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Helicobacter pylori cagA and *vacA* Genotypes and their Relationships to Peptic Ulcer Disease and Non-Ulcer Dyspepsia

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Abstract: The aim of this study was to detect cytotoxin-associated gene A (*cagA*) and vacuolating cytotoxin gene A (*vacA*) genotypes of *Helicobacter pylori* and to study their relationships to the associated diseases. In the present analytical descriptive study *H. pylori* isolates were collected from 150 patients who underwent gastro duodenoscopy in Imam Khomeini Hospital of Tabriz, Iran. Of the patients 76 (50.7%) were males and 74 (49.3%) were females. The patients were divided into two groups. Group I consisted of 117 (78%) Non-Ulcerative Dyspepsia (NUD) patients and group II consisted of 33 (22%) Peptic Ulcer Disease (PUD) patients. Extracted DNA of *H. pylori* isolates were subjected to PCR tests to detect *cagA*, signal (s) and middle (m) regions of *vacA* genotypes. The designed primers revealed the presence of *cagA* gene in 125 (83.3%) of the isolates. Regarding *vacA* signal sequences 99 (66%) of our isolates revealed s1 type. The proportion of s1a, s1b and s1c subtypes were 76/150 (50.7%), 7/150 (4.7%) and 16/150 (10.6%), respectively while 40/150 (26.7%) presented as s2 type. In further analysis of the m region of *vacA*, m1 and m2 subtypes were detected in 49/150 (32.7%) and 81/150 (54%) of the isolates, respectively. The m1 subtype were further divided into m1a [41/49 (83.7%)] and m1b [(8/49 (16.3%)]. Thirty one isolates (20.7%) showed more than one *vacA* alleles in a single patient. Our results showed that isolates carrying the *cagA* gene were higher in PUD group than in NUD group, but did not substantiate statistically the role of *cagA* as a marker influencing increased virulence ($p>0.05$). Present findings also showed that s1 and s2 subtypes of *vacA* gene are markers which differentiate between PUD and NUD groups.

Key words: *Helicobacter pylori*, cytotoxin associated gene A (*cagA*), vacuolating cytotoxin gene A (*vacA*), Peptic Ulcer Disease, Non-Ulcer Dyspepsia

INTRODUCTION

For most of the last century the cause of peptic ulcer disease was thought to be stress related and the disease to be prevalent in hyperacid producers. The discovery that *Helicobacter pylori* was associated with gastric inflammation and peptic ulcer disease has revolutionized our view of the gastric environment and the disease associated with it (Chelimsky *et al*., 2004).

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Stomach infection with *H. pylori* is the second most common infectious disease of human (Hussain *et al*., 2004). *H. pylori* is a Gram negative, spiral shaped bacterium that colonizes the gastric epithelium and predisposes to sever disease such as duodenal ulcer and gastric cancer (Buhling *et al*., 2004). This association is extremely important, since, in total, gastric cancer is the second leading cause of cancer death in the world (Suerbaum and Pierre, 2002; Buhling *et al*., 2004) and for this reason it is classified as a class I carcinogen by World Health Organization (WHO) (Yamazaki *et al*., 2005).

H. pylori has infected more than half of the world population (Yuan *et al*., 2004), causing chronic gastritis in all infected humans and clinical disease in 10-15% of those infected (Suerbaum and Achtman, 2004). The organism persists life long in host unless effective treatment is performed (Thompson and Taylor, 2000). Human colonization, pathogenicity and the evolution of infection all depend on strain diversity and host-bacterium interactions (Ferrero, 2005). However it has long been known to be genetically highly diverse (Ando *et al*., 2002; Suerbaum and Achtman, 2004) and sequence diversity within *H. pylori* is greater than that of most other bacteria (Falush *et al*., 2003). There are also indications of significant geographic differences among strains (Mukhopadhyay *et al*., 2000; Al Qabadi *et al*., 2005) and distribution of allelic types of *H. pylori* (Gold *et al*., 2001).

