

***Helicobacter pylori* infection in Havana, Cuba. Prevalence and *cagA* status of the strains**

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There is a great paucity of information about *Helicobacter pylori* infection in the countries of the Caribbean basin. Almost no studies have been performed to determine the prevalence, antibiotic resistance or virulence factors of the bacterium. To measure the prevalence of *H. pylori* infection among patients attending endoscopy in three clinics in Havana, Cuba, to evaluate clarithromycin resistance, and to determine the *cagA* status of the strains obtained. Endoscopy was performed and biopsies were obtained from 117 successive patients attending the Institute of Oncology, the Institute of Gastroenterology, and the Calixto Garcia Hospital in Havana, Cuba. Biopsies were maintained at -70 °C before being cultured on three different media (two selective and one non-selective) and incubated for 7 days at 37 °C under a microaerobic atmosphere. The presence of *H. pylori* was identified by oxidase, catalase and urease activities. DNA was extracted, and PCR was performed with primers H2761676 which amplify a 397 bp fragment of the *cagA* gene. Clarithromycin susceptibility was measured by the gel diffusion method. The diagnoses of patients were: 1 gastric carcinoma; 19 duodenal ulcers; 8 gastric ulcers; and 89 non-ulcer dyspepsia, including (62) gastritis, (9) hiatal hernia, (2) biliary reflux, (1) gastric polyps, and (15) no abnormality. Among the 117 biopsies tested, 83 were *H. pylori* positive (70.9%). The *cagA* status determined for 35 cases gave a positive result in 31 cases (88.5%). Only 3% of the strains were resistant to clarithromycin. The prevalence of *Helicobacter pylori* infection in the symptomatic population of La Habana is the same as reported for other developing countries. Most strains were *cagA* positive and are likely harbour the *cag* pathogenicity island. The low resistance to clarithromycin in the strains studied probably reflects the low degree of use of the antibiotic in this population.

Keywords: *H. pylori*, prevalence, endoscopic diagnosis

Introduction

The infection caused by *Helicobacter pylori* plays an important role in the development of several digestive diseases and it has a worldwide distribution and is significantly more frequent in developing countries than in industrialized country. This microorganism has been recently accepted as related to active chronic gastritis, peptic disease (both gastric and duodenal ones), gastric adenocarcinoma and gastric lymphoma type MALT

(1-5). The phenotype and genotype study of *Helicobacter pylori* include putative bacterial virulence factors. The most common bacterial virulence factors studies are the *cag* pathogenicity island, for which *cagA* is a marker and the vacuolating cytotoxin VacA (6-8). Few studies have been performed in the Caribbean to determine the prevalence of *Helicobacter pylori* infection and to get an insight into the type of strains which are circulating (9-12).

Our aims were

- ✓ To determine the prevalence of *H. pylori* infection among patients attending endoscopy clinic in Cuba.
- ✓ To evaluate the *cagA* status of the strains obtained.
- ✓ To evaluate clarithromycin susceptibility.

Material & Methods

Biopsy specimens: We examined *H. pylori* strain isolated from 117 patients who were subjected to upper gastrointestinal endoscopy for clinical reasons at the Institute of Oncology, the Institute of Gastroenterology and Calixto García Hospital in Havana, Cuba. Patients had the following endoscopic diagnosis: gastric carcinoma (1); duodenal ulcer (19); gastric ulcer (8); non ulcer dyspepsia: 89 including erosive gastritis (62), hiatal hernia (9), biliary reflux (2), gastric polyps (1), no abnormality (15). All patients signed informed consent. During the endoscopic procedure three biopsy specimen (two the antrum and one from the corpus) and were maintained at -70°C before being cultured.

Culture of *H. pylori*: Biopsies specimens were placed in 0.5 mL of Brucella broth (Biomérieux) and homogenized with an electric tissue homogeniser Ultraturax; LaboModerne, Paris, France for 30s. Aliquots were placed for isolation on each of three different media (selective in house medium, pylori Agar Biomérieux, Marcy L'Etoile, France), and a non-selective Columbia medium (Oxoid Unipath, Basingstoke, Hants, UK) enriched with 10% human blood. All media were incubated for 7 days at 37 °C in a microaerobic atmosphere. They were identified by colony morphology, oxidase, catalase and urease activities. Clarithromycin susceptibility was measured by the gel diffusion method. Table 1 (13-19).

DNA extraction: A pool of colonies obtained from 35 patients were transferred to a microcentrifuge tube that contained 500 µl of sterile distilled water and centrifugated for five min at 10 000 g. The pellet was resuspended in 300 µl of extraction buffer (20 µM Tris/HCL, pH 8, 0.5% Tween 20) and proteinase K was added at final concentration of 0.5 µg/mL. The mixture was incubated at 55 °C for one hour. Finally the enzyme was inactivated by heating the sample for 10 minutes at 98 °C (20).

***cagA* gene detection:** Primers used for *cagA* typing were H276/676. For PCR of each sample, the mixture contained 25 pmol of each primer, 100 ng of genomic DNA, each deoxynucleoside triphosphate at a concentration of 100 µM, and 1U of Taq DNA. The reactions were performed in an automated DNA thermal cycler, with 30 cycles of denaturation 94 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 2 min (21,22).

PCR product detection: All amplified PCR products were resolved in 0.8% agarose gels, stained with ethidium bromide and detected under a short - wavelength UV Light source. We consider strain *cagA* positive when it show a product of 397 bp fragment in PCR reaction (22-24).

