TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2014) 44: 126-132
© TÜBİTAK
doi:10.3906/sag-1209-72

Detection of *cagA* and *vacA* genotypes of *Helicobacter pylori* isolates from a university hospital in Ankara region, Turkey

Ceren ERDOĞDU[1,*], Zeynep SARIBAŞ[2], Yakut AKYÖN YILMAZ[2]

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey

[2]Department of Clinical Microbiology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Received: 20.09.2012 • **Accepted:** 18.03.2013 • **Published Online:** 02.01.2014 • **Printed:** 24.01.2014

Aim: The *cagA* and *vacA* profiles and their association with clinical findings show a distinct geographical distribution. In the present study, we aimed to determine the *cagA* status and *vacA* allelic subtypes in strains isolated from a university hospital in Ankara and to evaluate their associations with histopathological and endoscopic findings.

Materials and methods: A total of 120 *H. pylori* strains from stock cultures positive for the *ureA* gene were randomly included in the present study. Of these strains, *cagA* and *vacA* allelic subtypes (s1a, s1b, s2, m1, m2) were examined by polymerase chain reaction.

Results: Of the 120 strains, 64 (53.3%) were *cagA*-positive. However, no significant relationship was found between clinical outcomes and *cagA* positivity. There were 38 (33.6%) strains that had *vacA* m1 and 74 (65.5%) that had *vacA* m2 region. Overall, 75 (70.1%) samples were classified as *vacA* s1a, 3 (2.8%) as *vacA* s1b, and 29 (27.1%) as *vacA* s2. There was no significant relationship between *vacA* genotypes and endoscopic findings. The predominant *vacA* genotypes were s1am2 (35.6%) and s1am1 (33.6%), with almost the same rates. Furthermore, *cagA* positivity was found to be significantly related with the *vacA* s1am1 genotype.

Conclusion: The *cagA* and *vacA* profiles of our study population are consistent with the Middle Eastern profile.

Key words: *Helicobacter pylori*, *cagA*, *vacA*

1. Introduction

Helicobacter pylori is a widespread microorganism and it has been reported that more than half of the world's population is infected with the bacterium (1). The prevalence rate was found to be 86% in Turkey (2). *H. pylori* colonises in the mucus layer of the human stomach and is well accepted as the causative agent of chronic gastritis, peptic ulcers, mucosa associated lymphoid tissue lymphoma, and gastric cancer (3). Several *H. pylori* virulence genes that play a role in the pathogenesis of the disease have been identified. The vacuolating cytotoxin and cytotoxin-associated protein, encoded by vacuolating cytotoxin A (*vacA*) and cytotoxin-associated gene A (*cagA*), respectively are important virulence determinants of *H. pylori* (4,5). The *cagA* is reported to be found in more than half of the *H. pylori* isolates. It is known that *cagA* is a marker for the cag pathogenicity island and is associated with increased IL-8 production, nuclear factor–kB activation, mucosal inflammation and development of peptic ulcers, atrophic gastritis, and gastric cancer (6). The *vacA* gene encodes an 87–93 kDa molecular weight VacA protein, which induces cytoplasmic vacuoles in mammalian cells. Unlike the *cagA*, all *H. pylori* strains possess the *vacA* gene but only half of them can produce biologically active toxin. This condition is mostly attributed to sequence differences in the signal region (s1, s2) and middle region (m1, m2). Any combinations of these allelic subtypes can be observed, although the *vacA* s2m1 genotype is uncommon (5). It has been reported that strains possessing *vacA* s1 and m1 genotypes are more virulent than those carrying *vacA* s2 and m2. Furthermore, type *vacA* s1a strains were reported to show more toxin activity than *vacA* s1b, while type s2 has no detectable toxin activity and is mostly found to be associated with ulcer disease (5,7,8).

It has been well accepted that the presence of *cagA* and the distribution of *vacA* allelic subtypes demonstrate a significant geographical variation (5). In the present retrospective study, we aimed to determine the *cagA* prevalence and *vacA* allelic subtype frequencies and their association with clinical outcomes in a region of Turkey.