In course of the previous years, this genetic diversity and variability have been intensively studied. However, the *cagA* gene differed dramatically between isolates from East Asia versus Europe and North America and particular variants of *vacA* were apparently also restricted to East Asia (Blaser, 1999; Zhou *et al*., 2004). The *vacA* gene which is present in virtually all *H. pylori* strains encodes a 139-140 kDa protein comprising a signal (s) sequence that drives the protein from the cytoplasm, a 45 kDa carboxyl terminal auto-transporter and an exported cytotoxin divided into two parts, a 37 kDa and a 58 kDa region encoded by the medium (m) region of the gene (Bernard *et al*., 2004). The s and m regions are highly divergent among *H. pylori* strains isolated from patients with different degrees of gastric diseases and from different geographic regions. The s region is divided into s1, which in turn subdivided into s1a, s1b, s1c and s2 (Xue-Jun *et al*., 2005). Polymorphism of the m region comprises two major types m1 and m2, which can be subdivided into m1a, m1b and m2a, m2b (Hou *et al*., 2000; Kim *et al*., 2001). The mosaic combination of s and m region allelic types determines the production of the cytotoxin and is associated with pathogenicity of the bacterium (Nogueira *et al*., 2001).

H. pylori infections cause very high morbidity and mortality and are of particular concern in developing countries, where *H. pylori* prevalence is as high as 90% (Dubreuil *et al*., 2002). In Iranian population the rate of infection is >80% (Mohammadi *et al*., 2003). In this study, we tested *vacA* and *cagA* genes by PCR method in gastric biopsies for detecting prevalence of these genes and their relationships to gastro duodenal diseases.

MATERIALS AND METHODS

Patients and Organisms

Biopsy specimens collected from patients with gastric disorders, during gastroduodenal endoscopy at Imam Khomeini hospital in Tabriz, Iran from January to June 2006 were tested for rapid urease production and bacteriologic culture onto supplemented Columbia agar under microaerophilic condition by gas pack (Anaerocult C) at 37°C for 5-7 days. *H. pylori* isolates were suspended in 1 mL sterile 0.9% NaCl solution and pelleted by centrifugation. The organisms were then stored in microtubes at -70°C. Endoscopic findings were also recorded and based on the information patients were divided into two groups. Group I patients had duodenal or gastric ulcer (Peptic Ulcer Disease, PUD) and group II patients had Non-Ulcer Dyspepsia (NUD).

DNA Extraction, Primers and PCR

DNA extraction was carried out by modified SDS-Proteinase K method using primers listed in Table 1. The PCR reagent mixture consisted of sterile redistilled H_2O (17.0 μL), 0.2 mM concentration of dNTPs (0.5 μL), 10x PCR buffer (2.5 μL), 1.5 mM $MgCl_2$ (0.75 μL), 0.5 mM concentration of each

Table 1: Primers used in this study

Region	Primer	Nucleotide sequence
*cag*A	CAGT-F	ACCCCTAGTCGGTAATGGG
	CAGT-R	GCTTTAGCTTCTGAYACYGC
*vac*A (s1)	VA1-F	ATGGAAATACAACAAACACAC
	VA1-R	CTGCTTGAATGCGCCAAAC
*vac*A (s1a)	SS1-F	GTCAGCATCACACCGCAAC
	SS1-R	CTGCTTGAATGCGCCAAAC
*vac*A (s1b)	SS3-F	AGCGCCATACCGCAAGAG
	SS3-R	CTGCTTGAATGCGCCAAAC
*vac*A (s1c)	S1C-F	CTCTCGCTTTAGTGGGGYT
	S1C-R	CTGCTTGAATGCGCCAAAC
*vac*A (s2)	SS2-F	CCTAACACGCCAAATGATCC
	SS2-R	CTGCTTGAATGCGCCAAAC
*vac*A (m1a)	VA3-F	GGTCAAAATGCGGTCATGG
	VA3-R	CCATTGGTACCTGTAGAAAC
*vac*A (m1b)	VAm-F3	GGCCCCAATGCAGTCATGGAT
	Vam-R3	CTGTTAGTGCCTAAAGAAGCAT
*vac*A (m2)	VA4-F	GGAGCCCCAGGAAACATTG
	VA4-R	CATAACTAGCGCCTTGCAC

F: Forward, R: Reverse

primers (0.5 μL), 1 U mL^{-1} of Taq DNA polymerase (0.2 μL) and template DNA (3 μL). The total volume of PCR mix was 25 μL. DNA amplification was carried out with initial denaturation at 94°C for 5 min, followed by 35 cycles of amplification (denaturation at 94°C for 45 sec, annealing at 57°C for 45 sec, extension at 72°C for 50 sec), ending with a final extension at 72°C for 7 min. Positive results for s1a, s1b, s1c, s1 and s2 were indicated by the presence of 190, 187, 213, 259 and 199 bp amplified DNA fragments, respectively and revealed by electrophoresis on a 1.2% agarose gel at 75 V for 60-120 min in 1 x TBE buffer. Each PCR assay included redistilled water as a negative control. The expected lengths of PCR products amplified with m1a, m1b and m2 primers were 290, 291 and 352 bp, respectively.

