Original Article
http://mjiri.iums.ac.ir

Medical Journal of the Islamic Republic of Iran (MJIRI)
Iran University of Medical Sciences

CagA and VacA genotypes in peptic ulcer disease and non-ulcer dyspepsia: a case-control study

Hashem FakhreYaseri[1], Mehdi Shakaraby[2], Hamid Reza Bradaran[3]
Seyed Kamran Soltani Arabshahi[4], Ali Mohammad Fakhre Yaseri[5]

Received: 19 February 2014 Accepted: 23 April 2014 Published: 28 September 2014

Abstract

Background: The cag pathogenicity island includes a number of genes, including cytotoxin-associated protein A (cagA) and vacuolating cytotoxin (vacA) genotypes, which are associated with bacterial virulence. Although the role of cagA and vacA in the virulence of Helicobacter pylori (H. pylori) is well-established in epidemiological studies, the relationship between the cagA and vacA genotypes in Iran has yet to be fully elucidated. This study compared the association between cagA and vacA genotypes between peptic ulcer disease (PUD) patients and non-ulcer dyspeptic (NUD) patients.

Methods: This case control study was done on 130 patients with positive H. pylori in histological and Giemsa reports. The case group comprised 65 PUD patients, and the control group included 65 NUD patients. The presence of the cagA and vacA genotypes was determined using polymerase chain reaction (PCR) on biopsy samples, taken via endoscopy.

Results: Both cagA and vacA genotypes were positive in 51.5% (17) of the PUD group and 20% (6) of the NUD group (p= 0.009), and both cagA and vacA genotypes were negative in 48.5% (16) and 80% (24) of the case and control groups, respectively (p= 0.03). CagA-positive H. pylori was detected in 41.5% (27) and 24.6% (16) of the case and control groups, respectively (p= 0.001), and vacA-positive H. pylori was found in 60% (39) and 46% (30) of the case and control groups, respectively.

Conclusion: Both cagA and vacA genotypes were more prevalent in the PUD patients than in their NUD counterparts among our Iranian samples. It is seems that the determination of these two genotypes in PUD patients is a good screening tool for patient selection for endoscopy and treatment.

Keywords: Dyspepsia, Peptic ulcer, Genes.

Cite this article as: FakhreYaseri H, Shakaraby M, Bradaran H.R, Soltani Arabshahi S.K, Fakhre Yaseri A.M. CagA and VacA genotypes in peptic ulcer disease and non-ulcer dyspepsia: a case-control study. *Med J Islam Repub Iran* 2014 (28 September). Vol. 28:104.

Introduction

Helicobacter pylori (H. pylori) is a Gram-negative spiral-shaped microaerophilic bacterium that colonizes the human gastric mucosa (1). It is estimated that the prevalence of H. pylori infection ranges between 20% and 80% in the world (2,3). Although not all persons infected with this organism develop gastroduodenal pathology (4), H. pylori is now well-recognized as the major etiologic agent of chronic gastritis, peptic ulcer disease (PUD), adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma (MALtoma) (5,6) .

Several virulence factors have been recognized so far. However, cytotoxin-associated protein A (cagA) and vacuolating cytotoxin (vacA) are known to have great potential to cause disease development. CagA is a highly immunogenic protein of 128 kDa, and vacA is a protein of 87 kDa which can vacuolize the gastric epithe-

1. (**Corresponding author**) Assistant Professor, Research Center for Gastroenterology and Liver Disease, Department of Internal Medicine and Gastroenterology, Firoozgar Hospital, Iran University of Medical Sciences, Tehran, Iran. hfyaseri@yahoo.com
2. Associate Professor, Department and Research Center of Immunology, Iran University of Medical Sciences, Tehran, Iran. m_shekaraby@yahoo.com
3. Associate Professor, Department of Epidemiology, Iran University of Medical Sciences, Tehran, Iran. baradaran.hr@iums.ac.ir
4. Professor, Department of Internal Medicine, Firoozgar Hospital, Iran University of Medical Sciences, Tehran, Iran. soltarab34@gmail.com
5. General physician, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. fyasseri@mihanmail.ir

lial cells. These proteins are produced by some H. pylori strains which are encoded by the cagA and vacA genes. Half of all cytotoxin-positive H. pylori strains have the cagA gene in their genome (7,8).

