

C-erb-B2 (Her2/neu) Expression Rate And its Association with Clinicopathologic Parameters in Gastric Cancer

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ABSTRACT

We aimed to investigate the frequency of c-erb-B2 expression rate and its association with clinicopathological parameters in our gastric carcinoma series to make a contribution to emerging data on this oncogene. One hundred thirty-nine gastric adenocarcinoma patients underwent total gastrectomy between years 2006 and 2011 were selected to the study. C-erb-B2 expressions were determined by using immunohistochemical and/or chromogenic in situ hybridisation methods in tumor tissues. A total of 16 (11.5%) gastric adenocarcinoma cases were found positive for c-erb-B2 expression. Out of 16 positive cases, 13 had intestinal type adenocarcinomas. C-erb-B2 overexpression was significantly associated with histologic subtype ($p=0.001$), and histologic tumor grade ($p=0.001$). The frequency of overexpression in intestinal subtype (21.7%) was comparable with the known rates of overexpression in breast cancer. This results indicate that C-erb-B2 expression may identify a subgroup in gastric carcinoma cases which may benefit from targeted treatment modalities among Turkish population.

Keywords: C-erb-B2, Gastric adenocarcinoma, Immunohistochemistry, Trastuzumab

ÖZET

Mide Kanseri C-Erb-B2 (Her2/Neu) Ekspresyon Sıklığı ve Klinikopatolojik Parametrelerle İlişkisi

Bu çalışma, mide kanseri nedeni ile gastrektomi uygulanmış hasta serimizde C-erb-B2 ekspresyonunun sıklığını belirlemek ve C-erb-B2 ekspresyonu ile klinikopatolojik parametreler arasındaki ilişkiyi araştırmak üzere gerçekleştirilmiştir. Çalışma 2006-2011 yılları arasında opere edilen 139 hastaya ait gastrektomi materyalinde yapılmıştır. Tümör dokularında C-erb-B2 ekspresyonlarının saptanmasında primer antikorlar ile ilgili prosedüre göre immunohistokimya ve/veya kromojenik in-situ hibridizasyon yöntemleri kullanılmıştır. Olguların 16 tanesinde (%11.5) C-erb-B2 ekspresyonu saptanmıştır. C-erb-B2 ekspresyonu görülen 16 tümörün 13 tanesi intestinal tip adenokanser olup, C-erb-B2 ekspresyonu ile tümörün histolojik tipi arasında istatistiksel olarak anlamlı bir ilişki izlenmiştir ($p=0.001$). Ayrıca C-erb-B2 pozitifliği ile histolojik grade arasında da kuvvetli bir ilişki saptanmıştır ($p=0.001$). İntestinal subtip mide kanserlerindeki saptanan pozitiflik oranı (%21.7), meme kanserlerinde görülen sıklığa benzer düzeydedir. Bu sonuçlar, mide kanseri olgularında c-erb-B2 ekspresyonunun, hedefe yönelik tedavi yaklaşımlarından fayda görebilecek bir hasta grubunu ayırt etmede kullanılabileceğini düşündürmektedir.

Anahtar Kelimeler: C-erb-B2, Gastrik adenokarsinom, İmmunohistokimya, Trastuzumab

INTRODUCTION

The human epidermal growth receptor 2 (Her2/neu) gene is located on chromosome 17 (q21) and codes a transmembrane receptor protein which is also known as C-erb-B2. Her2/neu is a member of the human epidermal growth factor receptor (EGFR) gene family and it is accepted to be involved in the regulation of cell growth, proliferation and survival.^{1,2} Overexpression of C-erb-B2 occurs in 10%-34% of invasive breast cancer patients and it is associated with aggressive behaviour, resistance to treatment with chemotherapeutics, and poor response to endocrine treatment. C-erb-B2 overexpression has also been observed in various types of cancers including colon, bladder, ovary, endometrium, lung, uterine cervix, head & neck, esophageus, and stomach carcinomas.^{2,3,4} Immunohistochemical (IHC) staining or DNA hybridisation methods such as fluorescence in situ hybridisation (FISH) and chromogenic in situ hybridisation (CISH), are commonly used to evaluate the C-erb-B2 overexpression/amplification in different tumor types. Among these methods, immunohistochemistry has advantages with shortest turn around times, being a cost effective method, and the relative simplicity in assessment of Her-2/neu expression. In IHC method, a semiquantitative scoring system, ranges from (+) to (+++) staining is widely accepted to evaluate Her-2/neu expression.

