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Original Research Article

Her2-Neu Expression Analysis in the Tumors of Stomach and Intestine

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Conflict of interest: Nil

Abstract:

Background: Tumors of stomach and intestine is the 5th most common malignancy and 3rd leading cause of cancer death worldwide. Most cases are diagnosed in advanced stages making treatment difficult. International regulatory agencies have recently approved trastuzumab therapy in locally advanced and metastatic gastric adenocarcinomas expressing Her2-Neu.

Objective: The aim of present study is analyzed the Her2-Neu expression analysis in the tumors of stomach and intestine and correlate that with clinicopathological characteristics.

Methodology: Tissue specimens obtained from the from 61 cases who were clinicoradiologically diagnosed with benign and malignant lesions of stomach and intestine. Sections were stained with Haematoxylin& Eosin (H&E) stains under strict quality assurance. Her2-Neu monoclonal rabbit antibody was used form immunohistochemical analysis. Data of Her2-Neu expression was correlated with the clinicopathological characteristics.

Results: A higher incidence of stomach and intestinal lesions was found in the age group beyond 30 years. A high male to female ratio was observed. The dominant site of involvement was intestine (55.7%) followed by the stomach (44.3%). Total 17 (28%) cases were diagnosed as benign and 44(72%) were diagnosed as malignant on histopathology. Out of 44 malignant cases diagnosed on histomorphology, the maximum cases 29(66%) were diagnosed as moderately differentiated adenocarcinoma. Among 44 malignant patients have 3+ expression of Her2-Neu, 14(88%) patients have 2+ expression and 14 (67%) patients have 1+ expression. The association of Her2-Neu expression in different histomorphological grades and types showed that higher grades of adenocarcinoma showed 3+ and 2+ Her2-Neu expression.

Conclusion: Her2-Neu overexpression was found to be statistically significantly correlated with middle age group and moderately and poorly differentiated gastric and intestinal tumors; they may be the candidates for Herceptin-based targeted treatment. To investigate the function of Her2-Neu as a stand-alone prognostic factor, more research with ample sample size is required.

Keywords: Her2-Neu, Histopathology, Benign, Malignant, Cancer, Tumor.

Introduction

The stomach and intestine are part of the alimentary system that is prone to the vast majority of both benign and malignant tumors of epithelial origin (polyps), with mesenchymal (leiomyoma, leiomyoblastoma, neurogenic, vascular and lipoma), neuroendocrine tumours and miscellaneous (Inflammatory Pseudotumors, Peutz Jegher's Polyps, Cystic Tumors). Benign tumors of stomach and duodenum are not common and constitute only 5–10% of all stomach tumors. The most common benign lesions in the stomach are polyps (epithelial tumors) and they constitute 75% of all benign stomach tumors [1]. According to WHO, cancer caused nearly 10 million deaths in 2020, out of which stomach was accounted for 1.09 million cases (in terms of new cases), and 769000 deaths due to stomach cancer worldwide. The highest incidence is found in Eastern Asia, Eastern Europe and South America. In all populations studied thus far, there is a male preponderance in gastric cancer incidence with an average male to female ratio of 2:1 [2, 3]. Every year approximately 990,000 people are diagnosed to have gastric cancer out of which about 738,000 patients die from this condition. Previously gastric cancer was only prevalent in the western countries and Japan but now it causes a major threat to Asian countries including India [4]. In 2001 the incidence of gastric cancer in India was 35,675 out of which 23,785 were men and 11,890 were women. The north

eastern and southern states of India have the highest incidence of gastric cancer [5]. The Her2-Neu (also known as erbB-2) oncogene is the second member of the ―epidermal growth factor receptor family. It is overexpressed in many different types of human cancers, including breast, ovarian, lung, gastric, and oral cancers. Overexpression of Her2-Neu in breast cancer has been associated with poor overall survival and has been shown preclinically to enhance malignancy and the metastatic phenotypes. Although discrepancies exist between different studies, Her2-Neu overexpression seems to induce chemo resistance in certain experimental conditions. Many studies have convincingly shown that repression of Her2-Neu suppresses the malignant phenotypes of Her2-Neu overexpressing cancer cells. These findings strongly suggest that Her2-Neu may serve as an excellent target for developing anticancer agents specific for Her2-Neu overexpressing cancer cells. Her2-Neu encoded p185 protein is a receptor tyrosine kinase that can be associated with multiple signal transduction pathways. However, it is not yet clear how a specific signal pathway may correspond to a specific biological response [6,7].

