An Adaptive Evolution of *Helicobacter pylori*: Role of the CagA Presence in the Outcome of *H. pylori* Eradication in Children

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**Abstract**

**Introduction:** The aim of the study was to determine the evolutionary role of the CagA presence in the outcome of eradication treatment of *H. pylori*.

**Materials and methods:** Sixty-seven pediatric dyspeptic patients (median age 13.7 years, range 5 to 17 years, male/female 24/43) underwent endoscopy for *H. pylori* presence. Gastric biopsy specimens were taken for histology and/or culture and one biopsy sample was used for CagA PCR determination. *H. pylori* positive patients were treated for 2 weeks by amoxicillin (50 mg/kg/day), bismuth subcitrate (8 mg/kg/day), nifuratel (30 mg/kg/day) plus omeprazole (1 mg/kg, once daily).

**Results:** Forty one of 67 children (61.2%) were *H. pylori* positive. Nineteen of 41 strains (46.3%) were CagA positive and 22 were CagA negative (53.7%). *H. pylori* was eradicated in 33 patients (80.4%). Among the patients with successful eradication 18 children were CagA positive, fourteen were CagA negative.

Thus, more patients with CagA-negative status had evidence of ongoing *H. pylori* infection (36.4% (8/22) versus 5.3% (1/19); \(\chi^2=4.08, \ p=0.0021\); Fisher’s exact test \(p=0.0238\)).

**Conclusion:** In our study the carriage of CagA-lacking strain was associated with failure of treatment. In our opinion, this phenomenon, related to extraordinary genome plasticity, not only allows the microbe to maintain balanced relationship with the host, but also to survive in antibacterial therapy conditions.

**Keywords:** Evolution; *Helicobacter pylori*; Children; Eradication; Nifuratel

**Introduction**

A resistance to antibacterial drugs is a classic demonstration of natural selection in microorganisms during the period of host-bacterial interaction and high intensity of antibiotic exposure [1]. Many bacterial pathogens probably have affected humans for more than 15,000 years [2]. It is obvious, that the host-bacterial interaction can potentially be accompanied with microevolution of bacteria during long-term colonization protracting for lifelong persistence. Furthermore, subclinical infections are more common than apparent disease; bacteria may modulate their virulence in ways that prolong the interactions with the host to optimize transmission [3]. Some of the genes associated with antibacterial resistance have a long evolutionary history of diversification that began well before the antibiotic era [5,6]. Environmental forces (the release of large amounts of antibacterials et cetera) might change natural ecosystem to alteration of the population dynamics of the microorganisms, including selection of resistance with consequences for human health that is difficult to predict [4]. There is an evidence that *Helicobacter pylori* (H. pylori) has been colonizing humans for more than ten millions years or longer [2]. *H. pylori* is a gram-negative microaerobic, spiral bacterium that colonizes stomachs of approximately over 50% of the world human population. *H. pylori* infection is associated with chronic gastritis and peptic ulceration development and is also considered to be a risk factor for development of gastric cancer [2]. There are many factors that might be important for treatment outcome, including age CagA status, clinical presentations (e.g. dyspepsia vs. duodenal ulcer) et cetera [5,6]. An important virulence determinant of *H. pylori* is a CagA gene product which is found in approximately 40% isolates obtained from children [6]. The CagA gene is located at one end of the cag pathogenicity island (PAI), an approximately 40-kilobase region that is incorporated into the *H. pylori* genome by horizontal transfer from an unknown natural source [7]. Most studies considered cagA as a marker of the cag PAI [8,9]. The CagA-positive strains are associated with a higher grade of gastric or duodenal inflammation and are more virulent than the strains lacking it. Furthermore, the CagA gene of *H. pylori* is assumed as responsible for signaling mechanisms that lead to development of gastric adenocarcinoma [10]. Deletion of cag PAI by an inductive signal or selective forces may promote the readaptation of the pathogen at different stages of infection [3]. Taking into consideration that CagA gene (and cag PAI) are the basic virulence factors, the excision of cag PAI may demonstrate that attenuating the virulence of *H. pylori* strain can be an evidence of development of favourable adaptation. The aim of the study was to determine the evolutionary role of the CagA presence in the outcome of eradication treatment of *H. pylori*.

**Materials and Methods**

Between January 2009 and February 2010, 67 pediatric patients with upper dyspepsia (median age 13.7 years, range 5 to 17 years, male/female 24/43) undergoing endoscopy in the Outpatient Department of Children’s Republican Hospital of Bashkortostan Republic (Ufa, Russian Federation) were investigated for *H. pylori* presence. Fully informed parental consent was obtained in all cases. Upper gastrointestinal endoscopy was carried out after lidocaine throat anaesthesia using Olympus XP 20 gastroscope (Japan). Gastric
CagA pylori eradication there were 18 children from in 32 (78.04%) patients overall. Among the patients with successful four-six weeks after stopping the treatment, the CagA status was determined by PCR after DNA extraction. The biopsy samples were ground with tissue homogenizer and resuspended in 300 µl of extraction buffer (20 mM Tris–HCl, pH 8, 0.5% Tween 20), and proteinase K (0.5 mg/ml) was added. The mixture was incubated for 1 hour at 56ºC. Finally, the enzyme was inactivated by boiling for 10 minutes [9]. The CagA status was determined by amplification of fragments of the CagA gene, as described by Govorun et al. [12]. A positive CagA status was defined as positive CagA PCR results with one of the two primer sets (eg Figure 1).

