Helicobacter pylori Infection in Rural and Urban Dyspeptic Patients from Venezuela

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Abstract. The goal of this work was to assess the *Helicobacter pylori* prevalence in a rural mestizo population and compare it to an urban population from Venezuela. The study was performed in gastric juice samples of 71 dyspeptic patients from Caracas (urban) and 39 from Tucupita (rural), in the Orinoco Delta region. *Helicobacter pylori* was detected by amplification of 16S rRNA, *glmM*, and *ureA* genes in 55.0% patients from urban and 87.2% from rural populations. *cagA* was found positive in 51% and 62% urban and rural patients, respectively. Non-*H. pylori Helicobacter* species were not detected in the urban population, but was found in 7.7% of patients in the rural study site. Frequency values of the 16S rRNA, *glmM*, and *ureA* genes were higher in the rural population. The odds ratio for each gene was 15.18 for 16S rRNA, 2.34 for *glmM*, 2.89 for *ureA*, and 1.53 *cagA*, showing significant differences except for *cagA* when gene frequency was compared in both populations. These results demonstrate a higher frequency of *H. pylori* and gastric non-*H. pylori Helicobacter* infection in a rural mestizo population with low hygienic standards as compared with city dwellers, representing a potential risk for the development of gastroduodenal diseases.

Helicobacter pylori is a pathogen that colonizes the human gastric mucosa and has been involved in severe gastroduodenal diseases such as peptic ulceration, gastric lymphoma, and gastric adenocarcinoma.¹ The development of disease is associated with particular bacterial virulence, host response, or environmental factors.² More than 50% of world's population carries *H. pylori*, with proportions as high as 90% in developing countries, with a decrease in the prevalence of the infection in some places such as northern European and North American countries.³ On the other hand, it has been confirmed in many populations studies that low-socioeconomic conditions and deficient hygiene practices are a risk for acquisition of the infection.⁴ In developed countries, perhaps due to higher living standards, infection in children has fallen dramatically to 8.9% whereas in developing countries it is 72.8%, being the reinfection rate significantly higher.⁵ In Venezuela, the prevalence of gastric infection by *H. pylori* has been reported ranging from 38% to 95% in the asymptomatic and symptomatic population in different localities, by using different detection methods.^{6–8} In our laboratory, we have validated the detection of *H. pylori* by polymerase chain reaction (PCR) in string-absorbed gastric juice compared with gastroesophageal biopsies obtained by endoscopy in an urban population from Caracas. These studies showed that the string-absorbed gastric juice test is as sensitive as detection in gastroesophageal biopsies taken by endoscopy, allowing an accurate diagnosis of *H. pylori* infection by a less invasive method,^{9–11} as was also validated in Lima, Perú by Velapatiño and others.¹²

The presence of *cagA* (cytotoxin-associated gene A) is considered to be a marker for the virulent *H. pylori* strains and the *cag* pathogenicity island (*cag*-PAI).^{2,13} The prevalence of *H. pylori* infection with strains carrying *cagA* varies among different populations in Venezuela. Previous studies of serological prevalence of *H. pylori* infection in Warao Amerindian

populations of the Orinoco Delta region showed values ranging from 65% to 84% for *H. pylori* whole cell and CagA antigens, and anti-*H. pylori* IgG antibodies.^{14,15} The aim of this study was to assess the prevalence of *H. pylori* infection and virulence-associated *cagA* gene in string-absorbed gastric juice samples in two groups of symptomatic mestizo populations. The study involved one group from Tucupita village in the Orinoco Delta region, carried out in November 2012 and another from the capital city of Caracas, carried out between July and November 2011. Patients consulting the Service of Internal Medicine from Materno Infantil Dr. Oswaldo Ismael Brito in Tucupita, and the Gastroenterology Unit of the Oncology Service Hospital of Instituto Venezolano de los Seguros Sociales (IVSS) in Caracas, with gastrointestinal symptoms of dyspepsia were recruited into this study; all volunteers signed an informed consent form and the Bioethical Committee of the Venezuelan Institute of Scientific Research approved the study. Patients who had previously received treatment were excluded. A total of 110 volunteers, 39 from Tucupita (age range = 21–62 years), and 71 from Caracas (age range = 17–70 years), were admitted in the study. Patients from Tucupita who attend this clinic come from areas with a low socioeconomic status and deficient hygienic conditions, representing a poor rural mestizo community. Patients from Caracas attending the IVSS Hospital had a higher socioeconomic level in terms of hygienic conditions, water supply and consumption. Gastric juice samples were collected using an Enterotest capsule (HDC Corp., Milpitas, CA). *Helicobacter* spp. and *H. pylori* infection in gastric juice was detected by PCR for all four 16S rRNA, *glmM*, *ureA*, and *cagA* genes, using genus- and species-specific primers. PCRs were performed using the “PCR Master Mix” kit (Promega Corp., Madison, WI), as described previously.¹¹ We considered *H. pylori* infection when at least three (16S rRNA, *glmM*, and *ureA*) of the four genes were present, and non-*H. pylori* helicobacters (NHPHs) infection when only 16S rRNA gene was present.¹⁶

Helicobacter pylori in the rural population was found positive in 87.2% (34/39) of patients, whereas it was found positive in 55.0% (39/71) of subjects living in the urban area. *Helicobacter pylori* infection in the rural mestizo population

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TABLE 1
Analysis data of PCR detection of *Helicobacter* and *Helicobacter pylori* in string-absorbed gastric juice

Population <i>n</i>	16S rRNA <i>n</i> (%)		OR	<i>glmM</i> <i>n</i> (%)		OR	<i>ureA</i> <i>n</i> (%)		OR	<i>H. pylori</i> pos <i>n</i>	<i>cagA</i> <i>n</i> (%)		OR
	Negative	Positive		Negative	Positive		Negative	Positive			Negative	Positive	
Urban 71	32 (45)	39 (55)	16S rRNA	34 (48)	37 (52)	<i>glmM</i>	33 (46)	38 (54)	<i>ureA</i>	39	19 (49)	20 (51)	<i>cagA</i>
Rural 39	2 (5)	37 (95)	15.18	11 (28)	28 (72)	2.34	9 (23)	30 (77)	2.89	34	13 (38)	21 (62)	1.53
	95% CI: 3.4, 67.9			95% CI: 1.1, 5.4			95% CI: 1.2, 7.0			95% CI: 0.6, 3.91			

CI = confidence interval; OR = odds ratio.

