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The EGFR expression in gastric mucosa of children infected with *Helicobacter pylori*

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ABSTRACT

Purpose: Epidermal growth factor receptor (EGFR) modulates balance between proliferation and apoptosis in gastric mucosa of the gastrointestinal tract. The aim of the study was to evaluate immunohistochemically the EGFR expression in epithelial and gland cells of antral mucosa in children infected with *Helicobacter pylori* (*H. pylori*).

Material/Methods: The study included 44 children, aged from 5 to 18 years (mean age 13±3.4 years) with dyspeptic symptoms, of whom 30 (68.2%) children were infected with *H. pylori*, 14 (31.8%) children constituted controls. Endoscopic and histopathological assessment of antral mucosa samples was performed according to the Sydney System. Samples taken from gastroscopy were prepared to evaluate EGFR expression in epithelial and gland cells of antrum mucosa according to the manual of a detection kit of EnVision+System–HRP (DAKO).

Results: In children *H. pylori* infected, the EGFR expression in epithelial cells of antral mucosa equaled on average 82.5±15 cells/mm2 and ranged from 45.0 to 98.0 cells/mm^2 as well as differed statistically significantly when compared to controls (10.2±5.0 cells/mm^2) ($p<0.001$). In children with *H. pylori* infection, the EGFR expression in gland cells of antral mucosa ranged from 2.0 to 85.0 cells/mm^2 (mean 25.7±22.6 cells/mm^2); was lower and differed statistically significantly from controls (54.2 ± 29.6 cells/mm^2) ($p<0.001$). In children *H. pylori* infected, there was a statistically significant difference ($p<0.001$) between the EGFR expression in epithelial and in gland cells of antral mucosa.

Conclusion: The increased EGFR expression in epithelial cells in comparison with gland cells of antral mucosa in children with *H. pylori* infection may suggest its role in regeneration processes of gastric mucosa.

Key words: EGFR, *Helicobacter pylori*, apoptosis, children

INTRODUCTION

EGFR (epidermal growth factor receptor) belongs to the family of membrane receptors, including ErbB1(EGFR), ErbB2/Her-2, ErbB3, ErbB4 and is a glycoprotein with a molecular mass of 170 kDa, built up of a single polypeptide chain, containing 1186 aminoacids [1]. The gene encoding EGFR is located on the chromosome 7, in the region 13 of a short chromosome anchor (p) and the region 22 of a long chromosome anchor (q) [2]. This receptor consists of the extracellular ligand binding region, the intracellular region with tyrosine kinase activity and the transmembrane region with a single hydrophobic anchor sequence, by which the receptor traverses the cell membrane a single time. The extracellular aminoterminal end is divided into four domains, including domain III responsible for ligand binding [3]. The ligands binding to EGFR are, apart from epidermal growth factor (EGF), transforming growth factor α (TGF-alpha), amphiregulin (AR), heparin–binding epidermal growth factor (HB-EGF), and betacellulin (BTC).

There are numerous known factors causing EGFR activation such as: P substance [4], bradykinine [5], thrombine [6], UV radiation [7], toxins produced by *Pasteurella multocida* [8], as well as bacteria such as *Salmonella typhimurium* [9]. Keates et al. [10] suggest that also *Helicobacter pylori* (*H. pylori*) bacterium is able to trigger EGFR phosphorylation.

Similarly, Higashi et al. [11] prove that intracellular protein CagA activates apathogenic pathway of Ras/MAPK (mitogen-activated protein kinase)/MAPK1 kinase (mitogen-activated protein kinase 1), which leads to the excessive EGFR-dependent cell proliferation.

The EGFR activation causes its homodimerisation and then heterodimerisation, which in turn causes autophosphorylation of five tyrosine radicals (Tyr 1173, 1148, 1086, 1068, 992) of this receptor [12]. This results in the activation of tyrosine kinase triggering numerous pathogenic pathways with the contribution of the Ras/Raf/ MAPK1 kinase and phospholipase C, phosphotydiloinositolo-3-kinase, activating proliferation and inhibiting apoptosis [13].

