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HER2 Positive Gastric Carcinomas and Their Clinico-Pathological Characteristics

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Abstract

BACKGROUND: HER2 protein expression in gastric carcinoma, in correlation with existing, acknowledged prognostic factors which include the parameters that determine the TNM stage of the disease, could become the basis for ongoing research in the field of molecular targeted and personalised therapy.

AIM: To determine the expression of the HER2 protein in gastric carcinoma and to correlate the expression of a HER2 protein with clinicopathological characteristics of the disease.

MATERIAL AND METHODS: The data of HER2 protein expression and the parameters of the TNM classification were obtained from the histopathological reports of the Institute of Pathology in Skopje, and for the clinical stage we used patient's files from the University Clinic for Abdominal Surgery in Skopje.

RESULTS: The analysis of the correlation of HER2 protein expression and TNM classification parameters pointed out a significant correlation between HER2 protein expression and intragastric localisation of gastric carcinoma (P = 0.005), and the tumour grade of differentiation (P = 0.034). There was also a positive correlation between HER2 protein expression pattern and positive lymph nodes in patients with gastric carcinoma (P = 0.03). The expression pattern of HER2 +++ was significantly more common registered in patients with positive lymph nodes (P = 0.03)

CONCLUSION: The expression of HER2 protein could represent a biological marker with prognostic and predictive value in patients with gastric carcinoma. Considering the high mortality rate in patients with gastric carcinoma and lack of international standardised therapeutic approach, research of the role and significance of HER2 overexpression and Trastuzumab therapy may prove useful in the development of new therapeutic strategies.

Introduction

Gastric carcinoma is an aggressive disease that has a daunting impact on global health. Despite the decline in incidence and mortality rate in recent years, gastric carcinoma remains one of the leading causes of cancer-related deaths worldwide, especially in developing countries. According to the recent statistical database, gastric carcinoma, with 930,000 new diagnosed and 700,000 diseased per year, is included with 8% out of 10% cancer-related deaths per year among the world population [1] [2] [3].

Because the most patients present with advanced disease the survival rate in patients with gastric carcinoma remains low, besides the evolution of new and sophisticated surgical techniques and the development of supplementary preoperative, neoadjuvant and adjuvant chemotherapy protocols. Recognition of complete recovery after surgical treatment is present only in early stages of diagnosed gastric carcinoma. According to the fact that, even in developed countries, primary detection of gastric carcinoma is in the nonresectable stadium of the disease, the systemic therapy is the main option for treatment that will only prolong the duration of survival [4] [5] [6]. In spite of the surgical treatment and

systemic/adjuvant chemotherapy, survival rate in patients with advanced stage of gastric carcinoma remains low, as a consequence of which the medical treatment of patients in advanced stage of gastric carcinoma demands novel therapeutic possibilities.

Understanding the molecular basis of cancer will facilitate the development of novel molecular target therapies, which interfere with different signal cascades involved in cellular proliferation, differentiation and survival. For this purpose, new research reports for the influence of new biomarkers such as microRNA, microsatellite instability, different types of cytokines (IL1, IL6, IL10, IL11, TNF, X12), CyclinD, Bcl2, p53 and other, including the HER2 protein in carcinogenesis, and as e target molecules for new therapeutic modalities are more often published [7] [8] [9] [10] [11] [12] [13] [14] [15] [16] [17] [18].

The data about HER2 protein expression are dual, and the reported results in the literature are different, as a consequence of which the evaluation of HER2 protein expression values in gastric carcinoma in correlation with other clinicopathological prognostic factors is currently ongoing process [7] [15] [16] [17].

HER2 protein expression in gastric carcinoma, in correlation with existing acknowledged prognostic factors which include the parameters that determine the TNM stage of the disease, could become the basis for ongoing research in the field of molecular targeted and personalised therapy.

This research aims to determine the correlation between tissue expression of the HER2 protein and the stage of the disease, the parameters of TNM gastric carcinoma classification and the histologic grade of gastric cancer.

