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PREVALENCE OF GENOTYPES IN *HELICOBACTER PYLORI* ISOLATES FROM PATIENTS IN EASTERN TURKEY AND THE ASSOCIATION OF THESE GENOTYPES WITH CLINICAL OUTCOME

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ABSTRACT

There is not much information available regarding the prevalence of the genotypes of *Helicobacter pylori* isolates in Turkey, particularly in eastern Turkey. The aims of this study were to detect the prevalence of different genotypes of *H. pylori* in Turkish patients with gastrointestinal complaints and to determine the relationship of these genotypes with clinical outcome and sex. One hundred forty *H. pylori* isolates were examined for the presence of its genotypes by the PCR. We found that the prevalence of *vacA*s1, *vacA*s2, *cagA, cagE, iceA1, iceA2* and *babA2* genes were 88.6%, 11.4%, 71.4%, 35.7%, 41.4%, 58.6%, and 62.1%, respectively. The most predominant *vacA* subtype was s1a (81.4%). The most *vacA* allelic combination detected were *vacA*s1m1 (65.2%) and s1m2 (53.9%) in patients with peptic ulcer and gastritis, respectively. The only *vacA*s1 isolate was significantly associated with gastritis and peptic ulcer ($p<0.05$). The *vacA*s1a, ml, slml and *babA2* genes were significantly associated with peptic ulcer ($p<0.05$), whereas m2 gene was significantly associated with only gastritis ($p<0.05$). The difference between sex and genotypes was statistically significant among the *cagA*, *vacA*s1, *iceA2* and *babA2* genes. This study reported for the first time the prevalence of *H. pylori* genotypes in patients with gastrointestinal complaints in eastern Turkey. Further studies are needed to understand epidemiological importance of the genotypes of *H. pylori* isolates in this region and the association between the virulence genes and clinical outcome in different regions.

Key words: *Helicobacter pylori*, prevalence, patient, genotypes, PCR.

INTRODUCTION

Helicobacter pylori (*H. pylori*) which infects more than half of the world's population, a major etiological agent in development of gastritis (G), peptic ulcer (PU) and gastric carcinoma (3). Scientists have been shown that several genes, such as the vacuolating cytotoxin (*vacA*), cytotoxin associated gene A (*cagA*), cytotoxin associated gene E (*cagE*), induced by contact with epithelium (*iceA*) and blood adhesion binding antigen (*babA2*) have been determined and these genes may play important roles in the pathogenesis of *H. pylori* infection (22, 24).

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The *cagA* gene being a marker for the presence of the cag pathogenicity island (*cag*PAI) of approximaely 40 kb was the first gene found to be present in *H. pylori* strains (7) and its presence is associated with a more severe clinical outcome, such as PU, atrophic G, and gastric cancer (GC) (4, 5). The *cagA* induces interleukin-8 (IL-8) production and mucosal inflamation (4, 5). The *cag*PAI contains a gene known as *cagE*, is one of the marker genes in *cagI* of the *cag*PAI and it is required for translocation and phosphorylation (9, 28). The *cagE* gene was found to be associated with a more severe clinical outcome (11).

The *vacA* gene is present in all *H. pylori* strains and contains at least two variable parts (a hypervariable signal sequence and a middle region allele) (2). Among the *vacA* subtypes, subtypes s1a, s1b, s1c and s2, and m1, m2a and m2b have been identified (30). Although all strains of *H. pylori* include the *vacA* gene, they vary in their ability to produce cytotoxin (8). Type m1 strains show more toxic activity than m2, type s1a is more active than s1b, and type s2 is less active than s1 (2).

The recently discovered *iceA* gene exists in two main allelic variants of the gene, *iceA1* and *iceA2* (21). *iceA1* is upregulated upon contact of *H. pylori* with the gastric epithelium (21) and is a marker for PU disease (31).

Blood adhesion binding antigen A, encoded by the *babA2* gene has been exhibited to mediate binding activity between bacterial adhesin and human Lewis-b blood group antigens on gastric epithelial cells (14). Although three *babA* alleles have been identified (*babA1, babA2* and *babB*), only the *babA2* gene product is necessary for Lewis b binding activity (14).

