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CORRELATION BETWEEN HER2 AND EBV EXPRESSION IN GASTRIC CARCINOMA

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Abstract

Introduction**:** The understanding of the etiopathogenesis and the molecular basis of gastric carcinoma will facilitate the development of novel molecular target therapies, which interfere with different signal cascades involved in cellular proliferation, differentiation and survival.
The aim of this paper was to determine the correlation between HER2 and EBV expression in patients with gastric carcinomas.

Material and methods**:** Eighty patients with gastric carcinoma surgically treated were included in the study. Data of HER2 protein expression were obtained from the archived histopathological reports of the Institute of Pathology in Skopje. For detection of EBV, immunostainings were performed on tumor tissue and the peripheral nontumor gastric mucosa.

Results: The results of this study confirmed a significant association between HER2 and EBV expression (p=0.041). The value of the Spearman correlation coefficient (R = - 0.258) indicated a negative, indirect connection of HER2 and EBV expression, which was confirmed as statistically significant (p = 0.02).

The HER2 expression in gastric carcinomas was significantly associated with EBV expression, and the expression of HER2 was significantly more common in EBV negative cancers.

Keywords: gastric carcinoma, HER2, EBV, immunohistochemistry

Introduction

Gastric carcinoma is an aggressive disease that 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 [1-3].
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 the advanced stage of gastric carcinoma demands novel therapeutic possibilities.

The understanding of the etiopathogenesis and 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. Over the last decade, a large number of data have been published on the association of gastric carcinoma with Epstein - Barr virus (EBV) that is believed to play a role in the carcinogenesis of this neoplasm. The percentage of EBV positive gastric carcinoma is uncertain, and the etiological importance has still not been elucidated [4-5]. The conducted meta-analysis of 70 studies that included a total of 15 952 cases of GC revealed that EBV positive gastric carcinomas differed from the other gastric carcinoma by gender distribution, anatomic localisation and surgically different anatomy, indicating that EBV-associated gastric carcinoma is a particular etiological entity [4]. Epidemiological studies from different regions and studies that contribute to defining the role of EBV in the carcinogenesis and progression of GC are useful for the development of new therapeutic modalities [5].

Furthermore, 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 as target molecules for new therapeutic modalities are more often published [6-11]. 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 an ongoing process [6-11]. 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.

The aim of this paper was to determine the correlation between HER2 and EBV expression in patients with gastric carcinomas.

Material and methods

Eighty patients with gastric carcinoma surgically treated at the University Clinic for Abdominal Surgery in Skopje were included in the study. The surgical material was analysed at the Institute of Pathology, Faculty of Medicine in Skopje. Before the surgical treatment, an imaging technique procedure, gastroscopy and preoperative evaluation and preparation were obtained. For every patient, a standard surgical procedure, according to the tumor localization with loco-regional and systemic lymphadenectomy was performed. Data of HER2 protein expression were obtained from the archived histopathological reports of the Institute of Pathology in Skopje.

Immunohistochemical stainings for HER2 expression 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 tumor 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 were additionally determined with Fluorescent in Situ Hybridization (FISH) method.

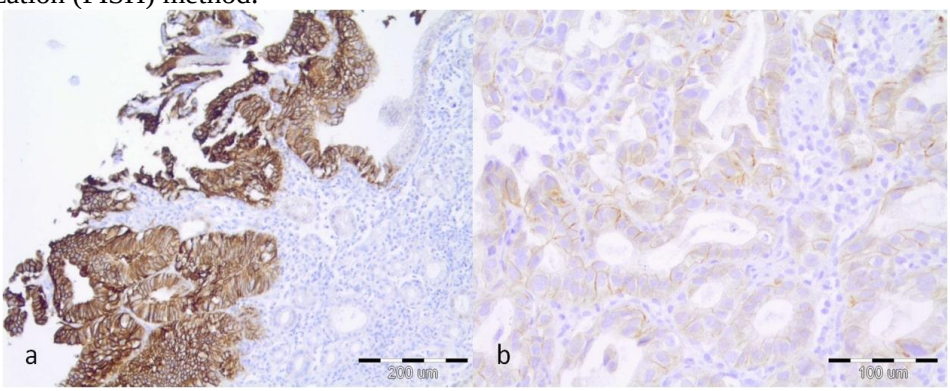

Figure 1. Immunohistochemical HER2 protein expression histological patterns. a) Her2+++ pattern of expression (x 100); b) Her2 ++ pattern of expression (x 200)

For detection of EBV, immunostainings were performed on tumor tissue and the peripheral nontumor gastric mucosa. A standard commercial control was used for immunostaining control. Immunohistochemical stainings for EBV were made with a standard procedure using Immunoperoxidase LSAB + system and specific primary monoclonal EBV-antibody (DAKO – Monoclonal Mouse. Anti-Epstein Barr Virus, LMP. Clones CS. 1-4. Code IR753). EBV expression was defined in 2 histological patterns, nuclear and cytoplasmic (Figure 2).

