THE ROLE OF CAGA STATUS ON HISTOLOGICAL VIRULENCE, CLINICAL PRESENTATION AND ERADICATION TREATMENT OUTCOMES IN HELICOBACTER PYLORI POSITIVE PATIENTS WITH FUNCTIONAL DYSPEPSIA

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ABSTRACT

Objective: *To investigate the role of cagA status in eradication treatment outcomes and clinical presentation in Hp positive patients with functional dyspepsia (FD).*

Material and Method: *In this double-blind, placebo-controlled trial, Hp infected patients with FD (unaware of the Cytotoxin Associated Gene A status assigned to receive seven days twice daily a lansoprazole-based triple therapy (49 patients) or lansoprazole alone (48 patients), randomly. Two months after the completion of treatment, Hp status was reassessed with the Carbon-14 Urea Breath Test (^{14}C-UBT) and symptoms were reassessed with a five-point Likert scale.*

Results: *The rate of treatment success was significantly higher among patients who became Hp-negative (34 of 41), as compared with among those who were still Hp-positive (24 of 56) at two months (82.9% vs. 42.9%; p<0.001), regardless of treatment groups. Cytotoxin Associated Gene A (CagA) gene positivity and cagA seropositivity were associated with marked chronic (p=0.021) and active (p=0.009) inflammation, respectively. CagA-PCR result (p=0.271) was positive 75.6% (31 of 41) of the patients who had Hp eradicated and 64.3% (36 of 56) of the patients with persistant infection. The rate of the cases positive for cagA-PCR was not significant between the patients who were considered as successfully treated (42 of 58) and those who were not (25 of 39) at two months (72.4% vs 64.1%; p=0.502).*

Conclusion: *Curing the infection in Hp positive patients with FD leads to relief of symptoms in short term follow-up. Although genetic presence and serologic detection of cagA is associated with the intensity of gastric inflammation, this virulence is not associated with clinical presentation and eradication treatment outcomes.*

Key Words: *CagA, H.pylori, eradication treatment, functional dyspepsia* **Nobel Med 2012; 8(1): 52-60**

HELİCOBACTER PYLORİ POZİTİF FONKSİYONEL DİSPEPSİLİ HASTALARDA *cag A* DURUMUNUN HİSTOLOJİK VİRÜLANS, KLİNİK VE ERADİKASYON TEDAVİSİ ÜZERİNDEKİ ROLÜ

ÖZET

Amaç: *Helicobacter pylori (Hp)* pozitif fonksiyonel dispepsili (FD) hastalarda cag A durumunun eradikasyon tedavi sonuçlarına ve klinik üzerine etkisini araştırmak.

Materyal ve Metod: Bu çift kör, plasebo kontrollu çalışmada Hp (+) fonksiyonel dispepsili hastalar (cag A durumu bilinmeyen) randomize şekilde 7 gün süre ile günde 2 kere lansoprazol içeren standart üçlü tedavi aldı (49 hasta) veya tek başına lansoprazol (48 hasta) aldı. Tedavi bitiminden 2 ay sonra Hp durumu Karbon 14 Üre Nefes Testi (^{14}C-UBT) ile, klinik durum ise 5 puanlı Likert ölçeği ile yeniden değerlendirildi.

Bulgular: Tedavi gruplarından bağımsız olarak 2. ayın sonunda Hp(-) (41 hastadan 34'ü) olanlarda halen Hp(+) (56 hastadan 24'ü) olanlara göre tedavi başarı oranı daha yüksekti (%82,9 karşı %42,9; p<0,001).

CagA geni pozitifliği ve CagA seropozitifliği belirgin kronik (p=0,021) ve aktif (p=0,009) inflamasyon ile ilişkili bulundu. CagA-PCR Hp eradikasyonu olan 41 hastadan 31'inde (%75,6) pozitif iken persistan infeksiyonu olan 56 hastadan 36'sında (%64,3) pozitifti (p=0,271). İkinci ayın sonunda başarılı tedavi edilenlerle (58 hastadan 42'si), edilmeyenler (39 hastadan 25'i) arasında cagA-PCR pozitiflik oranı açısından anlamlı farklılık yoktu (%72,4 karşın %64,1; p=0,502).

