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Original Article

Prevalence of *H. pylori* in gastric biopsy specimen in the southeastern region of Turkey

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Abstract

Introduction: *Helicobacter pylori* is a Gram-negative, microaerophilic bacterium that colonizes human gastric mucosa. Gastric ulcer, duodenal ulcer, chronic atrophic gastritis, mucosa-associated lymphoid tissue lymphoma, and stomach adenocarcinoma are associated with *H. pylori* as the etiological agent. Cytotoxin-associated gene A (*cagA*), which is one of the most important virulence factors of *H. pylori*, encodes a 120–145 kDa protein. The prevalence of *cagA* genes shows differences in *H. pylori* infections based on geographical area, and *cagA*-positive *H. pylori* strains play an important role in pathogenesis of gastric carcinoma.

Methodology: The aim of this study was to detect the prevalence of *cagA* and *vacA* genes in *H. pylori* isolates in adult patient groups in the southeastern region of Turkey. The presence of *H. pylori* was investigated in gastric biopsy specimens using the culture method, and polymerase chain reaction (PCR) analysis was performed to detect the presence of the *cagA* and *vacA* s1 genes.

Results: *H. pylori* was detected in 65% (84/129) of patients who had gastrointestinal complaints. The number of *vacA* s1 and *cagA* genes of isolates were 44 (74.5%) and 31 (52.5%), respectively.

Conclusions: *H. pylori* infection in southeastern region of Turkey with are comparable to those in developed countries. Patients with *cagA*- and *vacA*-positive *H. pylori* have a higher risk of severe inflammation and atrophy and should therefore be monitored for the development of gastric cancer.

Key words: *Helicobacter pylori*; *cagA*; *vacA* s1; polymerase chain reaction.

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Introduction

In many parts of the world, most gastric ulcer, duodenal ulcer, and chronic gastritis patients are infected with *H. pylori*. The organism plays a major etiological role in the development of gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and distal gastric cancer [1,2]. It has been reported that 50% of adults in developed countries and 80%–90% of the population in developing countries are infected with *H. pylori* [3,4]. Along with the antigen test on stools, urea breath test and endoscopic and histopathological examination are considered the gold standards for diagnosis [5].

There are different virulence factors affecting changes that may occur in the gastric mucosa of patients with *H. pylori*. Vacuolating cytotoxin A (vacA), cytotoxin-associated gene A (cagA) protein, and urease enzyme are the most important virulence factors of *H. pylori*. Recent studies have focused on investigating the relationship between virulence factors and clinical condition [6-8]. CagA protein is present 60%–80% in *H. pylori* strains and is generally accompanied by VacA [9].

The *H. pylori* status of the population of western and central Turkey is largely known, but the data from southeastern Turkey is not clear, so this study was planned and conducted to investigate the prevalence of *H. pylori* in urban and rural populations in the southeastern region of Turkey. The relationship between *H. pylori* genotypes and clinical outcome was evaluated by investigating the presence of *cagA* and *vacA* s1 genes in *H. pylori* strains isolated from patients with chronic gastritis in Diyarbakır Research and Training Hospital.

Methodology

Patients and sampling

A total of 129 gastric dyspeptic patients (64 male, 65 female) who were admitted to Diyarbakir Training and Research Hospital's Department of

Gastroenterology from December 2010 to July 2011, with ages ranging from 28–75 years, were included in this study. Gastric biopsy specimens were collected from the antrum and corpus of the stomach. No subjects had received treatment for *H. pylori* infection. The local ethics committee approved the protocol of genotype research.

H. pylori cultivation and identification

After biopsies were taken, samples were collected in *Brucella* broth medium (Becton Dickinson, New Jersey, USA) with 20% glycerol. All homogenized gastric biopsy specimens were inoculated onto *H. pylori*-selective agar medium (bioMérieux, Marcy l'Etoile, France). Inoculated plates were incubated at 37°C in an atmosphere of 5% O_2, 10% CO_2, and 85% N_2 for 5–7 days. Bacterial colonies were identified as *H. pylori* on the basis of colonial morphology, positive urease, catalase and oxidase tests, and Gram stain. All isolated *H. pylori* strains were stored at -80°C in *Brucella* broth medium with 20% glycerol.

