Investigation of the association between clinical outcome and the cag pathogenicity-island and other virulence genes of *Helicobacter pylori* isolates from patients with dyspepsia in Eastern Turkey

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Abstract

The aims of our work were to determine the presence of the cag pathogenicity-island (cag PAI) and other virulence genes of *Helicobacter pylori* recovered from patients with gastritis and peptic ulcer, and to investigate the correlation of these virulence genes with clinical outcome. The presence of the cagA, the promoter regions of cagA, cagE, cagT, and the left end of cag-PAI (LEC), cag right junction (cagRJ), the plasticity region open reading frames (ORFs), vacA and oipA genes among 69 *H. pylori* isolates were determined by polymerase chain reaction. Intact cag PAI was detected in only one (1.4%) isolate. The cagA gene was identified in 52.1% and 76.2% of isolates from patients with dyspepsia (gastritis and peptic ulcer), respectively. The plasticity region ORFs i.e. JHP912 and JHP931 were predominantly detected in isolates from peptic ulcer. Less than 25% of the isolates carried other ORFs. Types I, II and III were the most commonly found among the isolates. None of the isolates possessed type Ib, 1c, IIIb, IV and V motifs. The most commonly vacA genotypes were s1am1a and s1m2 in isolates with peptic ulcer and gastritis, respectively. The results confirmed that the prevalence of oipA (Hp0638) gene was 75% and 85.7% in patients with gastritis and peptic ulcer, respectively. Furthermore, vacA s1am1a positivity was significantly related to peptic ulcer (p < 0.05).

Key words: *Helicobacter pylori*, gastritis, peptic ulcer, cag pathogenicity-island, polymerase chain reaction.

Introduction

*Helicobacter pylori* (*H. pylori*) is a bacterial pathogen which can cause gastritis, peptic ulcer and gastric carcinoma (Cremonini *et al.*, 2001; Saunders *et al.*, 2005). Strains of *H. pylori* are classified into two types (types I and II) (Xiang *et al.*, 1995: Hofman *et al.*, 2000). Type I is a pathogenic form, correlates with severe disease status, expresses functional vacuolating cytotoxin A (vacA) and includes an approximately 40 kb cluster located at 3’ end of the cag pathogenicity island (cag PAI) (Censini *et al.*, 1996; Ikenoue *et al.*, 2001; Kersulyte *et al.*, 2000; Mattar *et al.*, 2007). Type II which is less virulent and includes a non-pathogenic form of vacA, lacks cag PAI (Censini *et al.*, 1996; Backert *et al.*, 2004).

The cag PAI is separated the two groups (cagI and cagII) by a novel insertion sequence called IS605 and these include at least 14 and 16 open reading frames (ORFs), respectively (Censini *et al.*, 1996; Akopyants *et al.*, 1998; Audibert *et al.*, 2001; Mattar *et al.*, 2007). The cytotoxin associated gene E (cagE) gene which is needed for the induction of interleukin (IL)-8 from gastric epithelial cells is located in the cagI (Censini *et al.*, 1996; Ikenoue *et al.*, 2001; Tan *et al.*, 2005). The cagT gene has been reported to be a marker of the cagII region (Mattar *et al.*, 2007) and correlates with severe clinical outcomes (Mattar *et al.*, 2007; Pacheco *et al.*, 2008).

Comparison of the genome sequence analysis of *H. pylori* 26695 and J99 strains demonstrated several regions of different G+C contents (Tomb *et al.*, 1997; Alm *et al.*,...
DNA extraction and Determination of the cag-PAI was ensured from each participant. The Ethics Committee at Firat University and informed consent of the cag right junction (cag) (Occhialini et al., 2000; Salih et al., 2007). In the J99 plasticity region (JHP914 to JHP951), the authors reported to observed to be 38 ORFs while 33 ORFs were not included in H. pylori 26695, and the majority of the ORFs encode putative proteins with unknown function (Occhialini et al., 2000). However, some of ORFs have been determined to share similarity to genes encoding proteins included in DNA replication (JHP919 and JHP931) and other functions (JHP941 and JHP951) (Occhialini et al., 2000; Salih et al., 2007).

Till date, we studied on the presence of several genes, such as cagA, vacA, cagE, induced by contact with epithelium (iceA) and blood adhesion binding antigen (babA2) among adults (Ozbey et al., 2012) and children (Ozbey et al., 2013) in Eastern Turkey. However, the data on identification of cag PAI and multiple virulence genes of H. pylori in Turkey is scarce. This study aimed to identify the presence of cag PAI and other virulence genes of H. pylori isolates from dyspeptic patients with gastritis and peptic ulcer in Elazig Province, the East of Turkey as well as to evaluate the relevance between the clinical outcome and the cag PAI and other virulence genes.