The EGFR receptor plays also a vital role in maintaining the integrity of the mucosa of the gastrointestinal tract [14]. It modulates the balance between the processes of proliferation and apoptosis in both normal and damaged mucosa of the gastrointestinal tract. Its presence regulates the growth, survival and as well as differentiation of epithelial cells. The tissue repair processes depend on this receptor since it is capable of stimulating proliferation [15], migration [16], and angiogenesis processes [17]. It is also responsible for the regulation of processes taking place in the normal mucosa as well as influences the pathophysiology of diseases connected with excessive cell proliferation e.g., cancers of the esophagus, stomach and intestines. Apart from the gastrointestinal tract, the EGRF expression increases in numerous cancerous cells e.g. cancer of the lungs, neck, head, prostate, urine bladder, pancreas and ovary.

The aim of the study was to evaluate immunohistochemically EGFR expression in epithelial cells and gland cells of antral gastric mucosa in children with *H. pylori* infection.

MATERIAL AND METHODS

The study included 44 children and adolescents aged from 5 to 18 years (mean age 13±3.4 years) with dyspeptic symptoms from the gastrointestinal tract e.g., chronic or recurring stomachaches, eating disorders, nausea and vomits which were the indication for gastroscopy. The study group consisted of 30 children (68.2%) with *H. pylori* infection (Hp+), confirmed by an rapid urease test and histopathological examination after exclusion of celiac disease and *Giardia lamblia* infection.

The control group included 14 children (31.8%) with functional disorders of the gastrointestinal tract, a negative patient's history and without symptoms indicating food allergy. *H. pylori* infection (negative urease test and negative histopathological examination) and *Giardia lamblia* infection (negative mucus cytology from duodenum-a brush swab in order to estimate trophozoite) were excluded.

Group with H. pylori infection

(Hp+) group –This group included 30 children (68.2%); 14 girls (it should be 31.8% from 30 children) and 16 boys (it should be 36.4% from 30 children), aged from 5 to 18 years (mean age 14±3.4 years), *H. pylori* infected, with a positive titre of specific IgG antibodies against *H. pylori* and a positive urease test. In the histopathological examination, chronic active gastritis, classified according to the modified Sydney System was determined in these children.

Control group (C)

Controls included 14 children (31.8%); 7 girls (it should be 15.9% from 14 children) and 7 boys (it should be 15.9% from 14 children) aged from 9 to 17 years (mean age 12.8±2.7 years) with a negative titre of specific IgG antibodies against *H. pylori* and normal gastric mucosa confirmed in the histopathological examination.

Methods

The kit "recom Well Helicobacter IgG" of Mikrogen GmbH firm was used to detect and identify directly IgG antibodies against *H. pylori* in the serum of the study children by means of the immunoenzymatic method ELISA. The concentration of IgG≥20 U/ml was considered positive.

Gastroscopy was performed in all 30 children with a positive titre of specific IgG antibodies against *H. pylori* and with dyspeptic symptoms from the gastrointestinal tract. Endoscopy was also performed in 14 children from the control group. Both 30 children with *H. pylori* infection and 14 children from the control group had not been treated before the gastroscopy against *H. pylori* infection. General anesthesia was applied during endoscopy in children below the age of 6 and older children, not cooperating. Pediatric size gastroscopes (Olimpus Q 145) were used. During gastroscopy a rapid urease test was performed (CLO-test=*H.pylori*), using kits produced by the Institute of Food and Nutrition in Warsaw.

Biopsy specimens were taken in gastroscopy according to the current standard. Gastric mucosa specimens obtained in gastroscopy were fixed in 10% formalin, next embedded in paraffin and cut off on the microton. Histopatological changes were evaluated by staining with hematoxyline and eosine (H-E). *H. pylori* bacilli were confirmed in the antral gastric mucosa samples when staining by the modified Giemsa's method.

