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RAPID COMMUNICATION

H pylori iceA alleles are disease-specific virulence factors

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Abstract

AIM: To characterize and compare genotype profiles of *H pylori* strains isolated from patients with chronic gastritis and duodenal ulcer in western part of Turkey.

METHODS: A total of 46 patients [30 chronic gastritis (CG) and 16 duodenal ulcer (DU)] who had undergone endoscopy because of dyspeptic complaints were studied. The antral biopsy specimens were evaluated for the presence of *H pylori* by rapid urease test and culture, and the genotype profiles were determined by real-time PCR.

RESULTS: The *cagA* gene was observed in 43 (93.5%) isolates. The *vacA* s1m2 genotype was the predominant subtype, found in 63.3% and 68.7% of isolates in patients with CG and DU, respectively. Twenty (66.6%) isolates from patients with CG were *iceA2* positive while the *iceA1* was predominant in those with DU (68.8%). In terms of the association of the *iceA* alleles to other genes, both alleles were significantly associated with the *cagA vacA* s1m2 genotype.

CONCLUSION: The prevalent circulating genotypes in CG and DU were *cagA vacA* s1m2 *iceA2* and *cagA vacA* s1m2 *iceA1* genotype, respectively. It was found that *cagA vacA* s1m2 genotype seems to be common virulence factors in both CG and DU while *iceA* alleles show specificity for gastroduodenal pathologies in this study.

Key words: *H pylori*; Virulence genes; Culture; Real-time PCR

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INTRODUCTION

Bacterial colonization of the gastrointestinal tract of humans and other animals is still an interesting matter of study for microbiologists and gastroenterologists. *H pylori* is a fastidious Gram-negative bacterium that colonizes in human gastric mucosa. It had been estimated that more than half of the world's population is infected with this organism[1]. *H pylori* infection is a major cause of chronic gastritis (CG) and the small number of patients develop severe complications such as duodenal ulcer (DU), gastric ulcer, gastric cancer, and gastric non-Hodgkin's lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). Variation in clinical outcomes has been attributed to differences in environmental factors and host genetics, together with bacterial genotypes[2,3]. In this regard, the high genetic variability that characterizes *H pylori* and the influence of particular virulence genes (especially *cagA*, *vacA*, and *iceA*) on clinical outcome of *H pylori* infection have been reported from different geographic regions[4-8].

A strain-specific *H pylori* gene, *cagA*, was considered as a marker for the presence of a pathogenicity island (*cag*-PAI) which encodes several proteins implicated in the pathogenesis of *H pylori*[9]. Some of the genes in the island encode a type Ⅳ secretion system, which can translocate the CagA protein into target cells. It is also reported that the other genes are particularly associated with epithelial cell responses such as higher level of IL-8 and increased leukocyte infiltration[10,11]. It has been thought that *cagA* positive *H pylori* strains were associated with a more severe clinical outcome of *H pylori* infection[9] although there was no association between *cagA* status and the outcomes in Asia[6,12,13].

The *vacA* gene encodes the vacuolating cytotoxin which causes damage in epithelial cells. The gene possesses two regions; a signal region (s1 and s2 alleles) and a midregion (m1 and m2 alleles)[14,15]. Although the *vacA* gene is present in every *H pylori* strain only about half of the strains produce the active cytotoxin because the production of cytotoxin is related to the mosaic combination of s and m allelic types. The *vacA* s1m1 genotype is thought to be associated with more severe pathologies[16].

The other virulence gene, *iceA* which has a significant

homology to a type II restriction endonuclease is also associated with *H pylori* infection[17]. Two allelic variants, *iceA1* and *iceA2*, have been identified. The *iceA2* positive strains have been reported more prevalant among patients with non-ulcer dyspepsia while *H pylori* strains possess *iceA1* allele have been more prevalant in peptic ulcer disease[5,17].

