An inverse relation between CagA+ strains of *Helicobacter pylori* infection and risk of erosive GERD

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ABSTRACT

الهدف : الهدف من الدراسة هو من أجل التحقق من اتحاد الإصابة بعدوى بكتيريا هيليكوباكتر بايلوري وسلالة عصياتها(سي ايه جي ايه) مع التهاب المريء الارجاعي .

الطريقة : أجريت الدراسة على مجموعة التحكم في الفترة ما بين (مايو2005م 2006–م) والمرضى المصابين بالتهاب المريء الارتدادي(مجموعة الحالة) وتمت مقارنة العمر وتطابق نوع الجنس للذين يعانون من أعراض مرض الارتداد المعدي المريئي ولكن مع كون نتائج تنظير الجهاز الهضمي العلوي طبيعية(مجموعة التحكم) بمستشفى الإمام الخميني بمدينة تبريز بإيران،تمت مقارنة معدلات الإصابة ببكتيريا هيليكوباكتر بايلوري ونتيجة العدوى الإيجابية(جي ايه آر دي) لها مع المجموعتين والمجموعات الفرعية مع الاختلاف في شدة التهاب المريء .

النتائج : تم إدراج 92 و 93 مريضا في مجموعة الحالة ومجموعة التحكم على التوالي . كان معدل الإصابة ببكتيريا هيليكوباكتر بايلوري أقل بشكل ملحوظ في مجموعة التحكم (81.5% versus 87.10%, p=0.29, odd ratio 0.654, 95% Confidence interval [CI] 0.293 to 1.495.
كان الالتهاب الإيجابي أكثر تكرارا في مجموعة التحكم (59.1% versus 40.2%, p=0.01, odd ratio 0.465, 95% CI 0.258 to 0.836) .
لم يكن هنالك فرقا ملحوظا بين شدة المجموعات الفرعية لهذا المرض(لبكتيريا هيليكوباكتر بايلوري) أو معدلات الالتهاب الموجبة(سي ايه جي ايه) p=0.30 و p=0.40 على التوالي) .

خاتمة : قد يكون لسلالة (سي ايه جي) الموجبة أثر وقائي ضد التهاب المريء الارجاعي .

Objective: The aim of this study is investigating the association of *Helicobacter pylori (H. pylori)* infection and its cytotoxic-associated gene A (cagA) strain with reflux esophagitis.

Methods: In a case-control setting (May 2005-2006), patients with reflux esophagitis (case group) were compared with age and gender matched people suffering from symptoms of gastroesophageal reflux disease with normal upper gastrointestinal endoscopic findings (control group) in Imam Khomeini Hospital, Tabriz, Iran. The rates of *H. pylori* and its cagA positive infections were separately compared between the 2 groups and the subgroups with different severity of reflux esophagitis.

Results: Ninety-two and 93 patients were enrolled in the case and the control groups. The rate of *H. pylori* infection was insignificantly lower in the case group (81.5% versus 87.10%, p=0.29, odd ratio 0.654, 95% Confidence interval [CI] 0.293 to 1.495). The CagA positive infections were found significantly more frequent in the control group (59.1% versus 40.2%, p=0.01, odd ratio 0.465, 95% CI 0.258 to 0.836). There was no significant difference between the severity subgroups of the disease for *H. pylori* (p=0.30) or cagA positive infection rates (p=0.40).

Conclusion: The CagA positive strains might have a protective effect against reflux esophagitis.

It is well known that *Helicobacter pylori (H. pylori)*, a common gastroduodenal pathogen is connected with ulceration, dyspepsia, and adenocarcinoma.[1,2] Many factors including the host and environment, as well as the specific strain of the organism may play a role in development of the infection.[3] Gastroesophageal reflux disease (GERD) is a common and widespread problem

that many people might ever have experienced during their life, so the issue has gathered diverse enthusiasm in recent decades.[4] Surprisingly, it has been noticed while the prevalence of H. pylori infection has been declining, the incidence of GERD has been increasing in developed countries.[5] Some series concluded that the eradication of *H. pylori* from patients with duodenal ulcers may provoke GERD. However, there is heterogeneity of this hypothesis in the literature.[6] The cytotoxic-associated gene A (cagA), which has been shown to encode a highly immunogenic protein CagA, has been introduced as a marker of the cagA pathogenicity island (PAI).[7] Our study was specifically aimed at assessing the association between *H. pylori* and its cagA strain and endoscopically proved reflux esophagitis in a case-control study of patients being investigated for GERD symptoms.