Genomic DNA extraction

At the time of DNA extraction, genomic DNA was obtained in all isolates using a bacterial genomic DNA extraction kit (NanoHelix, Daejeon, South Korea). A 399 bp fragment of the 16S rRNA gene from the culture was amplified by polymerase chain reaction (PCR) using the *Helicobacter* genus-specific primers HeliF (AAC GAT GAA GCT TCT AGC TTG CTA G) and HeliR (GTG CTT ATT CST NAG ATA CCG TCA T) [10]. The presence of *H. pylori* was confirmed by culture and PCR.

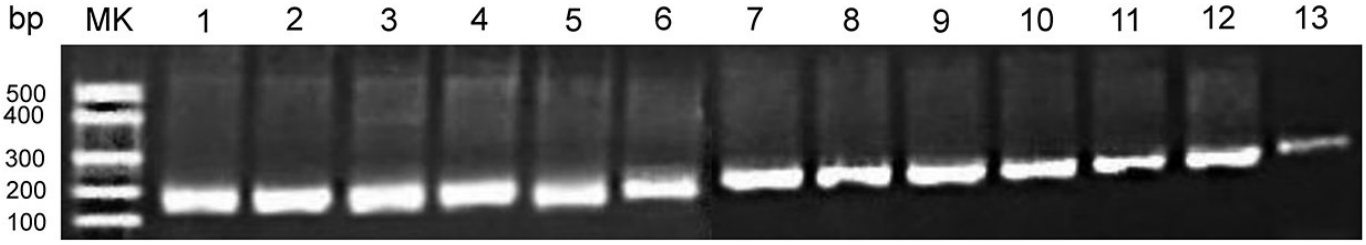
Analysis of cagA and vacA s1 status in H. pylori

PCR analysis was performed on *H. pylori* DNA samples to detect the presence of the *cagA* and *vacA* s1 genes. For detection of the *cagA* gene, primers CAGAF and CAGAR, which yield a fragment of 349 bp from the middle conservative region of the *cagA* gene, were used. For analysis of the *vacA* s1 region, primers VA1-F and VA1-R were used. Primers VA1-F and VA1-R yielded a fragment of 259 bp for s1 variants. The following protocol was used to amplify the genes: initial denaturation for 2 minutes at 95°C for 1 cycles; 20 seconds at 95°C for 30 cycles, 40 seconds at 52°C for 40 cycles, and 1 minute at 72°C; and a final elongation for 5 minutes at 72°C. After PCR, the amplified PCR products were electrophoresed in 1% agarose gels and examined under UV illumination (Figure 1). Specific primers were used to detect *cagA* and *vacA* s1 genes in all isolates (Table 1).

Results

A total of 84 (65%) *H. pylori* strains were isolated by culture examination of biopsy specimens from 129 patients (64 male, 65 female). These patients had complaints about acute or chronic gastritis. Genotyping was performed on 59 *H. pylori* strains isolated from 33 male and 26 female patients.

Figure 1. The s region was identified by amplification with primers VA1-F and VA1-R. The *vacA* s1 region produced a 259-bp amplicon and *cagA* region produced a 349-bp amplicon.



Lane Marker: 100-bp ladder; Lane 1,2,3,4,5,6: *H. pylori VacA* s1 band (259-bp) from patients; Lane 7,8,9,10,11,12,13: *H. pylori CagA* band (349-bp) from patients.

Table 1. Polymerase chain reaction (PCR) primers for amplification of *cagA* and *vacA* sequences.

Gene and DNA region amplified	Primer	Primer sequence (5'→3')	Size (bp) of PCR product (location)
cagA	CAGAF	GATAACAGGCAAGCTTTTGAGG	349 (1228–1576)[a]
	CAGAR	CTGCAAAAGATTGTTTGGCAGA	
vacA s1	VA1-F	ATGGAAATACAACAAACACAC	259 (797–1055)[b]
	VA1-R	CTGCTTGAATGCGCCAAAC	

[a]Nucleotide positions in the *cagA* gene of *H. pylori* ATCC 53726 (GenBank accession no. L117714); [b]Nucleotide positions in the *vacA* gene of *H. pylori* 60190 (GenBank accession no. U05676).

The *vacA* s1 gene was detected in 44/59 (74.5%) and *cagA* in 31/59 (52.5%) of the isolates. Both genes were found to be positive in 25/59 patients.

In patients with gastritis disease, 23/52 (44%) were positive for both *cagA* and *vacA* s1 type. Of the peptic ulcer (PU) disease strains, 3/7 were *cagA* positive (70%), and 6/7 strains were *vacA* s1 positive (Table 2).

Table 2. Association of *cagA* and *vacA* s1 gene positivity with gastric disease.