Previous studies have shown that strains which have cagA and produce cytotoxin protein are closely associated with PUD, albeit with different geographical prevalence rates (9- 11). Furthermore, the vacA-positive strain of H. pylori is known to be associated with PUD (12,13). Xiang et al. classified H. pylori strains in two main groups: type I strain had the gene coding for cagA and expressed cagA and vacA and type II strain did not have the gene coding for cagA and expressed neither cagA nor vacA. They reported rates of 56% and 16% for strain types I and II, respectively (9). Weel et al. demonstrated that H. pylori strains with the cagA and vacA genotypes were positive in 56.6% and 31.6% of patients with PUD and functional dyspepsia, respectively (12). Takata et al. found both cagA genotype and anti-vacA antibody in 62.9% and 7.8% of PUD and non-ulcer dyspeptic (NUD) patients, respectively (10). Elsewhere, Figueroa et al. reported that both anti cagA and vacA antibodies existed in 85% and 71% of patients with PUD and NUD, respectively (11). Moreover, Maeda et al. reported that there were both anti-cagA and vacA antibodies in 81%, 82%, and 72% of patients with gastric ulcer, duodenal ulcer, and NUD, respectively, thereby demonstrating that there was no significant difference between PUD and NUD groups (14).

For all the research in epidemiological studies into the role of the cagA and vacA genotypes in the virulence of H. pylori around the world, precious little information is available on the expression of these two genotypes in the H. pylori strain in Iran. The present study sought to compare the expression of cagA and vacA H. pylori virulence factors between a PUD group and an NUD group.

Methods

The study population was selected from dyspeptic patients admitted to Firoozgar Hospital, Tehran, Iran. According to the Leeds Medical School Criteria (15), 802 patients who had dyspeptic symptoms at least for two months with no underlying diseases were initially selected. All the patients provided informed consent and accepted to complete a standard questionnaire. Diagnosis was based on questionnaires and esophagogastroduodenoscopy (Fujinon ED-53 DEP) results. From this total, a case-control study was conducted on 130 patients, aged between 15 and 65 years. The case group was comprised of 65 PUD patients, and the control group consisted of 65 NUD patients. The case and control groups had no history of documented ulcer, previous H. pylori eradication, cigarette smoking, malignancy, underlying diseases, or esophagitis in esophagogastroduodenoscopy, and nor did they use proton pump inhibitors or antibiotics (from at least two months before endoscopy).

Peptic ulcer development depends on age and gender; accordingly, all the selected patients were matched accurately in terms of their age and gender. Four biopsy specimens were taken from the antrum and gastric body of each patient for histological study. All the specimens were stained by hematoxylin and eosin stain (H&E) or Giemsa after having been fixed overnight in buffered formalin, embedded in paraffin, and cut in five µm thickness. The specimens were evaluated by a pathologist. If at least five bacilli in each microscopic field were found, H. pylori was considered positive. Five milliliters of blood sample was taken from each patient and was sent to the Immunology Laboratory of Iran University of Medical Sciences for centrifuge and anti-cagA antibody measurement.

The genomic DNA was extracted from the biopsy samples using a DNA isolation kit for cells and tissues (Roche Applied Science Company) in accordance with the Manufacture's instruction and stored at -20° C. Two primers were designed comple-

Table 1. Characteristics of the primers used for the detection of cagA and vacA

Amplified region	Primer designation	Primer sequence	Product size (bp)
GlmM	GlmM-F	5′-AAGCTTTTAGGGGTGTTAGGGGTTT-3′	294
	GlmM-R	5′-AAGCTTACTTTCTAACACTAACGC-3′	
CagA1	CAG1-F	5′-GAT AAC AGG CAA GCT TTT GAG G-3′	349
	CAG1-R	5′-CTG CAA AAG ATT GTT TGG CAG A-3′	
CagA2	CAG2-F	5′-TTG ACC AAC AAC CAC AAA CCG AAG-3′	1385
	CAG2-R	5′-CTT CCC TTA ATT GCG AGA TTC C-3′	
VacA1	VAC1 – F	5′-CTG CTT GAA TGC GCC AAA C-3′	71
	VAC1 –R	5′-CAC AGC CAC TTT CAA TAA CGA-3′	
VacA2	VAC2 – F	5′-ATG GAA ATA CAA CAA ACA CAC-3′	480
	VAC2 - R	5′-CGT CAA AAT AAT TCC AAG GG-3′	