Endoscopic and histopathological assessment of gastric mucosa was performed according to the Sydney System. Each histological parameter of severity (mononuclear cell infiltration) and activity (neutrophils infiltration) of gastritis was graded on a four-point scale: 0-absent, 1-mild, 2-moderate, 3 severe.

All samples taken during gastroscopy were prepared to evaluate EGFR expression in epithelial and gland cells of antrum mucosa according to the manual of a detection kit of EnVision+System–HRP (DAKO). Microscopic preparations were dewaxed using xylene,

Table 1. **EGFR expression in epithelial cells of antral mucosa (number of cells /mm^2) with regard to the severity of inflammation in the study children.**

EGFR in epithelial cells of antral mucosa (number of cells /mm^2)							
Severity of inflammation	Number of patients (N)	Arithmetical mean ($\bar{x}$)	Minimum value (min)	Maximum value (max)	Median (Me)	Standard deviation (±SD)	Significance of differences (p)
Mild+ Moderate	18	84.7	45.0	98.0	87.5	14.6	p<0.001
Severe	12	79.2	4.0	97.0	79.0	15.4	p<0.001
Control (c)	14	10.2	1.0	21.0	11.0	5.0	–

p–statistical significance of difference with control group (c)

and then treated with a 7-minute-activity of the solution of Proteinase K-(DAKO/code S3020). The activity of endogenic peroxidase was blocked by 20-minute-incubation of microscopic preparations in 3% hydrogen peroxide. After 3-fold-washing of all preparations in the phosphate–buffered of physiological saline (PBS), they were incubated for 2 hours at the temperature of 37°C with EGFR in the dilution of 1:50. The 0.1% solution of bovine albumin (Sigma-Aldrich) was used to reduce binding of non-specific antibodies. The reaction was visualized with diaminobenzidine DAB (DAKO S3000, DAKO, Poland).

The EGFR expression was evaluated quantitatively in the antral gastric mucosa using the programming of Soft Imaging System Cell-(Olimpus firm). In each case, the number of cells with the EGFR expression was calculated in 4 fields of the preparation of the 1mm^2 area (Olympus CX41) under enlargement of 40.

Statistical analysis

Statistica 6.0 program was used to analyze statistically the study results. Arithmethic mean ($\bar{x}$), standard deviation (SD), median (M_e), minimum result (min) and maximum result (max) were calculated. The correlations between the study parameters were assessed by the U Mann-Whitney's test and the pair order Wilcoxon's test. The differences were considered as significant at $p \leq 0.05$.

The Bioethical Board of the Medical University of Białystok of Poland expressed consent for conducting the study (the number of the consent R-I-003/22/2006).

RESULTS

Screening examinations of IgG titre against *H. pylori* in children infected with *H. pylori* proved varied values, ranging from 23.9 U/ml to 376.0 U/ml (mean value 121.8± 81.9 U/ml). A statistically significant difference was determined between the serum concentration of IgG against *H. pylori* in children with *H. pylori* and controls (mean value 3.4±3.2 U/ml) (p<0.001).

The evaluation of histopathological changes in gastric mucosa of children with *H. pylori* infection proved moderate antral gastritis (56.7%) and severe antral gastritis (40%). Mild gastritis was determined in 3.3% of the examined. Antral gastritis of severe activity was found in 66.7% and of moderate activity in 30 % of children infected with *H. pylori.* Antral gastritis of mild activity was observed only in 3.3 % of children *H. pylori* infected.

In children with *H. pylori* infection, the EGFR expression in epithelial cells of antral mucosa equaled on average 82.5±15 cells/mm^2 and ranged from 45.0 to 98.0 cells/mm^2, being statistically significant when compared to controls (10.2±5.0 cells/mm^2) (p<0.001).

The highest expression of EGFR in epithelial cells of antral mucosa (mean 84.7±14.6 cells/mm^2), and statistically significant in comparison with controls (p<0.001) was determined in children *H. pylori* infected with mild and moderate gastritis. It ranged from 45.0 to 98.0 cells/mm^2. In children *H. pylori* infected with severe antral gastritis, the EGFR expression in epithelial cells of antral mucosa was on average slightly lower and equaled 79.2±15.4 cells/mm^2 (from 49.0 to 97.0 cells/mm^2), being statistically significant in comparison with controls (p<0.001) (*Tab. 1*).

