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Secretion of epidermal growth factor in saliva of duodenal ulcer patients; an association with *Helicobacter pylori* eradication and erosive esophagitis

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ABSTRACT

Purpose: Erosive esophagitis frequently develops after successful *Helicobacter pylori* eradication. Since salivary secretion of epidermal growth factor (EGF) plays a crucial role in the pathogenesis of gastroesophageal reflux disease, the current study objective was to find out whether erosive esophagitis development after *Helicobacter pylori* eradication is associated with the secretion of EGF in saliva.

Materials and methods: A total of 115 *H. pylori* infected patients (positive results of CLO-test, histology and serology) with a duodenal ulcer were recruited for the study. Gastroscopic examinations and saliva collections were performed twice, prior to *H. pylori* eradication and one year after its cessation. The salivary EGF was determined using a radioimmunological method.

Results: Salivary EGF secretion was lower in *H. pylori* infected subjects with erosive esophagitis than without (0.82±0.66 vs 1.70±3.49 ng/min, p=0.021). However, a year after successful *H. pylori* eradication, salivary EGF did not differ between the groups (2.17±2.06 vs 1.79±2.06 ng/min); the lack of difference was due to high peptide secretion in patients who developed erosive esophagitis after eradication.

Conclusion: Erosive esophagitis development following *H. pylori* eradication is not the result of decreased salivary EGF secretion.

Key words: EGF, esophagitis, *Helicobacter pylori* eradication, saliva

INTRODUCTION

Reflux esophagitis is a condition related to a disturbed balance between damaging agents of gastric origin (HCL, pepsin) and defensive ones (saliva, secretion of esophageal submucosal mucus glands). Epidermal growth factor (EGF), among many others, is a known factor protecting the esophagus against reflux gastric content. EGF has been found both in saliva and in esophageal submucosal mucus glands' secretion and its involvement in esophageal protection has been documented in studies conducted both in animals and humans [1-4]. EGF is a peptide which, on the one hand, improves protective properties of the mucus lining the esophageal epithelium and, on the other, accelerates esophageal epithelium regeneration in the case of its damage [3].

Erosive esophagitis frequently accompanies a duodenal ulcer and its occurrence increases after successful *Helicobacter pylori* eradication [5]. Since eradication of *H. pylori* alters the secretion and composition of saliva [6], the aim of the current study was to answer the question whether *H. pylori* eradication affects salivary EGF secretion, thus promoting erosive esophagitis.

Table 1. **Patients' data.**

Number	115
Gender (M / F)	89 / 26
Age (mean, range)	42.3 (18-70)
Smokers / non-smokers	76 / 39
Drinkers / non-drinkers	59 / 56

MATERIALS AND METHODS

Patients

A total of 115 patients of both genders, aged 18-70 years, with a duodenal ulcer and *H. pylori* infection (positive urease test and histology of endoscopic specimens of gastric mucosa as well as plasma anti-*H. pylori* IgG and / or IgA antibodies) were recruited for the study (*Tab. 1*). The qualifying criteria for inclusion were: duodenal ulcer niche 0.5-1.0 cm in diameter, good general health, normal basic laboratory test results and a low incidence of abdominal complaints. The exclusion criteria were: pregnancy, lactation, or drug intake (except for antacids) within 7 days preceding the gastroscopic examination. Also patients with a complicated duodenal ulcer, with a history of unsuccessful eradication therapy, who abuse alcohol (>100g ethanol/day), take non-steroid anti-inflammatory drugs or one or two antibiotics used for eradication therapy within the previous two months, and those with salivary glands abnormalities were excluded from the study.

Endoscopic examination and H. pylori testing

A gastroscopic examination was performed twice, prior to *H. pylori* eradication and one year after its cessation, by one endoscopist using a GIF V2 or GIF Q145 gastroscope. Gastric mucosa specimens were obtained from the prepyloric region and the corpus of the stomach: one for a urease test (CLO-test), and two for a histological examination. At the initial visit, 2 ml of venous blood was taken from each subject to measure the titre of IgG and IgA anti-*H. pylori* antibodies with enzyme-linked immunosorbent assay (*recom*Well *Helicobacter* IgG and *recom*Well *Helicobater* IgA, MICROGEN). Prior to therapy, the subject was considered infected if the serology and two tests for the presence of *H. pylori* in the gastric mucosa were positive. At the check-up (one year later), the patient was considered non-infected if the two tests for the presence of *H. pylori* in the gastric mucosa specimens were negative.