Sonuç: Hp (+) FD'li hastalarda infeksiyonun tedavi edilmesi kısa dönemli takipte semptomların azalmasına yol açmaktadır. CagA genetik varlığı ve serolojik olarak saptanmasının gastrik inflamasyonun şiddetiyle ilişkili olmasına karşın, bu virülans faktörü klinik görünüm ve eradikasyon sonuçları ile ilişkili değildir.

Anahtar Kelimeler: CagA, *H.pylori*, eradikasyon tedavisi, fonksiyonel dispepsi **Nobel Med 2012; 8(1): 52-60**

INTRODUCTION

Dyspepsia refers to persistent or recurrent pain or discomfort (early satiety, fullness, bloating, nausea) centered in the upper abdomen (predominant heartburn is kept out of definition). Most patients with dyspepsia do not have a biochemical or structural explanation for their symptoms. Such patients are diagnosed as having functional dyspepsia (FD).[1,2] The pathogenesis of FD is not well understood. It's pathophsiology remains incompletely understood and is likely to be multifactorial.[3,4] It has been proposed that if *Helicobacter pylori (Hp)* is a cause of FD, then eradication of Hp should lead to resolution of the symptoms.[5,6] However, the results of anti-Hp treatment in these patients are conflicting.[7-12]

Hp is a fastidious, gram negative, urease positive, spiral-shaped microaerophilic bacterium that causes chronic active gastritis, which can be lifelong, in almost every infected individual and infects up to 50% of the population in Western countries.[13,14] According to a large scaled epidemiologic study, Hp prevalance was found to be %82.5 over 18-years old population in Turkey.[15] Although Hp has been proved to be strongly associated with gastric ulcer, duodenal ulcer, atrophic gastritis, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer; only a small number of people with chronic Hp gastritis will actually develop such clinically relevant diseases.[16,17] The association of Hp with disease severity has been linked to a variety of strain-specific characteristics, such as the presence of the cytotoxin-associated gene A (cagA).[18,19] The cagA gene is present in about 70% of Hp strains in European countries and is component and an important marker of the cag pathogenicity island (cag PAI).[20-22] The presence of cag PAI, as reliably detected by cagA induces gastric epithelial cells to secrete interleukin (IL)-8 and other local proinflammatory cytokines.[20,22] CagA harboring strains seem to be more prevalent in peptic ulcers, atrophic gastric mucosa, and gastric carsinoma, especially MALT-lymphoma, than in gastritis alone or FD. This virulence effect of cagA positive Hp strains have been imputed to induction of inflammation.[23-26]

CagA positive strains of Hp, which cause more severe gastric inflammation, are more susceptible to antibiotics and are more often associated with peptic ulcer or malignancy than cagA negative strains. CagA positive strains have been found less frequent in patients with FD than in those with peptic ulcer.[23,27] This may be an explanation for the lower eradication rates in patients with FD than in patients with peptic ulcer disease. In this respect, cagA status may be associated with the severity, types of symptoms and moreover with the efficacy and the symptomatic benefit of eradication therapy in Hp infected patients with FD.[28,29]

MATERIAL and METHOD

Study Design: This randomized, double-blind, →

placebo-controlled study was carried out between September 2003 and September 2004 in Gülhane Military Medical Academy, Ankara, Turkey, according to the principles of good clinical practice and the Declaration of Helsinki. Written informed consent was obtained from all patients before enrollment and the study protocol was approved by the Local Ethics Committee.

Selection of Patients: Patients were eligible if they had dyspepsia (according to the Rome II criteria) for at least 12 weeks within 12 months; but had no clinical, biochemical, ultrasonografic and endoscopic evidence of current or previous organic disease likely to explain the symptoms.[1] Besides, all enrolled patients had at least mild (a symptom score of 1, according to a five-point Likert scale) epigastric pain or discomfort (or both) as their predominant symptom over the previous two months. Patients were excluded if they predominantly had symptoms of gastro-esophagial reflux disease or irritable bowel syndrome. On upper endoscopy, patients with gastric or duodenal ulcers, reflux esophagitis, Barrett's esophagus, esophagial erosions were excluded, although patients with gastric and duodenal erosions alone were included. Patients with comorbid systemic medical conditions (eg, chronic renal, or pulmonary, or liver disease, diabetes mellitus), pregnancy, known bleeding diathesis and taking oral anticoagulants were excluded. Patients with signs indicating serious disease (eg, anorexia, dysphagia, hematemesis, anemia, strong history of familial cancer) indicating serious disease were excluded. Patients were excluded if they were taking systemic corticosteroids, non-steroidal anti-inflammatory drugs (other than occasional use or low-dose aspirin) in the two months prior to the study. A history of Hp eradication treatment within the previous year was also one of the exclusion criteria. Patients were asked to stop taking histamine H_2-receptor antagonists, prokinetic agents, prostoglandins within two weeks; and proton pomp inhibitors, bismuth-containing compounds, antibiotics within two months before the study began.