Statistical Tests

The data were computed using SPSS statistical software (version 11.0) and analysed using Chi-square test. Any probability level less than 0.05 was considered statistically significant.

RESULTS

Patients and Bacterial Isolates

Bacterial isolates of *H. pylori* were collected from 150 patients who underwent gastro duodenoscopy at Imam Khomeini hospital of Tabriz, Iran. Of the patients 76 (50.7%) were males and 74 (49.3%) were females. The patients divided into two groups. Group I consisted of 117 (78%) Non-Ulcerative Dyspepsia (NUD), of which 87 patients with mean age of 40 years had gastritis and/or duodenitis and 30 patients with mean age of 39 years were unremarkable in endoscopy. Group II consisted of 33 (22%) Peptic Ulcer Disease (PUD) patients with mean age of 36 years, of which 18 (12%) had duodenal ulcers and 15 (10%) had gastric ulcers, one of which had malignant lesions.

Diversity of *cag*A and *vac*A Genes

The designed primers for *cagA* gene which were used to detect the entire 3' repeat regions revealed the gene in 125 (83.3%) of the isolates (Fig. 1). Isolates carrying the *cagA* 3' repeat region were more abundant in PUD patients (93.9%) than NUD ones (80.3%).

Regarding *vacA* gene, 99 (66%) of 150 isolates were s1 type. The proportion of s1a, s1b and s1c subtypes were 76/150 (50.7%), 7/150 (4.7%) and 16/150 (10.6%), respectively (Fig. 2). Forty

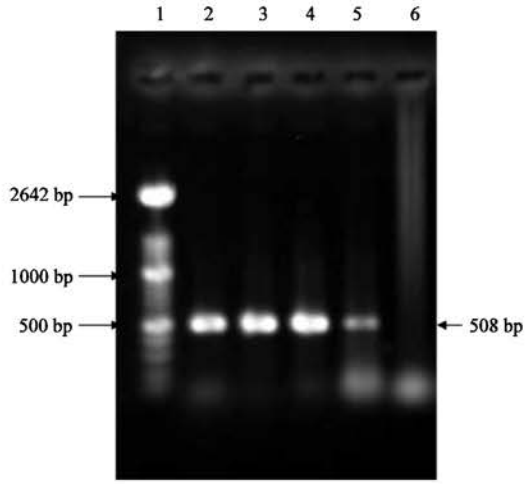

Fig. 1: 508 bp band of *cag*A in *H. pylori* isolates in gel electrophoresis (1.2% agarose), Lane 1: Size marker (100 bp DNA ladder) (2642, 1500, 1400, 1300, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100), Lanes 2-5: *cag*A positive *H. pylori* isolates 1, 44, 60, 72, respectively and Lane 6: Negative control

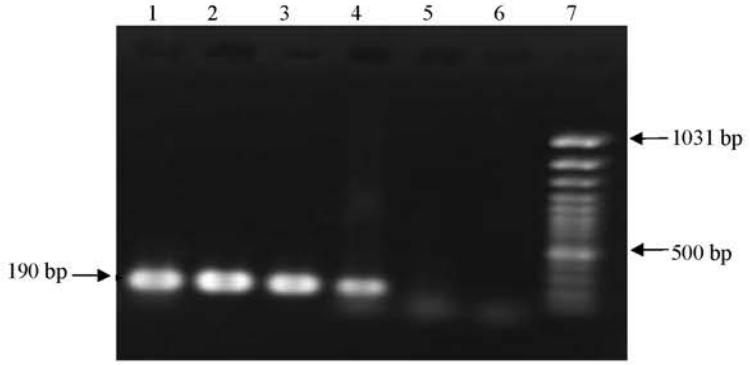

Fig. 2: 190 bp band of *vac*A in *H. pylori* isolates in gel electrophoresis (1.2% agarose), Lanes 1-4: *H. pylori* isolates 14, 93, 142 and 150, respectively (positive for s 1 a), Lanes 5 and 6: Negative controls and Lane 7: Size marker (100 bp DNA ladder)

isolates (26.7%) presented s2 type. The s1 type was found in 20 (87%) patients with PUD, compared to 63 (65.6%) of 96 NUD subjects ($p = 0.045$). The s2 type was found in 34.4% of NUD group, compared to 13% in PUD group ($p = 0.045$). Further analyses of *vacA* gene for m region, consisting of m1 and m2 subtypes, revealed m1 and m2 subtypes in 49/150 (32.7%) and 81/150 (54%), respectively. m1 subtype was further divided into m1a [41/49 (83.7%)] and m1b [8/49 (16.3%)] (Fig. 3). Distribution of *vacA* m1 and m2 types in PUD patients were 8 (34.8%) and 15 (65.2%) respectively, compared to NUD patients as 35 (36.5%) and 61 (63.5%), respectively ($p>0.05$). Distribution of *vacA* genotypes among PUD and NUD patients have been shown in Table 2.