* Correspondence: cerenozkul@hacettepe.edu.tr

2. Methods

2.1. Sample size

The subjects of this study were 120 previously untreated patients seen at outpatient clinics of the Gastroenterology Department of Hacettepe University between January 2000 and January 2009. A total of 120 *H. pylori* strains from stock cultures, which had been isolated between the years 2000 and 2009, were randomly included in the present study. The endoscopic and histopathological findings and demographical variables of the patients were reviewed retrospectively. Only 80 of the patients' data were available in terms of histopathological findings. The study was approved by the ethics committee.

2.2. Isolation of *H. pylori*

One antral biopsy specimen of each patient was used for the isolation of the *H. pylori*. Brain Heart Infusion Agar (Oxoid), which was supplemented with 7% horse blood and antibiotics (vancomycin 6 g/mL, trimethoprim 2.5 g/mL) was used for the culturing process. After the inoculation of biopsy samples, the plates were incubated in a microaerophilic atmosphere at 37 °C (BBL GasPak System). After the incubation period (48–72 h), colonies of *H. pylori* were identified both by urease, catalase, and oxidase test positivity and by observing characteristic microscopical morphology by Gram staining. The stock cultures were prepared from tryptic soy broth with 20% glycerol and glass beads and were stored at –80 °C until used.

2.3. PCR-based genotyping of *cagA* and *vacA*

DNA extraction was performed directly from stock cultures. A number of glass beads were taken into 300 µL of Tris-EDTA buffer (10 mM Tris HCl and 1 mM EDTA) and stored at –20 °C until processed. A standard cetyltrimethylammonium bromide (CTAB) genomic DNA isolation method was performed as previously described (9). The sequence of the primers used in this study is represented in Table 1.

In order to ascertain the presence of *H. pylori* DNA in stock cultures, the *H. pylori*-specific *ureA* gene was detected by polymerase chain reaction (PCR). A total of 120 samples positive for the *ureA* gene were further processed for the amplification of *cagA* and *vacA* alleles (s1a, s1b, s2, m1, and m2).

PCR reactions were performed with 25 µL of reaction mixture containing 2 µL of DNA template in a thermocycler device (MJ Research, Waltham, MA, USA). For the amplification of 411-bp fragments of the *ureA* gene, HPU1 and HPU2 primers were used (10). PCR consisted of 35 cycles of 1 min of denaturating step at 94 °C, 1 min of annealing step at 48 °C, and 1 min of elongation step at 72 °C, with a first cycle of 2 min at 95 °C and a final cycle of 5 min at 72 °C. For the detection of *cagA*, a 348-bp internal fragment of the gene was amplified using the F1 and B1 primers. The PCR program for *cagA* amplification was: 95 °C, 5 min, 1 cycle; followed by 35 cycles of 94 °C for 30 s (denaturation), 55 °C for 1 min (annealing), and 72 °C for 2 min (polymerisation); and 1 cycle at 72 °C for 5 min. The primers and conditions for the amplification of *vacA* allelic subtypes were those used by Atherton et al. (5). The amplified products were analysed on 2% agarose gel, stained with ethidium bromide, and visualised under UV light in order to determine the presence of specific fragments of the *cagA* and *vacA* allelic subtypes. *H. pylori* ATCC 43504 was used as a positive control. Each sample was studied twice to confirm the accuracy of the results.

Table 1. PCR primers used in this study.

Target	Primer name	Sequence of the primers (5' - 3')	Size of PCR products
ureA	HPU1 HPU2	5' GCCAATGGTAAATTAGTT 3' 5' GCCAATGGTAAATTAGTT 3'	411 bp
cagA	F1 B1	5' GATAACAGGCAAGCTTTTGAGG 3' 5' CTGCAAAAGATTGTTTGGCAGA 3'	348 bp
m1	VA3F VA3R	5' GGTCAAAATGCGGTCATGG 3' 5' CCATTGGTACCTGTAGAAAC 3'	290 bp
m2	VA4F VA4R	5' GGAGCCCCAGGAAACATTG 3' 5' CATAACTAGCGCCTTGCAC 3'	352 bp
s1a	SS1F VA1R	5' GTCAGCATCACACCGCAAC 3' 5' CTGCTTGAATGCGCCAAAC 3'	190 bp
s1b	SS3F VA1R	5' AGCGCCATACCGCAAGAG 3' 5' CTGCTTGAATGCGCCAAAC 3'	187 bp
s2	SS2F VA1R	5' GCTAACACGCCAAATGATCC 3' 5' CTGCTTGAATGCGCCAAAC 3'	199 bp

2.4. Statistical analyses

Statistical analyses were performed with SPSS 11.0 for Windows. In addition to the descriptive statistics, the groups were compared using the chi-square test. In the case of the presence of small data sets, the comparisons between the groups were performed by Monte Carlo exact test. $P < 0.05$ was considered statistically significant.