The previous studies focused on determining the genotype profiles of *H pylori* isolates in different clinical outcomes of *H pylori* infection in Turkey were limited and carried out in different geographic regions which has different environmental factors, and nutritional habits, together with different lifestyle[8,18-20]. Therefore, the aims of this study were to characterize and to compare the genotype profiles of *H pylori* isolates in patients with chronic gastritis and duodenal ulcer in the western part of Turkey.

MATERIALS AND METHODS

Patients and clinical samples

A total of 46 patients with dyspeptic complaints (30 women; mean age 49.5 and 16 men; mean age 46.9) who had undergone endoscopy in Pamukkale University Hospital were included. The exclusion criteria were briefly: treatment with antibiotics, non-steroidal anti-inflammatory drugs or proton pump inhibitors during the last 2 wk before endoscopy, having severe systematic disease, and uremic disease. The patients were divided into two groups as CG (n = 30) and DU (n = 16) according to the endoscopy reports. Written informed consent for participation was obtained from every patient before the study. The study protocol was approved in advance by the Human Institutional Review Board of Pamukkale University Medical School, and was performed in accordance with the Decleration of Helsinki. Antral biopsy specimes were evaluated for the presence of *H pylori* by rapid urease test and culture. The genotype profiles of *H pylori* isolates were determined by real-time PCR.

Rapid urease test and culture

For rapid urease test, the specimens were inoculated into the CLOtest (Kimberly Clarck, USA). A positive result was recorded when the color changed from yellow to pink within 24 h.

For bacterial culture, the biopsy specimens were inoculated on Brain Heart Infusion Agar (Difco) containing 7% horse blood and *H pylori* selective supplement (Oxoid-SR 147E). The agar plates were incubated under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) at 37℃ for up to 7 d. Colonies were identified as *H pylori* according to standard criteria including negative Gram's staining, typical cell morphology, and positive reactions for catalase, oxidase and urease.

DNA isolation from gastric biopsy specimens and real-time PCR

H pylori genomic DNA was extracted from the biopsy specimens using the QIAamp DNA mini kit (Qiagen, Istanbul) as described by the manufacturer. One hundred microliters of elution buffer was used to resuspend the DNA. The genomic DNAs were stored at 4℃ until used as a template in real-time PCR.

For real-time PCR, each reaction tube contained 2 μL of LightCycler FastStart Master SYBR Green I (Roche, Izmir), 12.4 μL of PCR-grade H_2O, 1.6 μL of 25mmol/L $MgCl_2$, 2 μL of a 10mmol/L concentration of primer set, and 2 μL of template DNA in a 20 μL PCR mixture. All oligonucleotide primers designed by Yamaoka *et al*[6] were used in real-time PCR and synthesized by TıbMolbiol (Berlin, Germany). The reaction protocol for *cagA* was as follows: an initial FastStart Taq DNA polymerase activation phase at 95℃ for 10 min; a 35 cycle amplification phase consisting of a 95℃ denaturation segment for 10 s, a 55℃ annealing segment for 5 s, and a 72℃ extension segment for 10 s. After completion of the amplification process, the reaction mixture was denaturated 95℃ for 0 s, held at 65℃ for 18 s, and then slowly heated to 95℃ at a ramp rate of 0.2℃ per second. The *cagA* real-time PCR protocol was used with little modifications for other oligonucleotide primers. At the end of the cycles, a cooling step at 40℃ for 30 s was performed for each reaction.

All runs were included one negative DNA control consisting of PCR-grade water and two or more positive controls (HP 26695, HP J99 and some clinical isolates, a gift from Dr. Yamaoka, Baylor College, Texas, USA).

Statistical analysis

The χ^2 test was used to compare differences in the prevalence of *H pylori* genotypes between groups. P values of < 0.05 were considered significant.

RESULTS

Patients were considered infected with *H pylori* infection if the biopsy specimens gave positive results in any one of the following tests: CLO test, culture, or real-time PCR. The rapid urease test and culture were positive in 78.2% and 86.9% of the specimens, respectively. Out of 46 specimens analyzed, all (100%) gave informative results by real-time PCR.