	Gastritis n (%)	Peptic ulcer n (%)	Total n (%)
cagA + strains	28/52 (54%)	3/7	31/59 (52.5%)
vacA s1 + strains	38/52 (73%)	6/7	44/59 (74.5%)

Discussion

Mucosal damage in stomach mucosa occurs in patients with *H. pylori*. *VacA* encodes a vacuolating toxin that is released by *H. pylori* and injures epithelial cells. VacA protein stimulates vacuolization in epithelial cells under *in vitro* conditions. These mechanisms have importance in the pathogenesis of stomach cancer.

With activation in low pH, VacA protein plays a role in connecting to epithelial cells. Recent studies have provided new insights into how VacA action at mitochondria might be functionally associated with cell death. VacA was demonstrated to disrupt mitochondrial dynamics [11-13]. Additionally, there was a significant correlation between *vacA* s1 type and enhanced chronic inflammation. The *vacA* s1 positivity rate was 74.5% in all patients.

When CagA protein secreted by *H. pylori* is injected into a host cell, toxic change is stimulated. CagA can disrupt signaling pathways by phosphorylation-dependent and -independent mechanisms, leading to abnormal proliferation, motility, and cytoskeletal change in gastric epithelial cells [14-16].

Strains that contain the *cagA* gene may lead to more severe clinic cases by stimulating cell transformation [16]. According to recent studies, there is an EPIYA-C containing region in *H. pylori* isolates that have *cagA* genes [17]. The EPIYA-C motif has a dual function in membrane association and tyrosine phosphorylation. It has been suggested that the EPIYA-C motif is a crucial therapeutic target of *cagA*-positive *H. pylori* infection [18].

Karrlson *et al*. studied *H. pylori* genotype in 155 gastric biopsy specimens. Their findings showed that two or more *cagA* EPIYA-C motifs are related to atrophy in gastric mucosa [19].

The course of *H. pylori* infection is almost certainly determined by a combination of host, bacterial and environmental factors, such as immune response, bacteria's virulence factors and the stomach acidic enviroment [20]. According to World Health Organization (WHO) statistics, risk for cancer development is doubled in persons infected with *H. pylori*. Additionally, *H. pylori* is reported as a class 1 carcinogen in gastric adenocarcinoma [21]. Risk for gastric cancer development is 2% among *H. pylori*-infected people [22].

The prevalence of *cagA*-positive *H. pylori* strains may vary depending on geography and patients' ages. Data from a study conducted by Gunn *et al*. [23] indicated that 91/120 (76%) isolates were positive for *H. pylori* strains by culture in England in 1998. Many other studies have also found that *cagA* is mostly accompanied by *vacA* genotype s1. Our findings are consistent with these results. Researchers in many studies have shown that the same factors (*cagA* and s1, i1 and m1 *vacA*) are associated with gastric cancer and precancerous intestinal metaplasia [24-28].

Also, Plummer *et al*. [29] researched the existence of the *cagA* gene in precancerous gastric lesions and found that the prevalence of *cagA* was 86% in the group with more severe lesions and 59% in the group with less severe lesions, based on 2,145 biopsy specimens in Venezuela.

Preneoplastic lesions were tracked by periodically conducting biopsies on 312 patients in Spain between 1988 and 2007. When results were evaluated, *H. pylori* strains harboring *cagA*, *vacA* s1, and *vacA* m1 genotypes were found more frequently in patients with more advanced gastric preneoplastic lesions [30].

H. pylori strains positive for the *cagA* gene (242/286; 85%) were detected in Alaskan patients between 1998 and 2005 [31]. Of these patients, 220 were native and 66 were non-native. The infection ratio of the isolates that had the *cagA* gene was 198/220 (90%) in natives and 44/66 (67%) in non-natives ($p < 0.0001$), and indicates geographical and ethnic differences. Researchers have reported that these results are close to those obtained from Europe and North America.

In an Italian population, *vacA* s1, i1, and m1 strains had all been reported to be significantly associated with gastric cancer (GC) [33]. Rhead *et al*. [32] reported similar findings in Iran. Basso *et al*. [33] studied the *vacA* signal, mid and intermediate region polymorphisms, *cagA* presence, and EPIYA-C segment number by PCR in 203 *H. pylori*-infected subjects (53 gastric cancer, 52 peptic ulcer [PU], and 98 gastritis) in

Italy. According to their results, *cagA*-positive strains were significantly associated with GC and PU. GC risk was further associated with the number of *cagA* EPIYA-C segments. An increasing number of EPIYA-C segments also increased the risk of intestinal metaplasia. In this study's findings, type s1 and i1 *vacA* alleles were also associated with GC and type i1 *vacA* with PUs and duodenal ulcers.