The prognosis is still dismal for many individuals with advanced stage stomach cancer when they first present. When compared to single- agent chemotherapy alone, improved treatment options for stomach cancer have improved overall survival. These include combination chemotherapy. In select tumors, additional tailored treatment at certain targets in cancer has been found to improve survival [8]. Several research have documented on the occurrence, therapeutic and prognostic consequences of Her2-Neu status in gastrointestinal carcinoma patients. This study is being done in an effort analyse the expression of Her2-Neu in gastrointestinal tumors of this region which will also help to achieve a better therapeutic outcome by early recognition and initiation of targeted therapy in the identified cases.

Methodology

Study design: The study was a single centric, prospective, observational and hospital-based study that was conducted in the Department of Pathology, Himalayan Institute of Medical Sciences (HIMS), Swami Ram Nagar, Dehradun. Total 61 samples were included for statistical purpose by convenient sampling.

Patients diagnosed with benign and malignant lesions of stomach and intestine clinicoradiologically were included in the study whereas patients who underwent surgery, chemotherapy or radiotherapy were excluded from the study. All the relevant clinical details of history, physical examination and investigation were recorded in the case reporting form. Signed informed consent of patients were taken.

Histochemical Protocol: Specimen was immediately put in 10% formalin, appropriately labelled for patient's name, gender, age. Excised biopsy/large resection specimen were received in the Department of Pathology and a specific ID number was given to each specimen. Grossing of specimen was done using standard protocols and measurements were recorded. Tissue processing was performed using Automated Tissue Processor. Paraffin blocks were made and Sections of 4–5micron thickness were cut with the help of microtome. Sections were placed in a water bath with a temperature of 5 degree below the melting point of paraffin wax. Cut ribbons of tissues were placed on albumenized glass slides. Sections were stained with Haematoxylin& Eosin (H&E) stains under strict quality assurance. All the sections from a particular case were examined. H&E-stained section was selected, having preserved morphology and internal control if possible. Tissue having necrosis and crush artefacts were avoided.

Her2-Neu immunostaining: For staining of Her2-Neu monoclonal rabbit antibody manufactured by Thermo scientific was used. Citrate buffer epitope retrieval method was used for antigen retrieval. Peroxidase blocking was done by incubating the sections in peroxidase blocking solution for 10 minutes. After peroxidase blocking, the sections were incubated in power block solution for 10 minutes to block the antibodies. Of the 2 sections one was incubated with human recombinant polyclonal antibody to Her2-Neu with purified human factor antibody to factor 8. The excess antibody was then wiped off. After this they were incubated with super-enhancer following the Horse-radish peroxidase (HRP) polymer and peroxidase substrate solution. The sections were counter staining with hematoxylin and mounted with DPX.

Statistical analysis:

Observations were statistically analysed using SPSS Software (Statistical Package for Social Sciences) version 23 and Microsoft Excel. Analysis of results were done by the use of tables, bar charts and pie diagrams. Categorical data and continuous data were expressed as frequencies and mean ± standard deviation or median respectively.

Association of categorical variables was analysed by using Pearson chi-square test, Kruskal Wallis test and Wilcoxon-Mann-Whitney U Test. A p value of <0.05 was considered as significant value.

Results

The youngest patient included in the study was 18 years of age and the maximum age group of the patients was between 70-80 years. Total 35 (57%)

males and 26 (43%) females presented with the stomach and intestinal lesions (Table 1).

Table 1: Sociodemographic determinants of the enrolled cases

Variable	Domain	Number	Percentage
Age groups	18-30 Years	4	6.6%
	31-40 Years	11	18.0%
	41-50 Years	13	21.3%
	51-60 Years	15	24.6%
	61-70 Years	9	14.8%
	71-80 Years	9	14.8%
Gender	Male	35	57
	Female	26	43

Out of total 61 patients 27(44%), had lesion involving the stomach whereas 34(56%) had intestinal involvement. Total 17(28%) cases were benign and 44(72%) were malignant on histomorphological diagnosis (Table 2).

