THE PREVALENCE OF HELICOBACTER PYLORI CAGA AND ICEA GENOTYPES AND POSSIBLE CLINICAL OUTCOMES

KIANOOSH DADASHZADEH[1*], MORTEZA MILANI[2, 3], MOHAMMAD H. SOMI[2]
[1]Department of Laboratory Sciences, Marand Branch, Islamic Azad University, Marand, Iran - [2]Liver and Gastrointestinal Diseases Research Center, Tabriz, Iran - [3]School of Advanced Medical, Tabriz University of Medical Sciences, Tabriz, Iran

ABSTRACT

Objective*: There is continuing interest in identifying Helicobacter pylori virulence factors that might predict the risk for symptomatic clinical outcomes. It has been proposed that iceA and cagA genes are such markers and can identify patients with peptic ulcers and gastric cancer.*

Methods*: To determine the prevalence of specific genotypes of H. pylori, clinical isolate of H.pylori obtained from 102 patients through endoscopic biopsies was cultured. The cagA alleles, iceA genotypes were determined by PCR.*

Results*: Distribution of cagA and clinical outcome was shown that the frequency of cagA-positive isolates in PUD, NUD and GC patients was 81.25% and 65.5% and 100%, respectively. Also the iceA1 allele was identified in 2 (100%) GC patients but iceA2 allele was not detected in these patients. Overall cagA and iceA1 alleles were detected in GC patients.*

Conclusions*: The cagA gene and iceA1 genotype was found to predominate in gastric adenocarcinoma patients, and also iceA2 genotype was also associated with PUD.*

Key words*: H. pylori - cagA - iceA.*

Received *May 30, 2015;* ***Accepted*** *November 02, 2015*

Introduction

The bacterium *Helicobacter pylori* colonize the gastric mucosa of approximately half the world's population. This gastric colonization induces chronic gastric inflammation in all infected individuals, but only 15-20% of infected patients develop gastric or duodenal ulcer (DU) and <1% develop gastric adenocarcinoma[1]. The prevalence of *H. pylori* infection in a population generally does not predict the incidence of serious clinical squeal, suggesting that host and pathogen genetic variation, as well as dietary and other environmental factors, play an important role. These factors analyzed in isolation have failed to provide adequate explanations for the variability in infection outcomes. However, Experience with other bacterial pathogens suggests that *H. pylori* strain-specific factors may influence the pathogenicity of different *H. pylori* isolates. This study have primarily focused on two groups of putative bacterial virulence factors, the cag pathogenicity island (for which cagA is a marker) and the *ice*A[2].

Several studies have suggested that *cag*A is a useful marker for the most virulent strains that are associated with peptic ulcer, atrophic gastritis and adenocarcinoma[2, 3]. The cag pathogenicity island (PaI) encodes a type IV secretory system and delivers cagA into the host cytosol where becomes phosphorylated on tyrosine residue. Phosphorylated *cag*A interacts with the phosphatase SHP-2 causing dephosphorylation of cortactin and cytoskeletal rearrangements forming the "hummingbird" phenotype[4]. Overall, the data support the notion that infection with a *cag*A-positive isolate increases the risk but does not predict the presence of a clinically significant outcome[4-6].

Recently, a novel putative virulence factor has been identified; the *ice*A (for induced by contact with epithelium) was suggested to have an association with peptic ulcer. The iceA gene has two main allelic variants, *ice*A1 and *ice*A2[7].The expression of iceA1 is up-regulated on contact between H. pylori and human epithelial cells, and may be related with peptic ulcer disease[8]. Van Doorn[9] reported that the *ice*A allelic type was independent of the *cag*A and vacA status, and there was a significant association between the presence of the *ice*A1 allele and peptic ulcer disease. Those researchers proposed that genotyping of *ice*A and *cag*A might offer an effective combination for identification of patients with peptic ulcers[10]. Their results were obtained from patients in Tabriz, northwest of Iran, and the search for virulence factors related to outcome of infection has been hampered by the fact that there appear to be differences in the predominant strain in circulation in different geographic regions. Thus, conclusions derived from data from a single geographic region may not be true for other geographic regions[10, 11].

In this study, we examined the *ice*A allele type in stains from our region and its relation with *cag*A status genotypes and clinical outcome.