Materials and Methods

Isolates

A total of 69 H. pylori isolates (48 cases of gastritis and 21 cases of peptic ulcer) obtained from Turkish dyspeptic patients attending Gastroenterology Unit of Firat University Hospital between May and December 2011 were analyzed for the presence of cag PAI and other virulence genes. Ethics approval was given by the Medical Ethics Committee at Firat University and informed consent was ensured from each participant.

DNA extraction and Determination of the cag-PAI and other virulence genes

DNA samples from H. pylori isolates were extracted by QIAamp DNA mini kit (Qiagen, Germany) according to the manufacturer’s guidelines.

PCR analyses were performed to amplify cagA, the cagA promoter region, cagE, cagT, and the LEC of the cag PAI, as described elsewhere (Ikenoue et al., 2001; Kauser et al., 2004) (Table 1).

Primers which designed by Kersulyte et al. (2000), Mukhopadhyay et al. (2000), Veralovic et al. (1991) and Kauser et al. (2005a) were used to determine the presence of the cag right junction (cagRJ), the plasticity region ORFs, vacA and oipA (Hp0638) genes (Table 1).

Amplification reactions were performed using 2XPCR Master Mix kit (#K01071, Fermentas) following the manufacturer’s instructions in touchdown thermal cycler (Hybaid, England) with PCR conditions shown in Table 1. Ten µL aliquot of each amplicon was expose to gel electrophoresis on a 1.5% agarose gel and visualised using a UV transilluminator.

Statistical analysis

Fisher’s exact and χ² tests were used to analyze significant differences between the cag PAI and other virulence genes of H. pylori isolates with the clinical outcome. A probability of less than 0.05 was evaluated significant.

Results

Table 2 shows the distribution of the cag PAI and other virulence genes of H. pylori isolates from cases of gastritis and peptic ulcer. The prevalence of LECI, LECII, cagE, the promoter region of the cagA and cagA were detected more (14.3%, 19%, 38.1%, 47.6% and 76.2%, respectively) in isolates from peptic ulcer. One isolate (1.4%; 1 of 69) was observed to possess the intact cag PAI.

Types I (6.3%), II (4.2%) and III (8.3%) were observed predominantly in isolates from gastritis. However, Ia (19%) and IIIa (23.8%) motifs were the most common types in peptic ulcer isolates. None of the isolates contained type Ib, Ic, IIIb, IV and V motifs. The most predominant plasticity region ORFs were JHP912 and JHP931 and these two ORFs were identified more in isolates from peptic ulcer. Less than 25% of the isolates carried other ORFs (JHP926, JHP933, JHP944, JHP945, JHP986). The vacA s1am1a was the most extensively vacA genotype found in isolates with peptic ulcer while s1m2 was the most predominant genotype in patients with gastritis. However, no vacAs1c, vacAm1b and vacAs2m1 genotypes were demonstrated in the current study. The oipA gene was observed in 75% of isolates with gastritis and 85.7% of isolates with peptic ulcer.

Assessing the association between the cag PAI and other virulent genes with clinical outcome, vacA s1am1a genotype was shown to be statistically significant with peptic ulcer (p < 0.05).

Discussion

Since its first identification by Censini et al. (1996) in 1996, the cag PAI part of the H. pylori genome has been widely studied so far (Olbermann et al., 2010; Rizzato et al., 2012).

