ORIGINAL ARTICLE

TJG
The Turkish Journal of Gastroenterology

Investigation of ErbB-2 Overexpression on Patients with Gastric Cancer in Eastern Anatolia of Turkey

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Background/aims: *In the present study, the frequency of ErbB-2 overexpression and its relationship with pathologic parameters on patients with gastric cancer in Eastern Anatolia of Turkey was studied.* ***Materials and Methods:*** *A total of 32 newly diagnosed patients were enrolled in the study. DNA isolation was performed on paraffinized tumor tissues obtained from patients by endoscopy or surgical resection. ErbB-2 overexpression was investigated from the isolated DNA by "Real Time Polymerase Chain Reaction".* ***Results:*** *ErbB-2 positivity was detected in five (15.6%) of 32 gastric cancer patients. The correlation between distant metastases and ErbB-2 positivity was found to be statistically significant (p=0.04). Additionally, no statistically significant correlation was noted between ErbB-2 positivity and parameters such as level of differentiation (p=0.7), the depth of tumor invasion (p=0.08), lymph node metastases (p=0.6), Lauren's classification (p=0.4), World Health Organization classification (p=0.3), tumor, node, metastasis staging (p=0.3) and tumor localization (p=0.2). Lymph node involvement was present in all ErbB-2 positive patients, the depth of tumor invasion was T3 (one case) and T4 (four cases) with the cardia being the most common location, which was remarkable, though not statistically significant (p>0.05). All ErbB-2 positive patients were detected with intestinal-type gastric cancer according to Lauren's classification and with the tubular-type according to World Health Organization classification.* ***Conclusion:*** *According to our findings, given the rates of ErbB-2 overexpression (15.6%) in gastric cancer, the investigation of ErbB-2 overexpression as an important biomarker in humanized monoclonal-antibody treatment in patients with gastric cancer was considered appropriate.*

Key words: Gastric cancer, ErbB-2, pathologic parameters, Turkey

Doğu Anadolu bölgesindeki mide kanserli hastalarda ErbB-2 overekspresyonunun değerlendirilmesi

Giriş ve Amaç: *Bu çalışmada, Türkiye'nin Doğu Anadolu Bölgesindeki mide kanserli hastalarda ErbB-2 overekspresyonunun sıklığı ve patolojik parametrelerle ilişkisini araştırdık.* ***Gereç ve Yöntem:*** *Çalışmaya, yeni tanı almış toplam 32 hasta alındı. Hastaların endoskopi veya cerrahi rezeksiyon ile alınan parafinize edilmiş tümör dokularından DNA izolasyonu yapıldı. İzole edilen DNA'lardan, "Real Time Polimeraz Zincir Reaksiyonu" ile ErbB-2 overekspresyonu araştırıldı.* ***Bulgular:*** *Otuziki mide kanserli vakanın beşinde (%15.6) ErbB-2 pozitifliği saptandı. Hastalardaki uzak metastaz ile ErbB-2 pozitifliği arasındaki ilişki istatistiksel olarak anlamlı bulundu (p=0.04). Vakaların, diferansiyasyon derecesi (p=0.7), tümör invazyon derinliği (p=0.08), lenf nodu metastazı (p=0.6), Lauren sınıflaması (p=0.4), Dünya Sağlık Örgütü sınıflaması (p=0.3), tümör, nod, metastaz evrelemesi (p=0.3) ve tümörün lokalizasyonu (p=0.2) ile ErbB-2 pozitifliği arasındaki ilişki istatistiksel olarak anlamlı değildi. İstatistiksel olarak anlamlı olmamasına rağmen(p>0.05), ErbB-2 pozitif olan vakaların hepsinde lenf nodu tutulumunun olması, tümör invazyon derinliğine göre T3 (bir vaka) ve T4 (dört vaka) olması ve lokalizasyon olarak kardiada daha fazla görülmesi dikkat çeken bulgulardı. ErbB-2 pozitif olan vakaların hepsi Lauren'a göre intestinal tip, Dünya Sağlık Örgütüne göre tübüler tipte görüldü.* ***Sonuç:*** *Sonuç olarak, mide kanserinde ErbB-2 overekspresyonu oranlarına (%15.6) bakıldığında, mide kanseri tanısı konulan hastalarda humanize monoklonal antikor tedavisi için önemli bir biomarker olan ErbB-2 overekspresyonuna bakılmasının uygun olacağı düşünüldü.*