3. Results

Of the 120 *H. pylori* strains, 84 (70%) of them had been isolated from children, whereas 36 (30%) of them were from adults; the mean ages of these groups were 12.37 ± 2.94 years and 42.13 ± 18.95 years, respectively.

All of the 120 strains included in the study were positive for the *ureA* gene. A total of 64 samples (53.3%) were found to be positive for *cagA*. However, there was no relationship between children and adults regarding *cagA* positivity ($P = 0.472$). Furthermore, no significant relationship was found between endoscopic and histopathological findings and cagA positivity ($P = 0.741$ and 0.554, respectively; Table 2).

Among 120 samples positive for *ureA*, neither *vacA* s nor *vacA* m regions could be amplified in 5 strains. In 8 samples the *vacA* s region and in 2 samples the *vacA* m region could not be determined. Both *vacA* m1 and m2 regions were detected together in 1 strain (0.9%). Thirty-eight (33.6%) strains had *vacA* m1 and 74 (65.5%) had *vacA* m2 region. Overall, 75 (70.1%) samples were classified as *vacA* s1a, 3 (2.8%) as *vacA* s1b, and 29 (27.1%) as *vacA* s2. There was no significant relationship between *vacA* genotypes and either endoscopic or histopathological findings.

When the results were evaluated in terms of overall *vacA* genotype, it was determined that *vacA* s1am1, s1am2, s1bm1, s1bm2, and s2m2 genotypes were detected in 33.6%, 35.6%, 1.0%, 1.9%, and 27.9% of the strains, respectively. None of the *H. pylori* strains were carrying the s2m1 genotype. The distribution of vacA genotypes according to endoscopic and histopathological findings is represented in Table 3.

Among *cagA* positive strains, 86.9% had the *vacA* s1a subtype, whereas only 11.5% strains had *vacA* s2 and 1.6% had *vacA* s1b subtypes. Furthermore, it was demonstrated that *cagA* positivity was significantly related with *vacA* s1am1 genotype ($P < 0.001$). In total, 48.3% of the *cagA* positive strains were classified as *vacA* s1am1 genotype, and 12.1% of them were classified as *vacA* s2m2 genotype (Table 4).

Table 2. The distribution of *cagA* according to different age groups and endoscopic and histopathological findings.

	cagA-negative (n = 56)	*cagA*-positive (n = 64)	P
Age groups			
Children (n = 84)	41 (48.8)	43 (51.2)	0.472*
Adult (n = 36)	15 (4.7)	21 (58.3)	
Endoscopic findings (n = 120)			
Normal mucosa (n = 8)	2 (25.0)	6 (75.0)	
Gastritis (n = 70)	34 (48.6)	36 (51.4)	
Gastroduodenitis (n = 23)	12 (52.2)	11 (47.8)	0.741**
Esophagitis (n = 8)	3 (37.5)	5 (62.5)	
Ulcer (n = 11)	5 (45.5)	6 (54.5)	
Histopathological findings (n = 80)			
Normal mucosa (n = 1)	1 (100.0)	0 (0)	
Gastritis (n = 60)	27 (45.0)	33 (55.0)	
Gastroduodenitis (n = 11)	6 (54.5)	5 (45.5)	0.554**
Esophagitis (n = 5)	2 (40.0)	3 (60.0)	
Ulcer (n = 3)	3 (100.0)	0 (0.0)	

*: Chi-square test. **: Monte Carlo exact test

Table 3. Relationship between *vacA* subtypes and endoscopic findings.

