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Helicobacter pylori infection and expressions of EGF, EGFR and c-erbB-2 proteins in gastric carcinoma

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Abstract: The family of epidermal growth factor (EGF, EGFR, c-erbB-2) plays a pivotal role in gastric cancer progression, invasion and metastasizing. *Helicobacter pylori* infection is known to contribute significantly to the formation and progression of gastric cancer. However, the mechanisms responsible for this process have not been yet elucidated. We analysed the relationship between *H. pylori* infection and expression of proteins belonging to the family of epidermal growth factor (EGF, EGFR, c-erbB-2). Fifty-five patients with gastric cancer were analysed for *Helicobacter pylori* infection. The expressions of EGF, EGFR, c-erbB-2 proteins were determined using an immunohistochemical method. No statistically significant correlation was found between the degree of *H. pylori* infection and the expressions of EGF, EGFR and c-erbB-2 in gastric cancer. However, c-erbB-2 expression in the main mass of tumour correlated with tumour expression of EGF and EGFR and with c-erbB-2 expression in local lymph nodes. The expression of c-erbB-2 in lymph nodes was statistically significantly related to the expressions of EGF and EGFR both in the main mass of tumour and in lymph nodes. The expression of EGF was found to correlate with EGFR in the main mass of tumour and the expression of EGF in lymph nodes was related to lymph node EGFR level. Our study did not confirm the relationship between *H. pylori* infection and the expression of epidermal growth factor in gastric cancer.

Key words: gastric carcinoma, *Helicobacter pylori*, EGF, EGFR, c-erbB-2

Introduction

According to the Polish Carcinoma Registry, gastric cancer is the second most common malignancy among men and the eighth in women [1]. The 5-year survival rate is only 18%. Before *Helicobacter pylori* was discovered, high incidence of gastric carcinoma had been associated with such risk factors as diet and lifestyle (ethnicity, nitrates and nitrites-rich diet, smoking and alcohol abuse). Carcinogenesis is a multistage process, which in gastric cancer begins with chronic inflammation of the gastric mucosa that develops into chronic atrophic inflammation (intestinal metaplasia) and eventually leads to dysplasia. A relationship has been found between *Helicobacter pylori* infection and chronic atrophic inflammation of the gastric mucosa. Thus, *H. pylori* may play a role in gastric carcinoma progression [2]. This hypothesis has been confirmed by a number of studies in the past several dozen years and approximately 35-60% of gastric cancers have been reported to be associated with *H. pylori* infection [3]. In the year 1994, World Health Organization (WHO) considered *H. pylori* to be class 1 carcinogen [4]. *Helicobacter pylori* is a gram-negative bacterium, 0.5 do 3 μm in size, known to colonize the gastric mucosa. It is elongated, spiral and has 3-7 long flagella at one end that allow movement. It has been estimated that this bacterium occurs in two-thirds of the world population. *H. pylori* is responsible for 80% of cases of gastric ulcer and 90% of duodenal ulcer. It shows resistance to gastric acid, and produces large amounts of urease, i.e. the enzyme catalysing degradation of urea to ammonium and carbon dioxide. Ammonium causes neutralization of hydrochloric acid (present in gastric juice) in a direct vicinity of the bacteria, thus enabling their survival. The harmful effect of *H. pylori* involves the release of toxins that initially cause mucosa inflammation; however, the process initiating

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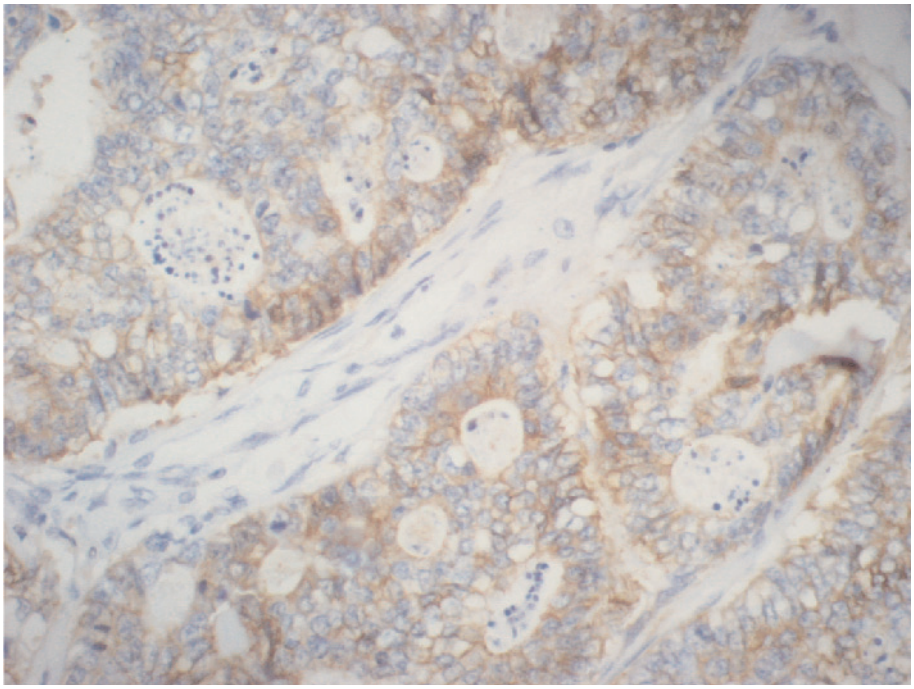

Fig. 1. Expression of EGFR in gastric cancer (original magnification ×40).