Material and Methods

One hundred and forty-nine patients with gastric carcinoma surgically treated at the University Clinic for abdominal surgery in Skopje were included in the study. The operative material was analysed at the Institute of Pathology, Medical Faculty in Skopje.

Before the surgical treatment at the University Clinic for Abdominal Surgery in Skopje, an imaging technique procedure, gastroscopy and preoperative evaluation and preparation were obtained. For every patient, a standard operative procedure, according to the tumour localisation with loco-regional and systemic lymphadenectomy was performed. Sixty-one patients underwent subtotal gastric resection with lymphadenectomy, and 88 patients underwent total gastrectomy with lymphadenectomy.

Following the surgical treatment, a substitution therapy in the post-operative period was applied, using different solutions. The substitution therapy includes correction of electrolyte disbalance with electrolyte solutions, correction of anaemia with transfusion, correction of hypoproteinemia with plasma and pure albumin solution, correction of coagulation factors deficiency with fresh frozen plasma and antibiotic therapy was performed if necessary. Every patient had a controlled postoperative dietary regime, antithrombotic prophylaxis and controlled analgesia for pain management.

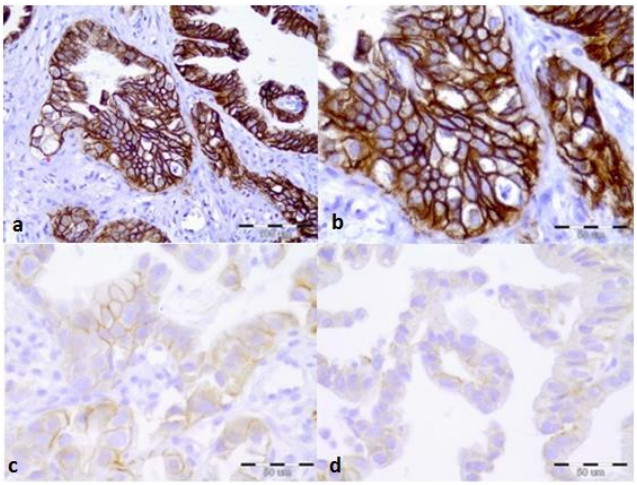

Figure 1: Immunohistochemical HER2 protein expression histological patterns. a) Her2+++ pattern of expression (x 200); b) same pattern of expression at higher magnification (x 400); c) Her2 ++ pattern of expression (x 400); d) Her2 + pattern of expression (x 400)

Data of HER2 protein expression and the parameters of the TNM classification (AJCC Cancer Staging 2017) were obtained from the archive histopathological reports of the Institute of Pathology in Skopje, and for the clinical stage, we used patient's files from the University Clinic for Abdominal Surgery in Skopje.

Immunohistochemical stainings were made with a standard procedure using Immunoperoxidase LSAB + system and specific primary monoclonal HER2-antibody (Ventana Medical Systems, Roche, and-HER2/neu Rabbit Monoclonal Primary Antibody Clone 4B5).

HER2 protein overexpression was defined in 4 histological patterns [18] (Figure 1):

0 No membranous staining or staining of < 10% of the tumour cells

+ Staining is weak or detected in only one part of the membrane in ≥ 10% of the cells

++ Moderate/weak complete or basolateral membranous staining in ≥ 10% of the cells

+++ Strong complete or basolateral membranous staining in ≥ 10% of the neoplastic cells

HER2 ++ and + expression are additionally determined with Fluorescent in Situ Hybridization (FISH) method

To make a correlation between tissue HER2 protein expression and the disease stage, parameters of the TNM classification and histologic tumour grade HER2 expression was carried out in 2 steps. The first step, the patients were divided into two groups, according to the HER2 protein expression, as patients with positive HER2 expression and patients with negative HER2 immunostaining, excluding the importance of protein expression scheme. In the second step, the same analyses were repeated, except for the fact that HER2 positivity was analysed according to the scheme mentioned above (+, ++, +++).