The "Asian paradox" that has been described by researchers might be explained by the widespread prevalence of weakly cytotoxic strains and correspondingly low frequency of *H. pylori*-associated diseases. However, here, the prevalence of strains with the *vacA* s1 region among Southeast Asia populations was very high in East Asian and Latin American populations, who have a higher risk of gastric carcinogenesis [34-36].

In the South Korean population (n = 225), the majority of *H. pylori* strains carry the *vacA* s1/i1/m1 allele and the *cagA* EPIYA-ABD allele. These facts may contribute to the high incidence of gastric maladies, including gastric cancer. Indeed, the finding that the majority of South Korean *H. pylori* strains carry the most toxic forms of *cagA* and *vacA* may explain the reason for the high prevalence of gastric disease and mortality among patients with gastric cancer in South Korea [37].

A total of 14 studies with a combined 1,281 patients (4 reports from Vietnam, 4 from Thailand, 5 from Malaysia, and 1 from Singapore) were included in the systematic analyses [38]. Of the 13 included studies, 93% of *H. pylori* strains were *cagA* positive. This genotype positivity rate was higher than the one found in our study. Both Western- and East Asian-type strains of *H. pylori* are found in Southeast Asia and are predominantly *cagA*-positive and *vacA* s1 type. However, the frequency of *H. pylori* infection in Turkey is lower than in the Asia region. In Southeast Asia, patients infected with *vacA* m1 type or *cagA*-positive strains have an increased risk of peptic ulcer disease. This *vacA* s1 genotype was highly prevalent in our patients who had peptic ulcers. The presence of *cagA* may be useful in prognosis.

In a study that included 450 patients with gastric symptoms, *H. pylori* was detected in biopsy specimens of 201 (45%) patients via PCR [39]. While 32% of patients had normal gastric mucosa, 68% had different gastroduodenal lesions. In this study, the prevalence of *cagA* gene was 52% (104/201), in which 97 were Western types and 3 were East Asian. The s1m1 genotype, which is a dominant *vacA* type, was predominantly detected in *H. pylori*-positive cases. Researchers associated higher and lower rates in previous studies conducted in different regions of Pakistan with geography.

In *H. pylori* strains recently isolated from biopsies of 46 patients, 37 (80.4%) of the 46 *H. pylori* clinical isolates were positive for *cagA* [40]. Additionally, the *cagA* gene was mostly accompanied by *vacA* s1m1 genotype in these isolates.

In Kuwait, *cagA*, *vacA* s1, and *vacA* s2 genes were found in 52.5%, 44.4%, and 39.4% of the patients, respectively, while 10.1% were positive for both *vacA* s1 and *vacA* s2 [41]. The *cagA* gene was found in combination with *vacA* s1 (*cagA* + *vacA* s1) in 31.3% of the patients. Patients with the *vacA* s1 gene alone also had a significantly more moderate-marked degree of chronic inflammation, while those with *vacA* s2 had significantly less chronic inflammation.

The presence of the *vacA* s1 genotype of *H. pylori* is associated with more severe chronic inflammation and atrophic gastritis. Our findings are consistent with this knowledge.

The prevalence of *H. pylori* infection was 58.9% in the Dominican Republic [42]. The *H. pylori* infection rate in patients with peptic ulcers (82.6%) was significantly higher than in patients with gastritis (54.5%). In this study, *cagA*-positive/*vacA* s1m1 genotype was the most prevalent. Patients with *cagA*-positive *H. pylori* could be at higher risk of severe inflammation and atrophy.

In Hacettepe University, Turkey, *cagA* positivity was detected in 58.6% (116/198) of *H. pylori* isolates obtained from biopsies from adults (n = 107) and children (n = 91) in 1997–2003. The positivity ratio was 62% (66/107) in adults and 55% (50/91) in children [43]. These results are similar to those in our study. According to the results obtained from 29 adults by Karaman *et al*., *cagA* was found in 19 isolates (65.5%) [6].

Conclusions

This study analyzed the relationship between gastritis and *vacA* s1 and *cagA* positivity and demonstrates the prevalence of *cagA* and *vacA* s1 in clinical isolates of *H. pylori* in the southeastern region of Turkey.

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