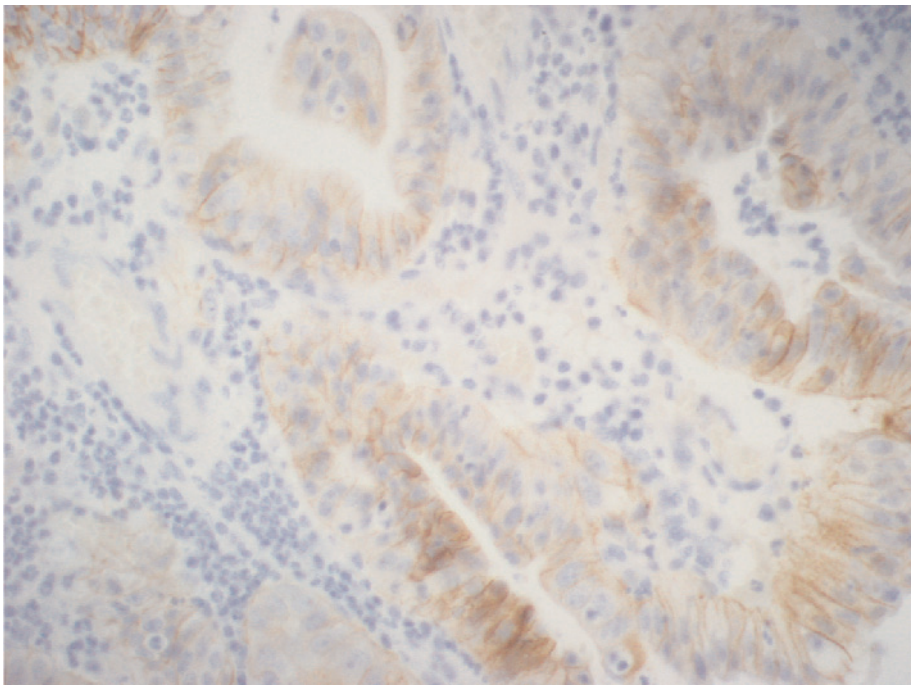

Fig. 3. Expression of c-erbB-2 in gastric cancer (original magnification ×40).

tumour formation and progression by *H. pylori* is still not fully elucidated [5,6]. There is a search for evidence whether *H. pylori* can affect the expression of the respective proteins also associated with the development of gastric carcinoma. EGF is suspected to play the major role in this mechanism [7].

The epidermal growth factor (EGF) is a single polypeptide built of 53 amino acids and playing an essential role in intercellular interactions. Cooperation between the epidermal growth factor of one cell and the receptor (EGFR) on the adjacent cell leads to some biological effects, including migration, growth or morphological changes in the cell. EGF stimulates the growth or exerts a trophic effect in many tissues, and plays an important role in proliferation, differentiation and maturation of embryonic cells. However, EGF and EGFR, apart from the key role they play in physiological development, also take part in neoplastic transformation. Increased expressions of EGF and EGFR have been observed in various malignancies, including gastric carcinoma [8]. Substantial evidence has been also provided for neoplastic oncogene HER-2/neu (cerbB-