Bp, base pair; GlmM, urea C gene used for the detection of H. pylori; F, forward; R, reverse; CagA1 and A2, two pairs of primers used for the detection of cag, which are called A1and A2; VacA1and A2, two pairs of primers used for the detection of vac, which are called A1and A2

mentary to the sequence located within the conserved region of the gene primers: cagA1 and cagA2. The primers F and R and their sequences as well as the sizes of the PCR products are depicted in Table 1.

The amplified products were evident as ethidium bromide-stained bands after Agarose gel electrophoresis. Moreover, 34 gbp band indicated the presence of the H. pylori cagA in the specimen, as is shown in Figure 1. The identification of the bacilli as H. pylori was confirmed via PCR for GlmM, a conserved gene formerly known as urea C (Fig. 2) (16).

The identification of vacA gene was confirmed by PCR using primers specific for its signal sequence with the pair of primers: vacA1 and vacA2. The characteristics of the primers are illustrated in Table 1. The amplified product was evident as above, and 480 gbp band indicated the presence of the H. pylori vacA in the specimen, as is shown in Fig. 3.

All the data were analyzed using SPSS software (version 18) after encoding for each patient. Age is shown with age ± standard deviation. The effects of Cag A and Vac A positive on the risk peptic ulcer developed were expressed as Odds ratios (ORs) with 95% confidence intervals (CIs) with reference of NUD subjects with H.Pylori infections. Proportions were compared by Fisher exact probability test and the chi-squared test. A P-value less than 0.05 was considered statistically significant

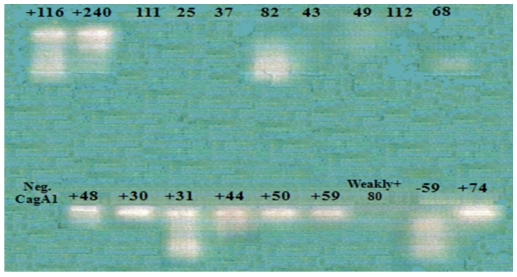

Fig. 1. CagA-positive and negative samples

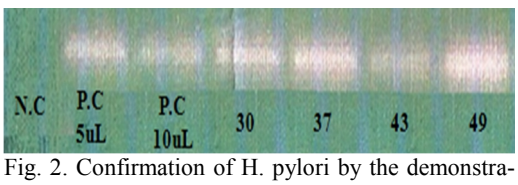

Fig. 2. Confirmation of H. pylori by the demonstration of the urea C gene on Agar electrophoresis

Results

On the basis of the Leeds Medical School Criteria, the case group included 65 PUD patients [including 37 (57%) females and 28 (43%) males at an average age of 41.6 ± 16.4 years (16 to 64)]. In the case group, gastric ulcer was detected in 27 (41.5%) patients [21 (77.8%) females and 6 (22.2%) males] and duodenal ulcer was found in 38 (58.5%) patients [16 (42.1%) females and 22 (57.9%) males]. The control group (NUD) group included 65 patients, comprised of 29 (45%) females and 36 (55%) males at an average age of 36.4 ± 10.8 years (18 to 60).

The overall frequency of the cagA gene was 43/130 (33%): 27/65 (41.5%) in the case (PUD) group and 16/65 (24.6%) in the control (NUD) group (p=0.001). The total frequency of the vacA gene was 69/130 (53%): 39/65 (60%) in the PUD group and 30/65 (46%) in the NUD group (p=0.25).