Study Protocol: Patients were asked to define their own duration of symptoms (three to six months, more than six to 12 months, more than 12 to 36 months, more than 36 months). Patients were classified according to the presence of the predominant (or most bothersome) single symptom. Thus, patients identifiying their predominant symptom as pain or discomfort in the upper abdomen were considered to have ulcer-like or dysmotility-like dyspepsia, respectively.

Patients who couldn't nominate their predominant complaint were considered to have nonspecific dyspepsia. Three biopsy-based methods (rapid urease test, histologic staining and polymerase chain reaction [PCR]) and a carbon-14 urea breath test (^{14}C-UBT) were used for the detection of Hp. The infection state was considered as positive when the results of at least two of these four tests were positive. Patients with FD whose Hp status was identified as positive (n=97) in this respect, were randomized to seven days of twice daily treatment with (30 mg lansoprazole, 1000 mg amoxicillin, and 500 mg clarithromycin) or (30 mg lansoprazole, and identical-appearing plasebo antibiotics). PCR assay was used also for the detection of cagA directly in gastric biopsy specimens. And an ELISA assay kit was used to test for anti-cagA IgG antibody in matching sera. The investigators and the patients were unaware of the cagA status and the treatment assignments until the study was fully completed. Compliance with treatment was excellent. None of the patients had treatment stopped because of adverse events. At base-line, we assessed the global overall severity of patients' worst symptoms of dyspepsia over the preceding two months by using a five-point Likert scale in which a score of 0 indicated no pain or discomfort and a score of 1 mild (can be ignored), a score of 2 moderate (cannot be ignored but does not influence daily activities), a score of 3 severe (cannot be ignored and occasionally limits daily activities), a score of 4 very severe (cannot be ignored and markedly limits daily activities) pain or discomfort. Two months after the completion of treatment, Hp status was reassessed with the ^{14}C-UBT and symptoms were reassessed with the same scale used at base-line. Treatment was considered successful if the patient reported having no pain or discomfort (a score of 0) and/or having a decrease of at least two points in the symptom score during the two months before the reassessment.

Endoscopy and Biopsy: Antral biopsy specimens were taken within 5 cm from the pyloric chanel for each of the three biopsy-based methods. The gastroscope (Olympus XQ30, Japan) and biopsy forceps were carefully cleaned and disinfected by immersion in a 2% glutaraldehyde (Cidex; Surgikos Ltd., Livingstone, Scotland) solution for 15 min, rinsed in water, and dried after each endoscopic session. Biopsy specimens for PCR assay were stored at -70ºC until processing.

Histologic Staining: Two biopsy specimens for histopatological examination were fixed in buffered 4% formalin overnight and were embedded in paraffin and cut into 5 µm sections perpendicularly to the mucosal surface. Hp status was based on the findings of examination of biopsy specimens using →

hematoxylin-eosin and toluidin-blue stains under light microscopy by a single experienced pathologist who was unaware of the data, including other tests for Hp and cagA status. The four Sydney morphological criteria (inflammation, activity, atrophy, intestinal metaplasia) were scored using a scale from 0 to 3 ('absent' to 'severe').[30]

Rapid Urease Test: One biopsy specimen was placed into a home-made semisolid 2% urea agar and incubated at room temperature. The colour reaction (to pink-red from yellow) was read after six hours.

PCR assay: Two antral biopsy specimens were used for PCR assays. Total genomic DNA of Hp was initially isolated directly in gastric biopsy specimens, as described earlier.[31] A set of primers (5' AAG CTT TTA GGG GTG TTA GGG GTT T 3' and 5' AAG CTT ACT TTC TAA CAC TAA CGC 3'), which target ureC gene, was used for the detection of Hp. Another set of primers (5' AAT ACA CCA ACG CCT CCA AG 3' ve 5' TTG TTG CCG CTT TTG CTC TC 3') was used for the determination of cagA status. The primers, PCR conditions and gel detection of amplified DNA products have been described in details before. DNA of Hp strain 17874 was used as a positive control. Lage et al. showed that the PCR assay targeting the ureC gene in biopsy specimens is at least as sensitive as culture for detecting Hp infection, and has a potential value for studying cagA.[32]