	s1a/m1 n (%)	s1a/m^2 n (%)	s1b/m n (%)	s1b/m^2 n (%)	s2/m^2 n (%)	Total n (%)
Endoscopic findings						
Normal mucosa	4 (57.1)	3 (42.9)	0 (0)	0 (0)	0 (0)	7 (100.0)
Gastritis	18 (30.5)	21 (35.6)	1 (1.7)	2 (3.4)	17 (28.8)	59 (100.0)
GD	6 (27.3)	7 (31.8)	0 (0)	0 (0)	9 (40.9)	22 (100.0)
Esophagitis	4 (66.7)	2 (33.3)	0 (0)	0 (0)	0 (0)	6 (100.0)
Ulcer	3 (30.0)	4 (40.0)	0 (0)	0 (0)	3 (30.0)	10 (100.0)
Total	35 (33.6)	37 (35.6)	1 (1.0)	2 (1.9)	29 (27.9)	104 (100.0)
Histopathological findings						
Normal mucosa	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gastritis	20 (36.4)	18 (32.7)	0 (0)	1 (1.8)	16 (29.1)	55 (100.0)
GD	3 (27.3)	6 (54.5)	0 (0)	0 (0)	2 (18.2)	11 (100.0)
Esophagitis	1 (25.0)	2 (50.0)	0 (0)	0 (0)	1 (25.0)	4 (100.0)
Ulcer	0 (0)	1 (33.3)	0 (0)	0 (0)	2 (66.7)	3 (100.0)
Total	24 (32.9)	27 (37.0)	0 (0)	1 (1.4)	21 (28.7)	73 (100.0)

GD: Gastroduodenitis.

Table 4. The relationship between *cagA* status and *vacA* genotypes.

	s1a/m1 n (%)	s1a/m2 n (%)	s1b/m1 n (%)	s1b/m2 n (%)	s2/m2 n (%)	P
cagA (+), n (%)	28 (48.3)	22 (37.9)	1 (1.7)	0 (0)	7 (12.1)	<0.001
cagA (–), n (%)	7 (15.2)	15 (32.6)	0 (0)	2 (4.4)	22 (47.8)	

4. Discussion

H. pylori genotypes and the prevalence of virulent bacterial genotypes, as well as their association with gastrointestinal diseases, show a distinct geographical distribution (5,11). Thus, in the present study we investigated the *cagA* prevalence and *vacA* allelic subtypes retrospectively and evaluated their relationships with clinical outcomes in strains that had been isolated at Hacettepe University Hospital. Table 5 summarises the *vacA* s and m genotype rates in the present study and in other studies from Turkey (7,12–17).

It has been known that *cagA* positivity rates and their association with clinical outcomes differ from region to region. In general, the *cagA* prevalence rate has been found to be between 50% and 60% in Middle Eastern countries, whereas in the East Asian countries almost all isolated strains are *cagA*-positive (18). In Turkey, Saltık et al. (19) reported the *cagA* positivity rate as 55.6% in 45 isolates and they found no significant difference between the *cagA* positivity and the severity of the gastroduodenal symptoms. Our present findings, regarding a determination of a *cagA* positivity rate of 53.3% and no association between endoscopic findings and *cagA* status, are similar to those of Saltık et al. (19). In another study from Turkey, Safak et al. reported the *cagA* prevalence rate as 36.3% (20). Such an association has previously been reported, especially in Western isolates (21). However, this relationship could not be determined in strains isolated from Asian countries (22,23). On the other hand, in a current study from Turkey, Karaman et al. reported a *cagA* positivity rate of 65.5% and they also found a significant relationship between *cagA* status and peptic ulcer disease (17).

The different combinations of *vacA* s and m regions identify the virulence characteristic of the *H. pylori* strains.

Table 5. The vacA s and m genotype rates of the present study and other studies from Turkey.

