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ORIGINAL ARTICLE

Association of *cagA+ Helicobacter pylori* strains with high urease activity and dyspepsia in Mexican adults☆

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Abstract
Introduction and aims: *Helicobacter pylori* (*H. pylori*) is associated with a higher risk of peptic ulcer and gastric cancer. The sole presence of the bacterium is not a determinant of clinical outcome, but rather the interaction of strain type and host factors determines the risk of disease. Our aim was to study the association between bacterial load, strain type, and gastric symptoms in *H. pylori*-positive subjects.
Materials and methods: In a community survey, a diagnostic ^{13}C-urea breath test for *H. pylori* was performed on 302 volunteers that were not taking antibiotics, antacids, or proton pump inhibitors one month prior to the test. The breath test produced 25 *H. pylori*-positive subjects, between 25 and 74 years of age, who then took a gastric symptoms survey and were tested for the presence of the *cagA* genotype in gastric juice, using the Entero-test®. Bacterial load was determined as a measure of urease activity, utilizing the delta over baseline (DOB) value, obtained in the ^{13}C-urea breath test.
Results: A total of 48% of the *H. pylori*-positive subjects were *cagA+*. A positive association was found between *cagA* status and high gastric urease activity ($p < 0.0001$) and the latter was significantly associated with the presence of symptoms ($p < 0.0001$).
Conclusion: Gastric urease activity was strongly associated with dyspeptic symptoms and *cagA+ H. pylori*. Elevated ^{13}C-DOB values could be used as indicators of a higher risk for gastric disease.

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PALABRAS CLAVE
Prueba de la ureasa en aliento;
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Helicobacter pylori

Asociación de cepas de *Helicobater pylori cagA+* con alta actividad de ureasa y dispepsia en adultos mexicanos

Resumen
Introducción y objetivos: La *Helicobacter pylori (H. pylori)* está asociada con un mayor riesgo de úlcera péptica y cáncer gástrico. La presencia de la bacteria no es un factor determinante para el desenlace clínico, sino que la cepa y otros factores del huésped interactúan para determinar el riesgo a adquirir la enfermedad. El objetivo del presente estudio fue investigar la asociación entre la carga bacteriana, el tipo de cepa y los síntomas gástricos en personas con positividad a *H. pylori*.
Materiales y métodos: En una encuesta dirigida a la comunidad se contactaron 302 voluntarios que no estuvieran tomando antibióticos, antiácidos ni inhibidores de la bomba de protones un mes antes del diagnóstico de *H. pylori* utilizando la prueba de la ureasa en aliento. Se seleccionaron 25 sujetos con edades entre los 25 y los 74 años, positivos a *H. pylori*, para una encuesta de síntomas gástricos y determinar la presencia del genotipo *cagA* en el jugo gástrico obtenido con Entero-test®. La carga bacteriana se determinó como medida de la actividad de la ureasa utilizando el valor ^{13}C-delta sobre el valor basal obtenido en la prueba de aliento.
Resultados: El 48% de los sujetos positivos a *H. pylori* fueron *cagA+*. Se encontró una asociación positiva entre el estado de *cagA* y la alta actividad de la ureasa gástrica ($p < 0.0001$), que además resultó significativamente asociada con la presencia de síntomas ($p < 0.0001$).
Conclusión: La actividad de la ureasa gástrica está fuertemente asociada con los síntomas de dispepsia y la presencia de *H. pylori cagA+*. Los valores elevados de ^{13}C-delta sobre el valor basal pudieran ser usados como indicadores de mayor riesgo de enfermedad gástrica.

Introduction and aims

Helicobacter pylori (*H. pylori*) colonization is a well-recognized risk factor for the development of gastric disorders in the human stomach,[1–3] even though most infected persons will remain asymptomatic throughout their lives. The seroprevalence of *H. pylori* infection in Mexico was reported at 66% and age was the strongest risk factor for infection.[4] According to the fourth Mexican consensus on *H. pylori*,[1] the association between functional dyspepsia and *H. pylori* infection is controversial. Several studies have suggested that the genetic variability of *H. pylori* and the host,[5] as well as environmental factors, determine clinical outcome.[6] *H. pylori* is genetically diverse and type I strains, which are cytotoxin-associated gene A-positive (*cagA+*) and secrete the vacuolating cytotoxin A (*VacA*), are the most virulent.[7,8] They are associated with duodenal ulceration,[9,10] abdominal pain, bleeding, active gastritis,[11] symptoms of dyspepsia,[12,13] DNA damage in the gastric mucosa,[14] and gastric carcinoma.[15,16]