There is not much information available on determination of the genotypes of *H. pylori* in Turkey, particularly in eastern Turkey where the overall incidence of G and PU are high. This study aimed 1) to detect the prevalence of the *vacA, cagA, cagE, iceA1, iceA2* and *babA2* genotypes in patients with G and PU, 2) to determine a possible association between clinical outcome and genotypes and 3) to identify any association between the genotypes and sex.

MATERIALS AND METHODS

Patients

A total of 140 *H. pylori* isolates (115 with G, 23 with PU, 2 with GC) identified by PCR from antral biopsies of 184 Turkish patients [17-92 years of age (average 49)] who underwent endoscopy at Firat University Hospital, Gastroenterology Department during 2009 and 2010. Approval of this study was obtained from the Medical Ethics Committee of Firat University. We received informed consent from all patients.

DNA Extraction

DNA samples was extracted by QIAamp DNA mini kit (Qiagen, Lot No: 11872534, Cat No: 51306) according to the manufacturer's instructions. The extracted DNA was stored at -20 ºC until used as template in PCR.

PCR analysis of genotypes in *H. pylori* isolates

The primers used in this study and PCR conditions are shown in Table 1. For the *cagA* gene subtypıng, primers CAGA-F and CAGA-R yielded a fragment of 298 bp of the *cagA* gene were used (13). For analysis of the *vacA* s region, primers VA1-F, VA1-R, SS2-F, and SS3-F were used (2, 23, 34). Primers VA1-F and VA1-R yielded a fragment of 259 bp or 286 bp in size for type s1 or s2 strains, respectively. For detection of the *vacA* m region, primers VAG-F and VAG-R generated a fragment of 567 bp for m1 variants and a fragment of 642 bp for m2 variants (23). For analysis of the *cagE* gene, primers CagE-F and CagE-R yielded a fragment of 508 bp of the *cagE* gene described by Tomasini *et al*. (28) were used. For detection of the *iceA* gene, primers *iceA*1-F, *iceA*1-R, *iceA*2-F, and *iceA*2-R were used (31). Primers *iceA*1-F and *iceA*1-R generated a fragment of 247 bp for the *iceA1* gene, and primers *iceA*2-F and *iceA*2-R generated a fragment of 229 or 334 bp for the *iceA2* gene (31). For analysis of the *babA*2 gene, primers BABA2-F and BABA2-R described by Sheu *et al*. (27) were used.

Amplification was performed in a reaction mixture (50 µl final volume) containing 25 µl 2XPCR Master Mix (Fermentas, K01071), 15 µl distilled water, 2.5 µl of each primer and 400 ng genomic DNA. The thermal cycling conditions performed with a touchdown thermal cycler (Hybaid, Middlesex, England). PCR product was analyzed on 1.5% agarose gel containing 0.5 µg/ml of ethidium bromide.

The DNAs of the HP 26695, HP J99 and some clinical isolates, provided by Dr. Yoshio Yamaoka from Michael E. DeBakey Veterans Affairs Medical Center, Houston, TX 77030, USA was used to confirm the PCR test as positive controls. Distilled water used as a negative control.

Table 1. Primers and PCR conditions used in our study.