^{14}C-UBT: After overnight fasting, a 37 kBq ^{14}C-urea capsule was swallowed for UBT. Breath samples were collected and counted using Heliprobe method. In this practical and low dose ^{14}C-UBT system, patients exhaled into a special dry cartridge system (Heliprobe BreathCard, Noster System AB Stockholm,Sweden) at 10 min. The activities of the cartridges were counted using a designated small Geiger-Müller counter system (Heliprobe-analyser). Results were expressed both as counts per minute (HCPM) and as grade (0=not infected, CPM <25; 1=equivocal, CPM 25-50; 2=infected, CPM >50), according to the counts obtained from the cartridges. If the result of ^{14}C-UBT was grade 1, thus confounding the interpretation of the test, the patient was considered to be infected. In a previous study, sensitivity, specificity, positive predictive value, negative predictive and accuracy of this ^{14}C-UBT was 100%, 76%, 88%, 100% and 91%, respectively.[33]

ELISA assay: Serum samples were taken from each patient after endoscopy, and were stored at -20°C until testing was performed. An ELISA kit (EUROIMMUN, Medizinische Labordiagnostika GmbH, Germany) using recombinant Hp cagA antigen was carried out to detect serum anti-cagA IgG. The result was expressed as the ratio of the optical density value of the sample to the threshold value (the cutoff optical density value of calibration serum 2). Since there is no international standard for IgG levels, this was quantitated by means of a standard curve calibrated in relative units per milliliter (RU/ml). Accordingly, serum level values ≥1 were considered seropositive, and those <1 were considered as seronegative.

Table 1: CagA status in subgroubs of functional dyspepsia

	ULD	DLD	NSD	Total
n(%)	42 (43.3%)	50 (51.5%)	5 (5.2%)	97 (100%)
cagA-PCR positivity	30 (71.4%)	35 (70%)	2 (40%)	67 (69.1%)
cagA seropositivity	35 (83.3%)	39 (78%)	2 (40%)	76 (78.4%)

ULD: ulcer-like dyspepsia; DLD: dysmotility-like dyspepsia; NSD: nonspecific dyspepsia
CagA: Cytotoxin Associated Gene A PCR: Polymerase Chain Reaction

Table 2: Base-line characteristics of 97 Hp positive patients with functional dyspepsia

	Triple therapy group (n=49)	Lansoprazole group (n=48)
Age (yr), mean±SD	38.18 ± 13.04	42.12 ± 12.69
Weight (kg/m^2), mean±SD	24.71 ± 3.85	25.43 ± 4.52
Female sex, n(%)	42 (85.7%)	36 (75.0%)
Smokers, n(%)	7 (14.3%)	13 (27.1%)
Alcohol users, n(%)	0 (0%)	1 (2.1%)
Caffeine users, n(%)	3 (6.1%)	2 (4.2%)
CagA-PCR positivity, n(%)	36 (73.5%)	31 (64.6%)
CagA seropositivity, n(%)	39 (79.6%)	37 (77.1%)
Duration of dyspepsia, n(%)		
3-6 months	11 (22.4%)	4 (8.3%)
>6-12 months	10 (20.4%)	11 (22.9%)
>12-36 months	11 (22.4%)	13 (27.1%)
>36 months	17 (34.7%)	20 (41.7%)
Dyspepsia subgroups, n(%)		
Ulcer-like	20 (40.8%)	22 (45.8%)
Dysmotility-like	27 (55.1%)	23 (47.9%)
Non-spesific	2 (4.1%)	3 (6.3%)

Statistical Analysis: Data were analysed with SPSS 10.0 (SPSS Inc., Chicago, Illinois, USA) statistical package. Descriptives were given either mean±SD or n(%) notation. Mann Whitney U tests were used for normally distributed data and also for the difference in mean symptom scores between the two treatment groups. Wilcoxon signed ranks test was used for comparison of pretreatment and posttreatment symptom scores in both treatment groups. Chi-square test or Fisher's exact probability test were used to analyze categorical variables, as appropriate. Correlations between the variables were evaluated by Kendall's tau-b test. All statistical tests were two-tailed. A p value of <0.05 was regarded as statistically significant. →