The *cagA*, *vacA* and *iceA* genotypes of *H pylori* isolates were determined by melting curve analysis of real-time PCR. The following isolates were also tested and found negative by real-time PCR: *Campylobacter jejuni*, *Escherichia coli*, *Salmonella enterica* serovar Enteritidis and *Staphylococcus aureus* (data was not shown). The melting temperatures for *cagA*, *vacA* s1, *vacA* s2, *vacA* m1, *vacA* m2, *iceA1*, and *iceA2* were 80.08℃, 86.44℃, 76.15℃, 82.85℃, 84.59℃, 83.06℃, and 79.02℃, respectively.

In this study, it was found that only three of 46 (6.5%) *H pylori* isolates were *cagA* negative, whereas the remaining 43 (93.5%) isolates were *cagA* positive. Of the *cagA* negative isolates, two were isolated from patients with CG. The prevalence of *cagA* gene was 93.3% and 93.75 in *H pylori* isolates in patients with CG and DU, respectively. There were no statistically significant differences between the *cagA* positivity and CG or DU (Figure 1).

All DNA extracts described in this study were positive for the *vacA* gene. For the *vacA* s and m region, 41/46

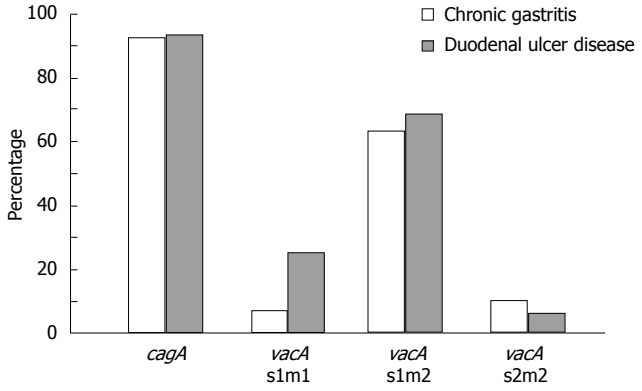

Figure 1 Percentage of distrubitions of *cagA* gene and *vacA* alleles in chronic gastritis and duodenal ulcer disease.

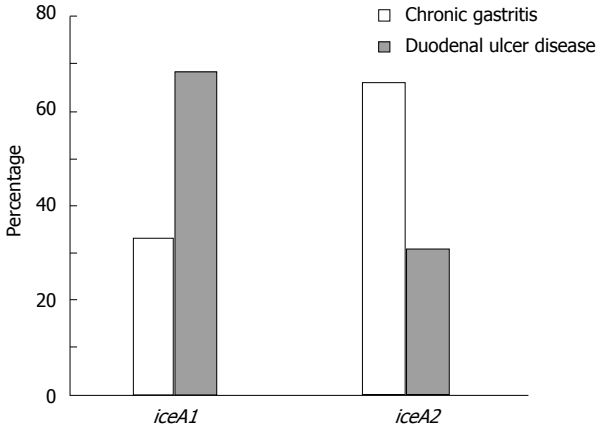

Figure 2 *iceA* alleles show specificity for chronic gastritis and duodenal ulcer disease.

(89.1%) isolates were the type s1 and 38/46 (82.6%) isolates were the type m2. The *vacA* s1m2 genotype was the predominant subtype, being found in 63.3% of the isolates in patients with CG and 68.7% of those with DU. No *vacA* s2m1 genotype was determined in this study (Figure 1). No correlation between the *vacA* genotype and both gastroduodenal pathologies was observed. The presence of the *vacA* s1m2 genotype in combination with *cagA* were 73.3% and 68.7% of the isolates in CG and DU, respectively.

In this study, the *iceA1* allele was detected in 45.7% (21/46) of the *H pylori* isolates, and the *iceA2* allele was detected in 54.3% (25) of the isolates. The *iceA1* allele was significantly associated with DU (68.8%, $P < 0.05$) while there was a significant relationship between *iceA2* allele and CG (66.6%, $P < 0.05$) (Figure 2).