Prevalence of *cagA/vacA* s1 type was 87% (20/23) in PUD patients compared to 63.5% (61/96) in subjects with NUD ($p = 0.045$). When *vacA* alleles analysed in this studied *H. pylori* isolates, 31 cases (20.7%) showed more than one *vacA* allele which 11 isolates (6.7%) contained mixed s subtypes

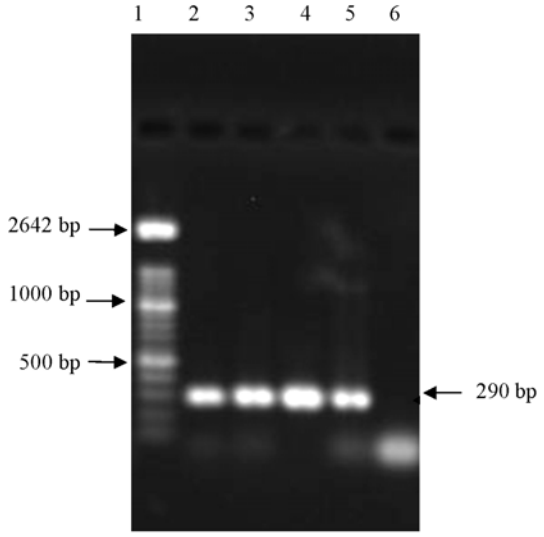

Fig. 3: 290 bp band of *vac*A (m1a) in *H. pylori* isolates in gel electrophoresis (1.2% agarose), Lane 1: Size marker XIV (100 bp DNA ladder, Roche), Lanes 2-5: *H. pylori* isolates 59, 60, 1 and 61, respectively (positive for m1a) and Lane 6: Negative control

Table 2: Distribution of *vacA* genotype among PUD and NUD patients

	PUD patients		NUD patients	
Region	No.	(%)	No.	(%)
s1	20	87°	63	5.6
s2	3	13°	33	34.4
m1	8	34.8	35	36.5
m2	15	65.2	61	63.5
s1/m1	7	30.4	29	30.2
s1/m2	13	56.5	34	35.4
s2/m1	1	4.3	6	6.2
s2/m2	2	8.7	27	28.1

PUD: Peptic Ulcer Disease; NUD: Non Ulcer Dyspepsia

Table 3: Multiple *vacA* subtypes from a single biopsy specimen in 31 PUD and NUD patients

vacA subtypes	No. in PUD patients	NUD patients
s1a, m1a+m2	4	5
s1a+s1c, m1a	1	4
s2, m1a+m2	-	4
s1c, m1a+m2	1	2
s1a+s1c, m2	3	-
s1a+s1b, m2	-	2
s1a, m1b+m2	-	2
s1b, m1a+m2	-	2
s1a+s1b, m1a	1	-

PUD: Peptic Ulcer Disease; NUD: Non Ulcer Dyspepsia

and 20 isolates (13.3%) displayed mixed subtypes. Multiple *vacA* subtypes from a single biopsy specimen has been shown in Table 3.

DISCUSSION

The geographic distribution of distinct *H. pylori* genotypes remains largely unknown and the prevalence of virulent bacterial genotypes in certain region, may have important epidemiological consequences (Saribasak *et al*., 2004). *H. pylori* strains that have the *cagA* gene express it and have been