Recently, trastuzumab, a monoclonal antibody against the C-erb-B2 receptor, has been demonstrated to prolong survival of the patients with breast cancer or advanced gastric cancer, by down regulating the C-erb-B2 receptor function.^{4,5} Since, patients with tumors that have C-erb-B2 overexpression benefit from trastuzumab therapy, it is essential to determine the C-erb-B2 status of tumors to use trastuzumab as an additional agent in treatment of related cancers.

Gastric carcinoma is one of the most common cause of cancer related deaths worldwide. It arises from the glandular tissue of the stomach lining. In its therapeutic management, a combined multidisciplinary approach such as surgery, chemotherapy and radiotherapy is required. After addition of trastuzumab to standard chemotherapy is reported to improve patient survival, C-erb-B2 expression status has been widely studied in gastric cancer.⁶ It is

implicated that the ratio of C-erb-B2 overexpression may also differ between the subtypes of gastric cancer.³ In the present study we aimed to investigate the frequency of C-erb-B2 expression in gastric carcinoma cases and to determine the relationship of overexpression with known prognostic parameters among Turkish population to make a contribution to the emerging data.

MATERIALS AND METHODS

One hundred and thirty-nine gastric adenocarcinoma patients that underwent total gastrectomy between years 2006 and 2011 were identified from the pathology laboratory archive comprising the specimens obtained from several hospitals; Medical Park Hospital, Anadolu Hospital, Medice Hospital, Medicabil Hospital to a private pathology laboratory (Patomer Pathology Laboratory) in Bursa, Turkey. Each patient gave written informed consent before the surgery and the study was conducted in accordance with Declaration of Helsinki and approved according to Turkish Ethical regulation. Routinely fixed (overnight in 10% buffered formalin) paraffin-embedded tumor samples were used in the study. H&E stained slides and the respective paraffin blocks were retrieved from the archive and were histologically revised. Subtyping of the tumors were made according to the World Health Organization (WHO) criterias. For immunostaining, sections were cut in 3 micrometer thickness. IHC staining was conducted using anti-C-erb-B2 antibodies (Biocare® Medical Cat. No: CME 342 AB, Clone EP1045Y 1/100 concentrated rabbit monoclonal, 4040 Pke Lane Concord, CA 94520 USA) according to manufacturer instructions.

To evaluate the expressions, a previously reported semiquantitative scoring system was carried out and specific membranous staining was taken under consideration.⁷ The slides were examined and scored by two experienced pathologists independently to avoid subjective bias. Evaluation of the results was performed by assigning a score of 0 to 3+ (Table 1). Representative samples of each staining score are demonstrated in Figures 1-4. Cases were divided into two groups according to immunostaining pattern:

Tumors with a score of 0 or 1+ were regarded as being C-erb-B2-negative (Group I), while score of 3+

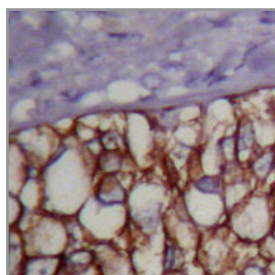


Figure 1. Membranous Her2/Neu expression on breast carcinoma used for positive control; Score 3 (x200 DAB Chromogen).

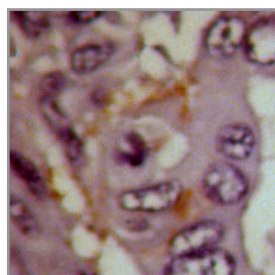


Figure 2. Focal weak Her2/Neu expression in gastric carcinoma; Score 1. (x400 DAB Chromogen).