Conflicting results have been obtained in studies on the prevalence of cagA gene in different geographical regions of the world. The prevalence of the cagA gene was 60-70% in Western countries (Rudi et al., 1998) but the prevalence in East Asian countries was detected to be found in more than 90% of cases (Maeda et al., 1998; Yamaoka et al., 1999). This study was similar to that reported in Turkey (Salih et al., 2007) and Western countries (Covacci et al., 1999; Arents et al., 2001) where cagA gene were observed
| Genes         | Primer | Oligonucleotide sequence (5’-3’)                  | PCR conditions                                                                 | Size (bp) of PCR product | References                              |
|--------------|--------|--------------------------------------------------|-------------------------------------------------------------------------------|--------------------------|------------------------------------------|
| *cag* PAI    |        |                                                  |                                                                               |                          |                                          |
| *cagA1*      | cagA-F1 | AACAGGCAAGTAGCTAGGCC                              | 94 °C for 5 min (initial denaturation)                                        | 701                      |                                          |
|              | cagA-R1 | TATTAAGCGTGTTGGTGGT                              |                                                                               |                          |                                          |
| *cagA2*      | cagA-F2 | GATAACAGGCAAGCTTNGA                               |                                                                               | 349                      |                                          |
|              | cagA-R2 | CTGCAAAAGAATTTGGCGA                               |                                                                               |                          |                                          |
| *cagAP1*     | cagAP-F1 | GTGGGTAAATATGTAATAGC                             | 90 °C for 30 s; 52 °C for 30 s                                               | 730                      | (Ikenoue et al., 2001; Kauser et al., 2004, 2005a) |
|              | cagAP-R1 | CTGCAAAAGAATTTGGCGA                               | 70 °C for 1 min (40 cycles)                                                   |                          |                                          |
| *cagAP2*     | cagAP-F2 | CTACTTGTCCACACCATTTT                              | 70 °C for 10 min (final extension)                                            | 1181                     | (Ikenoue et al., 2001; Kauser et al., 2004, 2005a) |
|              | cagAP-R2 | CTGCAAAAGAATTTGGCGA                               |                                                                               |                          |                                          |
| *cagE*       | cagE-F1 | GCGATTGTTATCTGCATTTAT                              | 94 °C for 10 min (final extension)                                            | 329                      |                                          |
|              | cagE-R1 | GAAGTGGTTAAAATAATCAATGCCC                          |                                                                               |                          |                                          |
| *cagT*       | cagT-F1 | CCATGTTTATACGCTGTTG                              | 94 °C for 10 min (final extension)                                            | 301                      |                                          |
|              | cagT-R1 | CATCACCACACCTTGTGAT                               |                                                                               |                          |                                          |
| LECI         | LEC-F1 | ACATTGTGCTAAATAAACGCC                             | 94 °C for 10 min (final extension)                                            | 384                      |                                          |
|              | LEC-R1 | TCTCATGTTGCATTTATGCT                              |                                                                               |                          |                                          |
| LECII        | LEC-F2 | AATAGGCTTTGTGGCATAGA                              |                                                                               | 877                      |                                          |
|              | LEC-R2 | ATCTTATGCTTCTTTATTCT                              |                                                                               |                          |                                          |
| *cag* right junction |        |                                                  |                                                                               |                          |                                          |
| *cagF4584* F (1) | GTTAATACAAAGGTTGGTTCTCCAAATACT                    | 94 °C for 30 s, 52 °C for 30 s                                               | 1000/800                 | (Kersulyte et al., 2000; Kauser et al., 2005a) |
| *cagR5280* R (3) | GGTTCAGGCATTCCCTCTAAATC                           |                                                                               | 400                      |                                          |
| *cagF4584* F (1) | GTTAATACAAAGGTTGGTTCTCCAAATACT                    | 94 °C for 30 s, 52 °C for 30 s                                               | 1000/800                 | (Kersulyte et al., 2000; Kauser et al., 2005a) |
| *miniIS605* R (8) | CGGTTAAGGATTGGTTTATTCCCTTTT                   | 94 °C for 10 min (40 cycles)                                                   | 350                      |                                          |
| *fcn unk* F (6) | TGGATTTACTCTGCTATGACT                             | 94 °C for 10 min (40 cycles)                                                   | 350                      |                                          |
| *cagR5280* R (3) | GGTTCAGGCATTCCCTCTAAATC                           | 72 °C for 1 min (30 cycles)                                                   | 350                      |                                          |
| *cagF4584* F (1) | GTTAATACAAAGGTTGGTTCTCCAAATACT                    | 94 °C for 30 s, 52 °C for 30 s                                               | 1000/800                 | (Kersulyte et al., 2000; Kauser et al., 2005a) |
| *miniIS605* R (8) | CGGTTAAGGATTGGTTTATTCCCTTTT                   | 94 °C for 10 min (40 cycles)                                                   | 350                      |                                          |
| *IS606-1692* F (5) | CCATTATTGCTGCTCTCA                                 | 72 °C for 1 min (30 cycles)                                                   | 350                      |                                          |
| *cagR5280* R (3) | GGTTCAGGCATTCCCTCTAAATC                           | 72 °C for 1 min (30 cycles)                                                   | 350                      |                                          |
| *cagF4584* F (1) | GTTAATACAAAGGTTGGTTCTCCAAATACT                    | 94 °C for 30 s, 52 °C for 30 s                                               | 1000/800                 | (Kersulyte et al., 2000; Kauser et al., 2005a) |
| *Xins* R (7) | CGCTCCTCTCTTGTCTGCTG                              | 94 °C for 10 min (40 cycles)                                                   | 350                      |                                          |
| Plasticity region |        |                                                  |                                                                               |                          |                                          |
| *JHP912* F   | CAATAGCTTGGCTACGCCTT                              |                                                                               | 624                      |                                          |
| ORFs         |        |                                                  |                                                                               |                          |                                          |