In children with *H. pylori* infection, the EGFR expression in gland cells of antral mucosa ranged from 2.0 to 85.0 cells/mm^2 (mean 25.7±22.6 cells/mm^2); was lower and differed statistically in comparison with controls (54.2±29.6 cells/mm^2) (p<0.001).

The EGFR expression in gland cells of antral mucosa was significantly lower in children with *H. pylori* infection with mild and moderate gastritis than in controls and ranged from 2.0 to 82.0 cells/mm^2 (mean 28.2±23.3 cells/mm^2); differed statistically significantly when compared to controls (p<0.01).

In children with *H. pylori* infection and severe gastritis of antral mucosa, the mean expression of EGFR in gland cells equaled 22.1±21.9 cells/mm^2 (range from 2.0 to 85.0 cells/mm^2) and was statistically significant in comparison with controls (p< 0.003) (*Tab. 2*).

The comparison of the EGFR expression in epithelial cells and gland cells of antral mucosa in children with *H. pylori* infection with regard to the inflammation severity proved that its expression was higher in epithelial cells than in gland cells.

In children *H. pylori* infected with mild and moderate antral gastritis, a statistically significant difference (p<0.002) was determined between the EGFR expression in epithelial cells (84.7±14.6 cells/mm^2), and in gland cells of antral mucosa (28.2±23.3 cells/mm^2). There was also a statistically significant

Table 2. **EGFR expression in gland cells of antral mucosa (number of cells /mm²) with regard to the severity of inflammation in the study children.**

EGFR expression in gland cells of antral mucosa (number of cells /mm²)							
Severity ofinflammation	Number of patients (N)	Arithmetical mean ($\bar{x}$)	Minimum value (min)	Maximum value (max)	Median(Me)	Standard deviation (±SD)	Significance of differences (p)
Mild+ Moderate	18	28.2	2.0	82.0	20.5	23.3	p<0.01
Severe	12	22.1	2.0	85.0	16.0	21.9	p<0.003
Control (c)	14	54.2	10.0	95.0	51.5	29.6	–

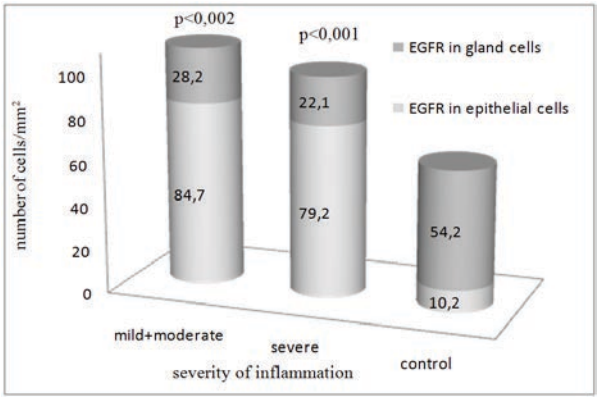
p–statistical significance of difference with control group (c)

Table 3. **EGFR expression in epithelial cells of antral mucosa (number of cells /mm²) with regard to the activityof the inflammation in the study children.**

EGFR expression in epithelial cells of antral mucosa (number of cells /mm²)							
Activity of inflammation	Number of patients (N)	Arithmetical mean($\bar{x}$)	Minimum value (min)	Maximum value (max)	Median(Me)	Standard deviation (±SD)	Significance of differences (p)
Mild+ Moderate	10	85.7	59.0	96.0	87.5	10.9	p<0.001
Severe	20	80.9	45.0	98.0	85.5	16.6	p<0.001
Control (c)	14	10.2	1.0	21.0	11.0	50	–

p–statistical significance of difference with control group (c)

Figure 1. **EGFR expression in epithelial cells and gland cells of antral mucosa (number of cells /mm²) with regard to the severity of inflammation in the study children.**



p–statistical significance of difference with control group (c)

(p<0.001) difference between this receptor expression in epithelial cells (79.2±15.4 cells/mm²) and gland cells (22.1±21.9 cells/mm²) in the study children with severe antral gastritis (*Fig. 1*).