	Sample size (n)	No detected genotype*	*vacA* s1 n (%)	*vacA* s2 n (%)	*vacA* m1 n (%)	*vacA* m2 n (%)
Present study	120	16	78 (72.9)†	29 (27.1)	38 (33.6)	74 (65.5)
Saribasak et al., 2004 (7)	65	1	55 (94.8)	3 (5.2)	8 (22.2)	28 (77.8)
Aydin et al., 2004 (12)	98	0	87 (88.8)	11 (11.2)	50 (51.0)	48 (49.0)
Salih et al., 2007 (13)	21	3	20 (100.0)	0 (0)	12 (66.7)	6 (33.3)
Erzin et al., 2006 (14)	91	0	81 (89.0)	10 (11.0)	38 (41.8)	53 (58.2)
Bolek et al., 2007 (15)	44	0	41 (93.2)	3 (6.8)	24 (54.5)	20 (45.5)
Umit et al., 2008 (16)	57	2	32 (64.0)	18 (36.0)	23 (43.4)	30 (56.4)
Karaman et al., 2011 (17)	29	0	22 (75.9)	7 (24.1)	16 (55.2)	13 (44.8)

*: Either vacA s or m regions could not be determined.
†: Both vacA s1a and s1b were established together.

It has been reported that type s1m1 strains produce a higher cytotoxin activity in vitro than type s1m2 strains, while s2m2 strains, which are considered as less virulent strains, produce no detectable cytotoxin. Thus, identification of the *vacA* profiles of the isolated strains and evaluation of these subtype combinations together with the clinical outcome of the patients is of significant importance.

In accordance with the previous reports from Turkey, we found *vacA* s1a as the predominant *vacA* s subtype with a rate of 70.1% (7,13,24). According to the reports from East Asian countries, all *H. pylori* strains isolated from East Asian countries are *vacA* s1 and *cagA*-positive, where the gastric cancer incidence has been reported in higher rates (25). This finding supports the idea that the strains with *vacA* s1 genotypes secrete the toxin more effectively from the cell and strains with both *vacA* s1 and *cagA* cause more serious gastrointestinal symptoms (8). Moreover, most of the *vacA* s1 strains are also *cagA*-positive (26). In the present study we found that 52 of the 59 *cagA*-positive strains were carrying the *vacA* s1 type, consistent with the literature. There was no association between the *vacA* s1 genotype and endoscopic findings. Our results are consistent with other reports from Turkey (7,12). However, in their study, Erzin et al. (14) reported an association between *vacA* s1a positive strains and duodenal ulcer and gastric cancer.

The association between the *vacA* m region and clinical outcome is still unclear. Such an association was reported in a few studies from Eastern and Middle Eastern countries (27). Salehi et al. (28) reported the *vacA* m1 and m2 rates as 40% and 60%, respectively, in an Iranian population. They also found an association between *vacA* m1 genotype and gastroduodenal pathology. We found the *vacA* m2 genotype as predominant with a rate of 65.5%. Our findings are in contrast with the results of Erzin et al. (14) and Aydin et al. (12), who reported the *vacA* m1 and m2 genotypes with an almost equal presence. However, Saribasak et al. (7) reported the predominant *vacA* m subtype as m2 (77.8%) and found no association between *vacA* m genotypes and the development of peptic ulcer. According to our present results, the *vacA* profile of Turkey is similar with those of Middle Eastern countries, where the *vacA* s1 and m2 subtypes have been reported to be predominant subtypes in many studies (28,29).

According to the results of the large metaanalysis study by Sugimoto et al. (30), in the combination of *vacA* s and m region genotypes, the predominant genotype was found to be *vacA* s1m2 (44.8%) in the North Middle East considering data from Turkey, Iran, and Iraq. In the same study, the overall rates of *vacA* s1m1, s2m1, and s2m2 were reported as 34.0%, 1.7%, and 19.5%, respectively. However, no previous studies had examined the subtypes of *vacA* s1 from these countries. In the study from Turkey by Erzin et al. (14), the *vacA* s1 subtypes were also examined, and they reported the *vacA* s1am2 as predominant. Consistently with the data from Middle East countries and from Turkey, we found the predominant *vacA* genotype as s1am2 (35.6%). However, the frequencies of *vacA* s1am1 and s1am2 were almost same, with the rates of 33.6% and 35.6%, respectively. Moreover, we found the rate of *vacA* s2m2, the less virulent type, as 27.9%. None of the strains were carrying the *vacA* s2m1 genotype, consistent with the literature (5).

According to the previous reports from Asian countries, the predominant strains were reported to be *vacA* s1cm1 and s1cm2 (11,31). The *vacA* s1c subtype was first identified by Van Doorn et al. (32) in strains isolated from East Asian countries. Since there were no reports

from Turkey and Middle Eastern countries regarding the presence of the *vacA* s1c subtype, it was not investigated in the present study.