considered more virulent than *cag* negative strains (Chelimsky *et al*., 2004), variation in the size of the *cagA* protein is related to the presence of a variable number of repeat sequences in the 3' region of the gene (Yamaoka *et al*., 1998; 1999a). Studies of the primary gene structure of the 3' region of *H. pylori* isolates showed differences markedly from East Asia and Western isolates (Yamaoka *et al*., 1998). The prevalence of *cagA* positive *H. pylori* varies from one geographic region to another, e.g., 45% in Sri Lanka (Fernando *et al*., 2002), 53% in Kuwait (Al Qabadi *et al*., 2005), 78% in Turkey (Saribasak *et al*., 2004), 67% in the Netherlands, 79% in Ethiopia, 81% in the United States (Asrat *et al*., 2004), 90% in Hong Kong (Wong *et al*., 2001), 94% in Malaysia (Ramelah *et al*., 2005), 96.3% in China and 97% in Korea. In this study, PCR primers used for detection of the *cagA* gene were aimed at the 3' part of the gene and could detect in 125 (83.3%) of the isolates. We found that isolates carrying the *cagA* 3' repeat region were more abundant in PUD patients (93.9%), whereas, it was 80.3% in NUD patients. These findings do not substantiate the role of the *cagA* as marked marker for increased virulence, because of similar high *cagA* gene positivity in *H. pylori* strains isolated from two different diseases and this finding is in agreement with studies from East Asia (Ramelah *et al*., 2005). Differences in *cagA* genotype may be useful for molecular epidemiology and may provide a marker for differences in virulence among *cagA*-positive *H. pylori* strains.

Regarding *vacA* genotype, the majority of the Portuguese strains (72%) contained type s1a (Van Doorn *et al*., 1998). s1a and s1b subtypes were equally present in France, Italy and North America (Van Doorn *et al*., 1999). The predominant *vacA* subtype in East Asia, e.g., in Korea (80.4%) and Hong Kong (95.8%) was the s1c allele (Kim *et al*., 2001; Wong *et al*., 2001). In this study, of the specimens with a single *vacA* genotype, the *vacA* s1 detected in 69.7 and the predominant subtype was s1a (54.6%), which is similar to those found in the Dutch study. The *vacA* s1a genotype in German *H. pylori* strains revealed a strong association with PUD (Rudi *et al*., 1998). Also another study reported that *vacA* s1 genotype was associated with PUD in Netherlands (Yamaoka *et al*., 1999b). De Gusmao *et al*. (2000) were also demonstrated a strong association between the presence of s1 allele and duodenal ulcers in Brazilian children. In contrast, no correlation was found between s1 allele and severe gastro duodenal disease in Sri Lanka (Fernando *et al*., 2002). Data from Asia suggest no correlation between genotypes of *H. pylori* and peptic ulcer (Kim *et al*., 2001; Azuma *et al*., 2004). In the present study, the prevalence of s1a genotype in PUD patients was higher than that in the NUD patients, which underlines the presence of this genotype in PUD patients.

Isolates belonging to type s2 express the *vacA* protein only in a small amount (Rudi *et al*., 1998). Xue-jun *et al*. (2005) and did not encounter *vacA* s2 isolates in China and Kim *et al*. (2001) reported it only in one out of 76 isolates in Korea (Kim *et al*., 2001). Other studies from East Asia including China, Hong Kong, Japan and Thailand reported less prevalence for *vacA* s2 (Van Doorn *et al*., 1999). Arabs of Middle East countries equally infected with the s1 and s2 alleles, whereas African Arabs (Egyptians, Somalian) showed s2 type more frequently than s1 type (Al Qabadi *et al*., 2005). In German isolates the allele s2 was found in the NUD group at a much higher frequency (31%) than that in the PUD group (4%) (Strobel *et al*., 1998). Saribasak and colleagues reported *vacA* s2 genotype in 3 of 65 patients (5%) in Turkey (Saribasak *et al*., 2004). The *vacA* s2 type was reported from Europe, America and Australia in 14.5, 22 and 29%, respectively (Van Doorn *et al*., 1999). In our study, the *vacA* s2 genotype was found in 36 of 119 (30.3%) patients, which was 13.04% (3/23) in PUD group and 34.38% (33/96) in NUD group. Therefore present results indicate more prevalence of s2 genotype in NUD group than the PUD group ($p = 0.045$). These findings are in accordance with those found in a previous study in Iran (Mohammadi *et al*., 2003).

There are marked differences between prevalence of *vacA* m genotypes in different regions. Van Doorn and colleagues reported that m1 and m2 subtypes were approximately equally prevalent in Europe and North America (Van Doorn *et al*., 1999). Of 59 *H. pylori* isolates, Atherton and coworkers found *vacA* m1 and m2 in 22 (37.3%) and 37 (62.7%) of isolates, respectively (Atherton *et al*., 1995). One study showed that 67% of Hong Kong strains were *vacA* m2 subtype (Wong *et al*., 2001). The *vacA* m1 genotype was detected in 12% of patients, while the m2 genotype was detected in 43% of isolates from Turkey (Saribasak *et al*., 2004). No significant relationship between *vacA* m region types

and the presence of PUD was found by Han *et al*. (1998). In present study, the *vacA* m1 and m2 genotypes were detected in 8 (34.8%) and 15 (65.2%) PUD patients, whereas they were found in 35 (36.5%) and 61 (63.5%) NUD patients, respectively ($p>0.05$).These findings indicate that the *vacA* middle region types were not independently associated with the occurrence of PUD.

