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RAPID COMMUNICATION

Analysis of serum antibody profile against *H pylori* VacA and CagA antigens in Turkish patients with duodenal ulcer

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Abstract

AIM: To investigate the frequency of seropositivity against CagA, VacA proteins and to determine their independent effects on the development of duodenal ulcer (DU) in Turkish patients.

METHODS: The study was designed as a prospective one from a tertiary referral hospital. Dyspeptic patients who were referred to our endoscopy unit for upper gastrointestinal endoscopy between June 2003 and March 2004 and diagnosed to have DU or nonulcer dyspepsia (NUD) were included. Biopsies from the antrum and body of the stomach were taken in order to assess the current *H pylori* status by histology, rapid urease test and culture. Fasting sera were obtained from all patients and *H pylori* status of all sera was determined by IgG antibodies using an enzyme-linked immunosorbent assay (ELISA) kit. All seropositive patients were further analysed using Western blot assays detecting IgG antibodies against CagA and VacA proteins. The χ^2 test was used for statistical comparison of the values and age-sex adjusted multiple regression analysis was used to determine the independent effects of CagA and VacA seropositivities on the development of DU.

RESULTS: Sixty-three patients with DU and 62 patients with NUD were eligible for the final analysis. Seropositivity for anti-CagA was detected in 51 of 62 (82%), and in 55 of 63 (87%) patients with NUD and DU, respectively (P = no significance), and seropositivity for anti-VacA was found in 25 of 62 (40%) and in 16 of 63 (25%) patients, with NUD and DU, respectively.

CONCLUSION: These findings suggest that none of these virulence factors is associated with the development of DU in the studied Turkish patients with dyspepsia.

Key words: *H pylori*; Western blot; CagA; VacA; Duodenal ulcer

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INTRODUCTION

Since the discovery of *H pylori* in 1983, the diagnosis and treatment of upper gastrointestinal disease have changed greatly. A strong association has been established between colonization of the gastric mucosa by *H pylori* and various benign and malignant gastroduodenal diseases including chronic active gastritis, gastric ulceration and duodenal ulcer, gastric adenocarcinoma and gastric lymphoma of mucosa-associated lymphoid tissue type[1]. The World Health Organization and International Agency for Research on Cancer consensus group stated in 1994 that there is sufficient epidemiologic and histologic evidence *H pylori* can be clarified as a definite carcinogen[2].

However, not all infected subjects develop disease complications and such a wide spectrum of diseases associated with *H pylori* infection may depend on the heterogeneity of *H pylori* and/or the host response to the same *H pylori* strain[3]. Besides immunological factors in the host, there are specific virulence determinants in *H pylori* strains that influence the outcome of the infection. It has been suggested that both possession of the *cagA* gene and production of a vacuolating cytotoxin encoded by the *vacA* gene, are linked with the

increased pathogenecity of *H pylori* strains. The *cagA* gene encodes a 120-140 kDa protein CagA, and is a part of a large pathogenecity island[4]. Strains expressing the CagA protein have been found to induce more severe inflammation, a higher degree of gastric atrophy, a higher incidence of duodenal ulcer and gastric adenocarcinoma of intestinal type[5,6]. The *vacA* gene is present in all *H pylori* strains, however it is expressed in only 50%-65% of them, thus inducing vacuolization of cells *in vitro*[7-9]. This *vacA* gene encodes a 81-91 kDa protein VacA, which has been shown to provoke the formation of vacuoles in gastric epithelial cells[4].

Turkey is a developing country with a very high prevalance of *H pylori*. A Turkish study dealing with asymptomatic children, using the UBT as the diagnostic test, found that one child out of two under 11 years of age is infected with *H pylori*[10]. Another serology based Turkish study in asymptomatic subjects indicates that about 70% of adults have antibodies against *H pylori* in our population[11]. These data obviously indicate that infection with *H pylori* is a major health problem in our country. The aim of the current study was to evaluate the frequency of cagA and vacA seropositivity in Turkish patients with deudenal ulcer (DU) and in controls with non ulcer dyspepsia (NUD) and to determine their independent effects on the development of DU.

MATERIALS AND METHODS

A total of 125 patients (63 with DU and 62 with NUD serving as a control group) who were referred to the Endoscopy Unit of Istanbul University, Cerrahpasa Medical Faculty between June 2003 and March 2004 were included. Inclusion criteria were the indication of endoscopy for the study of dyspeptic symptoms. Exclusion criteria were as follows: age under 18, previous gastric surgery and *H pylori* eradication treatment, consumption of antibiotics a month prior to the study, consumption of antisecretory drugs, bismuth salts or sucralfate two weeks prior to the study. A history of bleeding and coagulation disorders that are contraindications for biopsy sampling was also the reason for exclusion.The study was approved by the Ethics Committee of Istanbul University, Cerrahpasa Medical Faculty and all patients gave their written informed consent to participate in the study.