Atherton et al. found histologic evidence of higher *H. pylori* density in the gastric mucosa colonized by *cagA+* strains than in the epithelia colonized by *cagA−* strains.[17] Other authors have also found a significant relationship between the density of *H. pylori* colonization and the presence of *cagA+* and *vacAs1* strains,[18–20] suggesting the importance of bacterial load determination as a risk predictor of gastric disease. Perri et al. and Zagari et al. proposed the use of ^{13}C-delta over baseline (DOB) as a predictor of the intragastric bacterial load and severity of *H. pylori* gastritis in patients referred for endoscopy.[21,22] Matthews et al. also found that ^{13}C-DOB values were significantly higher in symptomatic subjects with moderate and severe antral gastritis, compared with those that had mild gastritis or no inflammation.[23] However, they found no correlation between *H. pylori* load, measured through bacterial culture and ^{13}C-DOB values.[23] In children with dyspeptic symptoms, Machado et al. also reported that ^{13}C-DOB does not estimate the severity of histologic measurements of bacterial colonization.[24] The lack of correlation may be due to the differing virulence of *H. pylori* strains, or to bacterial density estimates (through bacterial culture[22] and histologic estimation[24]), which are not indicative of actual mucosal *H. pylori* load.

The non-invasive ^{13}C-urea breath test (UBT) is the most sensitive and specific test for determining the presence of *H. pylori*.[1] It uses the ^{13}C-DOB value as the cut-off criterion, which is indicative of urease activity, and therefore, of bacterial load. Virulent *H. pylori* strain determination is made possible through complementary molecular or immunologic analyses. We hypothesized that a higher ^{13}C-DOB value, in addition to indicating bacterial load, would also indicate the presence of the *cagA+* genotype. Therefore, the primary aim of our study was to investigate the association between gastric urease activity (^{13}C-DOB), symptoms of dyspepsia, and *H. pylori cagA+*, in an open population. We also explored the association of sociodemographic variables with the presence of *H. pylori* and determined the variables that best explained the ^{13}C-DOB value.

Materials and methods

Study populations

In a community survey, 302 persons not taking antibiotics, antacids, or proton pump inhibitors for the past month, were recruited in Northern Mexico (Hermosillo, Sonora) to take a diagnostic urea breath test for *H. pylori* (within the time frame of June to November 2004). Twenty-five of those individuals were *H. pylori*-positive and their ages ranged from 25 to 74 years (mean age 34 years). They were randomly chosen to fill out a gastric symptoms survey and provide a gastric juice sample, using the Entero-test® (Enterotest HP, HDC Corporation, San Jose, CA, USA).

The subjects were clinically symptomatic, presenting with 2 or more symptoms of dyspeptic disease, such as epigastric pain, epigastric burning, postprandial fullness, early satiety, bloating, belching, nausea, and vomiting. The chronicity, variety, or intensity of symptoms were not considered in the symptom questionnaire.

Sociodemographic data

Educational level was evaluated, according to the following scale: 1, elementary school; 2, middle school; 3, high school; 4, semiprofessional; 5, professional; 6, postgraduate. Family income was expressed in monthly minimum wage units. Based on data in the literature, a score for the risk of presenting with *H. pylori* related to socioeconomic condition was calculated. Educational level, employment, family income, housing characteristics (construction material of the house, ceiling, and floor, presence and type of sewage disposal system, household drinking water), overcrowding (3 or more persons sharing one bedroom), the presence of domestic animals inside or outside the house, and animal breeding were all considered in the score. Characteristics associated with a high risk for presenting with *H. pylor*i were coded numerically (no risk: 0–2; moderate risk: 3–5; high risk: >5).

Detection of *Helicobacter pylori* status

H. pylori status was determined through the ^{13}C-UBT. Breath samples were collected before, 30 min after, and 45 min after the intake of 50 mg of ^{13}C-labeled urea, together with natural orange juice, to provide acidic gastric conditions. The ^{13}C-UBT test has 98% sensitivity. The urease secreted by *H. pylori* in the stomach hydrolyses urea to release $^{13}CO_2$ from the ingested labeled urea, which then enters into the body's bicarbonate pool and is excreted in breath. We measured the $^{13}CO_2/^{12}CO_2$ ratio through isotope ratio mass spectrometry (BreathMAT Plus, 1998, Finnigan MAT GMBH, Bremen, Germany) and expressed the results as intensity ratios $^{13}CO_2/^{12}CO_2$, 45/44). The ^{13}C-DOB values considered positive for *H. pylori* were those $\geq$3.5‰.