H. pylori positive patients were treated with a two-week course of amoxicillin (50 mg/kg/day), bismuth subcitrate (8 mg/kg/day), nitirufate (30 mg/kg/day) plus omeprazol (1 mg/kg, once daily) [13]. The criterion for eradication of H. pylori was the negative breath ammonia test (Helic test, AMA, Saint-Petersburg, Russia) in 4-6 weeks after the treatment completion. Also compliance was estimated in both groups at the end of the treatment and was graded as excellent if over 80% of the provided drugs had been used, fair if 60-80% had been used and poor if less than 60% had been used. Statistical analysis was performed by using the SAS 9.3 statistical package (SAS Institute, 2011) [14]. The significance of difference in categorized data was tested by the chi-squared test and Fisher’s exact test. For the both tests, a P-value under 0.05 was considered to have statistical significance. Estimated odds ratios (OR) and 95% confidence intervals (95% CI) were calculated as well.

Results
Forty one of 67 children (61.2%) undergoing endoscopy were H. pylori culture positive initially. Nineteen of 41 strains (46.3%) were CagA positive (group I) and 22 were CagA negative (53.7%) (group II). Four-six weeks after stopping the treatment, H. pylori was eradicated in 32 (78.04%) patients overall. Among the patients with successful eradication there were 18 children from CagA-positive sample (group I) and 14 from CagA-negative sample (group II). Thus, more patients in group II (CagA-negative status) had evidence of ongoing H. pylori infection (36.4% (8/22) versus 5.3% (1/19); χ²=4.08, p=0.0423). Nine children from both groups (21.96%) remained H. pylori positive. So, all of the unsuccessfully treated patients were CagA-negative initially; with the exemption of one CagA-positive carrier. It seems that the carriage of CagA-lacking strains of H. pylori are associated with unsuccessful eradication according to our data (OR=10.28; 95CI, 1.148 to 92.173). Importantly, compliance was equal in both groups, no patients dropped out the study (over 80% of the provided drugs had been used by all patients).

Discussion
From a biological point of view, the explanation of relationship between H. pylori eradication outcome and CagA status can have many reasons:

1. CagA-positive strains grow faster and would be more susceptible to antibiotics with the interfering metabolism dividing cells than CagA-negative ones, which may be in stationary phase;
2. Strain, expressing CagA, enhance production of proinflammatory cytokines and increase inflammation in gastric mucosa (and mucosal blood flow respectively) which may be favourable for antibiotics diffusion;
3. Proinflammatory cytokines are potent inhibitors of gastric acid secretion and can potentiate efficacy of PPI action [9,15].

Earlier, we found inverse negative relationship between CagA carriage and rdxA gene deletion in H. pylori strains (p=0.0001) [16]. Also it is well known that, alterations in the rdxA gene of H. pylori are required for moderate metronidazole resistance [17]. Similar data was presented by other authors as well [18]. Therefore, absence of cag PAI can be a risk factor for metronidazole resistance and plays a crucial role in successful eradication of H. pylori [19]. However, since we did not include metronidazole to eradication regimen in our particular study its resistance role is unconsidered. Results of the present study can be considered as an illustration of successful adaption of genome of H. pylori to environmental influences (antibiotics, host physiology). From our point of view, the evolutionary forces are capable to demonstrate a strong influence on the variable region of genes of virulence (CagA in this case) with low biological cost to the host and the microbe. The strains of H. pylori lacking the cag PAI, or parts of it, have lost the island through transformation with an empty site or through deletion mutations [20]. It is possible that this particular phenomenon associated with genome plasticity not just allows the microbe to maintain balanced relationship with the host, but also to survive in antibacterial therapy conditions.

The obtained results seem to reveal the source of genetic diversity among the strains and differences in risk for outcome of gastroduodenal pathology among human carriers of H. pylori [21]. Possibly, successful adaption to evolutionary forces also contributes to extraordinary chronicity of H. pylori infection [22]. Natural selection which helps to fix particular mutants or recombinant genotypes with beneficial alleles could turn a host-pathogen interaction into almost symbiotic relationship. As it was shown by P. Ewald, for agents of disease that are spread directly from person to person, low virulence is more preferable, as it allows the host to remain active and contact with other potential hosts [23]. An organism that kills his host (lethal outcome of peptic ulcer or gastric cancer) may never obtain a new host, so natural selection would seem to favour lower virulence. The colonies of H. pylori with functional cag PAI could be potentially more harmful to...
humans than those lacking it, at least due to the fact that gastric cancer is a highly lethal disease.

Undoubtedly, the study of virulence determinants of *H. pylori* that have been subject to natural selection in particular populations can provide new understanding of mechanisms in effective eradication of this infectious agent in pediatric and adult patients worldwide.

Unfortunately, our study has several important limitations. Further research on the evolutionary role of the CagA presence in the outcome of eradication treatment of *H. pylori* is needed with larger sample sizes. To generalize the results, we should have included more participants at different levels since our study involved relatively low number of patients. Also it is obvious that, the PCR on cagA gene is an indirect evidence of a presence or absence of the pathogenicity island and the post hoc fallacy might have been remained since the loss of cagA gene is not only one risk indicator of the unfavourable outcome. However, strength for interpretation of the current results is derived from the fact that our research is the only one study in the given field from the middle part of Russia so far. Our study provides supplemental information to help resolve the worldwide conundrum over the evolutionary role of the CagA presence in the outcome of *H. pylori*-associated disease. Despite all the limitations in our work, we regard further investigations of evolutionary role of CagA carriage are necessary.

**Conclusion**

Our data again confirmed the opinion of a number of researchers on higher survivability of *H. pylori* European strains, not carrying functional CagA in intensive antibacterial therapy conditions [9,10]. Further studies are needed to assess the role of the evolutionary process in which bacteria are losing their virulence factors for better survivability in symbiosis with humans.

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