Anahtar kelimeler: Mide Kanseri, ErbB–2, patolojik parametreler, Türkiye

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Manuscript received: 12.03.2012 **Accepted:** 02.10.2012

Turk J Gastroenterol 2013; 24 (3): 211-217
doi: 10.4318/tjg.2013.0641

INTRODUCTION

Growth factors and growth factor receptors, particularly with tyrosine kinase activity, enhance the flow of signal transmission that plays a role in the growth control and regulation of various genes related to growth and proliferation. The ErbB gene family consists of four homologous gene families: ErbB-1 (EGFR, HER1), ErbB-2 (HER2/Neu, c-erbB-2), ErbB-3 (HER3) and ErbB-4 (HER4). Overexpression of the ErbB receptor has both prognostic and therapeutic significance. Since good clinical outcomes have been achieved with humanized antibody (trastuzumab) developed against ErbB-2 in breast cancer, the investigation of HER2/Neu expression and use of HER2/Neu antibody in the treatment of other solid tumors has become a current issue (1, 2).

ErbB-2 overexpression in gastric cancer was first demonstrated by immunohistochemical (IHC) staining in 1986 (3). Subsequently, a number of studies have been conducted on ErbB-2 gene amplification and/or overexpression in gastric carcinoma. ErbB-2 expression has been reported at rates of 8% to 28% in various studies (4-11). ErbB-2 gene amplification in gastric carcinoma is associated with an intestinal-type histology and poor survival (12,13). A case report and cell line and xenograft model studies have shown that trastuzumab has a growth inhibitory effect in ErbB-2 amplified and/or overexpressed gastric carcinomas (2). Positive results of a large-scale phase III trial on the efficacy of trastuzumab have been reported in patients with gastric carcinoma with ErbB-2 expression (14). Therefore, the evaluation of ErbB-2 gene amplification status in patients with gastric carcinoma has become important in determining the eligibility for trastuzumab therapy.

ErbB-2 status in tumor samples can be determined using various methods. Immunohistochemistry (IHC), Western blotting, and enzyme-linked immunosorbent assay are currently used to measure HER-2 protein expression; and HER-2 gene amplification can be detected by Southern blotting, fluorescence in situ hybridization (FISH), chromogenic in situ hybridization, and real-time quantitative polymerase chain reaction (RT-PCR). In practice, IHC and FISH methods are more often used. IHC is faster and more economical, but is highly dependent on quality control and is difficult to standardize. FISH is regarded as the standard in evaluating the presence of ErbB–2 gene amplification. However, it is expensive in terms of equipment and is labor intensive. Furthermore, FISH is not valuable when tissue samples are not well fixed. To overcome these limitations, alternative methods such as real-time RT-PCR are used experimentally in patients withgastric carcinoma; and the reported results have shown relatively good concordance rates (2).

In the present study, we aimed to investigate the frequency of ErbB-2 overexpression and its relationship to pathologic parameters in gastric cancer, which is commonly seen around Eastern Anatolia in Turkey.

MATERIAL and METHODS

This study was conducted according to the ethical principles of the Declaration of Helsinki. Approval for the study was obtained from the Atatürk University Medical School ethics committee. All patients provided written informed consent upon enrollment.

Thirty-two patients who were diagnosed with gastric cancer in the departments of General Surgery and Internal Medicine at Atatürk University Medical School were enrolled in our study. All patients were newly diagnosed and treatment-naive.