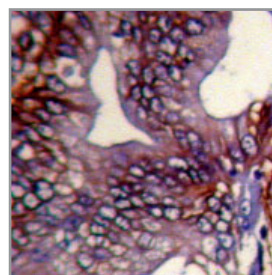


Figure 3. Focal strong membranous expression in gastric carcinoma. Staining in more than 10% of tumor cells ; score 2. (x200 DAB Chromogen).

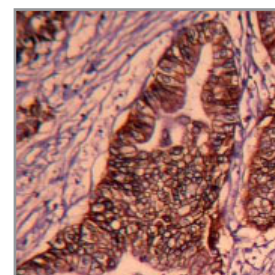


Figure 4. Strong membranous expression in gastric carcinoma. Staining in more than 50 % of tumor cells; score 3 (x100 DAB Chromogen).

indicated positive expression of C-erb-B2 (Group II). For cases with 2+ C-erb-B2 expression, CISH assays were performed to determine the final score.

Standard 5 μ m thick tumor sections were treated according to CISH kit procedure (ZytoDot® 2C amplification and visualization kit and SPEC HER2 \ CEN17 Probe kit, ZytoVision® GmbH, Bremerhaven, Germany). Ratio of green dots, corresponding to Her2 gene, to red dots, representing the centromeric region of chromosome 17, were calculated after evaluating at least 100 tumor cells per tumor. Scores higher than 2 were accepted as amplification of Her2 gene. Tumors with other scores were considered as not amplified. According to CISH results, tumors with Her2 amplification were included in group II (positive cases), whereas, tumors that showing no amplification of Her2 gene were included in group I (negative cases).

Statistical Analysis: Continuous variables were given as mean \pm standard deviation; categorical variables were defined as percentages. Continuous variables were compared by Student t-test and the χ^2 tests were used for analysing the differences in categorical variables between two groups. All p values were two-tailed, and statistical significance was defined as $p < 0.05$. The SPSS statistical software (SPSS 15.0 for windows, Inc., Chicago, IL, USA) was used for all statistical calculations.

RESULTS

Mean patient age was 59.1 ± 14 years old (range 27 - 89 years). Out of 139 cases, 79 (56.8%) were diagnosed with diffuse type and a subset of 60 (43.2%) patients were diagnosed with intestinal type. In immunohistochemical examination, 113 (81.3%) of 139 cases were negative for C-erb-B2

Table 1. Classification and Groups of cases according to c-erb-B2 expression status after immunohistochemistry and CISH assays.

Score	Pattern	Classification	N	Group
0	No staining or membrane staining in <10% of the tumor cells;	NEGATIVE	113	Group I
1+	Faint/barely perceptible membrane staining in >10% of the tumor cells, cells stained in part of the membrane;	NEGATIVE	8	
2+	Weak to moderate staining of the entire or basolateral membrane staining in >10% of the tumor cells;	EQUIVOCAL CISH (-)	2	Group II
2+	Weak to moderate staining of the entire or basolateral membrane staining in >10% of the tumor cells;	EQUIVOCAL CISH (+)	4	
3+	Moderate to strong staining of the entire or basolateral membrane staining membrane in >10% of the tumor cells.	POSITIVE	12	

Table 2. Distributions of patient demographics and pathological characteristics of tumors with respect to c-erb-B2 expression, and comparison with other studies.