Table 2: Site of involvement and histological impression

Variable	Domain	Number	Percentage
Site of Involvement	Gastric	27	44.3%
	Intestinal	34	55.7%
Histological Impression	Benign	17	28%
	Malignant	44	72%

Out of 44 malignant cases diagnosed on histomorphology, maximum cases 29(65.9%) were diagnosed as moderately differentiated adenocarcinoma whereas only 1 case was diagnosed as signet ring adenocarcinoma. Others included 1 patient diagnosed as Liposarcoma of intestine (Table 3).

Table 3: Histological Diagnosis

Histological Diagnosis	Number	Percentage
Poorly Differentiated Adenocarcinoma	5	11.3%
Moderately Differentiated Adenocarcinoma	29	65.9%
Well Differentiated Adenocarcinoma	3	6.8%
Mucin Secreting Adenocarcinoma	5	11.3%
Signet Ring Adenocarcinoma	1	2.2%
Others	1	2.2%
Total	44	100%

Out total cases graded as negative (n=19); 8 (42%) cases were benign and 11 (58%) were malignant. However, in higher grades i.e. 2+ (n=16), 14 (88%) cases were malignant and 2 (13%) cases were benign. Out of the total cases graded as 3+ (n=5); All 5 (100%) were malignant lesions (Table 4).

Table 4: Her2-Neu expression analysis as per histological Impression

Her2-Neu Expression	Histological Impression			P Value
	Benign	Malignant	Total	
Negative	8 (42%)	11 (58%)	19 (100%)	0.0593
1+	7 (33%)	14 (67%)	21 (100%)	
2+	2 (13%)	14 (88%)	16 (100%)	
3+	0 (0%)	5 (100%)	5 (100%)	
Total	17 (28%)	44 (72%)	61 (100%)	

The maximum number of cases were graded (1+) and (2+) (n=14) in malignant cases.

Out of all the carcinomas; moderately differentiated carcinoma shows a varied expression in grading 13 cases being graded as (2+); 7 cases as (1+) and 2 cases as (3+). The (3+) grade was maximum in patients diagnosed as poorly differentiated (n=3) followed whereas (2+) grade was maximum in patients diagnosed as moderately differentiated i.e. 13 (45%) and 1+ was seen maximum in Well differentiated (n=2), Mucin secreting adenocarcinoma (n=3) and one case of Signet ring adenocarcinoma (Table 5 and Figure 1-2).

Table 5: Her2-Neu expression analysis as per histological diagnosis

Histological Diagnosis	Her2-Neu Expression					Fisher's Test
	Negative	1+	2+	3+	Total	
Poorly Differentiated Adenocarcinoma	1 (9%)	1 (7%)	0	3 (60%)	5 (11%)	P= 0.069
Moderately Differentiated Adenocarcinoma	7 (64%)	7 (50%)	13 (93%)	2 (40%)	29 (66%)	$\chi 2$= 23.649
Well Differentiated Adeno-carcinoma	1 (9%)	2 (14%)	0	0	3 (7%)	
Mucin Secreting Adenocarci-noma	0	3 (21%)	2 (14%)	0	5 (11%)	
Signet Ring Adenocarcinoma	0	1 (7%)	0	0	1 (2%)	
Others	1 (9%)	0	0	0	1 (2%)	
Total	11 (100%)	14 (100%)	14 (100%)	5 (100%)	44 (100%)	