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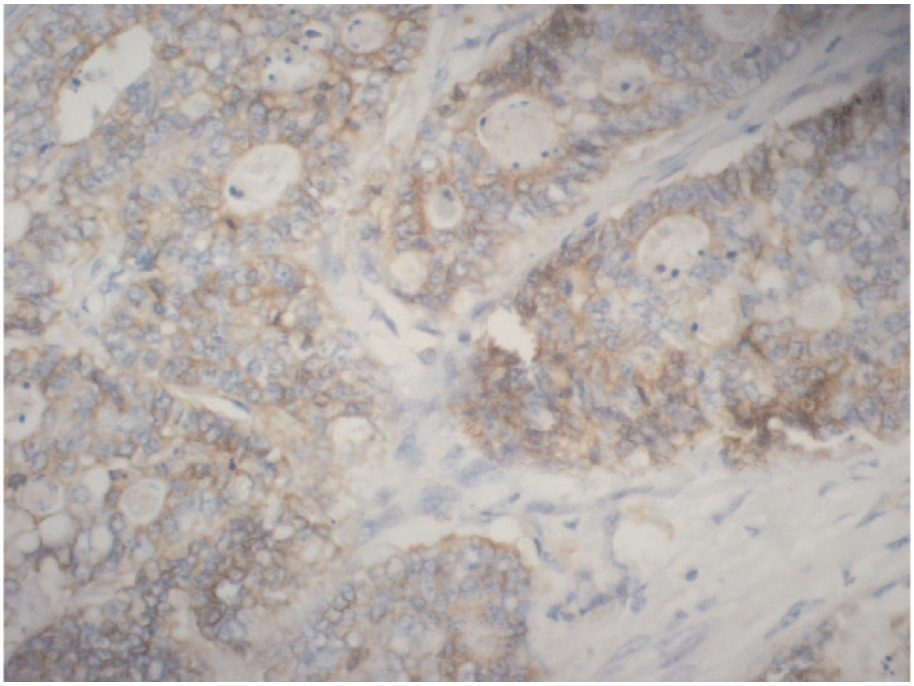

Fig. 2. Expression of EGF in gastric cancer (original magnification ×40).

2), which encodes transmembrane glycoprotein closely related to the epidermal growth factor receptor, whose overexpression has also been observed in gastric carcinoma [9].

The current study objective was to assess the expressions of the epidermal growth factor (EGF), the epidermal growth factor receptor (EGFR) and c-erbB-2 (HER-2/neu) in patients with gastric carcinoma in correlation with *Helicobacter pylori* infection.

Materials and methods

Material. Fifty-five patients treated for gastric carcinoma in II Department of General Surgery and Gastroenterology, Medical University of Białystok in the years 1991-2003 were recruited to the study.

Endoscopic specimens were stained with Giemza solution for the presence of *Helicobacter pylori*. The inflammation and the degree of *Helicobacter pylori* infection were classified according to the Sydney system [10]. The degree of infection was assessed using the scale: 0 -lack of infection, 1- low, 2 – moderate, 3 – high.

Immunohistochemistry. Immunohistochemical investigation was performed according to the following protocol. Fifty-five tumour specimens and twenty-eight local lymph node samples, formalin-fixed and paraffin-embedded, were cut on a microtom into 5µm sections. The sections were deparaffinized in xylenes and hydrated in alcohols. In order to expose the antigen, the sections were heated in a microwave for 15 min in citrate buffer (pH 6.0). To block endogenous peroxidase, incubation was performed with 0.5% hydrogen peroxide in methanol. Next, they were incubated with monoclonal antibodies against c-erbB-2 (Novocastra, NCL-c-erbB-2-316) overnight, with EGF (EGF-Sigma, clone EGF-10, Sigma-Aldrich, Poland) for 60 min at room temperature and with EGFR (EGFR, clone H11, Dako, Poland). The reaction was conducted using the ABC technique with a Novostatin Super ABC Universal Kit (Novocastra Laboratories Ltd.) Colour reaction for peroxidase was performed with chromogen DAB (DAKO S3000, DAKO, Poland) (Fig. 1,2,3).

Evaluation of samples. Protein expression was assessed using a semiquantitative method and the reaction was labelled as:

- (-) – lack of expression of EGF, EGFR, c-erbB-2 or reaction present in <30% of cells in the main mass of tumour and in metastatic lymph node
- (+) – expression of EGF, EGFR, c-erbB-2 in >30% of cells in the main mass of tumour and in metastatic lymph node

Positive reactions were calculated in at least 500 tumour cells in each tissue section using a light microscope (×400).

Statistical analysis. Statistical analysis was based on χ^2 test and exact Fisher's test. Correlations between the expressions of the respective proteins were calculated using Spearman's correlation coefficient test. The value of $p<0.05$ was considered statistically significant.

Results

Of 55 patients with gastric carcinoma, 28 did not have H pylori infection, 4 patients had low degree infection, 9 moderate and 14 high. No statistically significant correlation was found between the degree of *H. pylori* infection and the expressions of EGF, EGFR and c-erbB-2 in gastric carcinoma (Table 1). However, statistically significant correlations were observed between the respective proteins. The expression of c-erbB-2 protein in the main mass of tumour correlated with tumour expressions of EGF and EGFR and with c-erbB-2 expression in local lymph nodes. The expression of c-erbB-2 in the lymph node was statistically significantly correlated with the expressions of EGF and EGFR both in the main mass of tumour and in the lymph node. The expression of EGF was also found to correlate with the expression of EGFR in the main

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mass of tumour and the expression of EGF in the lymph node was observed to correlate with EGFR expression also in the lymph node (Table 2).