DISCUSSION

Several epidemiological studies have been reported the influence of particular virulence genes (especially *cagA*, *vacA*, and *iceA*) on clinical outcome of *H pylori* infection in different geographic regions[4-8]. This study was designed to characterize and to compare the genotype profiles of *H pylori* strains isolated from patients with chronic gastritis and duodenal ulcer in western part of Turkey.

In this study, the prevalence of *cagA* gene was 93.3% and 93.75 in *H pylori* isolates in patients with CG and DU, respectively. The result obtained from the isolates in the patients with DU is in agreement with other studies carried out in patients with DU in Turkey (85%-89%)[8,18]. However, the *cagA* prevalence in total is similar to those reported in Asia[6,12,21] and Ireland[22] but higher than those in Western countries[5,23,24]. Interestingly, the high prevalence of *cagA* (93.3%) in isolates of patients with CG was observed. The presence of *cagA* is known as a predictive marker for *cag*-PAI. Although intact *cag*-PAI was associated with the development of gastroduodenal pathology[25] it was also recently reported that this island was not intact in many strains across the world[26]. Therefore, the high prevalence of *cagA* in CG could not be explained precisely, a possible reason to explain this phenomenon is that the *cagA* positive strains with nonintact PAI might likely carry deleted or nonfunctional virulence genes[25].

The *vacA* gene was detected in all strains. The *vacA* s1m2 genotype predominant irrespective of the clinical outcome was also predominant in strains from Western countries including Turkey[4,18,22,27]. Aydin F *et al*[19] reported the *vacA* s1m1 genotype was the common *vacA* genotype in Black sea region located in Asian part of Turkey. The prevalence of *vacA* s1m1 genotype in our study was 6.6% and 25% of the strains in patients with CG and DU, respectively. The assesment of *vacA* gene mosaicism found only three out of the four possible combinations, the s2m1 mosaicism being never detected in our series, which was typically observed in strains from Western country including Turkey[18,19,22,24]. In this study, the *cagA vacA* s1m2 genotype was predominant genotype, and there was no significant relationship between both of the pathologies, indicating that the genotype was common virulence factors of *H pylori*.

Although the function of *iceA* gene is not clear, it is known that the expression of the gene is induced by contact between *H pylori* and the epithelial cells of the stomach[17]. The previous studies focused on the *iceA* genotyping in Turkey was limited. One of them reported that there was no significant association between the *iceA* genotype and clinical outcome of *H pylori* infection[20] while the other was found the *iceA* allele was significantly higher among patients with gastric cancer when compared to patients with non-ulcer dyspepsia[18]. In present study, most *H pylori* strains (66.6%) isolated from patients with chronic gastritis had *iceA2* allele while *iceA1* allele was predominant in those with duodenal ulcer disease (68.8%). This difference was statistically significant. In terms of the association of the *iceA1* allele to DU, there is an agreement with the previous studies carried out in the USA[6], China[28], and Netherland[5] but in contrast to the results reported from Japan[6]. The high prevalence of the *iceA2* allele in patients with CG was also observed in the USA[6]. Such differences the discrepant results between the *iceA* alleles and the clinical outcome of *H pylori* infection could be explained by the genetic heterogenity or to differences in the geographic locations as were previously reported for the other virulence genes[29,30].

In summary, the prevalent circulating genotypes in CG and DU in western part of Turkey were *cagA vacA* s1m2 *iceA2* and *cagA vacA* s1m2 *iceA1* genotype, respectively. It was also found that *cagA vacA*s1m2 genotype seems to be common virulence factors in both chronic gastritis and duodenal ulcer disease while *iceA* alleles show specificity for gastroduodenal ulcer disease. To understand the pathogenesis of the infection, the population genetics of *H pylori* together with host response including genetic predisposition and immune response, and environmental factors should also be considered.

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