It has been demonstrated that *H. pylori* carries only a single copy of *vacA* (Atherton *et al*., 1995) and detection of multiple genotypes implies the presence of multiple strains in a clinical sample (Kim *et al*., 2001). The presence of multiple *vacA* genotype in the present study was 20.7%. The presence of multiple *H. pylori* strains, as determined by random amplification of polymorphic DNA from isolates, has been reported earlier (Van Doorn *et al*., 1998). The frequency of multiple *vacA* genotypes in a single biopsy specimen appears to be more in Portugal (29%) than in the Netherlands (8%) (Van Doorn *et al*., 1998). Prevalence of multiple *vacA* genotypes in this study (20.7%) can be compared with results reported from Brazil (13%), Chile (32%), Ethiopia (8%), Korea (18%) (Asrat *et al*., 2004), China (20.5%) (Xue-jun *et al*., 2005) and Hong Kong (8.3%) (Wong *et al*., 2001).This phenomenon may relate to the prevalence of *H. pylori* infection among the populations in each country. The risk of co-infection or super infection with multiple strains may be higher in countries with a high prevalence of *H. pylori* infection than in countries with low prevalence (Van Doorn *et al*., 1998). It is unknown whether infection with multiple strains increase the risk of serious clinical implications, such as the development of ulcers and gastric cancer. However, in this study, infection with multiple strains in PUD group (30.3%) was higher than that of NUD group (17.9%).

Finally 56.5% of the isolates in PUD patients contained the combination of s1 (s1a) and m2. This result is in accordance with other findings in Tehran (49%) (Mohammadi *et al*., 2003) and China (61.5%) (Xue-jun *et al*., 2005), but different from the results found in the United States, where the genotypes s1/m1 and s2/m2 are more common (Atherton *et al*., 1995).

We conclude that *cagA* gene was higher in PUD group than in NUD group, but did not substantiate statistically the role of this gene as a marker influencing increased virulence ($p>0.05$). Present findings also indicated that *vacA* s1 and s2 genotypes were markers that differentiate between PUD and NUD group, whereas m1 and m2 markers did not provide any additional differentiation between these two groups. However, the s1/m2 especially s1a/m2 genotype is the most prevalent genotype in *H. pylori* strains isolated from PUD patients.

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REFERENCES

Al Qabadi, A., A.S. Mustafa, I. Siddique, A.K. Khajah, J.P. Madda and T.A. Junaid, 2005. Distribution of *vacA* and *cagA* genotypes of *Helicobacter pylori* in Kuwait. Acta Trop., 93 (3): 283-288.

Ando, T., R.M. Peek, D. Pride, S.M. Levine, T. Takata and Y.C. Lee *et al*., 2002. Polymorphisms of *Helicobacter pylori* HP0638 reflect geographic origin and correlate with *cagA* status. J. Clin. Microbiol., 40 (1): 239-246.

Asrat, D., I. Nilsson, Y. Mengistu, E. Kassa, S. Ashenafi and K. Ayenew *et al*., 2004. Prevalence of *Helicobacter pylori vacA* and *cagA* genotypes in Ethiopian dyspeptic patients. J. Clin. Microbiol., 42 (6): 2682-2684.

Atherton, J.C., P. Cao, R.M. Peek, N.K.R. Tummuru, M.J. Blaser and T.L. Cover, 1995. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. J. Biol. Chem., 270 (30): 1771-1777.

Azuma, T., S. Kato, W. Zhou, S. Yamazaki and A. Yamakawa, 2004. Diversity of vacA and cagA genes of *Helicobacter pylori* in Japanese children. Aliment. Pharmacol. Ther., 20 (1): 7-12.

Bernard, M., A. Cappon, G.D. Giudice, R. Rappuol and C. Montecucco, 2004. The multiple cellular activities of the *vacA* cytotoxin of *Helicobacter pylori*. Int. J. Med. Microbiol., 293 (7-8): 589-597.

Blaser, M.J., 1999. In a world of black and white, *Helicobacter pylori* is gray. Ann. Int. Med., 130: 695-697.