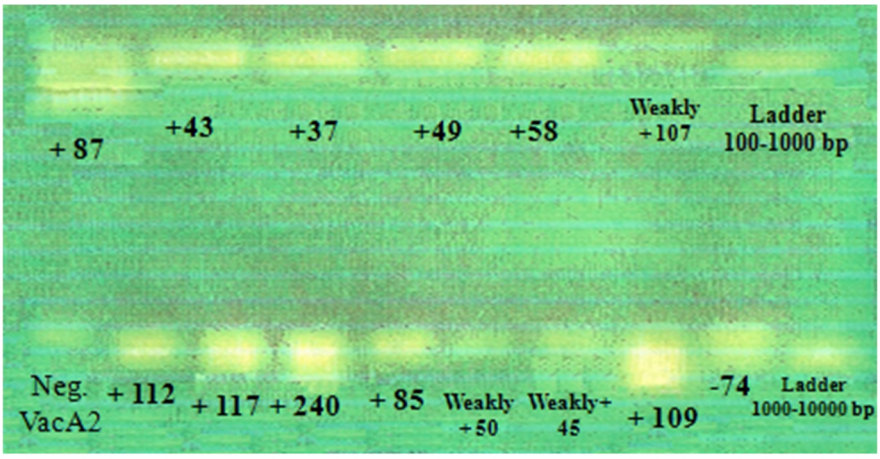

Fig.3. VacA-positive and negative samples

Table 2. Relation between endoscopic findings and genotypes

Genotype	Number of cases (%)		p
	(Case)PUD	(Control)NUD	
CagA+VacA+	17(51.5)	6(20)	0.009
CagA-VacA-	16(48.5)	24(80)	0.03

PUD, peptic ulcer disease; NUD, non-ulcer dyspepsia; CagA, cytotoxin-associated protein A; VacA, vacuolating cytotoxin A;+,positive;-,negative

The cagA and vacA genes were both positive in 17/33 (51.5%) and 6/30 (20%) of the patients in the case and control groups, respectively. The rate was, therefore, significantly higher in the case group than in the control group (odds ratio: 4.25, 95%CI: 1.25-13.06; p=0.009). These results are presented in Table 2.

Discussion

To the best of our knowledge, this study is the first Iranian case-control study to date to examine the relationship between both cagA and vacA genotypes of H. pylori in patients with or without PUD. Our results showed that 51.5% of the patients in the case (PUD) group had both cagA and vacA genotypes and that this rate was higher than that (20%) in the control (NUD) group. Although smokers and patients receiving acid-inhibiting medications or nonsteroidal anti-inflammatory drugs were excluded from this study, the results were similar to those reported by previous studies (9, 10 and 12). Therefore, this could be a reliable method for the diagnosis of PUD. Our results also demonstrated a correlation between the two genotypes of H. pylori, i.e., cagA and vacA, and PUD.

In contrast to the virtual absence of any research in Iran into the expression of the two genotypes of H. pylori strains, the literature abounds with information elsewhere. In 1995, Xiang et al. classified H. pylori strains in two main groups: type I strain had the gene coding for cagA and expressed cagA and vacA and type II strain did not have the gene coding for cagA and did not express either cagA or vacA. Additionally, the authors reported rates of 56% and 16% for types I and II, respectively (9). In 1996, Weel et al. showed both cagA and vacA-positive H. pylori strains in 56.6% and 31.6% of patients with PUD and functional dyspepsia, respectively (12). In 1998, Takata et al. found both cagA genotype and anti-vacA protein antibody in 62.9% and 7.8% of patients with PUD and NUD, respectively (10). In 2002, Figueroa et al. reported both anti cagA and vacA protein antibodies in 85% and 71% of patients with PUD and NUD, respectively; they believed that these results were due to the high prevalence of both cagA and vacA-positive H. pylori strains in their country (11). In 1998, Maeda et al. demonstrated both anti-cagA and vacA antibodies in 81%, 82%, and 72% of patients with gastric ulcer, duodenal ulcer, and NUD, respectively, and concluded that the difference

between the PUD and NUD groups was not significant (14).

These results can be explained by the high prevalence of type I strain of infection in Japan.

Most of the studies in this field have measured serum anti-cagA and vacA antibodies or vacA activity in vitro or in patients. Some studies have shown that the vacA activity and protein can occur even when the cagA or vacA genotypes cannot be detected and that there are some strains that can produce inactive or small amounts of the vacA protein. The use of gene-specific primers in epidemiological studies is the best method to demonstrate the cause and effect of the virulence factors in infectious diseases because the vacA protein can be detected in the absence of the expression of the cagA and vacA genotypes or because some strains can produce small amounts of this protein (9- 11 and 14).