Methods. *Patients.* Patients with symptoms of GERD persistent longer than 3 months were selected in the gastroenterology clinic of Imam Khomeini Hospital, Tabriz, Iran during May 2005-2006. These symptoms consisted of subjective complaints of heartburn and food or acid regurgitation at least once per week and longer that 3 months. Endoscopies were performed by the same gastroenterologist and the patients with erosive esophagitis without any other localized upper gastrointestinal (GI) lesion were assigned to the case group. The disease was endoscopically graded from A to D according to the Los Angeles classification system.[8] The control group consisted of patients with GERD symptoms but without any endoscopically recognized local lesion in the esophagus, stomach, and duodenum. The 2 groups were matched according to gender and age. The exclusion criteria for the 2 groups were pregnancy, history of gastric or the lower esophageal sphincter surgery, past or present peptic ulcer disease, presence of erosion in the stomach or duodenum, hiatal hernia greater than one centimeter in diameter, gastric outlet obstruction, upper GI bleeding, scleroderma, esophagitis secondary to infection, drug, and other causes, and history of taking proton pump inhibitors, histamine 2 receptor blockers over the 2 weeks and Bismuth and antibiotics over the last 4 weeks.

Detecting H. pylori infection and CagA strains. The presence of *H. pylori* infection was assessed by rapid urease test (RUT) and immunology. Any positive RUT or immunology result was considered as *H. pylori* infection. The gastric biopsy specimens for urease test were endoscopically taken from the antrum 2 cm from pylorus. For immunologic test, 2 cc of patient blood was centrifuged and the serum stored at -20°C. Anti *H. pylori* IgG titer was measured by a semi-quantitative kit through an immunoenzymatic reaction on the solid phase. Anti-cagA antibody titers (IgG) were determined

in the 2 groups without prior knowledge of *H. pylori* or esophagitis status by use of the cagA enzyme-linked immunosorbent assay (ELISA) kit. Standard charts were used to interpret the results on arb/ml unit. Anti-cagA IgG titer greater than 20 arb/ml was assumed as a positive serologic result. This study was approved by the Ethics Committee of Tabriz University of Medical Sciences. All patients signed the informed patient consent.

Statistics. The rate of *H. pylori* infection was compared between the age-categorized subgroups (younger than 40 years, 40-60 years, 60 years, and older) of the case and the control patients. The rates of *H. pylori* and its cagA strain infection were compared between the case and the control groups. These comparisons were repeated between the severity subgroups in the case patients and between the mentioned subgroups and the control patients. The mean titer of serum cagA antibody (arb/ml) was compared between the case and the control groups. Data were analyzed with the SPSS statistical software package (version 11.0, SPSS Inc, Chicago). Continuous variables were expressed as mean and categorical data were shown as frequency and percent. The contingency table (Chi square and Fisher's exact tests where applicable) and the Independent samples T test employed for comparisons. The *p* values below 0.05 were considered statistically significant. The odds ratio and the 95% CI were used as estimates of the risk.

Results. One hundred and eighty-five patients (93 patients in the case group and 92 people in the control group) were enrolled. The mean age of the participants was 43.1±15.5 years in the case group and 42.7±14.4 years in the control group (*p*=0.40). The 2 groups were matched for the gender (47 [51.1%] males and 45 [48.9%] females in the case group, 46 [49.5%] males and 47 [50.5%] females in the control group; *p*=0.82). Patients were divided into 3 groups according to their age (**Table 1**). The rate of *H. pylori* infection has been shown in each group and according to their gender. The rate of *H. pylori* infection in the case and control groups is summarized in **Table 2**. There is no significant difference (*p*=0.29, odds ratio 0.654, 95% CI 0.293 to 1.495). The rate of infection in different severity subgroups of the case patients has also been shown in **Table 2**. Note that there was no patient in group D. This rate is lower in the more severely affected subgroups (B+C) but not in a significant manner (*p*=0.39, odds ratio 1.604, 95% CI 0.538 to 4.787). As shown in **Table 2**, the rate of *H. pylori* positive patients in subgroup C is significantly lower than that in the control group (*p*=0.01). The rate of cagA strain positive patients was 92 (49.7%) cases in the whole studied population. As shown in **Table 2**, this rate was significantly lower in the case group (*p*=0.01,

odds ratio 0.465, 95% confidence interval 0.258 to 0.836). Comparing the rate of cagA strain positive patients in group A with the more severely affected subgroups (B+C) revealed no significant differences (*p*=0.83, odds ratio 1.098, 95% CI 0.475 to 2.538) (**Table 2**). As shown in **Table 2**, the rate of cagA strain positive patients in subgroup A is significantly lower than that in the control group (*p*=0.02). This rate was lower in more severely affected subgroups (B and C) as well, however they were not significant (**Table 2**). The mean titer of serum antibody against cagA was 42.2 arb/ml in the case group and 38.6 arb/ml in the control group (*p*=0.186).

