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Prevalence of *Helicobacter pylori cagA* and *vacA* genotypes in a population from Northeastern Mexico with chronic gastritis and intestinal metaplasia

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Helicobacter pylori **is associated with the development of a variety of gastroduodenal diseases. In this study, we evaluated the prevalence of *H. pylori cagA* and *vacA* genotypes from a Northeastern Mexico population. DNA was extracted from 135 gastric biopsies from patients with gastric disease: 110 with chronic gastritis (CG) and 25 with intestinal metaplasia (IM). Polymerase Chain Reaction (PCR) was used to detect *cagA* and *vacA* (*s1, s2, m1, m2*) genes of *H. pylori*. The study showed that the dominant genotypes were *cagA vacA s1/m1* 43 (31.8%), followed by *cagA vacA s2/m2* 18 (13.3%) and *cagA vacA s1/m2* 3 (2.2%). This study did not find any presence of the genotype *vacA s2/m1*. For the genotype *cagA vacA s1/m1*, a significant association was found between its presence in patients with IM compared with patient with CG ($P < 0.05$). In conclusion, the results show that the predominant genotype in the Northeastern Mexico population was *cagA vacA s1/m1*, which was found to be significantly associated with patients with IM.**

Key words: Gastric biopsies; *Helicobacter pylori*; *cagA; vacA*; Chronic Gastritis; Intestinal Metaplasia.

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INTRODUCTION

Helicobacter pylori, a microaerophilic Gram-negative bacterium that infects more than 50% of the world's population, is considered to be the main causative agent for most gastric disease (Van Doorn et al., 1998; Ahuja and Sharma, 2002; Atherton, 2006). It is recognized that intestinal metaplasia (IM) is an important risk factor for gastric cancer, accounting for more advanced stage of chronic gastritis (CG) (Wyatt et al., 1991). Currently there is this believed to be a strong association between the presence of *H. pylori* and IM, as precursor agents of gastric cancer.

The genetic characteristics of *H. pylori* and regional geographic conditions determine the type of strains that infect the population (Yamaoka et al., 2008). Therefore, it is of great interest to conduct molecular studies of the prevalent strains in each region and to determine the relationship to the severity of the lesions that they cause. Among the virulence factors described in *H. pylori* are the cytotoxin-associated gene A (*CagA*) encoded by the *cagA* gene and the vacuolating cytotoxin *VacA* encoded by the *vacA* gene, which have been related to the pathogenicity of the bacterium (Van Doorn et al., 1998; Atherton et al., 2003). The aim of this study was to determine the prevalence of *cagA* and *vacA* genes (*s1, s2, m1, m2*) from gastric biopsy samples from patients with gastric disease in Durango, Mexico, a region with a high incidence of these diseases (National Epidemiological Vigilance System, 2010). In addition, we investigated the correlation between the presence of these genes and the severity of lesions presented by the patients to determine whether these genes could be associated with pathogenic strains causing pre-cancerous lesions in this population.

MATERIALS AND METHODS

Subjects and clinical samples

Approval of the study was granted by the ethics committee of the IMSS Regional General Hospital Zone No. 1 from Durango. The 135 patients included in this study were diagnosed by histology as *H. pylori* positive (74 women and 61 men) with an average age of 57.15 ± 15.31 years (ranging from 14 to 85). These patients were referred to the Department of Gastroenterology at the Regional General Hospital Zone 1 of the Mexican Social Security Institute (IMSS) in the city of Durango, Mexico. During each endoscopic procedure, two antral and two corpus mucosal biopsy specimens were obtained from each patient for histological evaluation. The samples were classified according to the Sydney System (Dixon et al., 1996). Three biopsy specimens, two from the antrum and one from the corpus were examined by PCR for the presence of the genes *cagA* and *vacA* (*s1, s2, m1, m2*).