			Group2, Her2/neu positive n=16		Group1, Her2/neu negative n=123		Our study	Tanner (3)	Kim (10)	Xie (19)
			n	%	n	%	p value	p value	p value	p value
Age			16	11,5	123	88,5				
			55±13	60±14	0.173	0,17	NS*	0,019		
Sex	Male	82	7	8.5	75	91.5	0.188	0,43	NS*	0,131
	Female	57	9	15.8	48	84.2				
Histologi- cal type	Intestinal	60	13	21.6	47	78.4	0.001	0,0051	0,001	–
	Diffuse	75	3	4	72	96				
	Mixed	4	0	0	4	100				
Histologi- cal grade	Grade 1	4	0	0	4	100	0.001	–	0,001	0,476
	Grade 2	56	13	23.2	43	76.8				
	Grade 3	79	3	3.7	76	96.3				
Lymph Node Status	pN0	23	2	8.7	21	91.3	0.113	–	–	0,472
	pN1	43	2	4.6	41	95.4				
	pN2	43	9	21	34	79				
	pN3	30	3	10	27	90				
Staging	pT1	4	1	25	3	75	0.754	0,42	NS*	–
	pT2	16	2	12.5	14	87.5				
	pT3	92	11	12	81	88				
	pT4	27	2	7.4	25	92.6				
* NS: Not significant										

expression (score 0), and 8 (5.8%) cases showed incomplet membraneous staining in less than 10% of tumor cells and they were scored as 1 (+). A total of 121 cases, consisted of 8 tumors with 1 (+) staining and 113 unstained tumors were included to group I. Twelve (8.6%) cases revealed strong complete membraneous staining in more than 10% of tumor cells. These 12 cases were included in group II. In the rest 6 (4.3%) cases, there was weak to moderate staining of the entire or basolateral membrane staining in >10% of the tumor cells. These 6 cases were scored as 2 (+) immunohistochemically. In CISH assay, 4 out of 6 cases showed amplification whereas 2 of them did not. Those cases showing amplification in CISH assay were included in gro-

up I, while 2 of 6 cases showing no amplification were included in group II. After CISH results, the number of C-erb-B2 negative (group I) cases were 123 (88.5%) and the number of C-erb-B2 positive (group II) cases were 16 (11.5%).

Patient demographics and pathological tumor characteristics with respec to C-erb-B2 expression results in our study group are summarised in Table 2. Thirteen out of 16 (81.3%) tumors in group II were intestinal type gastric adenocarcinomas, whereas 3 tumors (18.7%) were of diffuse type adenocarcinomas. Of 60 intestinal type adenocarciomas, 13 (21.6%) cases showed Her2/neu expression, whereas only 3 (4.0%) of 75 diffuse gastric carcinomas were found positive. Intestinal type tumors showed

approximately five times more positivity than diffuse type did. The difference of C-erb-B2 overexpression rates between intestinal and diffuse type carcinomas was statistically significant ($p=0.001$). When compared to histological grade I or III cancers, the proportion of C-erb-B2 overexpressing tumors were also significantly higher in histological grade II tumors ($p=0.001$). The association between C-erb-B2 overexpression and lymph node involvement was not statistically significant. Also we did not observe an association between pathological tumor stage and C-erb-B2 overexpression. Distributions of mean patient age were similar for all groups, and C-erb-B2 overexpression did not differ between both gender groups.

DISCUSSION

Although the prevalence of gastric adenocarcinoma has decreased in the last decade, it is still the second most common cause of cancer death after lung cancer worldwide.⁸ Despite advances in management of gastric carcinoma, the outcome is poor, especially for patients with advanced stage disease. Therefore, targeted therapies have been introduced to improve the response rates. Her2/neu gene is one of the potential targets and attracted the researchers because its amplification is common in various solid tumors.⁹ The demonstration of C-erb-B2 overexpression in breast cancer provided new insights in treatment of these cancers. Blockage of C-erb-B2 receptors help the cessation of growth signaling pathway in cancer cells, and prevents the tumor proliferation. Trastuzumab, an antibody against these receptors, is widely used in treatment of breast cancers with C-erb-B2 overexpression.