Primarily, pathology samples of patients with gastric cancer were investigated to determine appropriate paraffin blocks. For the isolation of DNA, paraffin blocks were used in samples containing more than 30% tumor content. According to the recommendations of our medical biology laboratory and the guidance of Dr. Zeydanli DNA Isolation Kit From Paraffin-embedded Tissue, paraffinized tumor tissues were cut into sections of 5-7 µm and deparaffinized. DNA extraction was performed on deparaffinized samples with the DNA isolation kit [Dr. Zeydanli DNA Isolation Kit from Paraffin-embedded Tissue, Turkey (DZDNA)]. Tumor histology, level of differentiation, depth of tumor invasion and lymph node status were determined. The presence of distant metastases, tumor, node, metastasis (TNM) stage and localization of the gastric cancer were obtained from patients' records.

Quantitative measurement of the ErbB-2 oncogene from DNA extracts of tumor tissues was performed by using Light Cyler 2.0 (LC). The RT-PCR device was provided by Roche Diagnostic systems. Before processing, LC RT-PCR device calibration was checked with the kit that includes Calibrator DNA as a positive and negative control template

provided by Roche Diagnostic systems (Roche, Mannheim, Germany). LC HER2/neu DNA Quantification Kit was used for the quantitative measurement of the ErbB-2 oncogene. Results of samples analyzed by the LC RT-PCR program were transferred to "Quantification Software".

The ratio of targeted HER2/Neu gene copy number to the reference gene copy number was calculated for each sample. If the ratio was <2, HER2/Neu oncogene amplification was considered to be negative, whereas the ratio was ≥2, HER2/Neu oncogene amplification was defined as positive.

Statistical analysis

The data were expressed as number, percentage, mean and standard deviation, and data analysis was performed using a computer program. Analysis of categorical variables was performed by Chi-square test. A value of $p < 0.05$ was regarded as statistically significant.

RESULTS

The patients' characteristics

Patient characteristics are shown in Table 1. The mean age of the patients was found to be 58.4±12.38 (32-84) years. Seventeen patients (53.1%) were male and 15 patients (46.9%) female. The histopathologic diagnosis of 32 (100%) patients was adenocarcinoma.

Distant metastases was detected (liver, adrenal gland, periton, ovary, pancreas) in 11 (34.3%) patients.

The tumors were more localized in the cardia (40.6%) and antrum (34.3%), and were more poorly-differentiated (65.7%) and moderately-differentiated (28.1%). Regarding lymph node involvement: 8 patients (25%) were detected to be N0 (no lymph node metastases), 10 (31.3%) N2 (the number of metastatic lymph nodes is between 7-15) and 9 (28.1%) N3 (the number of metastatic lymph nodes is higher than 15).

The quantitative results of ErbB-2 oncogene and distribution measured by RT-PCR

The ratio of ErbB-2 oncogene measured by RT-PCR to the reference gene ranged between 0.21 and 15.6. The mean value was 2.03±3.01. ErbB-2 amplification was found to be positive in five (15.6%) of the cases and negative in 27 (84.4%).

The relationship between ErbB-2 oncogene and prognostic factors

Table 2 shows the relationship between pathological characteristics of the patients and ErbB-2 amplification. The relationship between distant metastases and ErbB-2 positivity was found to be statistically significant ($p=0.04$). Distant metastases were present in four ErbB-2 positive patients, whereas no distant metastases were observed in one patient.

The relationship between ErbB-2 positivity and parameters such as the level of differentiation ($p=0.7$), the depth of tumor invasion ($p=0.08$), lymph node involvement ($p=0.6$), Lauren classification ($p=0.4$), World Health Organization (WHO) classification ($p=0.3$), TNM staging ($p=0.3$) and location of the tumor ($p=0.2$) were not found to be statistically significant. On the other hand, lymph node involvement was present in all ErbB-2 positive patients. The tumors were T3 (one case) and T4 (four cases) and the cardia was a more common location, though not statistically significant ($p>0.05$).

DISCUSSION

Since good clinical outcomes were achieved by anti HER2/Neu antibody in breast cancer, the investigation of HER2/Neu expression in other solid tumors has become a current issue. In numerous studies so far, ErbB-2 overexpression was detected in varying rates in some tumors (i.e; breast, colon, bladder, ovarian, endometrial, lung, uterine cervix, head-neck and gastrointestinal system tumors) (15-25).