DNA extraction and genotyping

Genomic DNA was extracted using the NucleoSpin® Tissue kit (Macherey-Nagel, Düren, Germany) according to the protocol provided by the supplier. In all cases, PCR amplification was carried out in a 25 µl reaction mixture using: 1X PCR buffer, 0.2 mM dNTP mix, 0.5 mM of sense and antisense oligonucleotides, 1.5 mM $MgCl_2$, 2.5 U of Taq DNA polymerase and 50 to 100 ng of genomic DNA. PCR conditions for amplification of the *cagA* and *vacA* genes (and their *m* and *s* allelic regions) were as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 7 min. The oligonucleotides used are shown in Table 1. Oligonucleotides for the *H. pylori ureA* gene were used as controls for the integrity and specificity of the DNA. In each experiment, both positive and negative controls, with DNA from *H. pylori* ATCC J99 and without template DNA, were included.

Statistical analysis

Differences in gene frequencies between CG and IM patients were analyzed using the X^2 test. Values of $P < 0.05$ were considered to be statistically significant.

RESULTS

In this study samples were analyzed from 135 gastric biopsies of which 110 (81.4%) were from patients with CG and 25 (18.5%) were from patients with IM. The *H. pylori*-specific *ureA* gene was observed in all patients (Figure 1). The *cagA* and *vacA* genes showed amplification bands of 180 bp and 678 bp respectively (Figure 2). Of the 135 patients, 67 (49.6%) were *cagA*-positive, 107 (79.2%) were *vacA*-positive, and 60 (44.4%) patients were both *cagA*- and *vacA*- positive (Table 2). For the alleles *vacA s1/s2* and *vacA m1/m2* amplification bands were observed at 259/286 bp and 567/642 bp respectively (Figure 3). The most common allelic combinations for the *vacA* gene were *vacA s1/m1* (54.0%: 73/135) and *vacA s2/m2* (28.1%: 31/135). Only 4 patients (2.9%) were detected with the genotype *vacA s1/m2*. In two patients both the *s1 and s2* genotypes were detected suggesting mixed infection with two strains. For the combination of the *cagA* and *vacA* genotype, the predominant genotype was *cagA vacA s1/m1* (31.8%: 43/135), followed by *cagA vacA s2/m2* (13.3%: 18/135) and *cagA vacA s1/m2* (2.2%: 3/135) (Table 2).

Of the patients with CG, 45.4% (50/110) were *cagA*-positive, while 68.0% (17/25) of the patients with IM were *cagA*- positive. The presence of the *cagA* gene was higher in patients with IM than in those with CG, and this difference was statistically significant ($P < 0.05$). Of the patients with CG, 80.9% (89/110) were *vacA*- positive, while 72% (18/25) of patients with IM were *vacA*- positive (Table 3). The detection of the genotype *vacA s1/m1* in patients with IM (60%: 15/25) was not significantly different from patients with CG (52.7%: 58/110) ($P > 0.05$). The genotypes *vacA s2/m2* (25.5%: 28/110) and *vacA s1/m2* (2.7%: 3/110) had a higher prevalence in patients with CG than in those with IM, but this difference was not significant (Table 3).

The genotype *cagA vacA* s1/m1 was detected in 25.5% (28/110) and 60.0% (15/25) of the patients with CG and IM respectively, and this difference was statistically signi-

Table 1. Primers used for typing of *H. pylori ureA*, *cagA,* and *vacA* (*s* and *m*) genes of gastric biopsy samples.

Region	Oligonucleotide	Sequence (5'-3')	Size (bp)	Reference
ureA	*ure*A-1.SE-F *ure*A-3M.SE-R	gcggctgaattgatgcaagaagg gctcgcaatgtctaagcgtttaccgaa	350	Ryberg et al., 2008
cagA	*CagA*-2.SE *CagA*-3.AS	gaaatttggggatcagcgttacc tcctgcaaaagattgtttgg*caga*	180	Ryberg et al., 2008
vacA	*vacA*-1.SE *vacA*-3.AS	caatcgtgtgggttctggagc gccgatatgcaaatgagccgc	678	Monstein and Jonasson et al., 2001
vacA s1/s2	VAI-sF VAI-sR	atggaaatacaacaaacacac ctgcttgaatgcgccaaac	*s1*-259/*s2*-286	Chattopadhyay et al., 2004
vacA m1/m2	VAG-mF VAG-mR	caatctgtccaatcaagcgag gcgtcaaaataattccaagg	*m1*-567/*m2*-642	Chattopadhyay et al., 2004