Materials and methods

Patients

A total of one hundred two *H. pylori* isolates were obtained from gastric biopsies of patients with gastritis, peptic ulcer and gastro esophageal reflux diseases undergoing endoscopy. This study was approved by the ethical committee of regional Medical Research of Tabriz University of Medical Sciences and all patients provided written informed consent for this research.

H. pylori Culture and extraction of Genomic DNA

Briefly gastric biopsy samples were homogenized and cultured onto Brucella agar supplanted with 5% sheep blood and antibiotics (Vancomycin, Amphotericin B and Trimethoprim). Culture plates were incubated at microaerophilic condition, 37 ºC and high humidity for 5-7 days. Organisms were identified as H. pylori based on colony morphology, gram staining and positive oxidase, catalase and urease tests. Genomic DNA of total H. pylori isolates was extracted by using CTAB method[10] and stored at -20 ºC.

Detection of *cag*A and *ice*A genes

In this study PCR was used to detect the *H. pylori* specific ureC gene for confirmation of *H. pylori* isolates, the virulence-associated *cag*A structure and the presence of *ice*A gene. All primer sets were selected from the published literatures (Table 1). PCR reactions were performed in a volume of 50 μL containing10 mmol/L Tris-HCl, 1.5 mmol/L MgCl2, 0.2 mmol/L of each deoxynucleotide, 25 pmol of each primer and 2.5 units of Taq polymerase (Geneone, Germany). Thermal cycler program consisted the following steps; initial denaturation at 94ºC for 3 min followed by 35 cycles repetitions of 30 seconds at 94 ºC (denaturation), 30 seconds at 58 ºC for *cag*A and *glm*M, 57 ºC for *ice*A1 and 48 ºC for *ice*A2 (annealing) and 30 seconds at 72 ºC (extension) and final extension step was 3 min at 72 ºC.

Gene	Primer	Nucleotide sequence	size (bp)	References
*ure*C (*glm*M)	Hp-F	GGATAAGCTTTTAGGGGTGTTAGGGG	294	Ko et al., 2008
	HP-R	GCTTACTTTCTAACACTAACGCGC		
*cag*A	*cag*A-F	AGGGATAACAGGCAAGCTTTTGA	352	Van Doorn et al., 1998
	*cag*A-R	CTGCAAAAGATTGTTTGGCAGA		
*ice*A1	*ice*A1-F	GTGTTTTTAACCAAAGTATC	247	Ko et al., 2008
	*ice*A1-R	CTATAGCCASTYTCTTTGCA		
*ice*A2	*ice*A2-F	GTTGGGTATATCACAATTTAT	229	Ko et al., 2008
	*ice*A2-R	TTRCCCTATTTTCTAGTAGGT		

Table 1: Primers used in this study.

Statistics analysis

Data were analyzed by SPSS version 16. The Pearson X2 test was used to evaluate the relationship between individual genotypes and a variety of diseases.

Results

Of the 102 patients infected with *H. pylori*, 84 patients with non-ulcer diseases, 16 patients with peptic ulcer disease and 2 patients with gastric cancer. The mean age of the patients was 34±19 years (gender ratio M/F: 1.05).There was no significant difference between the mean age of patients with and without ulcers.

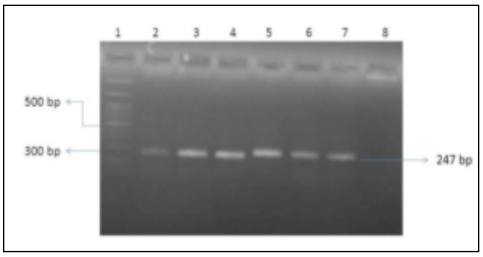

Figure 1: Amplified products of iceA1 gene by PCR
Lane 1: 100- 3000 bp DNA ladder, Lane 2-7 clinical isolates of iceA1 positive, Lanes 8: negative control.

In this study the distribution of *cag*A and clinical outcome was analyzed statistically and it was observed that the frequency of *cag*A-positive isolates in PUD, NUD and GC patients was 81.25% and 65.5% and 100%, respectively (Table 2).

Overall, *ice*A1 was detected in 41 isolates and iceA2 in 13 isolates. Seven isolates (6/9%) were positive for both iceA1 and iceA2, while 56 isolates (54/9%) did not yield any PCR product for iceA.

