Vacuolating Cytotoxin A (*vacA*) Gene in Peptic Ulcer Disease and Non-ulcer Dyspepsia

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ABSTRACT

***Background*:** Vaculating cytotoxic A (*vacA*) gene is one of the multiple *Helicobacter pylori* genotypes that produce a cytotoxin protein (VacA).This gene is a major cause of chronic peptic ulcers and gastric cancer. The aim of this study was to determine the correlation between *vacA* gene with peptic ulcer disease (PUD) and non-ulcer dyspepsia (NUD).

***Methods*:** This was a case control study of 130 patients, aged 16-64, with positive *H. pylori* in histological and Giemsa reports. The case and control groups included 65 PUD patients and 65 NUD patients, respectively. The presence of the *vacA* gene genotypes was determined using polymerase chain reaction (PCR) on biopsy samples, taken by endoscopy.

***Results*:** In the case group, gastric ulcer was detected in 41.5% (27) of the participants; of whom, 77.8% (21) were female; and duodenal ulcer was found in 58.5% (38) of the participants, of whom,42.1 % (16)were female. The control group (NUD) included 65 patients; of them,45% (29) were female and the average age was 36.4 ±10.8 years (18 to 60).The total frequency of the *vacA* gene was 53% (69/130),with 60% in the PUD and 46% in the NUD groups (Odds ratio:1.75,95% CI:1.42-2.12,P=0.25)..

***Conclusion*:** The *vacA* gene alone could not be a reliable diagnostic marker for discriminating peptic ulcer disease from non-ulcer dyspepsia in the Iranian population under study.

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Introduction

Helicobacter pylori is a gram-negative bacterium that colonizes the stomach of 50% of the world's population. The prevalence of *H. pylori* in the Iranian population is (90%) more than the developing countries (70%-86%). Most individuals harboring *H. pylori* remain asymptomatic. It is now clear that chronic infection with *H. pylori* is the major cause of peptic ulcer disease and an important factor in gastric adenocarcinoma development (1).

The *vacA* gene is polymorphic and contains the signal sequence *s* (*s*1or*s*2), a middle region *m* (*m*1or*m*2) and the more recently identified intermediate (*i*) and deletion (*d*) regions. The vaculating cytotoxin A (VacA) protein represents one of the major secreted virulence factors of *H. pylori*. The VacA protein is a potential candidate for the virulence of *H. pylori* as well as the cause of peptic ulcer disease. However, *vacA* gene positive strains have intact *cag* Pathogenicity Island (PAI), but the *vacA* gene responsibility in inducing peptic ulcer is still controversial (2-7). It was reported that *vacAs*1 is the most common allel in the Iranian population (1), and more prevalent in peptic ulcer disease than non-ulcer dyspepsia. However, some researchers did not find a significant association between *vacA* gene and peptic ulcer disease as a marker of etiologic factor (8-9). Therefore, we aimed to determine whether a correlation existed between *vacA* gene and peptic ulcer disease and whether the *vacA* gene alone could discriminate peptic ulcer disease from non-ulcer dyspepsia in our region.

This study was a case-control study. Based on the Leeds Medical School criteria (10), 130 patients, aged16 -69 were selected from the patients who referred to the Endoscopy Ward of Firoozgar Hospital with the chief complaint of dyspepsia of over two- month duration. All the patients provided informed consent and accepted to complete a standard questionnaire. An expert endoscopists performed esophagogastroduodenoscopy (EGD) for all the patients in the same center. From the participants, 130 patients with dyspepsia, with or without peptic ulcers were selected and included in the study. We based the eligibility of the patients on the results of the questionnaire and EGD. Those patients who had no history of proved ulcer, previous *H. pylori* eradication, cigarette smoking, malignancy, and other underlying diseases in EGD, and nor did they use proton pump inhibitors or antibiotics (least two months prior to endoscopy) were excluded. Four biopsy specimens were taken from the antrum and gastric body for the histological study and *H. pylori* detection. All the formalin-embedded specimens were fixed and stained with Hematoxylin and Eosin Stain (H&E), or Giemsa. An experienced pathologist evaluated the specimens. When at least five bacilli were found in each microscopic field, the patient was considered positive for *H. Pylori*.

The genomic DNA was extracted from the biopsy samples and stored at -20 °C using a DNA isolation kit for cells and tissues (Roche Applied Science Company) according to the manufacturer's instruction. Two primers were designed complementary to the sequence located within the conserved region of the gene primers: *vacA1* and *vacA2*. The primers sequences are: *vacA1, F:* 5´-CTG CTT GAA TGC GCC AAA C-3´and R: 5 -CAC AGC CAC TTT CAA TAA CGA-3´; *vacA2, F:* 5´-ATG GAA ATA CAA CAA ACA CAC-3´ and R: 5´-CGT CAA AAT AAT TCC AAG GG-3´ (11) were used. The amplified products were detected after agarose gel electrophoresis, and a 480 bp (base pair) band indicated the presence of the *H. pylori-vacA* in the specimen.

We analyzed data using SPSS software (Version18). Age was shown with age ± standard deviation. The effects of *vacA* gene positive on the risk of peptic ulcer were expressed as Odds ratios (ORs) with 95% confidence intervals (CIs) with reference of NUD subjects with *H. Pylori* infections. Proportions were compared by the

Mantel-Haenszel chi-squared test with Yates correction or Fisher exact probability test. A *P*-value of less than 0.05 was considered statistically significant.