From each patient four antrum and three corpus biopsies were collected for histology (two antrum and one corpus biopsy specimens), rapid urease test (one antrum and one corpus biopsy specimens) and culture (one antrum and one corpus biopsy specimens). One antrum and one corpus biopsy specimens were placed in a CLOtest (Ballard Medical Products, Draper, Utah, USA), maintained at room temperature and read at 1 h and 24 h after sampling. Biopsy specimens were processed for histological examination according to the standard procedure. Hematoxylin and eosin staining as well as a special staining for *H pylori* (Giemsa) were performed. All biopsy samples were examined by the same pathologist (S.E), who specialises in digestive diseases.

Biopsy specimens used for bacterial culture were placed in 2 mL of phosphat-buffered saline at 4℃ and then smeared on the surface of *H pylori* agar (Biomerieux, Lyon, France) plate. Isolates were identified as *H pylori* by Gram stain morphology and the positivity of urease, catalase and oxidase.

Table 1 Demographic characteristics of the patients included

Endoscopic finding	*n* (%)	Range	Male/Female (%)	% Hp (+)
Normal	62 (49.6)	36.90 ± 12.36 (18-68)	20/42 (32/68)	93
Duodenal ulcer	63 (50.4)	43.14 ± 16.27 (18-80)	39/24 (62/38)	97

A patient was classified as *H pylori*-positive if the culture alone or both histology and rapid urease test were positive in the presence of a negative culture and as *H pylori*-negative only if all these tests remained negative.

Fasting serum samples were obtained from all patients and stored at -20℃ until assayed. The *H pylori* status of all sera was determined by enzyme-linked immunosorbent assay (ELISA; Euroimmune, Lübeck, Germany) of anti-IgG as described by the manufacturer. The cut-off value for the ELISA was 20 U/mL, antibody concentrations less than this value were considered seronegative, whereas values more than 20 U/mL were assessed as seropositive. All seropositive patients were further analysed with the Western blot technique. We used commercially available Western blot kits (Euroimmun, Lübeck, Germany) to qualitatively detect IgG antibodies against VacA (95 kDa) and CagA (120 kDa).

Continuous variables, one-way analysis of variance between the groups were calculated using. Categorical variables were analyzed with χ^2 test and age-sex adjusted multiple logistic regression analysis was used to determine the independent effects of different virulence factors on the development of DU. $P < 0.05$ was considered statisticallly significant. All the statistical analyses were performed using SPSS 11 for Windows.

RESULTS

A total of 158 patients were enrolled in the study. Eleven patients (7%) did not give their serum samples although their written informed consent was obtained, and 22 of the remaining 147 (15%) patients were seronegative by anti- IgG ELISA. These 33 patients were excluded from the final analysis.

A total of 125 patients (62 with NUD, 63 with DU) with a mean age of 40.04 ± 14.74 years (range 18-80 years) were eligible for the final analysis. Demographic characteristics of the patients are summarized in Table 1. The patients were classsified as *H pylori* positive when the culture and/or histology plus rapid urease test gave positive results. When all three tests gave concordant negative results the patient was considered negative for *H pylori*. According to these criteria, 6 of 125 (5%) patients had discordant results and 113 of the remaining 119 (95%) patients were positive for *H pylori*.

Table 2 Percentage of seroreactivity to CagA and VacA antigens detected by Western blot in different groups of patients *n* (%)

	Anti-CagA IgG	Anti-VacA IgG
NUD (*n* = 62)	51/62 (82)	25/62 (40)
DU (*n* = 63)	55/63 (87)	16/63 (25)
P	0.432	0.076
Total (*n* = 125)	106/125 (85)	41/125 (33)

NUD : Non-ulcer dyspepsia; DU : Duodenal ulcer.

The percentages of seroreactivity to CagA and VacA antigens detected by Western blot in different groups of patients are summarized in Table 2.

The percentage of seroreactivity to different antigens were as follows: anti-CagA with DU 51 of 62 (82%) patients, with NUD and in 55 of 63 (87%) patients with DU ($\chi^2 = 0.617$; $P = 0.432$), anti-VacA in 25 of 62 (40%) patients with NUD and in 16 of 63 (25%) patients with DU ($\chi^2 = 3.158$; $P = 0.076$).