Carcinogenesis in gastric adenocarcinomas, which includes genetic alterations such as the activation of oncogenes and the inactivations of tumor suppressor genes, is a multistep process occurring in many other cancer types.¹⁰⁻¹² It has been reported that gastric adenocarcinomas may also show C-erb-B2 overexpression, providing a new choice in treatment of gastric adenocarcinomas. Thus, demonstration of C-erb-B2 receptor status in gastric carcinomas has been thought to be essential in treatment of these cancers. Since gene amplification is well correlated with protein expression levels, IHC methods are preferentially used to determine the C-erb-

B2 receptor proteins in tumor cells. In situ DNA hybridisation methods such as fluorescence in situ hybridisation (FISH) and chromogenic in situ hybridisation (CISH) assays are also used to detect Her2/neu amplification.^{13,14}

In the present study, the C-erb-B2 status was assessed by the IHC staining in 139 gastric tumors. A total of 121 tumors were found negative for C-erb-B2 expression, whereas, 12 cases were positive by IHC staining. For the remaining 6 cases, reevaluation was done by using CISH method. C-erb-B2 amplification was observed in 4 of 6 cases. Out of 16 cases with C-erb-B2 overexpression, 13 had intestinal type adenocarcinomas and 3 had diffuse type adenocarcinomas. C-erb-B2 overexpression rates in gastric carcinomas range from 8.2% to 53.4%.⁶ In our series, this rate was 11.5% which is concordant with previous studies. Discrepancies in reported C-erb-B2 overexpression rates may be caused by the usage of different antibody clones, alternative scoring systems, or heterogeneous staining of tumor tissues. More importantly, it is speculated that evaluation of C-erb-B2 IHC staining is more subjective in gastric cancer than in breast cancer because of higher background staining. However, it usually gives the information of C-erb-B2 expression status of these tumors.³ At least, negative cases may be identified by IHC staining and selection for hybridisation methods may be facilitated. Immunohistochemically moderate or weak positive cases may be confirmed by the hybridisation methods.

Since, gastric carcinomas showing C-erb-B2 overexpression are associated with several genetic alterations and characterised by DNA amplification, membraneous protein expression may reveal a distinct subgroup with specific clinicopathologic characteristics and different therapeutic approach requirements.^{10,15-19} Therefore, we were also interested in the relationship between clinicopathologic characteristics and C-erb-B2 expressions in gastric carcinomas. Tanner et al., reported that gastric carcinomas with C-erb-B2 overexpression were associated with the well or moderately differentiated histology.³ Our results confirm the association between C-erb-B2 overexpression and histological grade in gastric cancer. We found the proportion of C-erb-B2 positive tumors in moderately differentiated carcinomas as six-fold of the high grade tumors ($p=$

0.001). Although this difference was also evident between low and moderate grade tumors, the small number of well differentiated tumors in our study group was insufficient to make a such conclusion between both groups.

C-erb-B2 overexpression was also significantly associated with intestinal type histology in our patient cohort. The rate of C-erb-B2 positive cases in intestinal type gastric carcinomas were 21.6%, which is comparable with the rates of invasive breast cancer. However, further research is warranted to evaluate Trastuzumab sensitivity in C-erb-B2 positive intestinal type gastric cancers among our population. On the other hand, the striking difference in C-erb-B2 overexpression rates between diffuse and intestinal subtypes suggest that different molecular alterations may play a role in pathophysiology of this heterogeneous disease. Unfortunately, the low incidence of overexpression found in diffuse type carcinomas suggests that a limited number of patients may benefit from Trastuzumab therapy, whereas, the vast majority of the patients with this more aggressive subtype of gastric carcinoma will probably lack this treatment choice.

Although mean patient age of group I was slightly lower than group II patients, the difference was not significant. A slight but not significant difference in C-erb-B2 overexpression rates was also observed between two gender groups. Lymph node involvement rates and tumor stages were similar for both groups.

In conclusion, results of our study indicate that C-erb-B2 overexpression is mainly limited to intestinal subtype, and occurs in a moderately differentiated substantial part of the gastric carcinoma cases among Turkish population. It will be expected that the frequent overexpression of C-erb-B2 found in intestinal subtype would have an impact on treatment modalities of this tumor subtype. Therefore, further investigations in larger patient cohorts are needed to clarify its prognostic and therapeutic importance.

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