Buhling, F., G. Koch, T. Wex, A. Heimburg, M. Vieth, A. Leodolter and A. Roessner, 2004. Simultaneous detection and differentiation of anti-*Helicobacter pylori* antibodies by flow microparticle immunoflurescence assay. Clin. Diag. Lab. Immunol., 11 (1): 131-136.

Chelimsky, G., S.S. Blanchard and S.J. Czinn, 2004. *Helicobacter pylori* in children and adolescents. Med. Clin., 15 (1): 53-66.

De Gusmao, V.R., E.N. Mendes, D.M. De Magalhaes Queiroz, G.A. Rocha, A.M.C. Rocha, A.A.R. Ashour and A.S.T. Carvalho, 2000. *vacA* genotypes in *Helicobacter pylori* strains isolated from children with and without duodenal ulcer in Brazil. J. Clin. Microbiol., 38 (8): 2853-2857.

Dubreuil, J.D., G.D. Giudice and R. Rappuoli, 2002. *Helicobacter pylori* interactions with host serum and extracellular matrix proteins: Potential role in the infectious process. Microbiol. Mol. Biol. Rev., 66 (4): 617-629.

Falush, D., T. Wirth, B. Linz, J.K. Pritchard, M. Stephens and M. Kidd *et al*., 2003. Traces of human migrations in *Helicobacter pylori* populations. Science, 299 (5612): 1582-1585.

Fernando, N., J. Holton, D. Vaira, M. DeSilva and D. Fernando, 2002. Prevalence of *Helicobacter pylori* in Sri Lanka as determined by PCR. J. Clin. Microbiol., 40 (7): 2675-2676.

Ferrero, R.L., 2005. Innate immune recognition of the extracellular mucosal pathogen *Helicobacter pylori*. Mol. Immunol., 42: 879-885.

Gold, B.D., L.J. Vandoorn, J. Guarner, M. Owens, D. Pierce-Smith and Q. Song *et al*., 2001. Genotypic, clinical and demographic characteristics of children infected with *Helicobacter pylori*. J. Clin. Microbiol., 39 (4): 1348-1352.

Han, S.R., H.J. Schreiber, S. Bhakdi, M. Loos and M.J. Maeurer, 1998. *VacA* genotypes and genetic diversity in clinical isolates of *Helicobacter pylori*. Clin. Diag. Lab. Immunol., 5 (2): 139-145.

Hou, P., Z.X. Tu, G.M. Xu, Y.F. Gong, X.H. Ji and Z.S.H. Li, 2000. *Helicobacter pylori vacA* genotypes and *cagA* status and their relationship to associated diseases. World J. Gasteroenterol., 6 (4): 605-607.

Hussain, M.A., K. Farhana, A.K.H. Aleen, T. Santosh, M.H. Chittoor and A.H. Niyaz, 2004. Implications of molecular genotyping of *Helicobacter pylori* isolates from different human populations by genomic fingerprinting of enterobacterial repetitive intergenic consensus region for strain identification and geographic evolution. J. Clin. Microbiol., 42 (6): 2372-2378.

Kim, S.Y., C. W. Woo, Y.M. Lee, B.R. Son, J.W. Kim and H.B. Chae *et al*., 2001. Genotyping *cagA, vacA* subtype, *IceA1* and *BabA* of *Helicobacter pylori* isolates from Korean patients and their association with gastroduodenal disease. J. Korean Med. Sci., 16 (5): 579-584.

Mohammadi, M., A. Oghalaie, N. Mohajerani, S. Massarrat, M. Nasiri and M. Bennedsen *et al*., 2003. Prevalence of *Helicobacter pylori* vacuolating cytotoxin and its allelic mosaicism as a predictive marker for Iranian dyspeptic patients. Bull. Soc. Pathol. Exot., 96 (1): 3-5.

Mukhopadhyay, A., D. Kersulyte, J.Y. Jeong, S. Datta, Y. Ito and A. Chowdhury *et al*., 2000. Distinctiveness of genotypes of *Helicobacter pylori* in Calcutta, India. J. Bacteriol., 182 (11): 3219-3227.

Nogueira, C., C. Figueiredo, F. Carneiro, A.T. Gomes, R. Barreira and P. Figuira *et al*., 2001. *Helicobacter pylori* genotypes may determine gastric histopathology. Am. J. Pathol., 158 (2): 647-654.

Ramelah, M., A. Aminuddin, H. Alfizah, M.R. Isa, A.Y. Jasmi and H.J. Tan *et al*., 2005. *CagA* gene variants in Malaysian *Helicobacter pylori* strains isolated from patients of different ethnic groups. FEMS. Immunol. Med. Microbiol., 44: 239-242.