ErbB-2 overexpression in gastric cancer was first demonstrated by IHC staining in 1986 (3). ErbB-2 overexpression in gastric cancers exhibits some similarities to those in breast cancer (26). ErbB-2 expression has been reported at rates of 8% to 28% in various studies (4-11). ErbB-2 positivity was found to be 22.1% in the ToGA study with 3807 gastric cancer patients in which 130 centers from various countries participated (14). In our study, ErbB-2 overexpression was found in 15.6% of 32 gastric cancer patients. The rate of ErbB-2 overexpression in our study is generally consistent with other studies.

The results of studies that investigate the relationship between ErbB-2 overexpression and prognostic factors (the depth of tumor invasion, lymph node metastases, histological type, distant metastases, serosal invasion, and growth pattern) vary

Table 1. The patients' histopathological and clinical characteristics

Case	Loc. Diff.	Degree of Class.	Lauren Class.	WHO Invasion	Tumor Node	Lymph	Met.	Stage Expression	ErbB-2
1. Case	Cor.	Poorly	İntestinal	Musinos	Serosa	Yes	No	4	Negative
2. Case	Ant.	Poorly	Intestinal	Musinos	Serosa	Yes	No	3B	Negative
3. Case	Cor+Car	Poorly	Intestinal	Tubuler	Serosa	Yes	No	3B	Negative
4. Case	Ant.	Poorly	Diffuse	Musinos	Serosa	No	No	2	Negative
5. Case	Car.	Mod.	Intestinal	Musinos	Serosa	Yes	No	4	Negative
6. Case	Car.	Mod.	Intestinal	Tubuler	Serosa	Yes	SB+C	3B	Negative
7. Case	Ant.	Poorly	Diffuse	Tubuler	Serosa	Yes	No	4	Negative
8. Case	Ant.	Well	Diffuse	Tubuler	Submu.	No	No	1A	Negative
9. Case	Ant.	Poorly	Intestinal	Tubuler	Serosa	Yes	L	3A	Negative
10. Case	Cor.+Car	Poorly	Intestinal	Musinos	Serosa	Yes	P+O	4	Positive
11. Case	Ant.	Mod.	Intestinal	Tubuler	Serosa	Yes	No	4	Negative
12. Case	Ant.	Mod.	Intestinal	Tubuler	Submu.	No	No	1A	Negative
13. Case	Ant.	Mod.	Mixed	Tubuler	Serosa	Yes	L	3A	Negative
14. Case	Cor.	Poorly	Intestinal	Tubuler	Serosa	Yes	No	4	Negative
15. Case	Car.	Well	Intestinal	Tubuler	Muskuler	No	No	1B	Negative
16. Case	Ant.	Poorly	Intestinal	Tubuler	Serosa	Yes	No	4	Negative
17.Case	Car.	Poorly	Intestinal	Tubuler	Serosa	Yes	No	2	Negative
18.Case	Cor.	Poorly	Intestinal	Tubuler	Serosa	Yes	No	3B	Negative
19. Case	Car.	Poorly	Diffuse	Tubuler	Serosa	Yes	P	3B	Negative
20. Case	Car.	Poorly	Intestinal	Tubuler	Serosa	Yes	S	4	Positive
21. Case	Car.	Mod.	Intestinal	Tubuler	Serosa	Yes	P	4	Negative
22. Case	Car.	Poorly	Diffuse	Tubuler	Serosa	Yes	No	4	Negative
23. Case	Car.	Poorly	Intestinal	Tubuler	Serosa	Yes	L+O	2	Negative
24. Case	Cor.+Ant	Poorly	Intestinal	Tubuler	Serosa	Yes	L	4	Positive
25. Case	Cor.	Poorly	Intestinal	Tubuler	Serosa	No	No	4	Negative
26. Case	Car.	Mod.	Intestinal	Tubuler	Serosa	Yes	No	3A	Positive
27. Case	Cor.	Poorly	Intestinal	Musinos	Serosa	No	No	3A	Negative
28. Case	Car.	Poorly	Mixed	Musinos	Serosa	No	No	2	Negative
29. Case	Ant.	Mod.	Intestinal	Tubuler	Serosa	Yes	No	3A	Negative
30. Case	Ant.	Mod.	Diffuse	Musinos	Serosa	Yes	Pan.	3B	Negative
31. Case	Car.	Poorly	Intestinal	Tubuler	Serosa	Yes	P	4	Positive
32. Case	Car.	Poorly	Intestinal	Tubuler	Serosa	Yes	No	4	Negative