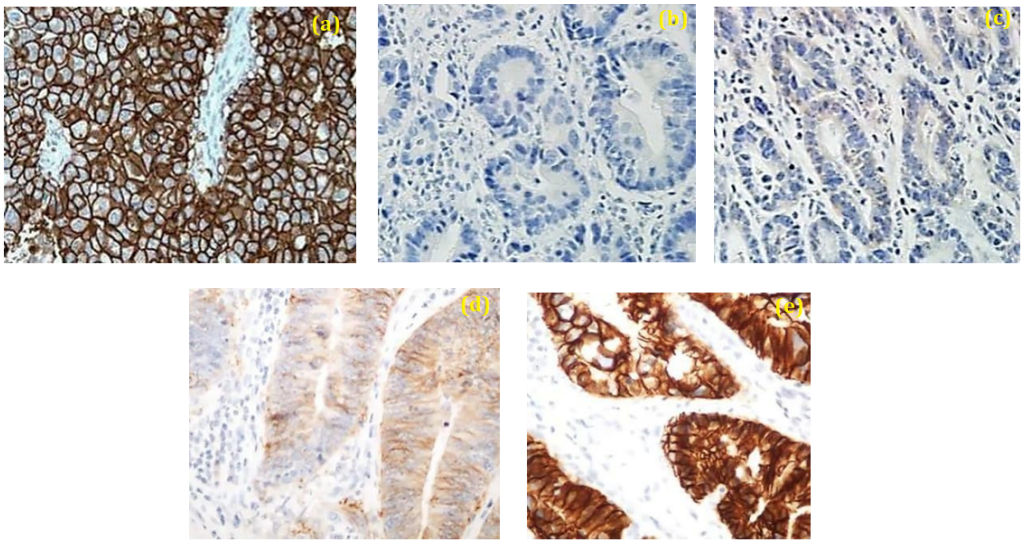

Figure 1: (a) Her2-Neu positive control (200X); (b) Her2-Neu negative staining; (c) Her2-Neu Score 1+ (100X); (d) Her2-Neu Score2+ (200X); (e) Her2-Neu Score 3+ (400X)

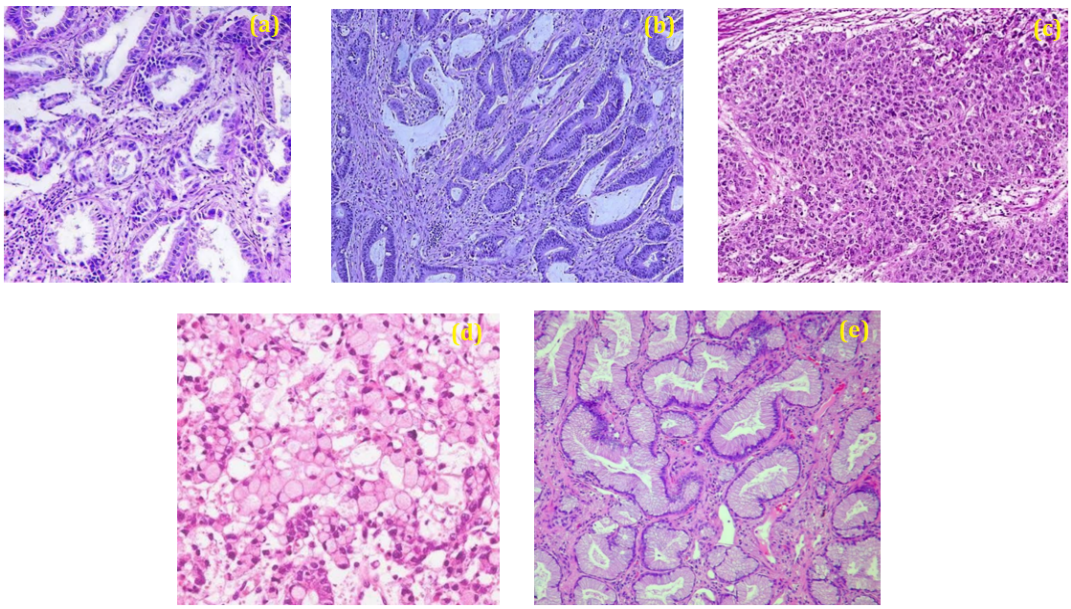

Figure 2: (a) Well differentiated adenocarcinoma (200X); (b) Moderately differentiated adenocarcinoma (200X); (c) Poorly differentiated adenocarcinoma (100X); (d) Signet ring adenocarcinoma (200X); (e) Mucin secreting adenocarcinoma (100X)

Discussion

Globally, colorectal cancer (CRC) is one of the leading causes of tumor-related mortality. Roughly 5 percent of patients with colorectal cancer have somatic HER2 mutations or amplified genes. For the treatment of HER2-positive metastatic colorectal cancer (CRC), the U.S. Food and Drug Administration approved trastuzumab and pertuzumab in conjunction with chemotherapy in 2019. With the availability of targeted medicines for HER2-positive patients that potentially enhance their prognosis, its approval represented a critical turning point in the treatment of colorectal cancer [9]. Because tumour cells overexpress Her2-Neu in contrast to normal cells, anti-Her2-Neu treatment can target malignant cells specifically. Although overexpression of Her2-Neu has been seen in other neoplasms, breast cancer has received the greatest research attention. Between 25 and 30 percent of breast tumors overexpress Her2-Neu. Her2-Neu positive status is linked to more aggressive illness and is a significant predictor of trastuzumab therapeutic response [10].