The *ice*A1 allele was identified in 2 (100%) GC patients but *ice*A2 allele was not detected in these patients. As shown in Table 2 the *ice*A1 allele was observed in PUD patients (43.75%) and in NUD patients (38.1%) while the prevalence of the *ice*A2 allele was observed in PUD (12.5%) cases and in NUD patients (13.1%); however, these differences were not statistically significant.

Genotypes	Number (%) of isolates			Total (n=102)	Pv
	NUD (n=84)	PUD (n=16)	GC (n=2)		
***ice*A1**	32 (38.1%)	7 (43.75%)	2 (100%)	41 (40/2%)	0.5
***ice*A2**	11 (13.1%)	2 (12.5%)	0	13 (12/74%)	1
***cag*A**	55 (65.5%)	13 (81.25%)	2 (100%)	70(68/63%)	0.07

Table 2: Relationship between clinical outcome and status of *cag*A and *ice*A

Combination of *ice*A, and *cag*A genotypes

We examined eight different combinations based on analysis of *cag*A (positive and negative), and the *ice*A type (*ice*A1 and *ice*A2) in patients with a single genotype (Table 3). We were unable to identify an association between these genotypes and clinical outcome.

***ice*A2**	***ice*A1**	***cag*A**	**cancer**	**ulcer**		**Total**
positive	positive	positive	GC	PUD	NUD	
"	"	positive		1	3	4
"	"	negative		1	2	3
"	negative	positive		0	4	4
"	"	negative		0	1	1
negative	positive	positive	2	5	17	24
"	"	negative		1	9	10
"	negative	positive		7	31	38
"	"	negative		1	17	18
Total			2	16	84	102

Table 3: Combination of *ice*A1, iceA2 and *cag*A genotypes and clinical outcome

Discussion

Gastric mucosa colonization by *H. pylori* leads to chronic gastritis, atrophic gastritis and is associated with several diseases such as peptic ulcers, gastric carcinoma, and MALT lymphoma[12]. However, there is an obvious difference between the number of infected and those who patients with clinical outcome. Although environmental and host factors are important, also previous studies show that the specific genotype of bacteria play an important role in the development of clinical symptoms[13].Thus infection with *H. pylori* specific genotypes such as cagA and iceA is related to more severe conditions, while other strains occur less pathogenic[14]. This study was designed to characterize the genotype of *H. pylori* from gastric biopsy specimens from patients with upper gastrointestinal diseases and the relationship with clinical outcome in northwest of Iran. The presence of the *cag*A, *ice*A1 and *ice*A2 genes were detected in *H. pylori* isolates.

Survey of previous studies showed that the *cag*A prevalence is different around the world[15]. As the prevalence of *cag*A gene in this study was 68%. Our result is in agreement with reports from Western countries[16], but lower than the East Asian countries where the cagA are present in more than 90% of cases[17]. The results of our study showed that cagA-positive isolates compared to *cag*A-negative isolates were more frequently isolated from PUD patients. While in NUD patients was the opposite, while this finding was not statistically significant ($pv > 0.05$). These findings are supported by previous studies[17, 18] and suggest that colonization with *cag*A-positive *H. pylori* strains associated with developing peptic ulcer disease.

Our results show that the prevalence of iceA1 and *ice*A2 genes in isolates was 39% and 13%, respectively. These results are in agreement with previous studies that the *ice*A1 gene was found to be prevalent in Japan, Korea and Netherlands patients[19-21]. However, several studies have reported different results, as the *ice*A2 gene was detected to be predominant genotype in these studies[22, 23]. It was found that iceA1 was significantly associated with peptic ulcer disease in polish and the USA[23, 24]. However, these reports have not been confirmed in other countries such as Korea and India[25, 26] in our study of total patients infected with *H. pylori*, two patients had gastric adenocarcinoma. The genotypes of strains isolated from these patients were *cag*A and iceA1 positive while these strains were negative for the *ice*A2 gene.