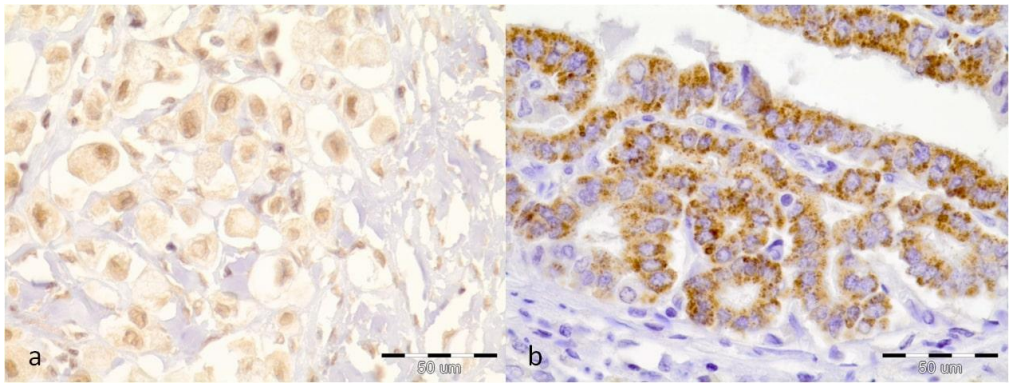

Figure 2. a) Nuclear positivity for EBV in signet cell gastric carcinoma (x 400); b) Cytoplasmic positivity for EBV in gastric cancer (x 40)

The correlation between HER2 and EBV expression was determined. Fisher's exact test was used for comparison of categorical variables. The degree of correlation between analyzed parameters was determined using Spearman's correlation coefficient. The statistical program SPSS for Windows, version 19.0 was used.

Results

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.

Expression of EBV in cells was nuclear and cytoplasmic. Nuclear expression was found in 15 (18.75%) cases and cytoplasmic in 4 (5%) cases. In 10 (66.66%) of positively stained cases, nuclear EBV expression showed patchy distribution in clusters of cells and the other 5 (33.33%) cases EBV positivity was diffuse in the tumor cells. The expression of EBV in the peri-tumor gastric mucosa showed patchy distribution. Positive expression of EBV protein was also detected in plasma cells present in the tumor stroma, or gastric submucosa of patients with GC. Positive immunostaining with the antibody against EBV was found in 19 (23.75%) of the total of 80 gastric carcinomas.

The results of this study confirmed a significant association between HER2 and EBV expression (p=0.041) (Table 1). In the group of 33 HER2-positive gastric carcinomas, there was no significant difference of the degree of HER2 expression between EBV-negative and EBV-positive carcinomas (p = 0.16) (Table 2). The value of the Spearman correlation coefficient (R = - 0.258) indicated a negative, indirect connection of HER2 and EBV expression, which was statistically significant (p = 0.02). The intensity of HER2 expression in gastric carcinomas was significantly associated with EBV expression (Table 3).

Table 1. Correlation between HER2 and EBV expression

HER2	**EBV**			**p-value**
	N	**Negative n (%)**	**Positive n (%)**	
negative	47	32 (68.09)	15 (31.91)	0.041sig
positive	33	29 (87.88)	4 (12.12)	

p (McNemar test)

Table 2. Correlation between pattern of HER2 and EBV expression

HER2 expression	EBV			p-value
	n=33	Negative n (%)	Positive n (%)	
low	8	6 (75)	2 (25)	0.16 ns
mild	4	3 (75)	1 (25)	
high	21	20 (95.24)	1 (4.76)	

p (Fisher exact test)

Table 3. Correlation between HER2 and EBV expression

variable	Spearman R	t–test	p-level
HER2 & EBV	-0.258	t=2.36	p=0.021sig

Discussion

In this study, no statistically significant difference between the pattern of HER2 expression in gastric carcinomas with negative and positive EBV expression was found (p = 0.16). However, the correlation made with Spearman's correlation coefficient showed an inversely proportional dependence (R = - 0.258) and p = 0.02, suggesting that the strength of HER2 expression in gastric carcinomas was significantly related to EBV expression.

The reported data about the overexpression of HER2 in gastric carcinoma were diverse, depending on characteristics of the analyzed groups and HER2 overexpression was reported in a range from 2% to 34% [11]. The difference in expression was dependent on the localization of the tumor in the stomach (gastroesophageal function or other localization), the histological subtype (diffuse, intestinal, mixed, and unknown), and differentiation of the tumor. 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 a positive correlation with the tumor size, serous invasion and lymph node metastases, and also with poor prognosis in patients for a 10-year survival period [11-18].

According to literature data, the EBV infection is associated with 2-16% of gastric carcinoma, but the published data, in general, refer to the role of EBV in carcinogenesis. Few data on the association of EBV with gastric carcinoma and its characteristics are available in the literature [19-22]. The prevalence of gastric carcinoma associated with EBV infection shows geographic variations [23] and is related to the lifestyle of patients. Thus, studies about EBV-associated gastric carcinomas are necessary and very actual [4, 5].

In conclusion, this research determined that HER2 expression in gastric carcinomas was significantly associated with EBV expression and the expression of HER2 was significantly more common in EBV negative cancers.

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