The relationship between the presence of *cagA* and the *vacA* s1 region was first described by Atherton et al. (5). In a recent study from Turkey, Nagiyev et al. (33) reported an association between more virulent *vacA* genotypes and *cagA* positivity. In the present study, as well, we found that 48.3% of the *cagA*-positive strains were carrying the more virulent *vacA* s1am1 genotype, whereas only 12.1% of those had the less virulent *vacA* s2m2 genotype. Thus, the *vacA* s1am1 genotype was found to be significantly associated with the presence of *cagA*. Moreover, among 28 patients harbouring more virulent *cagA*+ / *vacA* s1am1 isolates, 26 of them were in the gastritis, gastroduodenitis, or esophagitis group, indicating that the genotype *vacA* s1m1 and its association with the *cagA* positivity increase the risk of developing more severe gastric lesions. Such an association was also demonstrated by Andreson et al. (34) from Estonia, who reported the predominant genotype as *vacA* s1am1 and indicated a significant association of *cagA* positivity with *vacA* s1am1 and s1am2. In Turkey this association was reported by Karaman et al. (17). They found that all 16 strains with the s1m1 genotype were also *cagA*-positive.

In contrast with the studies reporting the association of clinical outcomes with *H. pylori* virulence genes, especially with *cagA* status, we did not show such an association of endoscopic findings with either *cagA* or *vacA* gene status. Interestingly, this is in agreement with the majority of other reports from Turkey, in which *cagA* prevalence rates were also found between 55% and 69% (17,19). Moreover, although the majority of the isolated *H. pylori* strains are *cagA*-positive in Asian countries, associations of *cagA* status and disease outcome are not encountered in this region (35). This association was also not demonstrated in studies conducted on Iranian populations (36,37).

In the present study, we aimed to determine the *cagA* prevalence, *vacA* subtypes, and their associations with clinical findings in Turkey. To the best of our knowledge, there are limited reports from this region regarding the *cagA* and *vacA* status of Turkey. This study may contribute to the regional findings, since the geographical data in this issue are of significant importance.

In conclusion, we determined the *cagA* prevalence rate as 53.3% and the *vacA* predominant subtype as s1am2. However, no association was determined between clinical outcomes and virulence genotypes. A significant association between *cagA* positivity and *vacA* s1am1 genotype was found, in accordance with the previous reports in the literature.

In the light of the present results, it can be said that the data from Turkey are consistent with the Middle Eastern profile regarding *cagA* positivity, *vacA* status, and their associations with clinical outcomes. Further studies with larger sample sizes are needed in order to clarify the *cagA* and *vacA* profile of Turkey.

References

1. Covacci A, Telford JL, Del Giudice G, Parsonnet J, Rappuoli R. *Helicobacter pylori* virulence and genetic geography. Science 1999; 284: 1328–33.
2. Sandikci MU, Doran F, Koksal F, Sandikci S, Uluhan R, Varinli S, Akan E. *Helicobacter pylori* prevalence in a routine upper gastrointestinal endoscopy population. Br J Clin Pract 1993; 47: 187–9.
3. Maeda S, Mentis AF. Pathogenesis of *Helicobacter pylori* infection. Helicobacter 2007; 12: 10–4.
4. Covacci A, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, Massone A, Papini E, Xiang Z, Figura N et al. Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc Natl Acad Sci USA 1993; 90: 5791–5.
5. Atherton JC, Cao P, Peek RM Jr, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vacA* types with cytotoxin production and peptic ulceration. J Biol Chem 1995; 270: 17771–7.
6. Brandt S, Kwok T, Hartig R, König W, Backert S. NF-κB activation and potentiation of proinflammatory responses by the *Helicobacter pylori* CagA protein. Proc Natl Acad Sci USA 2005; 102: 9300–5.
7. Saribasak H, Salih BA, Yamaoka Y, Sander E. Analysis of *Helicobacter pylori* genotypes and correlation with clinical outcome in Turkey. J Clin Microbiol 2004; 42: 1648–51.
8. Atherton JC, Peek RM Jr, Tham KT, Cover TL, Blaser MJ. Clinical and pathological importance of heterogeneity in *vacA*, the vacuolating cytotoxin gene of *Helicobacter pylori*. Gastroenterology 1997; 112: 92–9.
9. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus 1990; 12: 13–5.
10. Clayton CL, Kleanthous H, Coates PJ, Morgan DD, Tabaqchali S. Sensitive detection of *Helicobacter pylori* by using polymerase chain reaction. J Clin Microbiol 1992; 30: 192–200.
11. Van Doorn LJ, Figueiredo C, Mégraud F, Pena S, Midolo P, Queiroz DM, Carneiro F, Vanderborght B, Pegado MD, Sanna R et al. Geographic distribution of *vacA* allelic types of *Helicobacter pylori*. Gastroenterology 1999; 116: 823–30.
12. Aydin F, Kaklikkaya N, Ozgur O, Cubukcu K, Kilic AO, Tosun I, Erturk M. Distribution of *vacA* alleles and *cagA* status of *Helicobacter pylori* in peptic ulcer disease and non-ulcer dyspepsia. Clin Microbiol Infect 2004; 10: 1102–4.