Evaluation of the EGFR expression in epithelial cells with regard to the activity of inflammation of antral gastritis in children *H. pylori* infected showed its highest expression in children with gastritis of mild and moderate activity (mean 85.7±10.9 cells/mm²); it was statistically significant when compared to controls (10.2±5.0 cells/mm²) (p<0001).

In children with *H. pylori* infection with gastritis of severe activity of inflammation, the EGFR expression in epithelial cells of antral mucosa equaled 80.9±16.6 cells/mm² (range from 45.0 to 98.0 cells/mm²) and differed statistically significantly in comparison with controls (p<0.001) (*Tab. 3*).

The lowest mean expression of EGFR in gland cells of antral mucosa (22.4±20.6 cellsmm²) was revealed in children *H. pylori* infected with gastritis of severe activity of inflammation. It differed statistically significantly (p<0.001) when compared to the mean expression of EGFR in gland cells of antral mucosa in controls. In children *H. pylori* infected with gastritis of mild and moderate activity, the EGFR expression in gland cells of antral mucosa was on average 32.4±26.0 cells/mm² (from 10.0 to 82.0 cells/mm²) (*Tab. 4*).

Comparison of the EGFR expression in epithelial cells and gland cells in children *H. pylori* infected with regard to the activity of inflammation proved its higher expression in epithelial cells, similarly to the results referring to the severity of antral mucosa inflammation (*Fig. 2*).

In children *H. pylori* infected with gastritis of mild and moderate activity a statistically significant difference (p<0.001) was revealed between the EGFR expression in epithelial cells (85.7±10.94 cells/mm²)in comparison with its expression in gland cells (32.4±26.0 cells/mm²) of antral mucosa. Similarly, there was a statistically significant difference between this receptor expression in epithelial cells (80.9±16.6 cells/mm²) and gland cells (22.4±20.6 cells/mm²) in children *H. pylori* infected with gastritis of severe activity of inflammation (p<0.008) (*Fig. 2*).

DISCUSSION

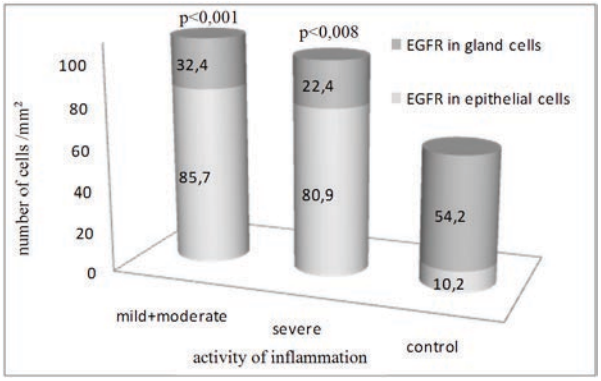
H. pylori is a main etiological factor of chronic gastritis in children, and peptic ulcer as well as the development of stomach cancer in adults. *H. pylori* elicits infiltration with neutrophils, eosinophils, lymphocytes and plasma cells. The

Table 4. **EGFR expression in gland cells of antral mucosa (number of cells /mm2) with regard to the activity of inflammation in the study children.**

EGFR expression in gland cells of antral mucosa (number of cells /mm^2)							
Activity of inflammation	Number of patients (N)	Arithmetical mean($\bar{x}$)	Minimum value (min)	Maximum value (max)	Median(Me)	Standard deviation (±SD)	Significance of differences (p)
Mild+ Moderate	10	32.4	10.0	82.0	22.5	26.0	NS
Severe	20	22.4	2.0	85.0	16.0	20.6	p<0.001
Control(c)	14	54.2	10.0	95.0	51.5	29.6	–

p−statistical significance of difference with control group (c)