Eradication therapy

All the patients were treated for 7 days with a set of omeprazole (Losec) 20mg bid, amoxicillin (Ospamox) 1000mg bid, and clarithromycin (Klacid) 500mg bid. For 14 consecutive days, omeprazole (20mg) was administered. Between therapy termination and the check-up, the subjects could only take antacids.

Saliva collection

Saliva was collected twice, one day after the initial gastroscopy and one hour before the final gastroscopic examination. Salivary samples were obtained in the morning hours, after overnight fasting and abstaining from fluids since midnight. Smokers were asked not to smoke in the hours preceding saliva collection. The samples were collected for 20 minutes. Due to substantial differences in saliva secretion in the first minutes of the examination, the samples obtained during the first 5 minutes were discarded.

The glass cup used for saliva collection was initially weighed. The procedure was repeated after a sample collection. After careful mixing, a 1 ml saliva sample was taken from the cup with an automatic pipette and then the cup was weighed again. Once the mass of the 1 ml sample was known, it was possible to determine its volume in the cup and calculate its secretion within one minute. Next, the saliva was frozen and stored at -20˚C.

Research techniques

The concentration of EGF was determined in defrosted saliva by means of a radioimmunological (RIA) method, using a commercially available kit (Amersham), as described earlier [7]. A standard curve was prepared with human recombinant EGF (Sigma). Samples were analyzed in duplicates. The intraassay and interassay variability was 4.2% and 7.5%, respectively.

Statistics

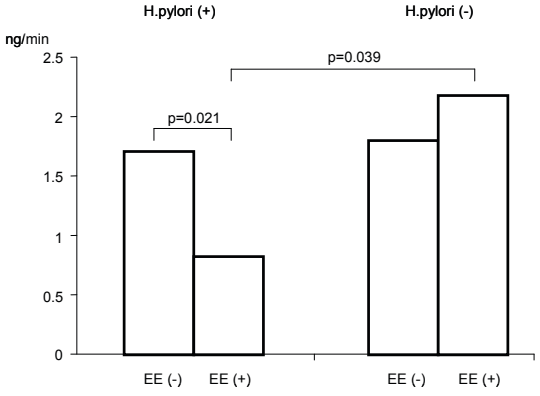
Results (mean ± SD) were subjected to statistical analysis using the Mann-Whitney U test or the Wilcoxon test. The study was approved by the Bioethical Committee of the Medical University of Białystok.

RESULTS

Of the 115 patients with duodenal ulcer recruited for the study, esophageal erosion was found in 13 (grade A – 10 patients, grade B – 3 patients; Los Angeles classification [8]). After a year, a check-up was performed on 81 patients; 21 subjects were excluded due to the intake of disallowed medications (mainly those inhibiting gastric secretion), 13 withdrew their consent for the study. In 64 of 81 patients, eradication therapy was successful. In 16 of 81, erosive esophagitis was detected during the second gastroscopy (grade A – 12 patients, grade B – 4 patients). In 12 subjects, esophagitis developed after successful eradication, in 4 it was also present before eradication therapy began; in the latter, eradication was also successful.

Prior to eradication therapy, secretion of salivary EGF was significantly lower in patients with erosive esophagitis than without (0.82±0.66 vs 1.70±3.49 ng/min, p=0.021) (*Fig. 1*). A year after successful eradication, differences in salivary EGF

Figure 1. **Salivary EGF secretion in *H. pylori* infected and non-infected subjects with regard to diagnosed erosive esophagitis (EE).**



secretion between the two groups were statistically insignificant (2.17±2.06 vs 1.79±2.06 ng/min) (*Fig. 1*). Since in patients with erosive esophagitis, EGF secretion was higher after *H. pylori* eradication than before therapy, the study patients were divided into 4 subgroups: 1) those with erosive esophagitis present both before and after eradication, 2) those with erosive esophagitis present before but not after eradication, 3) those with erosive esophagitis which developed after *H. pylori* eradication therapy, 4) those without erosive esophagitis before and after *H. pylori* eradication. According to this division, the lack of differences in salivary EGF secretion between patients with and without erosive esophagitis after eradication therapy may be due to the fact that peptide secretion was twice as high in patients with erosive esophagitis that developed after successful eradication as compared to those who had it before therapy (*Tab. 2*).