Age and sex adjusted multiple regression analysis disclosed that none of these factors was an independent risk factor for the development of DU ($P = 0.736$ and $P = 0.214$ for CagA and VacA, respectively).

DISCUSSION

H pylori infection is one of the most common chronic bacterial infections worldwide. Although most infected persons remain asymptomatic, 15% to 20% of *H pylori*-positive individuals develop peptic ulcer, gastric carcinoma, or mucosa-associated lymphoid tissue lymphoma[12]. However, it remains unclear why only a small number of infected patients develop such severe diseases. This phenomenon may be due to the differences in host genetics, environmental factors, and the virulence of bacterial strains.

H pylori strains are highly diverse[13,14]. Of the two main *H pylori* strains, type I produces a vacuolating cytotoxin, whereas type Ⅱ usually does not[15]. The genome of almost all type I strains has a gene coding for CagA, a highly immunogenic molecular weight protein, which is not present in most type Ⅱ strains. The *vacA* gene coding for vacuolating cytotoxin is polymorphic and present in all strains and the polymorphism of *vacA* accounts for the phenotypic differences between type I and type Ⅱ strains, both types synthesise a VacA protein which is active as a vacuolating cytotoxin in type I and inactive in type Ⅱ strains[15,16]. Infection with VacA-positive strains has been reported to be associated with particular gastroduodenal diseases[7,17-20] especially peptic ulcers[17, 20].

Approximately 60% of *H pylori* isolates harbor the *cagA* gene which encodes another putative virulence factor, CagA that can be variable in size (128-152 kDa)[21,22]. Serological and microbiological studies indicate that CagA-positive strains are associated with enhanced induction of local inflammatory response[23,24] and the presence of this protein has been linked to the development of peptic ulcer disease and gastric cancer[7,21,22,24-27]. However, there are geographic variations in prevalance of both VacA and CagA. Especially in East Asian countries very high prevalances of VacA and CagA-positive strains have been reported[28-30]. Based on these findings, Maeda *et al*[28] concluded that these virulence factors cannot be used as markers of particular gastroduodenal diseases. However, it was reported that the high prevalance of these factors may contribute to the characterization of *H pylori* infection in Japan.

Besides these, other reports suggest that neither VacA nor CagA positivities are associated with more serious gastroduodenal diseases. Yamaoka *et al*[31,32] examined a large number of strains from both Western and East Asian countries and constructed models to discriminate different clinical outcomes on the basis of the presence of *H pylori* putative virulence factors and concluded that none of these factors is helpful in predicting the clinical presentation.

Such conflicting results in the prevalance and clinical usefullness of these virulence factors have been observed in Turkey too. Regarding the CagA and VacA status, studies in dyspeptic patients from different regions of Turkey showed that the seropositivity is 62%-97% and 70%-76%, respectively[33-36]. However, only two of these studies[33,34] have found a significant correlation between the presence of anti-CagA antibodies and peptic ulcer. A very recent study from Turkey[37] displayed that 78% of dyspepsia patients harbour the *cagA* gene and that the *cagA* gene is significantly associated with peptic ulcer and gastric cancer. All these data indicate that the association between putative virulence factors and the development of particular gastrointestinal diseases in *H pylori*-infected individuals is still a worldwide matter of debate.

In the present study, we did not observe any significant differences in seropositivities of the studied virulence factors CagA and VacA between patients with DU and controls with NUD. The overall prevalence of CagA in seropositive patients was 85%, which is in concordance with other studies from Turkey[33-36], but the seropositivity of VacA was just 33%, a low percentage compared to previous studies[33,36], suggesting that this difference may be due to the use of commercially available different Western blot kits in these studies. We think that using VacA antigens from Turkish *H pylori* strains rather than the commercially available ones could lead to more conclusive results.

H pylori has a unique set of virulence factors, actively supporting its survival in the special ecological niche of the human stomach while VacA and CagA are the two major bacterial virulence factors involved in host cell modulation. Although several studies have been performed on this issue, results are still conflicting. It was reported that none of these putative virulence factors has disease specificity and that there is evidence that virulence is a host-dependent factor[38]. The primary factors responsible for the different patterns of gastritis in response to *H pylori* infection are environmental factors (e.g. diet) rather than the *H pylori* strain[38].

In conclusion, the fact that similar frequencies of CagA and VacA-positive *H pylori* strains were observed in all our dyspeptic patients, regardless of ulcer status, suggests that factors other than these may contribute to gastrointestinal pathology in patients with *H pylori* infection. Further studies are needed to eliminate the potential bias like the selection of controls with a large sample size including different diseases and ethnic groups in order to make the results robust.

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