Genes	Sequence (5′ → 3′)	Cycle Conditions	Size (bp) of Expected PCR product	Reference number
cagA	ATAATGCTAAATTAGACAACTTGAGCGA TTAGAATAATCAACAAACATCACGCCAT	94°C, 1 min; 60°C, 1 min; 72°C, 1 min (45 cycles)	298	13
vacA s1/s2	ATGGAAATACAACAAACACAC CTGCTTGAATGCGCCAAAC	94°C, 1 min; 52°C, 1 min; 72°C, 1 min (35 cycles)	259/286	2, 23
vacAs1a	GTCAGCATCACACCGCAAC CTGCTTGAATGCGCCAAAC	94°C, 1 min; 52°C, 1 min; 72°C, 1 min (35 cycles)	190	23
vacAs1b	AGCGCCATACCGCAAGAG CTGCTTGAATGCGCCAAAC	94°C, 1 min; 52°C, 1 min; 72°C, 1 min (35 cycles)	187	23
vacAs1c	CTCTCGCTTTAGTGGGGYT CTGCTTGAATGCGCCAAAC	94°C, 1 min; 52°C, 1 min; 72°C, 1 min (35 cycles)	213	34
vacA m1/m2	CAATCTGTCCAATCAAGCGAG GCGTCAAAATAATTCCAAGG	94°C, 1 min; 52°C, 1 min; 72°C, 1 min (35 cycles)	567/642	23
cagE	TTGAAAACTTCAAGGATAGGATAGAGC GCCTAGCGTAATATCACCATTACCC	94 °C, 1 min; 53°C, 45 s; 72°C, 45 s (35 cycles)	508	28
iceA1	GTGTTTTTAACCAAAGTATC CTATAGCCASTYTCTTTGCA	95°C 1 min; 57°C, 1 s; 72°C, 1 min (35 cycles)	247	31
iceA2	GTTGGGTATATCACAATTTAT TTRCCCTATTTTCTAGTAGGT	95°C 1 min; 57°C, 1 s; 72°C, 1 min (35 cycles)	229 or 334	31
babA2	CCAAACGAAACAAAAAGCGT GCTTGTGTAAAAGCCGTCGT	94°C, 1 min; 45°C, 1 min; 72°C, 1 min (30 cycles)	271	27

Statistical analysis

The Fischer's exact and χ^2 tests were used to compare the differences between *H. pylori* genotypes and clinical outcome and between the sex and genotypes. A p value of <0.05 was taken statistically significant.

RESULTS

The prevalence of *cagA vacA, cagE, iceA* and *babA2* genes are shown in Table 2. Because the number of patients with GC is very low, the relationship between *H. pylori* genotypes in patients with GC was not determined.

The *cagA* gene was determined in 100 (71.4%) of 140 isolates examined (Table 2). The *vacA* genes was found in all isolates we studied. All *vacA*m1 genotypes from patients were also *vacA*s1. The *vacA*s1a (81.4%) gene was found most frequently than *vacA*s1b (2.9%) and *vacA*s1c (4.3%). The most *vacA* allelic combination was s1/m2 (50.7%), followed by s1/m1 (37.9%) and s2/m2 (11.4%). In addition, the most common *vacA* allelic combination were *vacA*s1m1 (65.2%) and s1m2 (53.9%) in patients with PU and G, respectively. No *vacA*s2m1 genotype was observed in our study. Seventy-four (52.9%) isolates were found to be "triple positive" (*vacA*s1$^+$*cagA*$^+$*babA2*$^+$) (Table 2).

The prevalence of the *cagE* genotype was 50 (35.7%), and it was found more commonly in patients with PU. The *iceA* genes were found in all isolates we studied. The *iceA1* and

iceA2 genes were detected in 58 (41.4%) and 82 (58.6%) isolates, respectively. The *iceA1* gene was most frequently observed in patients (60.9%) with PU, whereas *iceA2* was most commonly found in patients (63.5%) with G. The *iceA2* isolates classified in two types according to PCR product size: 229 and 334 bp. The distribution of these two types were similar in the isolates examined (data not shown). The *babA2* gene was observed in 87 (62.1%) of all isolates studied (Table 2 and 3).

The presence of the only *vacA*s1 isolate was significantly associated with G and PU ($p<0.05$). The *vacA*s1a, ml, slml and *babA2* genes were significantly associated with PU ($p<0.05$), whereas m2 gene was significantly associated with only G ($p<0.05$). The *cagA* gene was significantly associated with s1, s1m1 and *babA2* genotypes ($p<0.05$) (Table 3).

The distribution of *H. pylori* genotypes and sex is shown in Table 4. The difference between sex and genotypes was statistically significant among the *cagA, vacA*s1, *iceA2* and *babA2* genes ($p<0.05$) (Table 4).

Table 2. The prevalence of virulence genes in *H. pylori* isolates.