| *JHP912* R   | GTTTAATGCTGCTGCTG                                |                                                                               | 991                      |                                          |
| *JHP926* F   | GATGAGCATAATCAG                                  |                                                                               | 991                      |                                          |
| *JHP926* R   | ACCCTTCAATACCGCTAGA                              |                                                                               | 991                      |                                          |
| Genes                           | Primer          | Oligonucleotide sequence (5'-3') | PCR conditions                                | Size (bp) of PCR product | References                  |
|--------------------------------|-----------------|---------------------------------|------------------------------------------------|--------------------------|-----------------------------|
| JHP931F                        | GTATTAGCGAAGTGCAATCAC |                                  | 94 °C for 5 min (initial denaturation)         | 1.133                    | (Mukhopadhyay et al., 2000) |
| JHP931R                        | GCTAATTTGTAGGCGGTAAGC |                                  | 72 °C for 1 min (initial denaturation)         | 94 °C for 1 min          | (Mukhopadhyay et al., 2000) |
| JHP933 F                       | GAGTGAGTTAGCGAACC |                                  | 72 °C for 1 min (35 cycles)                    | 708                      | (Mukhopadhyay et al., 2000) |
| JHP933 R                       | CTGTGCTTGCCTGCAAGG |                                  | 72 °C for 1 min (35 cycles)                    | 358                      | (Mukhopadhyay et al., 2000) |
| JHP944 F                       | CTATGAGTGAGAAATTAACGC |                                  | 72 °C for 7 min (final extension)              | 611                      | (Mukhopadhyay et al., 2000) |
| JHP944 R                       | CGCTCCATCCATCATTTTG |                                  | 72 °C for 7 min (final extension)              | 611                      | (Mukhopadhyay et al., 2000) |
| JHP945 F                       | CAATGCGACTAACAGCATAG |                                  | 72 °C for 7 min (final extension)              | 611                      | (Mukhopadhyay et al., 2000) |
| JHP945 R                       | CGCATATTGCGATCTTCTTG |                                  | 72 °C for 7 min (final extension)              | 611                      | (Mukhopadhyay et al., 2000) |
| JHP947 F                       | GATAATCTACGCGAAGG |                                  | 72 °C for 7 min (final extension)              | 611                      | (Mukhopadhyay et al., 2000) |
| JHP986 F                       | GCATGTCGCAATCGTAGC |                                  | 72 °C for 7 min (final extension)              | 611                      | (Mukhopadhyay et al., 2000) |
| JHP986 R                       | TGCATTTGCGATTTGCCTC |                                  | 72 °C for 7 min (final extension)              | 611                      | (Mukhopadhyay et al., 2000) |
| **vacA signal and middle regions** |                             |                                  |                                                |                          |                             |
| **vacA1 or vacA2**             |                 |                                  |                                                |                          |                             |
| VAIF                           | ATGAAAAAAACCTTTTAC |                                  |                                                | 259 (s1)                 | (Carrol et al., 2004)       |
| VAIXR                          | GCAATTGGCAAGTGATGTT |                                  |                                                | 286 (s2)                 | (Carrol et al., 2004)       |
| **vacA1a**                     |                 |                                  |                                                |                          |                             |
| SS1-F                          | GTCAGCATCACACGCAAC |                                  |                                                | 190                      | (Atherton et al., 1995)     |
| VA1-R                          | CTGCTTGAATGCGCCAAC |                                  |                                                | 190                      | (Atherton et al., 1995)     |
| **vacA1b**                     |                 |                                  |                                                |                          |                             |
| SS3-F                          | AGGCGCATTACCGCAAGG |                                  |                                                | 187                      | (Yamazaki et al., 2005)     |
| VA1-R                          | CTGCTTGAATGCGCCAAC |                                  |                                                | 187                      | (Yamazaki et al., 2005)     |
| **vacA1c**                     |                 |                                  |                                                |                          |                             |
| SIC-F                          | CTCTCGTATTATGGGGYTT |                                  |                                                | 213                      | (Yamazaki et al., 2005)     |
| VA1-R                          | CTGCTTGAATGCGCCAAC |                                  |                                                | 213                      | (Yamazaki et al., 2005)     |
| **vacA1m1a**                   |                 |                                  |                                                |                          |                             |
| VA3-F                          | GTCTAAAATGCGTGTATTG |                                  |                                                | 300                      | (Kersulyte et al., 2000;    |
| VA3-R                          | CCATTTGTCACCTGTAAGAC |                                  | 72 °C for 1 min (30 cycles)                    | 300                      | (Kersulyte et al., 2000;    |
| **vacA1m1b**                   |                 |                                  |                                                |                          | (Kauser et al., 2005a)      |
| VAm-F3                         | GCACCCCAATGCTCATGATGAT |                                  |                                                | 300                      | (Kersulyte et al., 2000;    |
| VAm-R3                         | GCTTATGCGTCTAAAGAAGCAT |                                  |                                                | 300                      | (Kersulyte et al., 2000;    |
| **vacA1m2**                    |                 |                                  |                                                |                          | (Kauser et al., 2005a)      |
| VA4-F                          | GGAACCCCAGGAAACATTG |                                  |                                                | 400                      | (Kersulyte et al., 2000;    |
| VA4-R                          | CATAATCGCGCTCTGCAAC |                                  |                                                | 400                      | (Kersulyte et al., 2000;    |
| **oipA**                       |                 |                                  |                                                |                          | (Veralovic et al., 1991;    |
| HP0638-F                       | GTTTTTGATGCTATGGGATT |                                  | 94 °C for 1 min; 52 °C 1 min;                  | 401                      | (Veralovic et al., 1991;    |
| HP0638-R                       | GTGCATCTCTATGGCTTTT |                                  |                                                | 401                      | (Veralovic et al., 1991;    |
**Table 2** - Distribution of the *cag* PAI and the other virulence genes of *H. pylori* isolates from cases of gastritis and peptic ulcer.