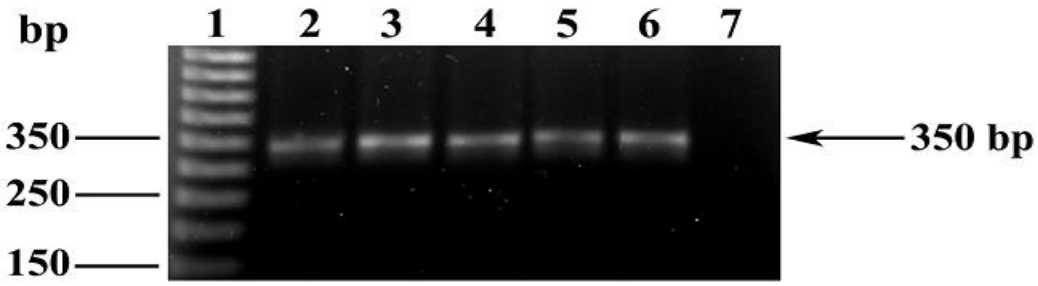

Figure 1. Identification of *H. pylori* from gastric biopsies samples by PCR. The image shows a representative electrophoresis gel from the amplification of the *ureA* gene (350 bp). Lane 1, MWM 50 bp ladder; lane 2, *H. pylori* reference strain ATCC J99; lanes 3-6, samples from biopsies; lane 7, negative control without template DNA.

ficant (P < 0.05). Meanwhile, the presence of the genotype *cagA vacA s2/m2* was detected in 13.6% (15/110) and 12% (3/110) of the patients with CG and IM respectively, but this difference was not significant. Only 1.8% (2/110) and 4% (1/25) of the patients with CG and IM respectively presented the genotype *cagA vacA s1/m2* (Table 3).

DISCUSSION

In this study 135 gastric biopsies were analyzed. The 49.6% prevalence of the *cagA* gene found in the population from Northeastern Mexico is in accord with other studies of Mexican, populations which report a prevalence of the *cagA* gene between 47.6 and 64% (Garza-Gonzalez et al., 2004; Torres et al., 2005; Paniagua et al., 2009). Studies of European and North American populations have reported a 60% prevalence of *cagA* (Ito et al., 1997), while in Asian countries the prevalence was over 90% for all isolates of *H. pylori* (Miehlke et al., 1996).

The 79.2% prevalence of the *vacA* gene differs from study report by Paniagua et al. (2009) which found 100% prevalence in clinical isolates of *H. pylori* obtained from patients with CG. For the allelic variants in the population studied, *vacA s1/m1* was the most prevalent (Table 2). These results differs from study report by Garza-González et al. (2004), who reported that *s2m2* strains were the most prevalent genotype in the north-eastern region of Mexico. In Europe and North America, the predominant genotype was *vacA s1*. For the *m* region the alleles *m1* and *m2* showed approximately the same prevalence in Europe and America, while *m2* was more dominant in Asian countries (Van Doorn et al., 1998).

The variant *vacA s2/m1* was not detected in this study.

The comparison among the *H. pylori* genotypes in the population studied showed that the dominant genotype was *cagA vacA s1/m1* (Table 2), which found detected in more patients with IM (60.0%) than with CG (25.5%) (P <