Virulence genes	Prevalence (n) (%)
*cag*A	100 (71.4)
*vac*As1	124 (88.6)
*vac*As1a	114 (81.4)
*vac*As1b	4 (2.9)
*vac*As1c	6 (4.3)
*vac*As2	16 (11.4)
*vac*Am1	53 (37.9)
*vac*Am2	87 (62.1)
*vac*As1/m1	53 (37.9)
*vac*As1/m2	71 (50.7)
*vac*As2/m2	16 (11.4)
*cag*E	50 (35.7)
*ice*A1	58 (41,4)
*ice*A2	82 (58.6)
*bab*A2	87 (62.1)
*vac*As1$^+$ *cagA* $^+$ *bab*A2$^+$	74 (52.9)

Table 3. The prevalence of the genotypes in 140 *H. pylori* positive patients with G and PU.

Genotypes	G (n = 115) (%)	PU (n = 23)(%)
*cag*A	79 (68.7)	19 (82.6)
*vac*As1	99 (86.1)*	23 (100)*
*vac*As1a	90 (78.3)	22 (95.7)*
*vac*As1b	4 (3.5)	0 (0)
*vac*As1c	5 (4.3)	1 (4.3)
*vac*As2	16 (13.9)	0 (0)
*vac*Am1	37 (32.2)	15 (65.2)*
*vac*Am2	78 (67.8)*	8 (34.8)
*vac*As1/m1	37 (32.2)	15 (65.2)*
*vac*As1/m2	62 (53.9)	8 (34.8)
*vac*As2/m2	16 (13.9)	0 (0)
*cag*E	39 (33.9)	10 (43.5)
*ice*A1	42 (36.5)	14 (60.9)
*ice*A2	73 (63.5)	9 (39.1)
*bab*A2	68 (59.1)	17 (73.9)*
*vac*As1$^+$ *cag*A $^+$ *bab*A2$^+$	59 (51.3)	13 (56.5)

G, gastritis; PU, peptic ulcer, * significant $p<0.05$

Table 4. The association between the sex of 140 *H. pylori*-positive patients and its genotypes.

Genotypes	Male (70) n (%)	Female (70) n (%)
*cag*A	53 (75.7)*	47 (67.1)
*vac*A s1	66 (94.3)*	58 (82.9)
*vac*A s2	4 (5.7)	12 (17.1)
*vac*A s1a	58 (82.9)	56 (80)
*vac*A s1b	3 (4.3)	1 (1.4)
*vac*A s1c	5 (7.1)	1 (1.4)
*vac*A m1	31 (44.3)	22 (31.4)
*vac*A m2	39 (55.7)	48 (68.6)
*vac*A s1/m1	31 (44.3)	22 (31.4)
*vac*A s1/m2	35 (50)	36 (51.4)
*vac*A s2/m2	4 (5.7)	12 (17.1)
*cag*E	28 (40)	22 (31.4)
*ice*A1	20 (28.6)	38 (54.3)
*ice*A2	50 (71.4)*	32 (45.7)
babA2	50 (71.4)*	37 (52.9)
*vac*As1$^+$ *cagA* $^+$ *bab*A2$^+$	45 (64.3)	29 (41.4)

* significant $p<0.05$

DISCUSSION

The number of studies related to genotypes of *H. pylori* in Turkey is seldom, and the data on the relationship of the genotypes and gastrointestinal diseases have been still disputable (8).

The *cagA* prevalence is different in every part of the world. The prevalence of *cagA* gene in this study was 71.4%.

This finding is in agrement with reports from Western countries (22, 24) but lower than reports from East Asian countries where the *cagA* are present in more than 90% of cases (33). In addition, the results of this study are consistent with previous reports (6, 29) which no association was found between *cagA* gene and PU. However, some studies have been reported that *cagA* gene was statistically associated with PU (10, 20, 26).

The different results have also been reported in studies related to the *vacA* gene of *H. pylori* strains. In the present study, the *vacA* gene was observed in all strains. Our finding was similar to previous reports from Turkey (10, 25, 26), Northern and Eastern European countries (32) where s1a gene was predominant genotype, but in contrast to a report from Korea (16). We detected a low prevalence of *vacA*s1b in this study which was contrast to pevious reports in Portugal, Central and South America (32). No *vacA*s1c genotype was determined except for the only one study reported in Turkey (18). In this study, the prevalence of the *vacA*s1c gene was found to be low. This may be related to low prevalence of GC in Turkey as stated in a study carried out by Erzin *et al.* (10).