Results & Discussion

H. pylori status of the Cuban population has not been explored. Endoscopy was performed and biopsies were obtained from 117 successive patients attending the Institute of Oncology, the Institute of Gastroenterology, and the Calixto García Hospital in Havana, Cuba. Biopsies were maintained at -70 °C before being cultured on three different media (two selective and one non-selective) and incubated for 7 days at 37 °C under a microaerobic atmosphere. The presence of *H. pylori* was identified by oxidase, catalase and urease activities. DNA was extracted, and PCR was performed with primers H276/676 which amplify a 397 bp fragment of the *cagA* gene. Clarithromycin susceptibility was measured by the gel diffusion method. The diagnoses of patients were: 1 gastric carcinoma; 19 duodenal ulcers; 8 gastric ulcers; and 89 non-ulcer dyspepsia, including (62) gastritis, (9) hiatal hernia, (2) biliary reflux, (1) gastric polyps, and (15) no abnormality. Among the 117 biopsies tested, 83 were *H. pylori* positive (70.9%). The *cagA* status determined for 35 cases gave a positive result in 31 cases (88.5%). Only 3% of the strains were resistant to clarithromycin (Table 1-2).

The prevalence of *H. pylori* infection in the symptomatic population of La Habana is the same as reported of other developing countries (9-12). Most strains were *cagA* positive and are likely harbour the *cag* pathogenicity island (Tabla 2). The low resistant to clarithromycin in the strains studied probably reflects the low degree of use of the antibiotic in this population (25-27).

Table 1. Growth of *Helicobacter pylori* isolated from biopsy specimens on direct culture

Endoscopic diagnosis	No. Positives/No. samples
Duodenal ulcer	15/19
Gastric ulcer	8/8
Gastric Carcinoma	0/1
Non-ulcer dyspepsia	
*Gastris	42/62
*Hiatal Hernia	8/9
*Biliary reflux	1/2
*Gastric polyps	1/1
*No abnormality	8/15

Table 2. Genotype *cagA* from Cuban isolated of *Helicobacter pylori*

Endoscopic diagnosis	No. isolated tested/ <i>cagA</i> +
Gastric ulcer	4/4
Non-ulcer dyspepsia	
*Gastritis	17/13
* Hiatal Hernia	3/3
* Biliary reflux	1/1
*Gastritis & Hiatal Hernia	2/2
*No abnormality	1/1
*Other diagnosis	7/7

Conclusion

- ✓ The prevalence of *Helicobacter pylori* infection in the symptomatic population of La Habana is the same as reported for other developing countries.
- ✓ Among the 117 biopsies tested, 83 were positive (70.9%).
- ✓ The *cagA* status determined for 35 cases gave a positive result in 31 cases (88.5%).
- ✓ Most strains were *cagA* positive and are likely harbour the *cagA* pathogenicity island.
- ✓ The low resistance to clarithromycin in the strains studied probably reflects the low degree of use of the antibiotic in this population.

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Infección por *Helicobacter pylori* en la Ciudad de La Habana, Cuba. Prevalencia de las cepas *cagA* positivas

Resumen

Existe una gran falta de información acerca de la infección por *Helicobacter pylori* en los países de la región del Caribe. Nuestros objetivos en este estudio fueron determinar la prevalencia, la resistencia a los antibióticos y los factores de virulencia de la bacteria. La medida de la prevalencia de la infección por *H. pylori* se determinó en un grupo de pacientes a los que se les practicó una endoscopia en tres centros hospitalarios de La Ciudad de La Habana, lo que nos permitió evaluar la resistencia a la claritromicina y la presencia de *cagA* + en las cepas obtenidas. De las endoscopias realizadas se obtuvieron 117 biopsias gástricas, procedentes de tres centros hospitalarios de La Ciudad de La Habana, Cuba: Instituto de Oncología, Instituto de Gastroenterología y el Hospital Calixto García. Las biopsias fueron mantenidas a -70°C para posterior cultivo en tres medios diferentes (dos selectivos y uno no selectivo) y su posterior incubación por 7 días a 37°C en una atmósfera de microaerofilia. La presencia de *H. pylori* fue identificada por la presencia de diferentes enzimas (oxidasa, catalasa, ureasa). Se realizó la extracción del DNA y la PCR, donde se utilizó el primer H2761676 y se amplificó con 397 fragmentos del gen *cagA*. La susceptibilidad a la claritromicina fue medida por el método de difusión en gel. Diagnóstico endoscópico: (1) cáncer gástrico; (19) úlcera duodenal; (8) úlcera gástrica; (89) dispepsias no ulcerosas, incluyendo (62) gastritis; (9) hernia hiatal; (2) reflujo biliar; (1) pólipo gástrico; (15) panendoscopias normales. Del total de 117 biopsias realizadas, 83 fueron positivas a la infección por *H. pylori* (70,9%). De las 35 cepas a las que se les realizó presencia de *cagA* + resultaron positivas 31 (88,5%). Solo el 3% de las cepas fueron resistentes a la claritromicina. La prevalencia de la infección por *H. pylori* en la población sintomática de La Ciudad de La Habana es la misma que la reportada en países desarrollados. La mayoría de las cepas fueron *cagA* positivas y son probablemente el puerto de la Isla de patogenicidad (*cagPAI*). La baja resistencia a la claritromicina en las cepas estudiadas, probablemente refleja la escasa utilización de este antibiótico en la población estudiada.

Palabras clave: *H. pylori*, prevalencia, diagnóstico endoscópico