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*Helicobacter pylori vac*A Genotypes in Shahrekordian (Iran) *H. pylori*-Positive Patients

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Abstract: Clinical outcome of *Helicobacter pylori* infection might be associated with specific virulence-associated bacterial genotypes. The distribution of different bacterial genotypes varies geographically. The aim of this study, is based on determining any associations between the *Helicobacter pylori vac*A research in causing gastritis and peptic ulcer, in Shahrekord, Iran. Gastric biopsies were collected from patients that were referred to endoscopy unit of Hajar hospital Shahrekord, Iran. *H. pylori* was detected in these biopsies by culture test. Then polymorphism of *vac*A gene was genotyped by Polymerase Chain Reaction (PCR). The *vac*A genotypes were significantly different among gastritis, peptic ulcer and gastric cancer patients. Also, the *vac*A s1 allele was more frequently identified than the *vac*A s2 allele among gastrointestinal patients. Furthermore, the severest genotype of *vac*A gene in these patients was s1/m2. The results of our study illustrated that s1/m2 genotypes were associated with peptic ulceration in patients that infected by *H. pylori*.

Key words: *Helicobacter pylori*, genotype, *vac*A

INTRODUCTION

The gram-negative bacterium *Helicobacter pylori* is a highly successful pathogen persistently colonizes the human stomach (Scott-Algood and Cover, 2006; Allen *et al*., 2005) and remains one of the most common infections of humans worldwide (E-Khayat *et al*., 2007). *H. pylori* infection occurs in approximately 50% of the world's population (Yucel *et al*., 2008; Anderson *et al*., 2002) and it's prevalence is estimated at around 25% in developed countries and at >80% in developing countries (Jafarzadeh *et al*., 2007; Lin *et al*., 2004).

In the absence of antibiotic therapy, *H. pylori* can persist in the human stomach for decades or for an entire lifetime (Scott-Algood and Cover, 2006). *H. pylori* induced gastric inflammation does not cause symptoms in most infected persons but is associated with an increased risk for development of duodenal and gastric ulcer diseases (Scott-Algood and Cover, 2006) and is an important risk factor for the development of gastric cancer and gastric lymphoma (Miciuleviciene *et al*., 2008). There is no doubt that the eradication of *H. pylori* infection leads to healing of duodenal ulceration and the risk of recurrence is greatly reduced (Tovey *et al*., 2006). Variation in the clinical outcome of *H. pylori* induced pathology is multifactorial, involving a complex interplay between the host immune responses, pathogen virulence factors and niche characteristics (Kamali-Sarvestani *et al*., 2006) several potential markers of pathogenicity have been described in *H. pylori* and some of them seem to be associated with more severe clinical outcomes of the infection (Miciuleviciene *et al*., 2008). Several genes have been identified that may play a role in the pathogenesis of *H. pylori*, such as CagA, *vac*A, iceA and babA (Kamali-Sarvestani *et al*., 2006).

There are 2 proteins of *H. pylori* recognized as most virulent that are crucial in the formation of lesions of gastric mucosa: *vac*A (vacuolating cytotoxin gene A) and CagA (cytotoxin-associated geneA). Both of them, take part in the colonization and modulation of inflammatory response and in the development of inflammatory changes, peptic ulcer and gastric carcinoma (Maciorkowska *et al*., 2007).

The cagA gene is part of the cag pathogenicity island, which is a region consisting of approximately 30 genes and this genes encode for an immunodominant protein that is injected via a type IV secretion system into epithelial cells and is capable of interaction with signal transduction pathways in epithelial cells leading to inhibition of apoptosis and a number of cellular changes (E-Khayat *et al*., 2007). The *vac*A is the important virulence factor of *H. pylori* (Roche *et al*., 2007). The *vac*A gene is highly polymorphic and consists of 2 possible signal regions s1 and s2 and 2 possible

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midregions m1 and m2. The production of the vacuolating cytotoxin is governed by the mosaic combination of s and m subtypes (E-Khayat *et al*., 2007). In this study we investigated *H. pylori* genotypes at molecular level in gastro-duodenal disease population within September 2007 to August 2008 in Shahrekord, Iran.

MATERIALS AND METHODS

Patient samples: *H. pylori* were isolated from gastric biopsy obtained after inform consents from patient of both sexes who had under gone gastrointestinal endoscopy. In total, 250 patients from Shahrekord, Iran who have been referred for upper gastrointestinal endoscopy to the endoscopy unit of Hajar hospital in Shahrekord were enrolled in this study. Gastric biopsies were obtained from antrum and corpus of the stomach of these patients and before long placed in urea medium in order to assay by Rapid Urease Testing (RUT). These biopsy specimens were used as the come on process.