Descriptive statistical methods were used for statistical analysis of the data. The rate of the interdependence of the analysed parameters was obtained with linear correlation. Statistical program SPSS for Windows 19.0 was used.

Results

The analysed group consisted of 149 patients, with the mean age of 65.19 ± 10.1, 108 (72.48%) of which were female and 41 (27.52%) male.

Clinical and histopathological characteristics of the patients and their cancers are shown in Table 1.

Table 1: Clinical and pathological characteristics of the analysed patients

Parameter	N (%)
Localization	
Cardia	61 (40.94)
Corpus	37 (24.83)
Antrum/Pylorus	51 (34.23)
T	
1	5 (3.6)
2	19 (12.75)
3	41 (27.52)
4	84 (56.38)
Nodal involvement	
Negative	36 (24.16)
Positive	113 (75.84)
Nodal status (TNM classification)	
0	36 (24.16)
1	27 (18.12)
2	30 (20.13)
3	56 (37.58)
Distant metastases	
No	136 (91.27)
Yes	13 (8.72)
Grade	
1	2.68)
2	64 (42.95)
3	81 (54.36)
Stage	
I	11 (7.38)
II	37 (24.83)
III	88 (59.06)
IV	13 (8.72)

The most common intragastric location of gastric carcinomas was cardia in 61 (40.94%) patients, followed by antral/pyloric carcinoma location in 51 (34.23%) patients and corpus location in 37 (24.83%) patients. According to the T state (local tumour growth), more than half of the examined patients 84 (56.38%) were in T4 state of the disease.

Presence of positive regional lymph nodes was detected in 113 (75.84) patients, and negative in 36 (24.16%) patients. The majority of the patients that comprised the analysed group had poor differentiated gastric carcinoma 81 (54.36%), and 88 (59.06%) were in Stage III of the disease.

Immunohistochemical staining with HER2 antibody showed HER2 protein expression in 44 (29.53%) carcinoma tissue, of which (6.71%) with HER2+, 7 (4.69%) with HER2++ and 27 (18.12%) with HER2+++ expression pattern.

Table 2: HER2 expression in analyzed patients

Variable	N (%)
Her2 expression (pattern)	
-	105 (70.47)
+	10 (6.71)
++	7 (4.69)
+++	27 (18.12)
Her2 positivity	
Negative cases	105 (70.47)
Positive cases	44 (29.53)

HER2 + expression pattern in gastric carcinoma was more frequent in female patients (27.59% vs 13.33%), while HER2+++ expression pattern was more frequently represented in male patients (73.33% vs 55.17%) in the group of 44 positive cases.

There was the insignificant difference in the distribution of HER2 protein expression according to the gender of the patients (P = 0.4).

Patients with positive HER2 protein expression in gastric carcinoma were with a median age of 65.5 ± 9.8 years, and patients with no HER2 protein expression were with a median age of 64.4 ± 10.7 years, without significant difference between the groups (P = 0.55).

Presence of HER2 protein was obtained in 16 (26.23%) carcinomas with a location in gastric cardia, 11 (29.735) in the corpus and 17 (33.33%) carcinomas with the antral/pyloric location, with no significant difference in HER2 protein expression, according to the location of gastric carcinoma (P = 0.71).

The analysis of different HER2 expression patterns showed:

HER2+ protein was more frequently present in gastric carcinomas with a location in the corpus (54.55%) about antrum/pylorus location (17.65%) and cardia (6.25%).

HER2++ expression was most frequently registered in carcinomas located in cardia (31.25%) and was absent in gastric corpus.

HER2+++ protein expression was more

frequent in antral/pyloric location, in correlation with cardia and corpus location (70.59%, 62.5%, 45.45% respectively).

Statistical analysis confirmed the presence of a significant difference in HER2 protein expression pattern according to the location of the gastric neoplasm (Table 3).