| *cag* PAI | Gastritis (n = 48) (%) | Peptic ulcer (n = 21) (%) |
|-----------|------------------------|---------------------------|
| LEC1      | 5 (10.4)               | 3 (14.3)                  |
| LEC2      | 3 (6.3)                | 4 (19)                    |
| *cagT*    | 17 (35.4)              | 7 (33.3)                  |
| *cagE*    | 16 (33.3)              | 8 (38.1)                  |
| *cagAP*   | 8 (16.7)               | 10 (47.6)                 |
| *cagA*    | 25 (52.1)              | 16 (76.2)                 |
| cagRJ region |                     |                           |
| Type I    | 3 (6.3)                | 1 (4.8)                   |
| Type Ia   | 0                     | 4 (19)                    |
| Type II   | 2 (4.2)                | 0                         |
| Type III  | 4 (8.3)                | 0                         |
| Type IIIa | 1 (2.1)                | 5 (23.8)                  |
| ORFs      |                       |                           |
| JHP912    | 25 (52.1)              | 14 (66.7)                 |
| JHP926    | 1 (2.1)                | 0 (0)                     |
| JHP931    | 15 (31.3)              | 9 (42.9)                  |
| JHP933    | 10 (20.8)              | 5 (23.8)                  |
| JHP944    | 8 (16.7)               | 3 (14.3)                  |
| JHP945    | 11 (22.9)              | 4 (19)                    |
| JHP986    | 6 (12.5)               | 1 (4.8)                   |
| *vacA* alleles |                 |                           |
| *vacAs1a* | 35 (72.9)             | 19 (90.5)*                |
| *vacAs1b* | 2 (4.2)               | 0 (0)                     |
| *vacAs2*  | 11 (22.9)              | 2 (9.5)                   |
| *vacAm1a* | 10 (20.8)              | 15 (71.4)*                |
| *vacAm2*  | 38 (79.2)              | 6 (28.6)                  |
| *oipA*    | 36 (75)                | 18 (85.7)                 |