Bacterial strains and culture: All *H. pylori* strains were grown on pH 6 trypticase brucella agar containing 5% sheep blood under microaerophilic conditions (5% O_2, 7% CO_2, 88% N_2) (Allen *et al*., 2000). After 5 days of culture on selective agar plates, the organisms were identified as *H. pylori* by Gram staining, colony morphology and positive oxidase, catalase and urease reactions.

DNA preparation: After culture, *H. pylori* colonies were pooled from the plates and washed by phosphate-buffered saline. *H. pylori* genomic DNA was prepared after bacterial cell lysis using SDS and proteinase K solution and phenol chloroform extraction (Sambrook and Russel, 2001).

Analysis of bacterial *vac*A genotypes: All primer sets used were selected from the published literature and were synthesized. The forward primer for signal sequence of *vac*A region was: VA2-F: 5'-CAATCTGTCCAATCAA GCGAG-3' and the reverse primer was: VA2-R: 5'-GCG TCTAAATAATTCCAAGG-3' The amplification of a 259 or 286 bp fragments were expected from genotype s1 or s2, respectively. The middle region of *vac*A gene was analyzed with forward primer as the sequence of VA1-F: 5'- ATGGAAATACAACAAACACAC-3' and Reverse primer as the sequence of VA1-R: 5'- CTGCTTGAATGC GCCAAAC-3', which amplified 570 bp fragments for m1 and 645 bp fragments for m2.

The PCR assay was performed in a 25 μL reaction mixture containing 100 ng of genomic DNA, 1 × PCR buffer (Promega, Madison WI), 2 mmol L^{-1} MgCl2, 0.2 mmol L^{-1} deoxynucleotide triphosphates, 1.5 unit of Taq polymerase (Promega) and 0.2 mmol L^{-1} of each primers. Cycling profile for amplification of *vac*A was one starting cycle at 95°C for 5 min, followed by 30 cycles at 94°C for 1 min, 1 min in 55°C for s region and in 52°C for m region and 72°C for 1 min. The final extension at 72°C was performed for 5 min. PCR products were examined by 2% agarose gel electrophoresis and photographed using an ultraviolet reflection analyzer.

Statistical analysis: The association between genotypes and the clinical symptoms was analyzed by using the Fisher's exact. The difference between the patient group such as gastritis, ulceration and adenocasinoma with bacterial was calculated by independence sample test.

RESULTS

We have detected *H. pylori* positive biopsy samples according to culture test. Out of 250 samples were collected in this study 178 specimens (71.2%) were grown on selective agar. *H. pylori* colonies were identified through *H. pylori* identification. Then genomic DNA was extracted from colonies of the bacterium.

The *vac*A gene was identified in all of the *H. pylori* strains (Miciuleviciene *et al*., 2008) and the primers on the allele s1, s2 and m1, m2 used in this study enabled the differentiation and characteristics alleles of s region and m region in *vac*A genotype isolated from examined *H. pylori* strains (Fig. 1). The most frequently observed *H. pylori vac*A gene signal region allele was s1 allele. Allele s1 of the signal region from the examined gene was determined in 143 specimens out of 178 DNA isolates (80.64%), whereas allele s2 found in 35 specimens (20.27%). *H. pylori vac*A gene m1 or m2 alleles of mid region were found in whole analyzed DNA.

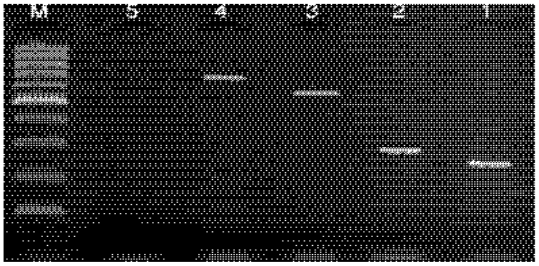

Fig. 1: Lane 1: 259 bp fragment related to s1 amplification. Lane 2: 286 bp fragment related to s2 amplification. Lane 3: 570 bp fragment related to m1 amplification. Lane 4: 645 bp fragment related to m2 amplification. Lane 5: negative control

Table 1: The association of clinical outcomes and molecular detection of *vac*A genotype

	NUD	GU	DU	Gastritis	Cancer
s1m1	4	9	15	10	-
s1m2	3	29	27	32	5
s2m1	4	-	-	3	-
s2m2	21	2	1	15	-