13. Salih BA, Abasiyanik MF, Ahmed N. A preliminary study on the genetic profile of cag pathogenicity-island and other virulent gene loci of *Helicobacter pylori* strains from Turkey. Infect Genet Evol 2007; 7: 509–12.

14. Erzin Y, Koksal V, Altun S, Dobrucali A, Aslan M, Erdamar S, Dirican A, Kocazeybek B. Prevalence of *Helicobacter pylori vacA*, *cagA*, *cagE*, *iceA*, *babA2* genotypes and correlation with clinical outcome in Turkish patients with dyspepsia. Helicobacter 2006; 11: 574–80.

15. Bolek BK, Salih BA, Sander E. Genotyping of *Helicobacter pylori* strains from gastric biopsies by multiplex polymerase chain reaction. How advantageous is it? Diagn Microbiol Infect Dis. 2007; 58: 67–70.

16. Umit H, Tezel A, Bukavaz S, Unsal G, Otkun M, Soylu AR, Tucer D, Otkun M, Bilgi S. The relationship between virulence factors of Helicobacter pylori and severity of gastritis in infected patients. Dig Dis Sci 2009; 54: 103–10.

17. Karaman M, Abacıoğlu H, Topalak OS, Simşek I. Molecular detection of *Helicobacter pylori vacA* and *cagA* genes in gastric tissue specimens of patients with peptic ulcer disease and non-ulcer dyspepsia. Mikrobiyol Bul 2011; 45: 11–20 (in Turkish with English abstract).

18. Rudi J, Kolb C, Maiwald M, Kuck D, Sieg A, Galle PR, Stremmel W. Diversity of *Helicobacter pylori vacA* and *cagA* genes and relationship to VacA and CagA protein expression, cytotoxin production, and associated diseases. J Clin Microbiol 1998; 36: 944–8.

19. Saltık İN, Demir H, Engin D, Ertunç ÖD, Akyön Y, Koçak N. The *cagA* status of *Helicobacter pylori* isolates from dyspeptic children in Turkey. Fems Immunol Med Microbiol 2003; 36: 147–9.

20. Safak B, Ciftci IH, Dilek FH, Uslan I, Cetinkaya Z, Asik G, Dilek ON. Prevalence of *cagA* and *vacA* genotypes of *Helicobacter pylori* isolated from Turkish patients with active or non-active chronic gastritis. Scand J Infect Dis 2010; 42: 6–7.

21. Kuipers EJ, Perez-Perez GI, Meuwissen SG, Blaser MJ. *Helicobacter pylori* and atrophic gastritis: importance of the *cagA* status. J Natl Cancer Inst 1995; 87: 1777–80.

22. Baghaei K, Shokrzadeh L, Jafari F, Dabiri H, Yamaoka Y, Bolfion M, Zojaji H, Aslani M, Reza Zali M. Determination of *Helicobacter pylori* virulence by analysis of the cag pathogenicity island isolated from Iranian patients. Dig Liver Dis 2009; 41: 634–8.

