	2
B/6211/17	46 yrs	F	2	Intestinal	End Bio	4.2X3.4X3 cm	Poorly					Not Present	Chr gas		1
B/5983/17	61 yrs	F	1	Diffuse	End Bio	4x3x2.2 cm	Poorly					Not Present	Ben gast glnd		1
B/7529/17	64 yrs	M	2	Intestinal	End Bio	8x4.4x2.7 cm	Poorly					Present	Den hyaliniza		1
B/4385/16	47 yrs	M	1	Diffuse	End Bio	11.3x5.2x3 cm	Poorly					Present	Chr gas		1
B/3763/16	53 yrs	M	2	Intestinal	End Bio	13x5.5x4 cm	Moderate					Not Present	Chr gas		1
B/1525/16	38 yrs	F	2	Mixed	End Bio	8x4.1x2 cm	Moderate					Not Present	Den hyaliniza		1
B/1051/15	68 yrs	M	2	Intestinal	End Bio	10.5x5.5x3 cm	Poorly					Not Present	Den hyaliniza		1
B/781-803/17	40 yrs	M	2	Diffuse	Gastrec Sp	9x6.3x5.5 cm	Moderate	Present	Not Pre	pT2N1M0	Stage IIA	Present	Chr gas	Positive	1
B/62/17	43 yrs	F	2	Intestinal	End Bio	6X5X2.8 CM	Moderate					Not Present	Chr gas		2
B/4520-21/17	65 yrs	M	2	Mixed	End Bio	4x3x2.2 cm	Poorly					Not Present	Chr gas		1
B/6013/16	58 YRS	M	1	Diffuse	End Bio	3.5X1X1 cm	Poorly					Not Present	Chr gas		3
B/3542/17	57 yrs	M	2	Intestinal	End Bio	3X4X2 cm	Moderate					Not Present	Chr gas		1
B/4683/17	63 yrs	F	1	Diffuse	End Bio	4.5x3x3 cm	Poorly					Not Present	Chr gas		2
B/3752/17	59 yrs	M	1	Intestinal	End Bio	3.7x4x0.7 cm	Moderate					Not Present	Chr gas		1
B/5652/17	52 yrs	M	1	Intestinal	End Bio	4.5X3X2.5 cm	Poorly					Not Present	Ben gast glnd		1