Among them m1 allele was shown in 129 specimens out of 178 samples (72.97%) and allele m2 in 49 specimens (27.02%) was detected. And the mid region type m1 was significantly more frequent in *H. pylori* strains with genotype s1 ($p<0.01$) and m2 alleles in most cases were detected in *vac*A s1 strains too. The *vac*A s1/m1 combination was found in 36 (20.27%) of typable strains; s1/m2 in 96 (54.05%) and s2/m2 was present in 39 (21.62%), although the combination of s2m1 is uncommon (E-Khayat *et al*., 2007) in this study it have been found in 7 specimens (4.05%). While estimating relationship between potentially virulent *H. pylori* strains and clinical outcomes, significant differences ($p<0.01$) were found between isolates from DU and CG patients. The *vac*A s1 allele without either the m1 or m2 allele was the most common genotype in patient with Peptic Ulcer Disease (PUD). Furthermore the *vac*A genotype in patient with gastric cancer was s1/m2. As it is shown in Table 1, 11.11% of the strains possess *vac*A s1/m1 genotype with NUD (Non Ulceration Disease) and 66.66% of this genotype was involved with PUD. Furthermore, 3.1 and 58.33% of s1/m2 strains was NUD and PUD, respectively. However s2 typing shows no significant correlation with PUD in this study. The relationship between the clinical symptoms and *H. pylori vac*A is shown in Table 1.

DISCUSSION

H. pylori infection is thought to play an etiologic role in several gastroduodenal diseases (Jafarzadeh *et al*., 2007). The present study, reports on the *vac*A genotypes of *H. pylori* that were identified in gastric biopsies.

The *vac*A gene was identified in all of the *H. pylori* strains (Miciuleviciene *et al*., 2008) and the clinical relevance of putative *vac*A genes of *H. pylori* is still a matter of controversy. This gene is considered to be an important virulence factor that contributes to epithelial cell injury and peptic ulceration in the host cells (Roche *et al*., 2007). Several studies, have demonstrated that the genotype varies among *H. pylori* strains isolated from different geographic regions and conclusions derived from one geographic region may not be true for others (Faundez *et al*., 2002).

Type s1 forms of *vac*A are active in many *in vitro* assays of toxin activity, whereas type s2 forms of *vac*A lack detectable activity in most *in vitro* assays (Allen *et al*., 2000; Tomasini *et al*., 2003). Infection with *H. pylori* strains containing type s1 forms of *vac*A is associated with an increased risk of gastric cancer or peptic ulcer disease compared to infection with strains containing type s2 forms of *vac*A (Tomasini *et al*., 2003; Cao and Cover, 2002) E-Khayat *et al*. (2007) reported, the s1 allele obtained from PUD patients expressed the *vac*A gene, while the transcription of the less virulent allele, s2 was detected in a few patients with PUD or gastritis who harbored this gene (E-Khayat *et al*., 2007). The *vac*A s2 allele was detected in <30% in the studied population from Europe, Central and South America and East Asia (Nimri *et al*., 2006). A study in Kuwait reported that *vac*A s1 and s2 types were detected in approximately equal numbers In biopsies obtained from patients (Al-Qabandi *et al*., 2005) and s1 alleles present almost identical percentage (65-100%) in North America and is observed in 77% of *H. pylori* strains in Eastern Asia and is the most frequently occurring subtype in *H. pylori* strains in Japan (Maciorkowska *et al*., 2007). Similarly, infection with *H. pylori* strains containing type m1 forms of *vac*A is associated with an increased risk of gastric cancer compared to infection with strains containing type m2 forms of *vac*A (Basso *et al*., 1998). The *vac*A m1 allele was more often observed (>80%) in South America, Portugal, Spain and Japan while in a study from China, the allele m2 of the midregion was the one most frequently observed (42.1% in children and 50% in adults) (Maciorkowska *et al*., 2007).

There are different allelic types of *vac*A, Based on the presence of a combination of the *vac*A s and m alleles. Some reports indicated that the *vac*A s1 allele was significantly associated with *vac*A m1 (Nimri *et al*., 2006). According to the previous studies, the type s1/m1 are toxic and strongly associated with duodenal ulceration, mostly in countries with a relatively low prevalence of *H. pylori* infection (Tovey *et al*., 2006; Faundez *et al*., 2002), for example the results of Shimoyama *et al*., show that all strains of Japanese population are s1/ml genotype (Shimoyama *et al*., 1998). But in this study, there was a significant correlation in combination of s1/m2 (54.05%), while in another study from Jordan, it was reported that *vac*A s1/m2 genotype was detected only in 7.7% of the *vac*A s/m combination (Nimri *et al*., 2006) and our study has revealed that s1/m2 strains are more characteristic for PUD. In general, *vac*A type s1m1 and s1m2 strains produce high and moderate levels of toxin, respectively, whereas s2/m2 strains produce little or no toxin (Van-Doorn *et al*., 1998). And in a study from Tennessee, all positive combination of *vac*A region were identified, with the exception of s2m1 (Atherton *et al*., 1995) and in this study the proportion of s2m1 was 4.05%.

CONCLUSION

In conclusion, results of our study suggest that s1/m2 genotypes are associated with peptic ulceration in patients from Shahrekord, Iran that infected by *Helicobacter pylori*.

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