23. Hussein NR, Mohammadi M, Talebkhan Y, Doraghi M, Letley DP, Muhammad MK, Argent RH, Atherton JC. Differences in virulence markers between Helicobacter pylori strains from Iraq and those from Iran: potential importance of regional differences in *H. pylori*-associated disease. J Clin Microbiol 2008; 46: 1774–9.

24. Kolaylı F, Karadenizli A, Bingöl R, Schneider T, Kist M. Differences of *vacA* alleles and *cagA* gene positivity of *Helicobacter pylori* strains isolated from two different countries: Turkey and Germany. Mikrobiyol Bul 2012; 46: 332–4 (in Turkish with English abstract).

25. Yamaoka Y, Kodama T, Kita M, Imanishi J, Kashima K, Graham DY. Relationship of *vacA* genotypes of *Helicobacter pylori* to cagA status, cytotoxin production, and clinical outcome. Helicobacter 1998; 3: 241–53.

26. Han SR, Schreiber HJ, Bhakdi S, Loos M, Maeurer MJ. *vacA* genotypes and genetic diversity in clinical isolates of *Helicobacter pylori*. Clin Diagn Lab Immunol 1998; 5: 139–45.

27. Wang HJ, Kuo CH, Yeh AA, Chang PC, Wang WC. Vacuolating toxin production in clinical isolates of *Helicobacter pylori* with different *vacA* genotypes. J Infect Dis 1998; 178: 207–12.

28. Salehi Z, Abadi AS, Ismail PB, Kqueen CY, Jelodar MH, Kamalidehghan B. Evaluation of *Helicobacter pylori vacA* genotypes in Iranian patients with peptic ulcer disease. Dig Dis Sci 2009; 54: 2399–403.

29. Rhead JL, Letley DP, Mohammadi M, Hussein N, Mohagheghi MA, Eshagh Hosseini M, Atherton JC. A new *Helicobacter pylori* vacuolating cytotoxin determinant, the intermediate region, is associated with gastric cancer. Gastroenterology 2007; 133: 926–36.

30. Sugimoto M, Zali MR, Yamaoka Y. The association of *vacA* genotypes and *Helicobacter pylori*-related gastroduodenal diseases in the Middle East. Eur J Clin Microbiol Infect Dis 2009; 28: 1227–36.

31. Zhou W, Yamazaki S, Yamakawa A, Ohtani M, Ito Y, Keida Y, Higashi H, Hatakeyama M, Si J, Azuma T. The diversity of *vacA* and *cagA* genes of *Helicobacter pylori* in East Asia. FEMS Immunol Med Microbiol 2004; 40: 81–7.

32. Van Doorn LJ, Figueiredo C, Sanna R, Pena S, Midolo P, Ng EK, Atherton JC, Blaser MJ, Quint WG. Expanding allelic diversity of *Helicobacter pylori vacA*. J Clin Microbiol 1998; 36: 2597–603.

33. Nagiyev T, Yula E, Abayli B, Koksal F. Prevalence and genotypes of *Helicobacter pylori* in gastric biopsy specimens from patients with gastroduodenal pathologies in the Cukurova Region of Turkey. J Clin Microbiol 2009; 47: 4150–3.

34. Andreson H, Lõivukene K, Sillakivi T, Maaroos HI, Ustav M, Peetsalu A, Mikelsaar M. Association of *cagA* and *vacA* genotypes of *Helicobacter pylori* with gastric diseases in Estonia. J Clin Microbiol 2002; 40: 298–300.

35. Kuo CH, Wu DC, Lu CY, Su YC, Yu FJ, Lee YC, Wu IC, Lin SR, Liu CS, Jan CM et al. Low molecular weight protein of *Helicobacter pylori* and its relation to gastroduodenal diseases. Hepatogastroenterology 2003; 50: 897–901.

36. Siavoshi F, Malekzadeh R, Daneshmand M, Ashktorab H. *Helicobacter pylori* endemic and gastric disease. Dig Dis Sci 2005; 50: 2075–80.

37. Jafari F, Shokrzadeh L, Dabiri H, Baghaei K, Yamaoka Y, Zojaji H, Haghazali M, Molaei M, Zali MR. *vacA* genotypes of *Helicobacter pylori* in relation to *cagA* status and clinical outcomes in Iranian populations. Jpn J Infect Dis. 2008; 61: 290–3.