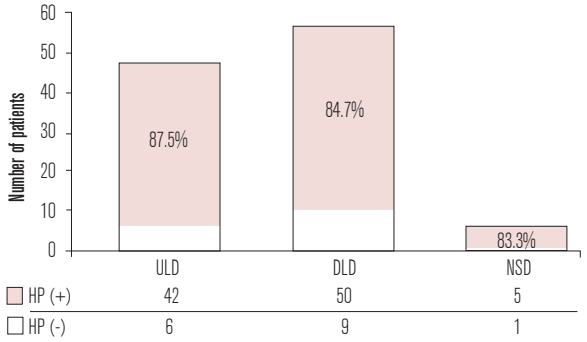

Figure 1. Helicobacter pylori status in supgroups of functional dyspepsia (ULD: ulcer-like dyspepsia; DLD: dysmotility-like dyspepsia; NSD: non-specific dyspepsia)

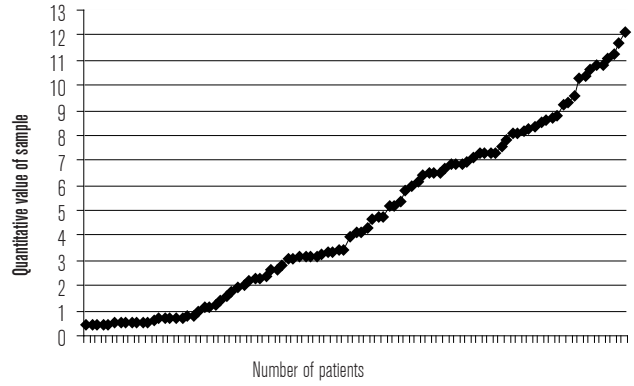

Figure 2. The quantitative values for anti-cagA IgG ELISA assay. Values ≥ 1 are seropositive (n=76), values < 1 are seronegative (n=21). Each point represents one sample.

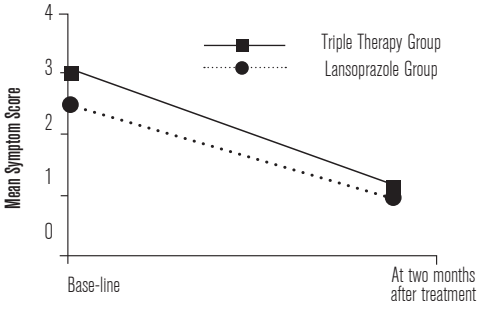

Figure 3. Mean symptom scores during base-line and at the two months follow-up visit

RESULTS

Prevalance: Of 113 patients with FD, 85.8% (n=97) were Hp-positive; 14.2% (n=16) were Hp-negative. The prevalance of Hp was 87.5% (42 of 48) in patients with ulcer-like dyspepsia; 84.7% (50 of 59) in patients with dysmotility-like dyspepsia; and 83.3% (5 of 6) in patients with non-specific dyspepsia (p=0.906) (Figure 1). The seroprevalance of anti-cagA IgG antibodies in the sera from Hp positive patients with FD was 78.4% (76 of 97). The antibody titers of Hp positive patients, in term of ELISA units are shown in Figure 2. CagA gene sequences were detected by PCR in 69.1% (67 of 97) of all Hp positive patients with FD.

CagA status and histological virulence: CagA-PCR positive patients had significantly greater inflammation scores, as compared with negative ones (p=0.021). CagA seropositive patients had significantly greater activity scores, as compared with seronegative ones (p=0.009). Furthermore, among cagA seropositive patients, anti-cagA IgG levels significantly correlated with both inflammation (p=0.001) and activity scores (p<0.001). None of the patients with Hp positive FD had atrophy; and only 5 of them had metaplasia.

CagA status and clinical presentation: The rates of the presence of the cagA (p=0.349) and the rates of cagA seropositivity (p=0.084) were similar when Hp positive patients were divided into subgroups (ulcer-like, dysmotility-like and nonspecific) based on the predominant complaint (Table 1). There was not statistically significant association between the score of pretreatment symptom severity and the rate of cagA-PCR positivity (p=0.192) or the rate of cagA seropositivity (p=0.035). However, among cagA seropositive patients, significant correlations were found between anti-cagA IgG levels and symptom severity (p=0.007).