Figure 2. **EGFR expression in epithelial cells and gland cells of antral mucosa (number of cells /mm^2) with regard to the activity of inflammation in the study children.**



p−statistical significance of difference with control group (c)

pathogenesis of chronic inflammation of the gastric mucosa remains incompletely understood [18]. There are numerous pathogenic pathways, through which *H. pylori* induces apoptosis in gastric mucosa, e.g., a mitochondrial pathway, in the course of which mitochondrial proteins from the family of Bcl-2 are activated. The proteins of Bcl-xl, Bcl-2 from the family of Bcl-2 inhibit apoptosis whereas Bax, Bak, Bad, Bid proteins activate apoptosis through the inhibition/activation of caspase cascade, respectively.

Another pathogenic pathway is a receptor mechanism taking place with the participation of EGFR or the family of tumor necrosis factor receptors – TNFr. The membrane receptor of CD95 also belongs to this family. In the course of *H. pylori* infection then EGFR activation inhibits apoptosis in gastric mucosa and stimulates proliferation. Kovalenko et al. [19] underline the importance of EGFR in the pathogenesis of chronic gastritis in children *H. pylori* infected.

Findings and reports referring to the EGFR expression in adults infected with *H. pylori* are predominant in the literature. Romano et al. [20] suggest in their studies that the exposure of gastric mucosa cells to *H. pylori* infection leads to an increase in the EGFR expression.

Coyle et al. [21] indicate that the EGFR activation is one of pathogenic mechanisms leading to the excessive proliferation of gastric mucosa and cancerogenesis in *H. pylori* infection. Caputo et al. [22] postulate that *H. pylori* bacterium-induced damage to the mucosa of the gastrointestinal tract triggers the compensative expression of EGFR that stimulates cell proliferation and inhibits apoptosis through the cascade activation of MAP kinase.

Similarily, Wong et al. [23] using an immunoenzymatic method of ELISA proved that damage to antral mucosa in *H. pylori* infection caused an increase in the EGFR expression. According to these authors, EGFR expression plays a significant role in repair processes of ulceration in the course of *H. pylori* infection.

Schiemann et al. [24] using the PCR method found a decrease in the expression of mRNA EGFR in gastric mucosa of adults with *H. pylori* infection. Zarrilli et al. [25] indicate that *H. pylori* bacterium could cause stomach cancer through an increase in EGFR expression that in turn stimulates proliferation and inhibits apoptosis.

Ashktorab et al. [26] proved that *H. pylori* infection caused the increased production of EGFR, which in turn resulted in the impaired balance between proliferation and apoptosis in gastric mucosa and was associated with a risk of neoplasm.

The immunohistochemical examinations carried out by Abe at al. [27] showed an increase in the EGFR expression in foveolar cells of gastric mucosa and parietal cells of gastric glands.

The comparison of the EGFR expression in epithelial cells and gland cells of antral mucosa in children with *H. pylori* infection taking into consideration the severity and activity of inflammation proved that this receptor expression was higher in epithelial cells than in gland cells.

Kovalenko et al [19] examining bioptates of gastric mucosa obtained from 44 children, aged from 7 to 15 years, infected with *H. pylori* with diagnosed clinically chronic gastritis indicated that chronic gastritis was characterized by the maximum EGFR expression correlating with the severity of gastric mucosa inflammation.

Our study concerning the EGFR expression in epithelial cells and gland cells of antral mucosa in children infected with *H. pylori* may suggest the role of this receptor in the regeneration of antral mucosa.

Scarce findings and reports referring to this problem indicate the need to carry out further studies in children with chronic *H. pylori*−induced gastritis.

CONCLUSIONS

1. A statistically significant difference was determined between the EGFR expression in epithelial cells and gland cells in children *H. pylori* infected compared to controls.
2. The increased EGFR expression in epithelial cells in comparison with gland cells of antral mucosa in children with *H. pylori* infection may suggest its role in regeneration processes of gastric mucosa.

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