Identification of *cagA+ Helicobacter pylori* strains

Gastric juice samples were taken from *H. pylori*-positive subjects after an overnight fast, using a string test (Entero-test® pediatric test, Enterotest HP, HDC Corporation, San Jose, CA, USA). The test consisted of a 90 cm length of nylon fiber enclosed in a 2.5 cm long weighted gelatin capsule that dissolves in the stomach. The string remained in the stomach for 1 h, after which it was retrieved via the oral route and placed in 15 ml of sterile saline for DNA isolation.

The string was vigorously shaken in the saline and centrifuged for DNA extraction from the resulting pellet, using the QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA, USA). DNA was subject to PCR amplification of the *glmM* and *cagA* genes, using the following available primers: cagA-F: 5′-ATAATGCTAAATTAGACAACTTGAGCGA-3′; cagA-R: 5′-AGAAACAAAAGCAATACGATCATTC-3′, product size: 120 bp[25]; glmM-F: 5′-GGATAAGCTTTTAGGGGTGTTAGGGG-3′; glmM-R: 5′-GCTTACTTTCTAACACTAACGCGC-3′, product size 300 bp.[26]

PuReTaq Ready-to-go™ PCR Beads (Amersham Biosciences, GE Healthcare Life Sciences, Pittsburg, PA, USA) were used for PCR amplification. Each PCR bead reconstituted to 25 μl contained 10 pmol of the cagA-F and cagA-R or glmM-F and glmM-R primers (Sigma-Genosys, USA), 200 μM of deoxy nucleoside triphosphates, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris–HCl, and approximately 100 ng of genomic DNA. Thirty-five cycles of 1 min at 95 °C, 1 min at 52 °C, and 1 min at 72 °C were performed. PCR products were visualized through standard procedure electrophoresis on 2% agarose gels.[27]

Statistical analysis

Data were analyzed using the 2007 Windows Number Cruncher Statistical System (Number Cruncher Statistical System for Windows, Kaysville, UT, USA). Descriptive statistics were used to characterize the population studied. A correlation analysis was performed to find associations between the sociodemographic variables and *H. pylori* infection markers, adjusting for sex (female, 0; male, 1) and age. To determine the variables that best explained the bacterial load (^{13}C-DOB), a multivariate model was developed and included the following variables in the selection process: family income, overcrowding, housing characteristics, *cagA+ H. pylori* strain (absent, 0; present, 1), and presence of symptoms (absent, 0; present, 1), adjusted for age and sex.

Results

Table 1 shows the sociodemographic characteristics of the participants. Only one of the 25 subjects identified as positive through the breath test was negative for the PCR amplification of *glmM*, the genetic marker of *Helicobacter*.

CagA+ H. pylori strains were found in 48% of the subjects. When divided by the presence of symptoms, 66% of the symptomatic subjects had *cagA+ H. pylori* strains, versus 33% of the asymptomatic subjects. The presence of *cagA+ H. pylori* strains was significantly associated with symptoms of dyspepsia ($r = 0.42$; $p = 0.003$) and negatively associated with educational level ($r = -0.44$; $p = 0.001$) (Table 2).

High gastric urease activity was associated with symptoms, given that 87.5% of the *H. pylori* positive participants

Table 1 Sociodemographic characteristics of the subjects.

Age (years)	34.5
Range	25–74
Sex, n	
Female	7
Male	18
Educational level[a]	6
Monthly family income (minimum wage)	5–10
Percentage of participants at risk of presenting with H. pylori *related to socioeconomic condition*[b]	
No risk	44
Moderate risk	44
High risk	12

[a] Educational level was evaluated using the following scale: 1, elementary school; 2, middle school; 3, high school; 4, semiprofessional; 5, professional; 6, postgraduate.

[b] Calculated as described in the Materials and methods section, considering educational level, employment, family income, housing characteristics, overcrowding, the presence of domestic animals inside or outside the home, and breeding animals. Characteristics associated with a high risk of presenting with *H. pylor*i were coded numerically (final scores were: no risk, 0–2; moderate risk, 3–5; and high risk, >5).

with high gastric urease activity (^{13}C-DOB values > 20‰) had symptoms of dyspepsia, versus 35% of the *H. pylori* positive subjects with low gastric urease (^{13}C-DOB values < 20‰) and 12% of the *H. pylori* negative subjects.

Gastric urease activity, which is a marker of *H. pylori* load, showed a significant association with the presence of dyspepsia ($r = 0.55$; $p < 0.0001$) and *cagA+ H. pylori* strains ($r = 0.53$; $p < 0.0001$) (Table 2).

The variables that best explained the ^{13}C-DOB value were the presence of symptoms ($p = 0.0003$), *cagA+ H. pylori* strain ($p = 0.0004$), and family income, adjusted for age and sex ($p < 0.01$) (Table 3).