*significant p < 0.05.

To be higher in peptic ulcer patients compared to gastritis. We confirmed that no relevance between the *cagA* and gastroduodenal disease in the present study which was in accordance with previous studies (Hussein et al., 2008; Baghaei et al., 2009). However, other studies (Gunn et al., 1998; Basso et al., 2008) represented an association.

Previous studies reported that strains which lack the *cagT* gene had a defective ‘molecular syringe’ (Rohde et al., 2003; Kauser et al., 2005b). We represented that isolates from gastritis and peptic ulcer carried *cagE* and *cagT* with almost similar proportion. In a study performed in England, most of strains obtained from ulcer patients retained the *cagE* and *cagT* (Kauser et al., 2005b). A previous study has shown that the *cagE* is a better marker of an intact *cag* PAI in Japanese strains (Ikenoue et al., 2001) which is in contrast with our findings. Kauser et al. (2004) and Matteo et al. (2007) described that a conserved LEC region was rearranged more in strains related to severe pathology worldwide.

The prevalence of the *cag* PAI varies in different geographical regions. There was only one report concerning the distribution of the *cag* PAI and the ORFs of *H. pylori* strains in Turkey (Salih et al., 2007). Previous reports showed that an intact *cag* PAI gene was highly observed in Japanese, Malaysia and Singapore strains, least found in European and African strains, and very poorly found in Peruvian, Indian, Iranian and Turkish strains (Kauser et al., 2004; Baghaei et al., 2009; Salih et al., 2007; Schmidt et al., 2010). Our results also support the findings (Baghaei et al., 2009; Rudi et al., 1998) indicated that an intact *cag* PAI gene was detected to be low prevalence in Iranian and Turkish strains. This could be due to geographical closeness, the similar condition of life and diet in Iran and Turkey (Baghaei et al., 2009). An intact *cag* PAI may be underestimated when a selective primers were used since *cag* PAI was encoded by ~ 40 kb gene (Schmidt et al., 2010).

Five main types (I, II, III, IV and V) were detected at the cag RJ region and scientists reported that the three types (I, II and III) were prevalent (Kersulyte et al., 2000). The authors indicated that type IIIa or type I were observed in 28.8% of the motifs in England strains and some of the European strains share similar profiles with the Asian strains (Kauser et al., 2005b). The results of the current study are also supportive of a previous study that Turkish strains showed to be predominant of types I, II and III which were not associated with the severity of the disease (Salih et al., 2007).

Among the plasticity region ORFs, JHP940 and JHP947 have been observed more in strains with gastric cancer (Occhialini et al., 2000). Our data is similar to the previous reports in Costa Rica, Netherlands and Turkey where the prevalence of JHP0945 was almost similar proportions between *H. pylori* isolates obtained from gastritis and peptic ulcer (Occhialini et al., 2000; de Jonge et al., 2004; Salih et al., 2007) but different from a study (Sugimoto et al., 2012) which demonstrated that the prevalence of JHP0945 was found to be higher in isolates with peptic ulcer. We observed that JHP0931 gene was not associated with clinical disease in the present work which was in consistent with a study in Costa Rica (Occhialini et al., 2000). However, Salih et al. (2007) found that JHP912 and JHP931 genes was significant association in cases with peptic ulcer in Turkey.

The *H. pylori* oipA which have great antigenic characteristics and increase the serum level of IL-8 besides the clinically important demonstration of peptic ulcer, is an important virulence factor (Yamaoka et al., 2002; Zambon et al., 2002; Kudo et al., 2004). We showed no significant correlation between the oipA gene and peptic ulcer, in contrast with a previous study (Salih et al., 2007) performed in Turkey.
In a study carried out in Turkey, the authors detected that the most predominantly genotype among type II isolates was s1/m2, but except for one patient with gastritis and gastric ulcer possessed s1/m2 genotype, all type I isolates had s1/m1 genotype (Nagiyev et al., 2009). This study showed that none of *H. pylori* isolates had vacA m1b genotype. Our study is concurrence with previous studies (Blaser et al., 1995; Salih et al., 2007) which reported that s1a/m1a was the most prevalent genotype among isolates with peptic ulcer. In contrast, s1c/m1b and s1a/m1b strains were the predominant genotypes in East Asian countries (Yamazaki et al., 2005). We found that the s1m2 strains were predominantly detected in isolates from gastritis. Our findings were similar to the previous reports in Turkey (Erzin et al., 2006; Nagiyev et al., 2009) where the vacAs1a strains showed to be significantly correlated with peptic ulcer.

In conclusion, this study suggests that *cagA*, *oipA*, JHP912, JHP931 and *vacA* s1am1a were the most common genes in isolates with peptic ulcer, and *vacA*s1am1a was significantly correlated with peptic ulcer. When considering the worldwide distribution of *H. pylori* as a common pathogen, further larger scale researches are necessary to be conducted in strains obtained from different geographical regions in order to assess the possible role of *cag* PAI and other virulence genes in different clinical outcomes which is correlated with *H. pylori* infections.

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