In this present study, a higher incidence of stomach and intestinal lesions was found in the age group beyond 30 years. Similar results were observed in other studies. In the study by Damle et al., the mean age of patients was 61.75 ± 12.07 years and 82% of patients were older than 50 years of age [10]. Patients age in the Patel et al. research varied in age from 21 to 81 years, with a mean age of 59.4 years. The largest number of cases were observed in those who were over 50 years old [11]. Patients in the Sukanya et al. research varied in age from 30 to 80 years, with the age group over 60 years old having the greatest frequency (41%) [12]. According to Abdi-Rad A et al. the incidence of gastric cancer was significantly low below the age of 40 years [13].

In the present study, a high male to female ratio was observed. Total 57% males and 43% females presented with the stomach and intestinal lesions. In the study by Abd El-Aziz, male predominance is observed, which is in consistent with the re study [13]. There were 24 females (21.05%) and 90 males (78.95%) in the study done by Damle et al. The male-to-female ratio was 3.75:1 [10].

Gastric adenocarcinoma was shown to be more prevalent in 27 males (57.4%) than in 20 females (42.6%) in the Patel et al. research, with a M: F ratio of 1.4: 1 [11]. According to Sukanya et al., there was a 2.3:1 male to female ratio in stomach cancer cases, with males being the major proportion [12]. Abdi- Rad A et al [13]; Abd El-Aziz et al [13]; Lei Y-y et al [14]; Roy et al [15]; including a meta-analysis have also reported similarly. Although Orosz et al [16]; Corless et al [17]; showed an equal male to female ratio or even a slight female predominance. Both in western world and other areas of globe, men are affected more than the females which are in par with our study.

The dominant site of involvement was intestine (55.7%) followed by the stomach (44.3%) in this study. According to Nadaf et al. study, the distal stomach accounted for 60% of the sites observed, with the gastro- esophageal junction coming in second with a percentage of 21.4%, and the proximal stomach with a percentage of 18.5% [18]. According to the Damle et al. study, the gastric cardia and gastroesophageal junction (42.9%) were the most frequently tumor site [10]. The antrum was the most often occurring site of the tumor in the Patel et al. research, accounting for 53.2% of cases. Less curvature in the stomach body followed in 14.9% of cases, and the gastroesophageal junction was involved in 12.7% of cases [11].

In present study, 17 (28%) cases were diagnosed as benign and 44(72%) were diagnosed as malignant on histopathology. Study done by Cherian et al showed 120(80%) cases being diagnosed as malignant lesions of gastrointestinal tract which is at par with our study [19]. Around 27(61.3%) malignant cases were intestinal whereas 17(38.6%) cases were from gastric region this was similar to the Damle et al study which showed that intestinal adenocarcinoma (83.3%) was the most common histological type of adenocarcinoma [10]. However, our results were discordant with the study conducted by Nadaf et al which showed 60% gastric malignancies which could be attributed to their larger sample size [18].

Out of 44 malignant cases diagnosed on histomorphology, the maximum cases 29(66%) were diagnosed as moderately differentiated adenocarcinoma; meanwhile 5(11.3%) cases were diagnosed as poorly differentiated and 3(6.8%) as well differentiated adenocarcinomas. The majority of the tumors in the Rajagopal et al. research was moderately differentiated (66.7%), followed by poorly differentiated (18.3%) and well differentiated (15%) types [5]. Our results were hence, in concordance with other studies.

In the current study, 5(100%) malignant patients have 3+ expression of Her2-Neu, 14(88%) patients have 2+ expression and 14 (67%) patients have 1+ expression. Only 13 individuals (11.40%) in the Damle et al. research had positive Her2-Neu staining (Score 3+). FISH confirmed that four patients (3.50%) with equivocal IHC results (2+) were overexpressed the Her2-Neu [10]. Her2-Neu (3+) was seen in 21.3% of the patients in the Patel et al. research, followed by equivocal (2+) in 10.6% of the cases and negative in 68.0% of the cases [11]. According to Matsusaka et al., 97.5% of IHC score 3+ cases (158 of 162 patients) and

47.3% of IHC score 2+ cases (61 of 129 patients) had positive FISH results [20]. He et al., examined 197 cases of gastric cancer, they found that 31 of the cases (15.74%) had Her2-Neu gene amplified by FISH, and 19 of the cases (9.64%) had Her2-Neu IHC staining scores of three or higher [21]. The Sukanya et al study grading method for Her2-Neu expression by immunohistochemistry in gastric cancer showed that it was negative (0+,1+) in 52 cases (74.3%), equivocal (2+) in 10 cases (14.3%), and positive (3+) in 8 cases (11.4%) [12].