Of the case group included 65 PUD patients; of whom, 37 (57%) were female, and their average age was 41.6 ±16.4 (16 to 64). The control (NUD) group included 65 patients; of whom 29 (45%) were female, and their average age was 36.4 ±10.8 years (18 to 60 years old), Table1. 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 (Odds ratio: 1.75, 95% CI: 1.42-2.12, P = 0.25), Table 2.

Table 1. Demographic information of 130 patients with ulcer and non-ulcer dyspepsia.

Findings	Number of Pateints (%)		Odds Ratio (95 % CI)	P-Value
	Peptic ulcer disease	Non-ulcer dyspepsia (%)		
Number of patients	65 (100)	65 (100)	-	-
Sex				
Female	37 (57)	29 (45%)	-	-
Male	28 (43%)	36 (55%)	-	-
Age (years)				
Mean±SD	41.6 ±16.4	36.4 ± 10.8	-	-
Range	16 to 64	18 to 60		
Ulcer				
Gastric	27 (41.5%)	-	-	-
Duodenal	38 (58.5%)	-	-	-
Genotypes				
cagA gene positive	27 (41.5)	16 (24.6)	4.25 (1.2513.06)	0.009
vacA gene positive	39 (60)	30 (46)	1.75 (1.42-2.12)	0.25

Our results revealed that 60% of the patients in the case (peptic ulcer disease) group had *vacA* gene and that this rate was lower than that (46%) in the control (non-ulcer dyspepsia) group. Although patients receiving acid-inhibiting medications or non-steroidal anti-inflammatory drugs, and smokers were excluded from the study, the results were similar to those reported by previous studies (9). Therefore, *vacA* gene alone could not be a reliable diagnostic marker for discriminating peptic ulcer disease from non-ulcer dyspepsia in the Iranian population. The effects of *vacA* allelic combination of *H. pylori* on clinical prognosis show geographic differences in Western and Eastern populations. The VacA protein represents one of the major secreted virulence factors of *H. pylori.* The toxin has two domains, p33 and p55, are cleaved after secretion from the bacteria. The p33 domain is responsible for pore formation rather than enzymatic activity, and the p55 domain is responsible for interaction with the host cell membrane. The VacA protein is capable of internalizing and inducing the formation of intracellular vacuoles due to the osmotic swelling of late endocytic compartments. A large variety of additional cytotoxic functions has been attributed to VacA in the recent years such as altering the endosomal function, inhibiting T-cell proliferation, internalizing and damaging mitochondria, and inducing apoptosis both in epithelial and immune cells. The *vacA* gene is present nearly in all *H. pylori* strains, including both toxin positive and negative isolates,but only 50% of strains induce toxic vacuolization in certain cell lines (6). Depending on the *vacA* gene,strains were further classified into three categories of high vacuolation (*s*1/*i*1/*m*1), low vacuolation (*s*1/i1/*m*2), and non-vacuolation (*s*2/*i*2/*m*2, *s*1/*i*2/*m*1, *s*1/*i*2/*m*2, and *s*2/*i*1/*m*2). The *cagA* gene contributes to the development of peptic ulcer disease, while the presence of the *vacA gene* is a risk factor for increased activity and gastritis severity (5).Based on the presence or absence of the cytotoxin-associated gene-pathogenicity island (*cag*PAI) and the vaculating cytotoxin (VacA), *H. pylori* strains were categorized as *type I* or *typeII*, respectively. The correlation between *cagA* gene and VacA protein expression is not yet clear. The researchers have shown functional antagonism between *vacA* and *cagA* gene in host cells in the processes of nuclear factor of activated T-cells (NFAT) and (2) epidermal growth factor (EGF) receptor signaling (2-4) that could cause morphological changes. Patients with peptic ulcer disease show an increase in Th1 and Th2 cellular responses. In contrast, the role of T-regulatory (T*reg*) cells appears as a protective factor against epithelial cell injury. T*reg* cells secrete IL10 is inversely correlated with IL-8 and NF-κB (nuclear factor kappa B) in

asymptomatic carriers (12). Moreover, the *cagA* gene inactivation has no effect on the expression of VacA or on the ability to induce IL-8. Robinson and colleagues reported that *H. pylori*-induced peptic ulcer disease is associated with inadequate regulatory T cell responses (3). Furthermore, Oertli et al. showed *gamma*-glutamyl transpeptidase and *vacA* gene (two *H. pylori* antigens) induced T*reg* cells in the gastric mucosa of mice (7). We previously argued that both *cagA* and *vacA* genotypes were more prevalent in the PUD patients than in their NUD counterparts among our Iranian samples (13). This study had some limitations. First, it did not assess factors such as the study population's socioeconomic status, diet, and immune system. In addition, *vacA* alleles and VacA protein of *H. pylori* were not studied and referral bias may have existed.

Conclusion

Our study revealed that the *vacA gene* assessment alone may not be sufficient to understand the virulence potential of a strain and an etiological factor of peptic ulcer disease. Therefore, determining multiple virulence factors, as *cagA* gene, requires understanding strain dependent disease contributions. However, this hypothesis should be studied in a large number of patients with dyspepsia, and *vacA* alleles and VacA protein should be assessed.

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Conflict of interests

The authors declare no conflicts of interest.

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