There have been some controversies with respect to the association between the cagA and vacA genotypes and gastrointestinal disorders in Iranian studies (17-23). Where most of these investigations have reported that the cagA gene is more prevalent in Iran than in European and Western countries (17,18), Siavoshi et al. showed that only 44% of H. pylori strains are cagA positive (19), which chimes in with our findings. In addition, some Iranian reports have demonstrated a relationship between the cagA genotype and gastrointestinal disorders (20,21), whereas others have detected no correlation (22,23).

It has been demonstrated that H. pylori carries only a single copy of the vacA gene. The risk of H. pylori with multiple strains may be higher in countries with high prevalence rates of H. pylori infection than in those with low prevalence rates (9,14).

Several studies have demonstrated that patients with duodenal ulcer are most often infected by strains which have the cagA and vacA genotypes. Consequently, it has been suggested that both virulence factors play a role in the pathogenicity of peptic ulcers (9). It has also been reported that only strains with both cagA and vacA genotypes can induce gastric injury in the mice (13).

The present study has some limitations, first and foremost among which is that it did not assess factors such as the study population's socioeconomic status, diet, and immune system. In addition, other virulence factors of H. pylori were not studied and there was referral bias in patient selection (14).

Conclusion

The present study showed that both H. pylori cagA and vacA genotypes were more prevalent in the PUD group than in the NUD group in our sample of Iranian patients. We would suggest that other gastrointestinal diseases be tested for both genes using gene-specific primers among Iranians, because the characteristics of a host's immune response or environmental factors may also play important roles in the pathogenesis of these diseases. It seems that the assessment of both genotypes in PUD patients is a better way of selecting patients with dyspepsia for endoscopy and treatment.

Acknowledgments

We thank the Department of Immunology of Pardis Hemmat, Iran University of Medical Sciences for their ELISA assay. Many thanks to the Gastroenterology, Endoscopy, and Pathology Wards of Firoozgar Hospital. We also acknowledge the contribution of S. Pourazar Dizagi and Amir Hossien Fakher Yaseri. This work was supported by the Research Center of Iran University of Medical Sciences.

References

1. Varbanova M, Malfertheiner P. Bacterial load and degree of gastric mucosal inflammation in Helicobacter Pylori infection. Dig Dis 2011;29:592-9.

2. Feldman RA. Epidemiology observation and open questions about disease and infection caused by Helicobacter Pylori in: Actman M, Suerbaum S, eds. Helicobacter Pylori: Molecular and cellular biology. Wymondham, United Kingdom, Horison.

Scientific press. 2001; 29: 51.

3. Rowland M, Kumper D, Daly L, O´ conner P, Vaughan D, Drumm B. Low rates of Helicobacter Pylori reinfections in children. Gastroentrology 1999;117: 336-41.

4. Moayyedi P, Soo S, Deeks J, et al. Systemic review and economic evaluation of Helicobacter Pylori eradication treatment for non-ulcer dyspepsia. BMJ 2000; 321:689.

5. Atherton J.C. The pathogenesis of Helicobacter Pylori-induced gastro-duodenal disease.Annu Rev Pathol Mech Dis 2006;1:69-96.

6. Kwong M.F, Tiing L.A. Epidemiology of Helicobacter Pylori and gastric cancer in Asia. J Gastroentrol and Hepatol 2010;25:479-86.

7. Effrosini G.P, Dionyssios N. Sgouras, K.S, Papadakos K.P, Se´bastien B, et al. CagA and VacA Polymorphisms Are Associated with Distinct Pathological Features in Helicobacter pylori-Infected Adults with Peptic Ulcer and Non-Peptic Ulcer Disease J Clinic Microbiol 2010;48(6):2237-9.

8. Olberman P, Josenhans C, Moodley Y, Uhr M, Stamer C, Vacterin M, Suerbaum S, Achtman M, Linz B. A global over review of the genetic and functional diversity in the Helicobacter Pylori Cag Pathogenicity Island. PLoS Genetics 2010;6(8):1-17.