Table 3: HER2 protein expression in gastric carcinoma according to the location

Localization	n	Her2 expression pattern			P-value
		+	++	+++	
Cardia	61	1 (6.25)	5 (31.25)	10 (62.50)	0.005**
Corpus	37	6 (54.55)	0	5 (45.45)	
Antrum/Pylorus	51	3 (17.65)	2 (11.76)	12 (70.59)	

**P < 0.05; **P < 0.01.*

We did not find significant differentiation in HER2 expression according to the local growth (T status) neither when analysed only HER2 negative cases with HER2 positive, nor when we analysed HER2 negative carcinomas with different HER2 positive patterns (+,++,+++) (P = 0.54, P = 0.63 respectively.

HER2 protein expression was insignificantly different in patients with gastric carcinoma with positive and negative lymph nodes. Additional analysis of different HER2 expression pattern regarding positive and negative lymph nodes showed that HER2 +++ was significantly more frequent in patients with positive regional lymph nodes (P = 0.03). HER2 +++ pattern was found in 33.33% of patients with negative lymph nodes and 68.57% of patients with positive lymph nodes.

Statistically, a significant difference was found in the carcinomas with different grade (G) in patients with positive and negative HER2 expression. More frequent HER2 expression was detected in patients with moderately differentiated carcinomas in comparison to the patients with poorly differentiated carcinomas (40.63% vs 20.99%, P = 0.034), (Table 4).

Table 4: Distribution of HER and HER negative patients according to the grade of carcinoma differentiation

Grade	n	Her2 expression		p-value
		Negative	Positive	
G1	4	3 (75)	1 (25)	0.034
G2	64	38 (59.38)	26 (40.63)	
G3	81	64 (79.01)	17 (20.99)	

**P < 0.05; **P < 0.01.*

Additional analysis of HER2 expression patterns (+, ++, +++) confirmed non-significant difference in HER2 expression patterns according to the tumor differentiation (P = 0.28), but there was a statistically negative significant correlation between HER2 expression pattern and tumor differentiation (P = 0.01), meaning that HER2+++ pattern was significantly related to well-differentiated carcinomas (Figure 2).

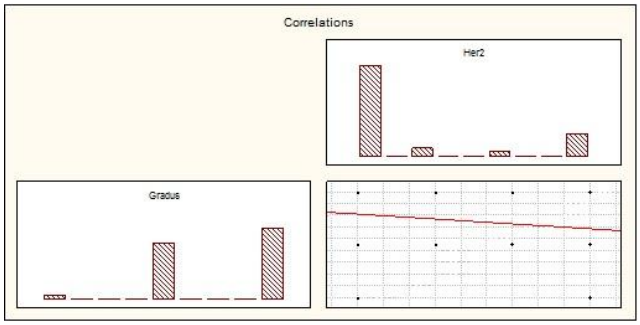

Figure 2: Correlation between the grade of differentiation and HER2 protein expression

HER2 protein expression was not significantly related to the Stage of the disease neither an analysis of positive and negative cases nor in HER2 expression patterns analysis (P = 0.56; P = 0.61, respectively).

Discussion

TNM classification of gastric carcinoma is the most valuable prognostic factor in patients with this disease. The classification contains the following elements: T – local tumour growth, N- lymph node involvement and M- the distant metastatic spread of the primary disease. The grade of differentiation of a tumour is also a part of the classification. However, there is a variable prognosis among the patients at the equal stage of the disease. Therefore, a request for revealing additional parameters for better identification of biological subgroups imposes itself. Biological predictive factors are obtained from genetic process, which is considered to be the key step in the development of gastric carcinoma (HER2, E-cadherin, EGFR, microsatellite instability, changes in few factor expression, including thymidylate synthase, beta-catenin, mucin-Ag, p53, COX-2, matrix metalloproteinases, and receptors for vascular endothelial factor) [7].