In conclusion, this study was show the prevalence of virulence genes *cag*A *ice*A1 and *ice*A2 in Northwest Iran. The *cag*A gene and *ice*A1 genotype was found to predominate in gastric adenocarcino-

ma patients, and also *ice*A2 genotype was also associated with PUD. It may be the size of sample in our study insufficient to predict of clinical outcome relationship with virulence genes in *H. pylori* infection. Despite of the results of some studies have shown that the iceA2 genotype was frequently found in patients with gastric carcinoma or duodenal ulcer. However, it is not easy to declare that *ice*A2 gene is considered as a protective factor in some area and that is associated with more severe diseases in other countries. This virulence gene could be used a molecular marker for bacterial pathogenesis.

Conclusions

The *cag*A gene and *ice*A1 genotype was found to predominate in gastric adenocarcinoma patients, and also *ice*A2 genotype was also associated with PUD.

References

1) Alam J, Maiti S, Ghosh P, De R, Chowdhury A, Das S, et al. Significant association of the dupA gene of Helicobacter pylori with duodenal ulcer development in a South-east Indian population. Journal of medical microbiology. 2012; 61(Pt 9): 1295-302.
2) Hobsley M, Tovey F. Helicobacter pylori: the primary cause of duodenal ulceration or a secondary infection? World Journal of Gastroenterology. 2001; 7(2): 149-51.
3) Sedaghat H, Moniri R, Jamali R, Arj A, Zadeh MR, Moosavi SGA, et al. Prevalence of *Helicobacter pylori* *vac*A, *cag*A, *cag*E, *ice*A, *bab*A2, and oipA genotypes in patients with upper gastrointestinal diseases. Iranian Journal of Microbiology. 2014; 6(1): 14-21.
4) Noto JM, Peek Jr RM. The *Helicobacter pylori* cag pathogenicity island. Helicobacter Species: Springer; 2012. p. 41-50.
5) Antonio-Rincón F, López-Vidal Y, Castillo-Rojas G, Lazcano-Ponce EC, Ponce-de-León S, Tabche-Barrera ML, et al. Pathogenicity island cag, vacA and IS605 genotypes in Mexican strains of *Helicobacter pylori* associated with peptic ulcers. Ann Clin Microbiol Antimicrob. 2011; 10: 18.
6) Terry CE, McGinnis LM, Madigan KC, Cao P, Cover TL, Liechti GW, et al. Genomic comparison of cag pathogenicity island (PAI)-positive and-negative Helicobacter pylori strains: identification of novel markers for cag PAI-positive strains. Infection and immunity. 2005; 73(6): 3794-8.
7) Galvis AA, Trespalacios-Rangel A, Otero W, Mercado-Reyes M, Poutou-Piñales R. Prevalence of *cag*A, *vac*A, *bab*A2 and *ice*A genes in *H. pylori* strains isolated from Colombian patients with functional dyspepsia. Polish J Microbiol. 2012; 61: 33-40.
8) Keshavarz Azizi Raftar S, Moniri R, Saffari M, Razavi Zadeh M, Arj A, Mousavi SGA, et al. The *Helicobacter pylori* Resistance Rate to Clarithromycin in Iran. Microbial Drug Resistance. 2014.
9) van Doorn LJ, Figueiredo C, Sanna R, Plaisier A, Schneeberger P, de Boer W, et al. Clinical relevance of the cagA, vacA, and iceA status of Helicobacter pylori. Gastroenterology. 1998; 115(1): 58-66.
10) Yamaoka Y, Kodama T, Gutierrez O, Kim JG, Kashima K, Graham DY. Relationship between *Helicobacter pylori ice*A, *cag*A, and *vac*A status and clinical outcome: studies in four different countries. Journal of Clinical Microbiology. 1999; 37(7): 2274-9.
11) Graham D, Genta R, Graham D, Crabtree J. Serum CagA antibodies in asymptomatic subjects and patients with peptic ulcer: lack of correlation of IgG antibody in patients with peptic ulcer or asymptomatic Helicobacter pylori gastritis. Journal of clinical pathology. 1996; 49(10): 829-32.
12) Dadashzadeh K, Milani M, Rahmati M, Akbarzadeh A. Real-Time PCR Detection of 16S rRNA Novel Mutations Associated with Helicobacter pylori Tetracycline Resistance in Iran. Asian Pacific journal of cancer prevention: APJCP. 2014; 15(20): 8883.
13) Kaklikkaya N, Cubukcu K, Aydin F, Bakir T, Erkul S, Tosun I, et al. Significance of *cag*A status and *vac*A subtypes of Helicobacter pylori in determining gastric histopathology: virulence markers of *H. pylori* and histopathology. Journal of gastroenterology and hepatology. 2006; 21(6): 1042-7.
14) Yamaoka Y. Mechanisms of disease: Helicobacter pylori virulence factors. Nature Reviews Gastroenterology and Hepatology. 2010; 7(11): 629-41.
15) Aziz F, Chen X, Yang X, Yan Q. Prevalence and correlation with clinical diseases of *Helicobacter pylori* cagA and vacA genotype among gastric patients from Northeast China. BioMed research international. 2014; 2014.
16) Yamaoka Y, Souchek J, Odenbreit S, Haas R, Arnqvist A, Borén T, et al. Discrimination between cases of duodenal ulcer and gastritis on the basis of putative virulence factors of *Helicobacter pylori*. Journal of clinical microbiology. 2002; 40(6): 2244-6.
17) Uchida T, Nguyen LT, Takayama A, Okimoto T, Kodama M, Murakami K, et al. Analysis of virulence factors of *Helicobacter pylori* isolated from a Vietnamese population. BMC microbiology. 2009; 9(1): 175.
18) Wu AH, Crabtree JE, Bernstein L, Hawtin P, Cockburn M, Tseng Cc, et al. Role of *Helicobacter pylori Cag*A+ strains and risk of adenocarcinoma of the stomach and esophagus. International journal of cancer. 2003; 103(6): 815-21.
19) Ito Y, Azuma T, Ito S, Suto H, Miyaji H, Yamazaki Y, et al. Sequence Analysis and Clinical Significance of theiceA Gene from *Helicobacter pylori* Strains in Japan. Journal of clinical microbiology. 2000; 38(2): 483-8.
20) Ko JS, Kim KM, Oh YL, Seo JK. cagA, vacA, and iceA genotypes of Helicobacter pylori in Korean children. Pediatrics International. 2008; 50(5): 628-31.
21) Shiota S, Suzuki R, Yamaoka Y. The significance of virulence factors in Helicobacter pylori. Journal of digestive diseases. 2013; 14(7): 341-9.
22) Aghdam SM, Sardari Z, Safaralizadeh R, Bonyadi M, Abdolmohammadi R, Moghadam MS, et al. Investigation of Association between oipA and iceA1/iceA2 Genotypes of *Helicobacter pylori* and