The majority of patients (61.7%) in the Rajagopal et al. research had a score of 0, while 26.7% had a score of 3+, indicating Her2-Neu positivity [5]. The expression of Her2-Neu was examined by IHC in 61 cases of stomach and intestinal lesions in the current investigation. 42 (68.86%) of the cases had Her2-Neu positive, which was consistent with findings from a prior study by Nadaf et al. that identified Her2-Neu positivity in 23% of cases of gastric and gastro-esophageal carcinomas [18]. In the past, 9.4% of Indian patients with stomach cancer had Her2-Neu positive expression, according to Sekaran et al. [22]. According to Lei et al. meta- analysis, Asian nations have a little greater proportion of positivity (19.52%) than European countries, which have a rate of 16.91% [14]. In a Chinese research, Shan et al. showed a frequency of 9.8% [23]. In a Japanese multicenter observational research, Matsusaka et al. found that high Her2-Neu expression rate of 15.6% [20]. Geographic variance has been seen among Indian research; Sukanya et al. from Tamil Nadu reported a 12% prevalence [12]. In their multicentric clinical study, Patil et al. found 7% frequency [24]. A median of 15% was found in 41 studies after a comprehensive evaluation a study conducted by Chua et al [25]. Gupta et al. reported a 24.5% frequency in Delhi population [26]. In the state of Orissa, Panda et al. found 18.7% frequency [27]. 15% was the frequency in the Damle et al. research conducted in a Keralan tertiary care facility [10].

In this study, the association of Her2-Neu expression in different histomorphological grades and types showed that higher grades of adenocarcinoma showed 3+ and 2+, Her2-Neu expression in 5(100%) poorly differentiated (grade III) and 14(88%) moderately differentiated adenocarcinomas (grade II) respectively. This was in concordance with a prior study by Farzand et al that showed out of the fifty patients, 33 patients showed Her2-Neu expression.

Out of these; twenty-six (52%) had grade-II (Moderately differentiated) malignancy, sixteen (32%) had grade-I (well differentiated), and eight (16%) had grade-III (poorly differentiated). Tumor grades were significantly correlated with overexpression of the Her2-Neu protein [28]. Regarding the morphological types of adenocarcinomas positive for Her2-Neu, they found 16 (48%) cases of adenocarcinoma (not otherwise specified) positive for Her2-Neu overexpression, among which 13 (81.25%) were from group C and 3 (18%) were from group A. Five(15%) cases positive for Her2-Neu overexpression were mucinous adenocarcinoma, out of which 4 (80%) were from group C and 1 (20%) was from group A. Five (15%) cases of positive Her2-Neu overexpression were of the signet ring variant of adenocarcinoma; 2 (40%) from group C and 3 (60%) from group A [28]. Similar observations were seen in a study by Kasochi et al, where compared to diffuse or mixed sub-types, intestinal type moderately differentiated tumors had a higher incidence of Her2-Neu overexpression [29].

Since there is a dearth of information in India about the survival and prognosis of patients with gastric cancer who express Her2-Neu, these findings may have practical implications for assessing the tumor prognosis in terms of the likelihood of relapse. The overall survival and disease-free survival for the patients with tumors expressing positive Her2-Neu expression were not examined which could be done in future studies. We also did not analyze the correlation of chemotherapy with the Her2-Neu expression. A further study with bigger sample size and longer follow up is needed and should be conducted to validate the finding of this study.

Conclusion

Our study findings concurred with a wide range of national and international literature that is available in the field. However, given the small number of patients in a tertiary care setting, care should be taken when interpreting the data. Her2-Neu overexpression was found to be statistically significantly correlated with middle age group and moderately and poorly differentiated gastric and intestinal tumors; they may be the candidates for Herceptin-based targeted treatment. To investigate the function of Her2-Neu as a stand-alone prognostic factor, more research with ample sample size is required. It is also necessary to investigate gastric and intestinal tumors that do not express Her2-Neu in order to validate any regional variations that may occur.

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