HER2 protein (HER2/neu, ErbB-2) is a 185-kDa transmembrane tyrosine kinase receptor, coded by HER2 proto-oncogene, located on the long arm of the 17-the chromosome. HER2 protein is a member of epidermal growth factor receptor family. This receptor family consists of 4 members: HER1 (also known as EGFR), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4), which share the same molecular structure on their extracellular domain for ligand binding and intracellular part with tyrosine-kinase (TK) activity (with the exception of HER3). HER2 does not bind to anyone knew ligand and is considered to be ligand-free co-receptor for all other members of ErbB family. In fact, HER2 presents a peripheral partner for hetero-dimerisation of other members of HER family. Ligand binding to HER family cellular receptor induces homo-

dimerisation and hetero-dimerisation of EGFR to other types of HER proteins (HER2 is a part of the hetero-dimerisation process). Various ligand binding activity for extracellular domain initiates a signal transmitting (transducing) cascade, which may have an impact on the cell proliferation, apoptosis, cell adhesion, migration, angiogenesis and differentiation [7] [8] [9] [19] [20].

Many authors suggest an important HER2 role in various cancer types development. HER2 expression and/or amplification is recorded in invasive type of breast carcinoma [19], endocrine cancer [20] colon carcinoma [21], bladder carcinoma [22], ovarian carcinoma [23], endometrial [24] and cervical carcinoma [25], lung carcinoma [26], head and neck carcinoma [27], esophageal [28] and gastric carcinoma [29].

The reported data about the overexpression of HER2 in gastric carcinoma are diverse, depending on characteristics of the analysed groups and HER2 overexpression is reported in a range from 2% to 34%. The difference in expression is dependent on the localisation of a tumour in the stomach (gastroesophageal function or other localisation), the histological subtype (diffuse, intestinal, mixed, and unknown), and differentiation of a tumour.

Although according to some authors, there is no correlation between over-expression of HER2 and the disease prognosis, other authors found an association between HER2 overexpression and worse prognosis. It is considered that HER2 expression is in positive correlation with the tumor size, serous invasion and lymph node metastases, and also with poor prognosis of the patients for the 10-year survival period [7] [14] [29] [30] [31] [32] [33] [34] [35] [36).

The results from this study confirmed a significant correlation between tissue expression of HER2 and intragastric tumour location, lymph node metastasis and grade of tumour differentiation. The correlation between HER2 expression and the grade of differentiation is determined as negative, which means that HER2 expression is significantly associated with the more differentiated carcinomas, i.e. well-differentiated carcinomas.

Trastuzumab (Herceptin, Genentech, San Francisco, CA), is a monoclonal antibody that interferes with HER2 receptor (EGFR 2 receptor blocker), which in combination with chemotherapy protocols improves the outcome and survival in patients with different types of carcinomas, including the gastric carcinoma [19] [37] [38], but yet some patient may develop resistance to therapy with this antibody. For that reason, ongoing research on HER2 receptor are still required [38] The results from The International randomized study carried out in 2010, (Trastuzumab for gastric cancer treatment), revealed that Herceptin human monoclonal antibody, trastuzumab antibody anti-HER2 significantly prolong the overall survival, in comparison to chemotherapy protocol alone, in patients with HER2 overexpression [20] [37] [38] [39]. So, this research may be the basis for continuous research of the importance of HER2 expression in gastric carcinoma, with scientific and also medical call practice contribution.

In conclusion, this research determined significant difference in HER2 expression in gastric carcinoma with antro/pyloric localiztion, in comparison to carcinoma located on gastric cardia or corpus.

Statistically significant negative correlation of HER2 expression in gastric carcinoma and histologic grade of a tumour is also cinfirmed. According to these results, HER2 expression is significantly corelated to more aggressive disease and possibility of poor outcome.

We also found significant correlation between HER2 expression pattern HER2+++ and metastasis in regional lymph nodes.

The correlation of HER2 expression and above mentioned parameters highlights the possibility of HER2 expression being a valuable biologic marker with prognostic importance in gastric carcinoma patients. Considering the high mortality rate in patients with gastric carcinoma and lack of international standardized therapeutic approach, research of the role and significance of HER2 overexpression and Transtuzumab therapy may prove useful in development of new therapeutic strategies and treatment possibilities.

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