Loc: Localization, Cor: Corpus, Car: Cardia, Ant: Antrum, Dif: Differentiation, Met: Metastasis, P: Periton, Pan: Pancreas, L:Liver, S: Surrenal, O: Over, SB: İncebarsak C:Colon, Mod: Moderately, Class: Classification

widely. ErbB-2 overexpression was found to be statistically significantly higher in gastric cancers which exhibit one of the following features: Well-differentiated, advanced stage, papillary-type, presence of liver metastases, serosal invasion or lymph node involvement (6, 26, 27). Sausa et al. (7) demonstrated that there was an association between ErbB-2 overexpression and tumor location and venous invasion. Jain et al. (28) also reported that there was a relationship between ErbB-2 positivity and intestinal type, tubular type and expansive-spread pattern. However, some studies reported that no significant relationship was detected between ErbB-2 overexpression and the depth of tumor invasion, lymph node involvement, venous invasion, the localization of the tumor and concluded that ErbB-2 was not a good predictor for prognosis (5, 12, 29, 30).

In the present study, the relationship between ErbB-2 positivity and distant metastases was found to be statistically significant (p=0.04). Distant metastases were present in four ErbB-2 positive patients, whereas no distant metastases were observed in one patient. The relationships between ErbB-2 positivity and depth of tumor invasion (p=0.08), lymph node involvement (p=0.6) and tumor location (p=0.2) were not found to be statistically significant in our study. Lymph node involvement was present in all ErbB-2 positive patients and the depths of tumors invasion were T3 (one

Table 2. The relationship between ErbB-2 positivity and Pathological parameters in patients.

Pathologic parameters Tumor Tissue	Groups	n	%	ErbB-2 Positive Tumor Tissue n	*p* value
Differantiation grade	Poorly	21	65.7	4	0.7
	Moderately	9	28.1	1	
	Well	2	6.2	0	
Depth of tumor invasion	T1	2	6.2	0	0.08
	T2	1	3.1	0	
	T3	19	59.4	1	
	T4	10	31.3	4	
Lymph node involvement	No	7	21.9	0	0.6
	Yes	25	78.1	5	
Lauren classification	Diffuse	6	18.8	0	0.4
	Intestinal	24	75	5	
	Mixt	2	6.2	0	
WHO	Mucinous	8	25.0	0	0.3
Classification	Tubular	24	75.0	5	
Distant metastases	Yes	11	34.3	4	0.04
	No	21	65.7	1	
TNM	Grade 1	3	9.4	0	0.3
Staging	Grade 2	4	12.4	0	
	Grade 3	11	34.4	1	
	Grade 4	14	43.8	4	
Tumor Localization	Antrum	11	34.4	0	0.2
	Cardia	13	40.6	3	
	Corpus	8	25.0	2	

case) and T4 (four cases), which were remarkable, though not statistically significant (p>0.05). The findings indicating that the tumor was localized to the cardia in three ErbB-2 positive patients, in the corpus/cardia in one patient and in the corpus/antrum in one case were also remarkable.

A vast majority of studies on gastric cancer patients have reported that ErbB-2 overexpression was more common in the intestinal-type according to Lauren's classification and in the tubular or tubulopapillary type according to WHO (6,7,10,13). Flejou et al. (30) demonstrated that all patients with ErbB-2 overexpression had gastric cancer of the intestinal-type according to Lauren's classification. In a study of 131 gastric cancer patients (62 cases with intestinal-type, 46 cases with diffuse-type, 20 cases with mixed-type) by Tanner et al. (13) ErbB-2 positivity was detected in 14 (21.5%) patients with intestinal-type tumors, in one patient (2.2%) with a diffuse-type tumor and in two patients (5.3%) with a mixed-type tumor. In a study of 248 gastric cancer patients (intestinal-type, 96 cases; diffuse-type, 127 cases; mixed type, 25 cases) by Kim et al. (2) ErbB-2 positivity was detected in 16 patients (16.6%) with the intestinal-type tumor, in one patient with (0.8%) the diffuse-type tumor and in two patients (8%) with a mixed-type tumor. In the ToGA study in which the efficacy of trastuzumab was investigated for the treatment of gastric cancer, the histopathology of the ErbB-2 positive patients was intestinal-type in 32.2%, mixed-type in 20.4%, and diffuse-type in 6.1%. The relationship between the histological type and ErbB-2 expression reached statistical significance (14).