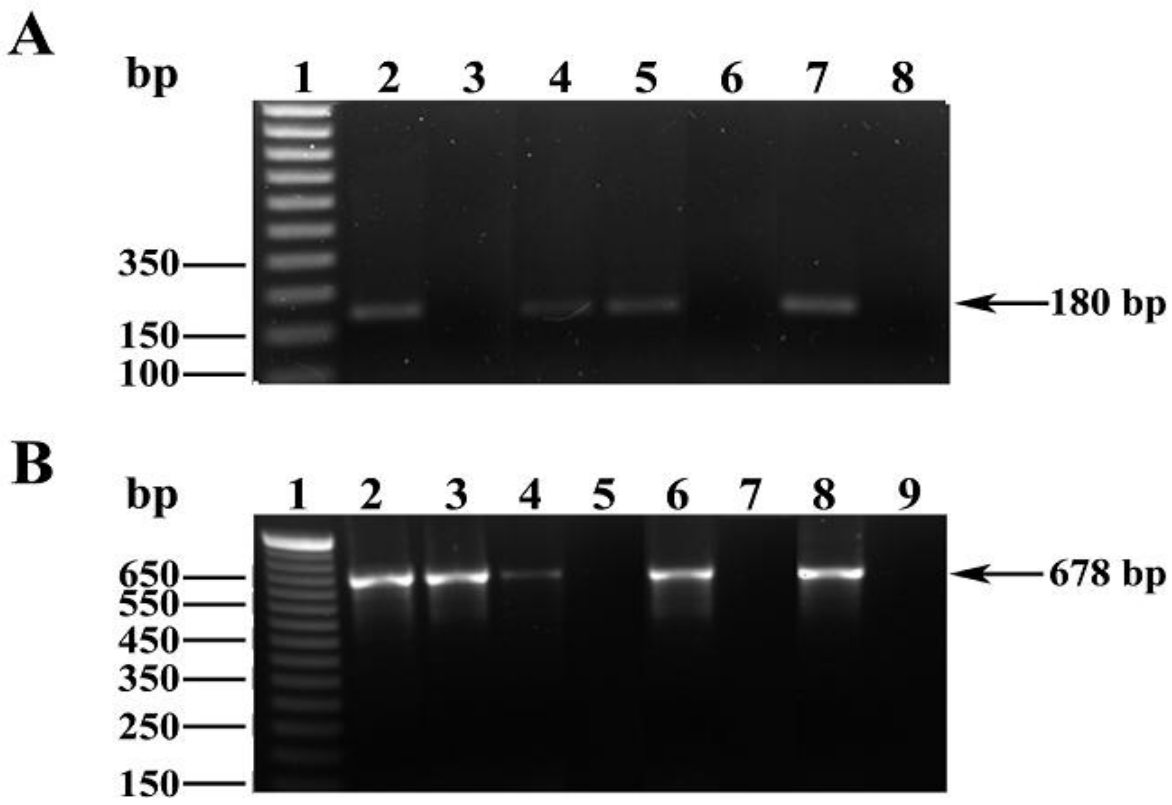

Figure 2. Genotyping of *H. pylori* from gastric biopsies samples by PCR. The image shows a representative electrophoresis gel from the amplification of the genes *cagA* (180 bp) and *vacA* (678 bp). A) *cagA*. Lane 1, MWM 50 bp-ladder; lane 2, *H. pylori* reference strain ATCC J99; lanes 3-7, samples from biopsies; lane 8, without template DNA. B) *vacA*. Lane 1, MWM 50 bp-ladder; lane 2, *H. pylori* reference strain ATCC J99; lanes 3-8, samples from biopsies; lane 9, negative control without template DNA.

Table 2. Prevalence of the genotypes of *cagA* and alleles of *vacA* (*s* and *m*) and the combination of both detected in samples from gastric biopsies from a Northeastern Mexico population.

Genotype	Prevalence (%)
cagA	67 (49.6%)
vacA	107 (79.2%)
s1/m1	73 (54.0%)
s2/m2	31 (28.1%)
s1/m2	4 (2.9%)
s2/m1	0 (0.0%)
cagA vacA	60 (44.4%)
cagA vacA s1/m1	43 (31.8%)
cagA vacA s2/m2	18 (13.3%)
cagA vacA s1/m2	3 (2.2%)
caga vacA s2/m1	0 (0.0%)