Discussion. This study aimed at evaluating the role of *H. pylori* and particularly the cagA strain in patients with reflux esophagitis. The rate of *H. pylori* infection was not significantly different between the patients with erosive GERD and nonerosive reflux disease (NERD). Similar results were reached regarding the severity of the disease. Up to now, conflicting data has been reported by studies evaluating the association between the reflux esophagitis and the presence of *H.*

pylori infection. In some series, the rate of *H. pylori* infection was lower in patients with GERD than that in the control population, proposing a protective effect of *H. pylori* against the GERD,[9] whereas in other studies the rate of infection of *H. pylori* was similar[10] or even higher[11] in patients with GERD. Small sample size, inaccurate methods of detecting *H. pylori* infection, or lacking of controls or inappropriate selection are the main flaws of relating studies. In Sharma and Vakil's[12] review article, a low incidence of *H. pylori* infection has been demonstrated in patients with erosive esophagitis comparing with their healthy controls, however, other studies challenged these results. A high prevalence of *H. pylori* infection in Iran justifies the high rate of infection in our case and control groups. The rate of infection was lower in patients with erosive esophagitis, but not in a significant manner. As mentioned earlier, there is an ongoing debate on this issue in the literature. Some consider a protective effect for *H. pylori* infection while others do not. Our results are in conformity with the later viewpoints. In a study by Raghunath et al,[13] this heterogeneity has been emphasized. Geographical differences has been assumed as a cause. Higher prevalence of *H. pylori* infection among patients with erosive esophagitis comparing the healthy cases in an Asian study is in favor of this supposition.[12] This means that in some areas the prevalence of infection is very high (almost one hundred percent in some areas), so considering a role for this bacteria may be impossible. For determining the interfering effects of overall prevalence of *H. pylori* infection, geographical differences, and the type of gastritis on this probable interaction, further studies should be carried out, again emphasizing on these confounding factors. In our study, there was not any difference between the *H. pylori* positive patients considering their age. Serrano et al,[14] reached the same result. There was an insignificant tendency of *H. pylori* infection to milder cases with reflux esophagitis in our study. Fujishiro et al[15] also did not find any differences between severity-based subgroups of GERD patients according to *H. pylori* infection rate. Cytotoxic-associated gene A has been proposed as one

Table 1 - Age-related categorization of the case (reflux esophagitis positives) and the control (reflux esophagitis negatives) patients and the rate of *H. pylori* infection.

Age	Group	Males	Females	*H. pylori* positive
			n (%)	
< 40 years	case	24 (53.3)	21 (46.7)	34 (75.5)
	control	21 (46.7)	24 (53.3)	38 (84.4)
40-60 years	case	14 (46.7)	16 (53.3)	26 (86.6)
	control	18 (50)	18 (50)	32 (88.8)
60 years ≤	case	9 (52.9)	8 (47.1)	15 (88.2)
	control	7 (58.3)	5 (41.7)	11 (91.6)
Total	**case**	**47**	**45**	**75 (81.5)**
	control	**46**	**47**	**81 (87.1)**

H. pylori - Helicobacter pylori

Table 2 - The rates of *Helicobacter pylori (H. pylori)* and its cagA strain infection in the case (reflux esophagitis positive) and the control (reflux esophagitis negative) groups and the severity-based subgroups of the case patients (A, B, and C) with their comparison.

Infection type	Control n=93	Case n=92	Control versus case	A n=40	Control versus A	B n=35	Control versus B	C n=6	Control versus C	B + C n=41	A versus B + C
	n (%)		P-value	n (%)	P-value	n (%)	P-value	n (%)	P-value	n (%)	P-value
H. pylori Positive	81 (87.1)	75 (81.5)	0.29	40 (78.4%)	0.17	31 (88.6%)	0.82	4 (66.7%)	0.01	35 (85.3%)	0.39
CagA Positive	55 (59.1)	37 (40.2)	0.01	20 (39.2%)	0.02	16 (45.7%)	0.17	1 (16.7%)	0.08	17 (41.5%)	0.83

*significance - *p* ≤ 0.05

of these virulent markers.[6] Thus, we considered this peculiar strain in our study. Cytotoxic-associated gene A strain positive patients were significantly higher in the case group with no differences considering the severity of the disease. Previous studies have demonstrated that carriage of cagA strain was inversely related to the severity of the reflux disease, indicating that the carriage of cagA strain has a protective effect against the esophageal complications of GERD.[16] Our results showed that the patients with erosive esophagitis may be protected by infection with the cagA strain. In some other studies, cagA strain colonization has been proposed as a defensive factor against erosive GERD, namely, its lower prevalence in patients with erosive esophagitis.[17] Pereira-Lima[10] showed a significant lower rate of infection with cagA strain infection in patients with more severe erosive esophagitis. In our study, small sample size in patients with more severe disease (B and C) may be the main cause of such consequences in this regard.

In conclusion, *H. pylori* infection with the cagA positive strain is potentially protective against the spectrum of gastroesophageal reflux disease. Considering these apparent paradoxes, our entire approach to the worldwide elimination of this organism, sometimes indiscriminately, will need critical reevaluation; is the bug all bad? Further, detailed studies need to be carried out to clarify the answer and many other intriguing relationships between *H. pylori* and esophageal disorders. In the current study, the prevalence of *H. pylori* was not different between the patients with and without reflux esophagitis. The CagA strain positive patients were significantly lower in the case group with no differences considering the severity of the disease.

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