All ErbB-2 positive patients were detected to have intestinal-type histology according to Lauren's classification, and tubular-type histology according to the WHO classification in our study. Although our results are consistent with the literature, no statistically significant relationship was observed between the histological sub-type (Lauren's and WHO classification) and ErbB-2 positivity (p>0.05).

Several studies on gastric cancer have indicated that ErbB-2 overexpression is more common in

well-differentiated adenocarcinomas compared to poorly-differentiated adenocarcinomas (29, 31). In a study on gastric cancer patients, Kimura et al. (12) found that there was a relationship between ErbB-2 overexpression and histological differentiation, and concluded that ErbB-2 overexpression was more common in patients with well-differentiated gastric adenocarcinomas. Allgayer et al. (32) reported that ErbB-2 overexpression was more common in patients with well-differentiated adenocarcinomas. However, the difference did not reach statistically significance.

In our study, ErbB-2 overexpression was observed to be more common in patients with poorly-differentiated adenocarcinoma. Of five cases in whom overexpression were detected, four were poorly-differentiated and one was moderately differentiated. However, no statistical difference was detected between ErbB-2 overexpression and the level of histological differentiation ($p>0.05$).

IHC staining is frequently used for the detection of ErbB-2 overexpression. Since conflicting results were obtained in studies performed with IHC staining, the use of FISH and RT-PCR methods was utilized to demonstrate ErbB-2 gene amplification. In a study of 375 patients with gastric adenocarcinoma by Ishikawa et al. (33) ErbB-2 overexpression was found to be 10.4% by IHC staining, and 5% by FISH. Kimura et al. (12) found a high correlation between FISH and IHC staining results in the same patient group with 83% sensitivity and 98% specifity. It has been suggested that FISH can be used for characterization of gastric carcinoma in ErbB-2 positive patients and IHC staining, and FISH or both can be used to determine suitable candidates for targeted antibody therapies. Rosai et al. (34) recommended that the evaluation be commenced with IHC staining and to finish the procedure if the staining score was 3+ or 0, while gene amplification by FISH was required when the score was 1+ or 2+. Kim et al. (2) investigated ErbB-2 overexpression in gastric cancer patients by using IHC, FISH and RT-PCR techniques. When the methods were compared, ErbB-2 overexpression was found to be 22% by IHC and 7.6% by FISH. The correlation between IHC staining and FISH results was found to be statistically significant. When the same cases were investigated by RT-PCR, the quantitative ErbB-2 positivity was found to be higher in cases in which overexpression was detected by FISH when compared to that in other cases. A high correlation was found between FISH and RT-PCR results with 100% sensitivity and 96.9% specificity.

In our study, ErbB-2 overexpression was investigated by RT-PCR without performing IHC staining or FISH. The positive rate of ErbB–2 gene expression was 15.6% in the tumor tissue of our patients. The results were consistent with those performed with RT-PCR.

In conclusion, given the rate of ErbB-2 overexpression in gastric cancer, it is appropriate to investigate ErbB-2 overexpression in gastric cancer patients as administration of monoclonal antibody treatment (trastuzumab) would be effective in ErbB-2 positive cases. Since it is considered that such a treatment would be an eligible option for at least 15-20% of patients, trastuzumab is believed to bring a new perspective to current neoadjuvant chemotherapy. This treatment may also increase the success of the surgical treatment and provide a complete recovery for many patients.

Acknowledgements: *This work was supported by the Ataturk University Scientific Research Projects Department (BAP-2007/88).*

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