Rudi, J., C.H. Kolb, M. Maiwald, D. Kuck, A. Sieg, P.R. Galle and W. Stremmel, 1998. Diversity of *Helicobacter pylori vacA* and *cagA* protein expression, cytotoxin production and associated diseases. J. Clin. Microbiol., 36 (4): 944-948.

Saribasak, H., B.A. Slih, Y. Yamaoka and E. Sander, 2004. Analysis of *Helicobacter pylori* genotypes and correlation with clinical outcome in Turkey. J. Clin. Microbiol., 42 (4): 1648-6851.

Strobel, S., S. Bereswill, P. Balig, P. Allgaier, H.G. Sonntag and M. Kist, 1998. Identification and analysis of a new *vacA* genotype variant of *Helicobacter pylori* in different patient groups in Germany. J. Clin. Microbiol., 36 (5): 1285-1289.

Suerbaum, S. and M. Pierre, 2002. *Helicobacter pylori* infection. N. Eng. J. Med., 347 (15): 1175-1185.

Suerbaum, S. and M. Achtman, 2004. *Helicobacter pylori:* Recombination, population structure and human migrations. Int. J. Med. Microbiol., 294 (2-3): 133-139.

Thompson, J.C., D.N. Taylor, 2000. *Helicobacter pylori*. Curr. Treat. Options Infect. Dis., 2: 283-293.

Van Doorn, L.J., C. Figueiredo, R. Rossau, G. Jannes, M.V. Asbroeck and J.C. Sousa *et al*., 1998. Typing of *Helicobacter pylori vacA* gene and detection of *cagA* gene by PCR and reverse hybridization. J. Clin. Microbiol., 36 (5): 1271-1276.

Van Doorn, L.J., C. Figueiredo, F. Megraud, S. Pena, P. Midolo and F. Carneiro, 1999. Geographic distribution of *vacA* allelic types of *Helicobacter pylori* in Hong Kong. Helicobacter, 116 (4): 823-830.

Wong, B.C., Y. Yin, D.E. Berg, H.H. Xia, J.Z. Zhang and W.H. Wang *et al*., 2001. Distribution of distinct *vacA*, *cagA* and *iceA* alleles in *Helicobacter pylori* in Hong Kong. Helicobacter, 6 (4): 317-324.

Xue-jun, C.H., Y. Jie and S.H. Yue-fang, 2005. Dominant *cagA/vacA* genotypes and coinfection frequency of *Helicobacter pylori* in peptic ulcer or chronic gastritis patient in Zhejiang province and correlations among different genotypes, coinfection and severity of the diseases. Chin. Med. J., 118 (6): 460-467.

Yamaoka, Y., T. Kodama, K. Kashima, D.Y. Graham and A.R. Sepulveda, 1998. Variants of the 3' region of the *cagA* gene in *Helicobacter pylori* isolate from patients with different *H. pylori*-associated diseases. J. Clin. Microbiol., 36 (8): 2258-2263.

Yamaoka, Y., H.T. El-Zimaity, O. Gutierrez, N. Figura, J.K. Kim and T. Kodama *et al*., 1999a. Relationship between the *cagA* 3' repeat region of *Helicobacter pylori*, gastric histology and susceptibility to low pH. Gastroenterology, 117 (2): 342-349.

Yamaoka, Y., T. Kodama, O. Gutierrez, J.G. Kim, K. Kashima and D.Y. Graham, 1999b. Relationship between *Helicobacter pylori iceA* and *vacA* status and clinical outcome: Studies in four different countries. J. Clin. Microbiol., 37 (7): 2274-2279.

Yamazaki, S.H., S.H. Kato, N. Mutsokura, M. Htani, Y. Ito and H. Suto *et al*., 2005. Identification of *Helicobacter pylori* and the *cagA* genotype in gastric biopsies using highly sensitive real-time PCR as a new diagnostic tool. FEMS. Immunol. Med. Microbiol., 44 (3): 261-268.

Yuan, J.P., T. Li, Z.H. Li, G.Z. Yang, B.Y. Hu and X.D. Shi *et al*., 2004. mRNA expression profiling reveals a role of *Helicobacter pylori* vacuolating toxin in escaping host defense. World J. Gastroenterol., 10 (10): 1528-1532.

Zhou, J., J. Zhang, C. Xu and L. He, 2004. *CagA* genotype and variants in Chinese *Helicobacter pylori* strains and relationship to gastroduodenal diseases. J. Med. Microbiol., 53 (3): 231-235.