Treatment Outcomes: The two treatment groups were well matched for demografic and clinical features. All dyspeptic patients belonged to the middle-class socioeconomic status with the similar annual income (Table 2). The rate of eradication of Hp was 61.2% (30 of 49) in the triple therapy group and 22.9% (11 of 48) in the lansoprazole group at two months (p<0.001) (Table 3). The rate of treatment success was higher, but not significant, among patients in the triple therapy group (33 of 49), as compared with among those in the lansoprazole group (25 of 48) (67.3% vs. 52.1%; p=0.150). However, the rate of treatment success was significantly higher among patients who were Hp negative (34 of 41), as compared with among those who were Hp positive (24 of 56) at two months (82.9% vs. 42.9%; p<0.001). The difference in mean symptom scores between the two treatment groups at baseline and two months after treatment was not significant. The scores in both groups were significantly lower than those at entry (p<0.001) (Figure 3). The rate of eradication and treatment success didn't differ according to the symptom subgroups (p=0.144 and 0.040, respectively) and to the symptom durations (p=0.483 and 0.902 respectively). The rate of treatment success correlated well with patients' ages, negatively (p=0.001) and there was also a positive correlation between the rate of treatment success and the score of pretreatment symptom severity (p=0.006).

CagA status and treatment outcomes: CagA-PCR →

was positive in 75.6% of the patients in whom Hp was eradicated (31 of 41) and 64.3% of the patients with persistant Hp infection (36 of 56) (p=0.271) (Figure 4). The rate of the cases positive for CagA-PCR was also not significant between the patients who were considered as successfully treated (42 of 58) and those who were not (25 of 39) (72.4% vs 64.1%; p=0.502). The rate of cagA seropositivity also, as the rate of cagA-PCR positivity, did not differ when patients were analyzed according to the Hp eradication or treatment success (Figure 5).

Analysis of the test results: When the results of cagA-PCR in 113 patients with FD were considered as true; sensitivity, specificity, positive predictive value, negative predictive value and accuracy of ELISA were determined as 94.0%, 67.4%, 80.8%, 88.6%, and 83.2%, respectively (Table 4).

The number of positive and negative results of four tests used for the diagnosis of Hp were shown in Table 5. We evaluated the number of true and false results of the new ^{14}C-UBT and of Hp-PCR according to the infection state as previously described. In this respect, sensitivity, specificity, positive predictive value, negative predictive and accuracy of ^{14}C-UBT was 99%, 88%, 98%, 93% and 97%, respectively; and of Hp-PCR was 92.8%, 100%, 100%, 69.6% and 93.8%, respectively (Table 6).

DISCUSSION

FD is a worldwide common clinical syndrome with unknown etiology.[34,35] The association between Hp infection and FD continues to be controversial.[29,36] The prevalence of Hp in FD varies and is 30 to 87% in Western countries and in Turkey.[15,28,35,37,38] The prevalence of Hp was 85.8% in our study population with FD.

Since, cagA positive strains induce more severe gastritis; it is logical to assume that the presence of these strains may be associated with clinical presentation including symptom severity in Hp positive patients with FD. Loffeld et al. showed that functional dyspeptic patients with cagA positive Hp strains have more dyspeptic symptoms and a higher symptom score than those with cagA negative strains.[39] However, in three other studies no significant difference was found in dyspeptic symptoms and dyspepsia subgroups among cagA positive and negative patients with FD.[40-42] Studies showed that cagA-positive strains cause greater antral histological changes in dyspeptic patients, but could not find a correlation between this virulence and intensity or type of symptoms.[43-45]

This study was aimed to investigate this relationship in only functional dyspeptics.

Table 3: Outcomes at two months after completion of treatment

	Triple therapy group (n=49)	Lansoprazole group (n=48)	p
Rate of Hp eradication, n(%)	30 (61.2%)	11 (22.9%)	<0.001
Treatment success, n(%)	33 (67.3%)	25 (52.1%)	=0.150

Table 4: Evaluation of the ELISA results, when the results of cagA-PCR assay considered as "true" in patients with functional dyspepsia

ELISA/cagA-PCR	cagA-PCR (positive result)	cagA-PCR (negative result)	Total
ELISA (positive result)	63	15	78
ELISA (negative result)	4	31	35
Total	67	46	113

cagA-PCR: Cytotoxin-associated gene A-polymerase chain reaction assay

Table 5: The number of the results of tests used for the diagnosis of Helicobacter pylori