Discussion and conclusions

The presence of *cagA+ H. pylori* strains was associated with *cagA* positivity and educational level ($r = -0.44$; $p = 0.001$). In other words, *cagA+ H. pylori* carriers had a lower educational level than *cagA− H. pylori* carriers.

The *cagA* gene is a marker of the cag-pathogenicity island, and its presence is associated with more severe pathologies.[11,28,29] The injection of CagA protein into gastric epithelial cells affects their spreading, migration, and adhesion, as well as other signal-transduction pathways related to proinflammatory responses, and induces interlukine-8 (IL-8) via the NF-κB signaling pathway.[29] We found an association between *cagA+ H. pylori* colonization and the presence of symptoms. Similar results were obtained by Loffeld et al., who found that *cagA+ H. pylori* patients had more dyspeptic symptoms than patients with *cagA− H. pylori* strains.[30]

Utilizing gastric urease activity as an indicator of colonization intensity or bacterial load,[22] we found that higher gastric activity correlated with greater symptoms, concurring with results reported by others.[17–22]

The main limitation of the present study was its small sample size. Nevertheless, to the best of our knowledge, it is the first analysis to investigate and show a positive association between ^{13}C-DOB and *cagA+ H. pylori* strains, suggesting that an elevated ^{13}C-DOB value could be indicative of pathogenicity. Another limitation of our study was the fact that the chronicity, variety, or intensity of dyspeptic symptoms were not included, preventing a more in-depth examination of the association between urease activity, *H. pylori* strain, and symptoms.

In conclusion, our data support the association between high gastric urease activity (elevated ^{13}C-DOB values), *cagA* positive colonization, and the presence of gastric symptoms. High ^{13}C-DOB values in patients could be a criterion for performing *H. pylori* genotype identification and deciding to initiate clinical treatment. Further studies aimed at establishing a possible cut-off value for pathologic risk are warranted.

Table 2 Correlation coefficients and p values from the correlation analysis (adjusted by age and sex) for associations between the sociodemographic variables and *H. pylori* infection markers.

	cagA+	Gastric urease activity (^{13}C-DOB)	Educational level	Family income	*H. pylori+*
Dyspeptic symptoms	0.42 $p = 0.003$	0.55 $p < 0.0001$	−0.29 $p = 0.04$	0.33 $p = 0.02$	0.51 $p = 0.0003$
cagA+		0.53 $p < 0.0001$	−0.44 $p = 0.001$	0.21 N.S.[2]	0.50 $p = 0.0002$
Gastric urease activity (^{13}C-DOB)			−0.19 NS	−0.08 NS	0.81 $p < 0.0001$
Educational level				−0.34 $p = 0.016$	−0.20 NS
Family income					0.12 NS

DOB: delta over baseline; NS: not significant.

Table 3 Multivariate regression analysis to assess gastric urease activity.

Dependent variable	Independent variable	$\beta \pm$ SE	p
Gastric urease activity (^{1}3C-DOB)	Intercept	-3.02 ± 5.06	0.55
	Symptoms	10.93 ± 2.7	0.0003
	cagA+ H. pylori strain	11.63 ± 3.02	0.0004
	Family income	-3.99 ± 1.45	0.0090
	Sex	-3.24 ± 2.38	0.1809
	Age	0.23 ± 0.12	0.0719

DOB: delta over baseline; $\beta \pm$ SE regression coefficient $\pm$ standard error.
$R^2 = 0.5396$ ($p < 0.0001$).

Ethical considerationsProtection of human and animal subjects

Our study was evaluated and approved by the Ethics Committee of the *Centro de Investigación en Alimentación y Desarrollo, A. C.*, based on the international standards stated in the Declaration of Helsinki and the General Health Law Regulation, related to the field of Health Research, of Sonora, Mexico.

Data confidentiality

The authors declare that they have treated all patient data with confidentiality and anonymity, following the protocols of their work center.

Right to privacy and informed consent

All participants signed written statements of informed consent, prior to enrollment. Strict data confidentiality was guaranteed, and the authors declare that the information contained in the study does not allow the identification of the participants.

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Authorship

M.F. Moreno-Ochoa participated in the data acquisition, analysis, and interpretation and approval of the final version of the manuscript. M.E. Valencia participated in the study conception and design, data analysis and interpretation, drafting of the article, and approval of the final version of the manuscript. G.G. Morales-Figueroa participated in the data analysis and interpretation and approval of the final version of the manuscript. S.Y. Moya-Camarena participated in the study conception and design, data analysis and interpretation, drafting of the article, and approval of the final version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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