Discussion

Proteins belonging to the family of the epidermal growth factor (EGF, EGFR, c-erbB-2) are thought to be involved in neoplastic formation and progression of gastric cancer. We found correlations between them, but not between *H. pylori* infection and EGF expression, unlike other authors. Cole *et al*. [7], who measured the levels of EGF and EGFR protein by flow cytometry, found that they doubled in *H. pylori* infection. Eradication of the infection reduced the levels of both proteins. This may be one of the pathomechanisms of gastric mucosa hyperproliferation and carcinogenesis associated with *H. pylori* infection, EGF overexpression and increased density of epidermal growth factor receptors (EGFR) on cells. Also Wong *et al*. [11] measured EGF and EGFR mRNA in gastric mucosa tissues and saliva using ELISA. Both EGF and EGFR mRNA were found to increase in the presence of *H. pylori* infection in the gastric mucosa. However, salivary EGF concentration was not found to be related to mucosa damage or *H. pylori* infection. Successful *H. pylori* eradication brought EGFR mRNA normalization, whereas with eradication failure the level remained high. It can be concluded that *H. pylori* may regulate EGFR expression in the gastric mucosa, which is vital in the treatment of *H. pylori* infection. When the infection is untreated, elevated levels of EGF and EGFR may stimulate cell proliferation and initiate a neoplatic process. An important study was conducted by Wallasch *et al*. [12], who presented a model in which *H. pylori* activates the EGFR signalling pathway, increasing IL-8 production and initi-

Table 1. Correlation between *H. pylori* infection degree and absence or presence of c-erbB-2, EGF and EGFR expression in gastric cancer.

Parameters		Degree of *Helicobacter pylori* infection				*p*
		0 (n=28)	1 (n=4)	2 (n=9)	3 (n=14)	
c-erbB-2	-	14	2	4	10	NS
	+	14	2	5	4	NS
EGF	-	16	2	6	6	NS
	+	12	2	3	8	NS
EGFR	-	11	3	3	8	NS
	+	17	1	6	6	NS

Correlation is significant at the level of p<0.05. NS – no statistical significance

Table 2. Correlations between the degree of *H. pylori* infection and the level of c-erbB-2, EGF and EGFR expression in tumour and lymph node of gastric cancer.

	H. pylori	c-erbB-2 tumour	c-erbB-2 node	EGF tumour	EGF node	EGFR tumour	EGFR node
H. pylori	---	-0.1441 N=55 p=0.294	-0.0734 N=28 p=0.705	0.0823 N=55 p=0.550	-0.0359 N=28 p=0.856	-0.1106 N=55 p=0.421	-0.0657 N=28 p=0.740
c-erbB-2 tumour	-0.1441 N=55 p=0.294	---	0.7346 N=28 p=0.000	-0.6867 N=55 p=0.000	0.2949 N=28 p=0.128	0.4667 N=55 p=0.000	0.2660 N=28 p=0.171
c-erbB-2 node	-0.0734 N=28 p=0.705	0.7346 N=28 p=0.000	---	-0.6714 N=29 p=0.000	0.4038 N=28 p=0.033	0.4213 N=28 p=0.023	0.4415 N=28 p=0.019
EGF tumour	0.0823 N=55 p=0.550	-0.6867 N=55 p=0.000	-0.6714 N=28 p=0.000	---	-0.2692 N=28 p=0.166	-0.4867 N=55 p=0.000	-0.2208 N=28 p=0.259
EGF node	-0.0359 N=28 p=0.856	0.2949 N=28 p=0.128	0.4038 N=28 p=0.033	-0.2692 N=28 p=0.166	---	0.0538 N=28 p=0.786	0.4778 N=28 p=0.010
EGFR tumour	-0.1106 N=55 p=0.421	0.4667 N=55 p=0.000	0.4213 N=28 p=0.023	-0.4867 N=55 p=0.000	0.0538 N=28 p=0.786	---	0.2208 N=28 p=0.259
EGFR node	-0.0657 N=28 p=0.740	0.2660 N=28 p=0.171	0.4415 N=28 p=0.019	-0.2208 N=28 p=0.259	0.4778 N=28 p=0.010	0.2208 N=28 p=0.259	---

Correlation is significant at the level of p<0.05. Significant correlation is marked in bold.

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ating neoplastic transformation of the gastric mucosa. Our study, however, did not confirm the hypothesis that *H. pylori* may increase the expression of EGF and EGFR, leading to gastric cancer progression. Broader investigations are necessary to elucidate the correlations between *H. pylori* infection and the expression of epidermal growth factor proteins. We also found no correlation between *H. pylori* infection and c-erbB-2 protein expression. The overexpression of c-erbB-2 protein is known to be associated with low differentiation and advancement of cancer and thus with worse prognosis in patients. Similarly, other researchers found no relationship between *H. pylori* and c-erbB-2 protein [13,14]. The current study did not confirm the hypotheses put forward by other researchers; however, in our study different methods were used for the evaluation of proteins and *H. pylori* infection.

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