The OR, its standard error, and 95% CI are calculated according to Altman.¹⁷

was higher than that thus far reported in other urban localities from Venezuela, which ranged from 49% in Caracas to 76% in Mérida; however, it is in accordance with a report from urban Lima, Peru (91%), shantytown residents.^{9–12} The frequency of genes was 16S rRNA 55.0%, *glmM* 52.0%, and *ureA* 54.0% for urban population, while that in the rural group was 95.0%, 72.0%, and 77.0%, respectively (Table 1). The frequency of appearance of all three genes in string samples taken from Caracas patients was nearly identical, whereas these values were higher for rural patients from Tucupita.

To assess the risk of infection between the two populations, we calculated odds ratios (OR) for the three genes. The OR values for each gene, between both populations were 15.18 for 16S rRNA, 2.34 for *glmM*, and 2.89 for *ureA*, all being statistically significant (Table 1). These results indicate a higher risk of *H. pylori* infection in the rural mestizo population in relation to the urban one and are in accordance with other observations in Bhutan, Jordan, and eastern Sudan where the *H. pylori* infection in rural populations was higher than in urban ones.^{4,18,19} The frequency of *H. pylori* infection may be linked to multiple factors among which bacteria, host, and environmental conditions play a role.

The *cagA* gene, as a marker for *cag*-PAI in *H. pylori* was detected in 51.0% of urban patients and 62.0% of the rural ones (Table 1). These results are in accordance with a previous report of *cagA* prevalence in gastric juice of 57% subjects from Caracas,¹¹ and within the reported range (50–86%) found in gastric biopsies of different localities from Venezuela.^{20–22} The prevalence range (50–86%) of *cagA* detection in different regions demonstrate a dominance of circulating *cagA* positive *H. pylori* strains, and its presence is commonly associated with the development of severe gastroduodenal damage, including gastric diseases and cancer.^{23,24} By analyzing the *H. pylori* detection profile we found a significant OR value of 10.66, when positive profile of all genes was compared with negative profile between urban and rural population (Table 2). This indicates that the difference for *H. pylori* infection in the rural population is due at low number of negative patients in rela-

tion to urban. Regardless of the infections mode, sanitary conditions, or geographic origin of the strains, the high incidence of *H. pylori* prevalence in the rural region represents a risk for the development of gastric diseases and/or cancer.

An interesting finding in this study was the presence of gastric non-*H. pylori* *Helicobacter* species in 7.7% (3/39) of the patients of rural group, whereas species other than *H. pylori* were not found in patients from the urban population (Table 2). This may suggest that difference in socioeconomic conditions, such as lack of appropriate hygiene practices and extremely limited sanitary facilities, associated to life style could play a role.^{12,25} Gastric NHPH such as *Helicobacter heilmannii* and *Helicobacter felis* have been found in gastric biopsies of symptomatic patients ranging from 0.1% to 0.25% in countries with a high level of socioeconomic development (Italy, Japan, and Germany), whereas a higher prevalence has been found in rural areas or developing countries such as southern China (2%) and Thailand (6.2%).^{26–28} The gastric NHPH infection has been associated with chronic gastritis in humans and pets (dogs, cats, and pigs). On the other hand, enterohepatic NHPH, such as *Helicobacter cinaedi*, *Helicobacter bilis*, *Helicobacter canis*, *Helicobacter brantiae*, *Helicobacter hepaticus*, and *Helicobacter fennelliae*, have been reported to be associated with other human diseases (bacteremia, cellulitis, and cutaneous diseases).²⁹ A lower prevalence of gastric NHPH infection has been reported in countries with a high level of socioeconomic development, whereas higher prevalence of infection is found in rural areas or developing countries.²⁷ PCR detection in gastric juice represents an alternative for a secure diagnosis of *H. pylori* and non-*pylori* *Helicobacter* infection, especially when studies are made in rural communities, without the requirement of an endoscopy to obtain gastroesophageal samples. These results demonstrate a high frequency of *H. pylori* (87.2%) and gastric NHPH (7.7%) infection in the gastric juice of a rural mestizo population with low hygienic standards, suggesting a higher potential risk for the development of gastroduodenal diseases in this population.

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TABLE 2
Helicobacter pylori and NHPHs infection status by PCR in the gastric juice

			Urban (<i>N</i> = 71)		Rural (<i>N</i> = 39)		OR
16S rRNA	<i>glmM</i>	<i>UreA</i>	<i>n</i>	%	<i>n</i>	%	Urban vs. rural
Neg	Neg	Neg	32	45.07	2	5.13	–
Pos	Pos	Pos	36	50.7	24	61.53	All neg/all pos
Pos	Neg	Pos	2	2.81	6	15.38	10.66
Pos	Pos	Neg	1	1.4	4	10.25	95% CI: 3.98, 28.58
Pos	Neg	Neg	0	0	3	7.69	–

CI = confidence interval; Neg = negative; NHPHs = non-*H. pylori* helicobacters; OR = odds ratio; Pos = positive.

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