9. Xiang Z, Censini S, Bayeli PF, Telford JL, Figura N, Rappuoli R, et al. Analysis of expression of CagA and VacA virulence factors in 43 strians of Helicobacter pylori reveals that clinical isolates can be divided into two major types and that CagA is not necessary for expression of the vacuolating cytotoxin Infect Immun. 1995;63:94-8.

10. Tohru T, Shuji F, Keizo A,Takuro S, Mitsuo O, Yoshiro , Junko O. Analysis of the expression of CagA and VacA and vacuolating activity in 167 isolates from patients with either peptic ulcers or non-ulcer dyspepsia. Am j Gastroentrology 1998; 93:1030-34.

11. Figueroa G, Troncoso M, Toledo M.S, Faudez G, Acuna R. Prevalence of serum antibodies to Helicobacter Pylori VacA and CagA and gastric diseases in Chile. J Med Microbiol.2002;51300-3004.

12. Weel Jan F.L, Rene W M Van der H, Yvette G, Philip R, Monique F, Jacob D, et al. The interrelationship between cytotoxin-associated gene A, vacuolating cytotoxin, and Helicobacter Pylori-related diseases. J Infect. Dis.1996;173:1171-5.

13. Marchetti M, Arico B, Burroni D, Figura N, Rappuoli R, Ghiara P. Development of a mouse model of Helicobacter pylori infection that mimics human disease. Science 1995;267:1655-8.

14. Maeda S, Ogura K, Yoshida H, Kanai F, Ikenoue T, Kato N, et al. Major virulence factors,VacA and CagA, are commonly positive in Helicobacter Pylori isolates in Japan. Gut 1998;42:338-43.

15. Moayyedi P, Duffett S, Braunhottz D, Mason S, Richards ID, Dowell AC, et al. The Leed´s Dyspepsia Questioner (LDQ); a valid tool for measuring the presence and severity of dyspepsia. Alimen. Pharmacol. Ther. 1998;12:1257-62.

16. J.J Lu, Perng C.L, Shyu R.Y, Chen C.H, Lou Q, Chang S.K, Lee C.H. Comparison of five PCR methods for detection of Helicobacter pylori DNA in gastric tissue.J Clin Microbiol 1999;37:772-4.

17. Talebkhan Y, Mohammadi M, Mohagheghi M.A, Vaziri H.R, Eshagh Hosseni M, Mohajerani N, et al. Cag A gene and protein status among Iranian Helicobacter Pylori strains. Dig Dis Sci 2009; 4(9):634-8.

18. Salehi Z, Halimi Jelodar M, Rassa M, Ahaki M, Mollasalhi H, Mashayekhi F. Helicobacter Pylori CagA and peptic ulcer disease in Iran.Dig Dis Sci 2009;54:608-13.

19. Siavoshi F, Malekzadeh R, Daneshmand M, Ashktorab H. Helicobacter Pylori endemic and gastric disease. Dig Dis Sci 2005;50: 2075-80.

20. Nawfal R.H, Mohammadi M, Talebkhan Y, Dorghi M, Letley D.P, Muhammad M.K, et al. Differences in virulence marker between Helicobacter Pylori strains from Iraq and Iran: potential importance of regional differences in H. Pylori–associated disease. J Clin Microbiol.2008; 46(5): 1774-9.

21. Kamali-sarvestani E, Bazargani A, Masoudian M, Lankarani K, Taghavi A.R and Saberifiroozi M. Association of Helicobacter Pylori CagA and VacA genotypes and IL-8 gene polymeras with clinical outcome of infection in Iranian patients with gastrointestinal diseases. World J Gastroentrology 2006; 12:5205-10.

22. Baghaeia K, Shokerzadeh L, Jafari F, Dabiria H, Yamaokab Y, Bolfiona M, et al. Determination of Helicobacter Pylori virulence by analysis of the Cag Pathogenicity Island isolated from Iranian patients. Dig and Liver Dis.2009;41(9):534-8.

23. Jafari F, Shokerzadeh L, Dabiri H, Baghaei K, Yamaoka Y, Zojaji H, et al. VacA genotypes of Helicobacter Pylori in relation to CagA status and clinical outcomes in Iranian population. Jpn Infect Dis 2008;61:290-3.