0.05) (Table 3). These results agree with those reported by González-Valencia et al. (2000) and Paniagua et al. (2009), who reported that *cagA vacA s1/m1* strains were the most prevalent genotype in Mexican patients. Studies in other populations in Latin American have found the *cagA vacA s1/m1* genotype is the most prevalent strain (Ortiz-Princz et al., 2010). Studies in Asian populations have found that the prevalent genotype was *cagA vacA s1/m2* (Miehlke et al., 1996; Ito et al., 1997). Several studies have found that the genotype *cagA vacA s1* are virulent strains correlating to their presence with ulcerogenic strains and associated to metaplasia and gastric adenocarcinoma (Zambon et al., 2003). In order to obtain more conclusive data, a study analyzing a larger population of patients with IM, CG, and other gastrointestinal disorders is needed to determine the association of *H. pylori* genotypes with pathogenic potential as well as their association with the severity of lesions. This type of study aims to determine which genotype could be used as a potential biomarker for pathogenic strains that causes pre-cancerous lesions in different regions.

In conclusion, this study detected the genotype *cagA vacA s1/m1* in 31.8% of the tested population. Furthermore, a statistically significant association was found between the presence of this genotype in patients with IM (60.0%) compared with patients with CG (25.5%) ($P < 0.05$).

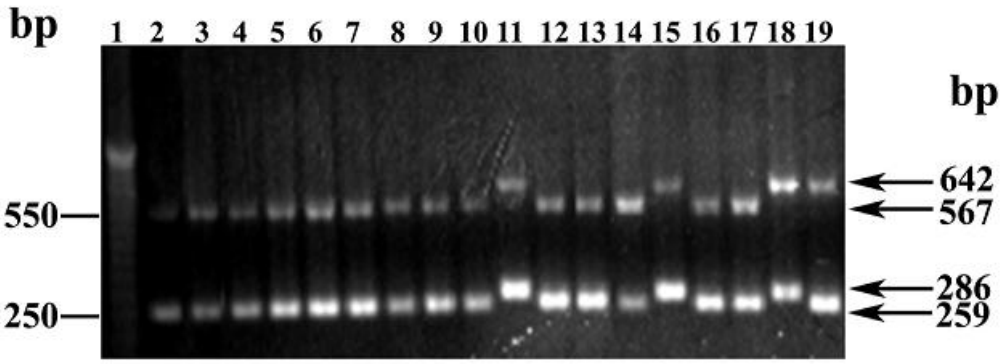

Figure 3. Genotyping of the different alleles of *vacA* (*s1/s2* and *m1/m2*) from gastric biopsy samples. The image shows a representative electrophoresis gel from the amplification of the genes *vacA s1/s2* (259/286 bp) and *vacA m1/m2* (567/642 bp) by PCR. Lane 1, MWM 50 bp-ladder; lane 2, *H. pylori* reference strain ATCC J99 (*vacA s1/m1*); lanes 3-10, 12-14, 16 and 17, gastric biopsy sample (*vacA s1/m1)*; lanes 11, 15, 18, gastric biopsy sample (*vacA s2/m2)*; lane 19, gastric biopsy sample *vacA (s1/m2)*.

Table 3. Correlation between the genotypes of *cagA* and alleles of *vacA* (*s* and *m*) from a Northeastern Mexico population.

Genotype	Pathologies		Total	P-*Value*
	CG* n = 110 (81.4%)	IM* n = 25 (18.5%)		
cagA	50 (45.4%)	17 (68.0%)	67	**0.042**
vacA	89 (80.9%)	18 (72.0%)	107	0.321
vacA s1/m1	58 (52.7%)	15 (60.0%)	66	0.510
vacA s2/m2	28 (25.5%)	3 (12.0%)	29	0.149
vacA s1/m2	3 (2.7%)	1 (4.0%)	4	0.735
vacA s2/m1	0 (0.0%)	0 (0.0%)	0	
cagA vacA				
cagA vacA s1/m1	28 (25.5%)	15 (60.0%)	43	**0.001**
cagA vacA s2/m2	15 (13.6%)	3 (12.0%)	18	0.828
cagA vacA s1/m2	2 (1.8%)	1 (4.0%)	3	0.504
cagA vacA s2/m1	0 (0.0%)	0 (0.0%)	0	

CG* Chronic gastritis, IM* intestinal metaplasia.

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