Gastric Cancer in Iran. Asian Pacific Journal of Cancer Prevention. 2014; 15(19): 8295-9.
23) Biernat MM, Gościniak G, Iwańczak B. Prevalence of *Helicobacter pylori cag*A, *vac*A, *ice*A, *bab*A2 genotypes in Polish children and adolescents with gastroduodenal disease. Postępy higieny i medycyny doświadczalnej (Online). 2014; 68: 1015.
24) Podzorski RP, Podzorski DS, Wuerth A, Tolia V. Analysis of the *vac*A, *cag*A, *cag*E, *ice*A, and *bab*A2 genes in Helicobacter pylori from sixty-one pediatric patients from the Midwestern United States. Diagnostic microbiology and infectious disease. 2003; 46(2): 83-8.
25) Kim JY, Kim N, Nam RH, Suh JH, Chang H, Lee JW, et al. Association of polymorphisms in virulence factor of *Helicobacter pylori* and gastroduodenal diseases in South Korea. Journal of gastroenterology and hepatology. 2014; 29(5): 984-91.
26) Dharne MS, Munot H, Pujari R, Kakrani AL, Patole MS, Shouche YS. *Helicobacter pylori cag*A, *vac*A and *ice*A genotypes in western Indian population of Maharashtra with varied gastroduodenal diseases. Indian journal of pathology & microbiology. 2007; 50(4): 740-8.

Acknowledgements
We would like to thanks Marand Branch, Islamic Azad University for the financial support of this research, which is based on a research project contract and the staff of hospitals and Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, for their help in conducting this study.

Correspoding author
KIANOOSH DADASHZASDEH
Department of Laboratory Sciences, Marand Branch, Islamic Azad University
Marand
(Iran)