Methods	No. of patients										Total
	12	64	15	8	6	3	1	1	2	1	113
Histopathology	–	+	+	–	+	–	+	+	–	–	
Urease test	–	+	–	+	+	–	+	–	–	+	
14C-UBT	–	+	+	+	+	+	–	–	+	–	
Hp-PCR	–	+	+	+	–	+	–	–	–	–	

^{14}C-UBT: Carbon-14 urea breath test, Hp-PCR: Helicobacter pylori-polymerase chain reaction

Table 6: Evaluation of the results of Hp-PCR and 14C-UBT, when the positivity of at least two of four tests considered Helicobacter pylori infection

Methods	True positives (n)	True negatives (n)	False positives (n)	False negatives (n)
^{14}C-UBT	96	14	2	1
Hp-PCR	90	16	0	7

^{14}C-UBT: Carbon-14 urea breath test, Hp-PCR: Helicobacter pylori-polymerase chain reaction assa

In our study, there was no difference in the prevalence of cagA positivity and cagA seropositivity when patients were divided into dyspepsia subgroups. Although cagA-PCR positive and cagA seropositive patients had significantly greater inflammation scores (p=0.021) and activity scores (p=0.009), respectively, as compared with negative ones; there wasn't either an association between the cagA status (in terms of cagA positivity and cagA seropositivity) and symptom severity. However, among cagA seropositive patients, higher anti-cagA IgG titers were associated significantly with both greater inflammation scores (p=0.001) and activity scores (p<0.001). And in these patients, higher anti-cagA IgG titers were correlated with significantly more severe pretreatment symptoms (p=0.007). Therefore, instead of determining the cagA status, the quantitative degree of systemic anti-cagA IgG response should be recommended for the evaluation of histological virulence and symptom severity and →

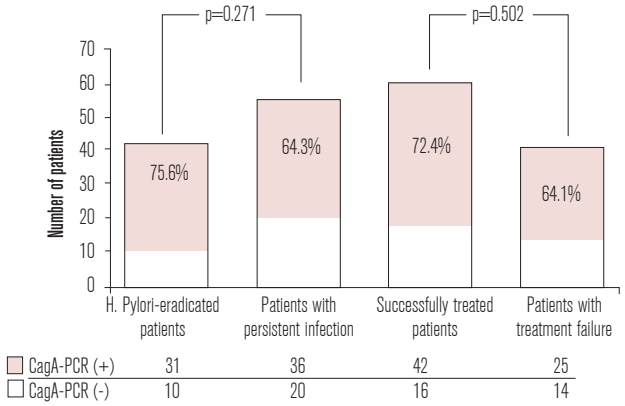

Figure 4. The results of cagA-PCR assay according to the eradication status and treatment success

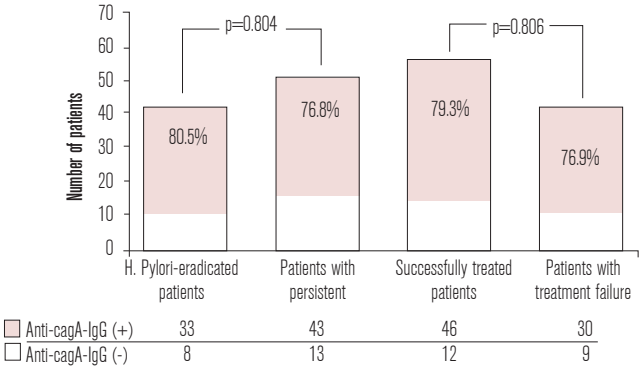

Figure 5. The results of ELISA assay according to the eradication status and treatment success

their relationship. Nevertheless, since no association was established with the treatment outcomes; we will not discuss anti-cagA IgG titers here any more.

It is also logical to assume that cagA positive Hp strains should be considered when evaluating the efficacy and clinical outcome of the eradication therapy in patients with FD. Broutet et al. have explained the role of the cagA gene on eradication outcome logically with it's inflammatory effects on gastric mucosa.[46] Consequent increased blood flow due to enhanced inflammation may facilitate the diffusion of antibiotics.