We found that no s1b, s1c and s2 genotypes associated with G and PU. Our data similar to previous reports in Turkey where *vacA*s1a strains were showed to be significantly associated with PU (10, 18), but different from a study in Turkey reported that *vacA*s1a strains were not found to be statistically associated with PU (26).

Our results supported the findings of the studies performed in Turkey and other countries (3, 6, 10, 18) where the s1m2 genotype was the most common gene combinations of the *vacA*, but contrary to studies (8, 26) reported that s1m1 genotype was the most predominant gene. The prevalence of s2m2 genotype was determined as 11.4% and all of s2m2 positive isolates were *cagA* negative. No s2m1 genotype was found in this study. This finding correlated confirm few data reported from other geographic regions of Turkey (6, 10, 18). On the other hand, this study confirmed the findings of previous reports (6, 8, 10, 29) where there was no significant association between *vacA*s1m2 genotype, G and PU disease.

The prevalence (35.7%) of *cagE* in this study is lower than other studies conducted in USA (64%) (22), Turkey (59.3%) (10), UK (71.2%) (15) and Thailand (88.4%) (8) but higher than a previous study in Turkey (28.6%) (25). In an attempt to detect association between the *cagE* gene and PU, Fallone *et al.* (11), Day *et al.* (9) and Erzin *et al.* (10) have reported a significant association between the *cagE* gene and PU which is contrast to our findings.

In contrast to the results reported in China, Japan, Korea and Netherlands where the *iceA1* gene was predominant (16, 20, 31), the *iceA2* gene was detected to be predominant genotype in this study. This result is in agreement with previous reports that the *iceA2* gene was found to be prevalent in Brazilian, European and American patients (1, 20, 22).

Our results were similar to a previous study (1) reported that the distribution of *iceA2* 229 and 334-bp amplicon was found to almost the same in *H. pylori* strains. The results of the present study are similar to previous studies that no significant association between the *iceA1* gene and PU have found in Brazilian and Turkish patients (1, 6, 10), but in contrast to those reported by Peek *et al.* (21) and van Doorn *et al.* (31), who showed the association between the presence of *iceA1* gene and PU. The *iceA2* was more frequently observed in males than in females. Similar result have been reported by Ashour et al. (1).

The prevalence of the *babA2* genotype in *H. pylori* strains varied in different countries of world. The prevalence of the *babA2* was 34-72% in Western countries (12, 19, 29) while it was 80-100% in Asian countries (16). We found a higher prevalence (62.1%) of *babA2* than the result (53.8%) reported in a previous study (10) in Turkey. Erzin *et al.* (10) had used the primers described by Gerhard *et al.* (12), we used the primers of Sheu *et al.* (27) which exhibited a high prevalence of *babA* (8). A low prevalence of the *babA2* gene was detected when the primers described by Gerhard *et al.* (12) were used (12, 20). These primers amplifies 832 bp (12) in a highly variable region, associated with false negative PCR due to allelic

variation (19). This study showed a highly significant association between the *babA2* gene and PU disease, concurring with the previous studies (12, 29). This state may be explained by allelic variation in the *babA* gene which could have a variable affect in the different geographic regions of a country (19).

Regarding the relationship with genotypes in each isolate, we found to be an association between the *cagA* stratus and the *vacA*s1 genotype in present study which was similar with previous findings (12, 26, 29, 32). In addition, our study showed significantly association between the *babA2* and the *vacA*s1 in contrast to the results reported by Erzin *et al*. (10) and Torres *et al*. (29).

In regard to the association with the distribution of *H. pylori* genotypes and sex in this study, the association was statistically significant among the *cagA, vacA*s1, *iceA2* and *babA2* genes. In a study conducted by Mansour and colleagues (17), there was no significant association between *cagA* gene and sex, but the association was statistically significant among the *vacA*, *iceA* and *oipA* genes (17).

This study reported the prevalence of the genotypes in *H. pylori* isolates in Elazig province located in the East of Turkey for the first time. However, the multicenter and large scale studies are needed to help us better understand epidemiological importance of this disease and the association between *H. pylori* genotypes and clinical outcome in different regions and populations.

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