DISCUSSION

The finding of *H. pylori* in the stomach has caused an evolution of opinions concerning the pathogenesis and treatment of peptic ulcer disease, and has simultaneously contributed to a better understanding of a number of phenomena associated with the pathogenesis of reflux esophagitis.

The current study was inspired by the following facts: 1) the key role of hydrogen ions refluxed from the stomach in the pathogenesis of esophagitis [9,10], 2) increased incidence of erosive esophagitis after *H. pylori* eradication from the stomach [5,11] and 3) a protective role of salivary EGF in the development of esophagitis [1,3,4]. The study involved patients with a duodenal ulcer, since these patients demonstrate high gastric secretion of hydrogen ions and relatively frequently develop erosive esophagitis after *H. pylori* eradication [5,11].

The causes of erosive esophagitis that develops in duodenal ulcer patients after *H. pylori* eradication are still not fully elucidated; one possibility could be changes in salivary secretion and composition [12-14]. The role of saliva in the protection of the esophageal mucosa has been frequently mentioned, with emphasis on a beneficial effect of salivary bicarbonate, mucin and EGF [1,12,15,16]. However, the role of salivary EGF in esophagitis that appears following *H. pylori* eradication from the stomach has never been investigated.

Earlier findings have indicated that in duodenal ulcer patients, saliva secretion, glycoprotein and bicarbonate contents increase a year after successful *H. pylori* eradication [6]. These results pertaining to the direction of changes are consistent with our present findings, although in the study cited above, the follow-up gastroscopy was not performed a year after therapy. Thus, it is unknown how many patients developed erosive esophagitis and whether there was a relationship of saliva secretion and its protective components with erosive esophagitis.

In erosive esophagitis, saliva secretion and salivary components that play a protective role against the refluxed gastric content in the esophagus may be reduced [1,16]. The current data seem to partly confirm this assumption. In patients with a duodenal ulcer and erosive esophagitis already present before antibacterial therapy, the secretion of salivary EGF was significantly lower than in the group without erosive esophagitis. If salivary EGF had a protective effect against the damaging action of acid on the esophageal mucosa, we should expect its reduced secretion in patients with erosive esophagitis developing after eradication therapy. However, we obtained the opposite result; salivary EGF secretion was the highest in these patients, although no statistically significant differences were observed in comparison to the remaining groups. This could mean that apart from the protective role of EGF within the esophagus, an increased salivary EGF secretion following *H. pylori* eradication does not prevent development of erosive esophagitis.

The increased incidence of erosive esophagitis in patients with a duodenal ulcer after *H. pylori* eradication has been described in a number of studies [5,11,17]. In these studies,

Table 2. **Salivary EGF secretion (ng/min) in duodenal ulcer patients before and after *H. pylori* eradication with regard to diagnosed erosive esophagitis (EE).**

		One year after completion of eradication therapy	
	Basal	success	failure
EE present both before and after treatment	(4) 0.50±0.13	(4) 1.24±1.16	-
EE present only before treatment	(3) 0.65±0.22	(1) 1.25	(2) 0.91±0.89
EE developed after treatment	(12) 2.12±2.29	(12) 2.49±2.23	-
No EE either before or after treatment	(62) 1.28±1.16	(47) 1.80±1.73	(15) 1.93±2.63

however, patients were not investigated for non-erosive esophagitis before *H. pylori* eradication, and thus the presence of this form of reflux disease prior to the eradication procedure cannot be excluded. This assumption may be supported by microscopic investigations of the esophagus of duodenal ulcer patients without endoscopic features of reflux disease; 70% of them had histological features of inflammation [18]. It can be thus assumed that some duodenal ulcer patients had undiagnosed esophagitis prior to *H. pylori* eradication. In some of them, the non-erosive form may thus have changed into an erosive one.

Gastroesophageal reflux disease symptomatology (heartburn/regurgitation) as well as 24h esophageal pH-monitoring test could be helpful in interpreting the obtained results; unfortunately, these points have not been placed in the study protocol.

Reflux esophagitis development after *H. pylori* eradication has a mild clinical course [19], perhaps due to a high secretory compensation from the salivary glands. Although the reflux esophagitis present before eradication and developing after this procedure is the same disease, different pathogenetic mechanisms may be involved; low saliva secretion and its protective components can promote erosive esophagitis in patients infected with *H. pylori*, but not in patients who develop erosive esophagitis after eradication.

Taking the above into consideration, it can be suggested that changes in salivary EGF secretion observed after *H. pylori* eradication do not have a negative impact on the protective salivary potential for the esophageal mucosa.

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