We found that cagA seropositivity or the presence of the cagA gene wasn't associated with the rates of Hp-eradication and symptomatic treatment success at short term follow-up after eradication of Hp in patients with any types of FD. In a previous study with a 12 months follow-up, Greenberg et al. also found no association.[47]

Recently, Xia et al. showed that pre-treatment systemic IgG antibody responses to specific virulence factors of Hp, including cagA (116 kDa), vacA (89 kDa), ureE (19.5 kDa), ureA (26.5 kDa), ureH (30 kDa) and 35-kDa proteins were not associated with the relief of symptoms 12 months after the eradication of Hp infection in patients with FD.[48]

As mentioned above, the question of whether to eradicate Hp in patients with FD is still debated.[12,29,36] However, we found that eradicating Hp infection led to relief of symptoms at short term follow-up in patients with FD, regardless of treatment groups. But, if the eradication rate of triple therapy group could have been as higher as expected, then its symptomatic success would also be more significant than the lansoprazole group. This result gave us a clue that longer courses of treatment (14-day treatment instead of 7-day treatment) might increase the rates of eradication and so, of symptomatic success in the population studied. Unexpected poor eradication rate of triple therapy might be due to antibiotic resistance in the study where patient compliance was excellent. In clinical studies, the eradication rate of Hp with lansoprazole is 0-25% with monotherapy.[49] We observed a high rate of eradication with lansoprazole alone (22.9%) in our study. Therefore, lansoprazole may be considered as the drug of choice in the triple therapies in Turkish population. Eradication rate and symptomatic benefit did not differ according to the symptom subgroups and to the symptom durations. Symptomatic benefit was more evident among patients with higher pretreatment symptom scores and among younger patients.

There is a close correlation between the presence of the cagA gene in the infecting strain of Hp and production of cagA protein.[19,21] Therefore, cagA protein can be used as a serological marker for virulence of Hp. CagA is a high (120-140 kDa) molecular weight protein and initiates a marked mucosal and systemic humoral immunological responses.[50,51] The presence of anti-cagA antibodies have been widely associated with increased risk for the development of peptic ulcer disease, atrophic gastritis, and gastric cancer. Expression of the CagA protein can be sensitively and specifically diagnosed by detecting antibodies to it. Therefore, serum immunoglobulin (IgG) antibodies to the cagA antigen may be reliable marker of cagA gene and even cag PAI.[21,22,51] For most of the 113 patients with FD in our trial, the presence of the cagA gene (which is directly detected in gastric biopsy specimens) considerably correlated with the presence of anti-cagA IgG antibody in the matching sera (when compared with the cagA-PCR assay, accuracy of the ELISA assay was 83.2%). In the study, the most observed discrepancy between the two tests was the results that cagA-PCR detected as negative, whereas ELISA detected as positive, accounting for the low sensitivity of ELISA assay. This discordant results may be explained by the absence of microorganisms in the sample used for PCR assay, or the presence of an in vivo Taq polymerase inhibitor.[52,53] Actually, of these 15 cases negative for cagA-PCR, seven cases (whose→

Hp status was identified as positive by at least two other tests) was also negative (false negative) for Hp-PCR, indicating the above explanations. Another possible explanation of the discrepancy between the genetic and phenotypic assays may be that most Hp positive patients with FD are infected with cagA positive and cagA negative organisms at the same time.[54,55] Most likely, in these patients infected by a mixed population of cagA positive and cagA negative Hp, the proportion of cagA positive Hp may be to small, leading to failure of the detection by PCR. However, there may be still sufficient CagA protein exported in vivo to induce a host response detected by ELISA.[39] It is also possible that one patient with only cagA negative organisms in his stomach tested cagA seropositive, may indicate a past infection with cagA positive strains. Immunological memory due to a past contact also may be used to interpret the detection of two cases of cagA seropositivity in 16 Hp negative patients. In addition, since only antral specimens were studied for PCR assays, this may not reflect the colonization of different areas of the stomach.[55,56]

An important critisizm about the present study may be the duration of time interval after eradication for symptom reassessment. A six-month or a one-year time interval could be more accurate for symptom score reassessment in long term. In that case success in the follow up of the patients would not catch up the present study. Anyway the present study provided important data at least in a short term follow up after eradication.

CONCLUSION

In conclusion, cagA positive Hp strains which cause more severe acute or chronic gastric inflammation, are not associated with clinical presentation and eradication outcomes in patients with FD. Based on our study findings, we finally recommend that patients with FD should be treated for Hp, especially if they are young and harboring severe symptoms. However, management strategies should not be based on the presence and absence of cagA. Detection of anti-cagA antibodies should be accepted as practical and reliable way of determining cagA status in further clinical trials which are needed to provide new insights into the possibility that certain subsets of patients with FD may benefit from treatment.

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