



## Seroprevalence of *Helicobacter pylori* infection in chagasic and non-chagasic patients from the same geographical region of Brazil

Soroprevalência da infecção por *Helicobacter pylori* em indivíduos chagásicos e não-chagásicos da mesma região geográfica do Brasil

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### ABSTRACT

**Introduction:** In this study, we evaluated the seroprevalence of *Helicobacter pylori* infection among chagasic and non-chagasic subjects as well as among the subgroups of chagasic patients with the indeterminate, cardiac, digestive, and cardiodigestive clinical forms. **Methods:** The evaluated subjects were from the Triângulo Mineiro region, Minas Gerais, Brazil. Chagasic patients showed positive reactions to the conventional serological tests used and were classified according to the clinical form of their disease. Immunoglobulin G antibodies specific to *H. pylori* were measured using a commercial enzyme-linked immunosorbent assay kit. **Results:** The overall *H. pylori* prevalence was 77.1% (239/310) in chagasic and 69.1% (168/243) in non-chagasic patients. This difference was statistically significant even after adjustment for age and sex (odds ratio = 1.57; 95% confidence interval, 1.02-2.42;  $p = 0.04$ ) in multivariate analysis. The prevalence of infection increased with age in the non-chagasic group ( $p = 0.007$ ,  $\chi^2$  for trend), but not in the chagasic group ( $p = 0.15$ ,  $\chi^2$  for trend). *H. pylori* infection was not associated with digestive or other clinical forms of Chagas disease ( $p = 0.27$ ). **Conclusions:** Our findings demonstrate that chagasic patients have a higher prevalence of *H. pylori* compared to non-chagasic subjects; a similar prevalence was found among the diverse clinical forms of the disease. The factors contributing to the frequent co-infection with *H. pylori* and *Trypanosoma cruzi* as well as its effects on the clinical outcome deserve further study. **Keywords:** *Helicobacter pylori*. Chagas disease. Chronic Chagas disease clinical forms. Co-infection.

### RESUMO

**Introdução:** No presente estudo, foi comparada a soroprevalência da infecção por *Helicobacter pylori* entre os indivíduos chagásicos e não-chagásicos, bem como entre subgrupos de chagásicos com as formas clínicas indeterminada, cardíaca, digestiva e cardiodigestiva. **Métodos:** Os indivíduos avaliados eram provenientes da região do Triângulo Mineiro, Minas Gerais, Brasil. Foram realizados testes sorológicos convencionais para diagnóstico da infecção pelo *T. cruzi* e os chagásicos foram classificados de acordo com a forma clínica. O diagnóstico de infecção por *H. pylori* foi estabelecido pela detecção de anticorpos IgG específicos utilizando-se um kit comercial de ELISA. **Resultados:** A prevalência da infecção por *H. pylori* foi 77,1% (239/310) no grupo de pacientes chagásicos e 69,1% (168/243) no grupo de não-chagásicos. Esta diferença foi estatisticamente significativa mesmo após ajuste para idade e sexo (OR = 1,57; 95% CI, 1,02-2,42;  $p = 0,04$ ) na análise multivariada. A prevalência da infecção aumentou de acordo com a idade no grupo não-chagásicos ( $p = 0,007$ ,  $\chi^2$  for trend) mas este aumento não foi observado no grupo dos chagásicos ( $p = 0,15$ ,  $\chi^2$  for trend). Não houve associação da infecção por *H. pylori* com a forma digestiva ou com qualquer outra forma clínica da doença de Chagas ( $p = 0,27$ ). **Conclusões:** Foi demonstrado que pacientes chagásicos apresentam maior prevalência da infecção por *H. pylori* quando comparados com não-chagásicos, independente da forma clínica da doença. Os fatores que contribuem para a frequente co-infecção *Helicobacter pylori* e *Trypanosoma cruzi*, bem como seus efeitos na evolução clínica das doenças associadas devem ser melhor estudados.

**Palavras-chaves:** *Helicobacter pylori*. Doença de Chagas. Formas clínicas da doença de Chagas crônica. Co-infecção.

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### INTRODUCTION

Chagas disease, a tropical disease strongly associated with poor socioeconomic conditions, is endemic in rural Latin America. However, cases of Chagas disease have been reported throughout the world in the past few decades due to the increase in migrations towards non-endemic countries<sup>1</sup>. An estimated 10 million people are infected, mostly in Latin America, with the causative agent *Trypanosoma cruzi*, which is mainly transmitted by blood-sucking triatomine bugs<sup>2</sup>.

The clinical course of Chagas disease is highly variable. In a substantial proportion of the individuals who survive the acute phase, the disease enters the chronic phase that generally starts with a long period of clinical latency called the indeterminate form, which lasts for 10-30 years or throughout life<sup>3</sup>. Approximately one-third of these infected patients will develop serious lesions in the heart and/or digestive tract, characterizing the other clinical forms of chronic Chagas disease (i.e., cardiac, digestive, or cardiodigestive).

Likewise, the gram-negative bacterium *Helicobacter pylori* persists in most individuals, after being acquired mainly in childhood, as a chronic infection throughout life without complications, although a small percentage of infected individuals will develop severe gastroduodenal disease. Currently, *H. pylori* infection is recognized as the main cause of chronic gastritis and is directly linked to the development of peptic ulcer disease as well as gastric carcinoma and MALT lymphoma<sup>4</sup>. Interactions between bacterial virulence, host-related and environmental factors influence the clinical outcome in infected individuals, whereas concurrent infection with other pathogens may also potentially exacerbate or reduce disease severity due to the modulation of the gastric inflammatory process<sup>5-8</sup>.

Before the discovery of *H. pylori*, the chronic gastritis commonly seen in chagasic patients, most

often in those with the digestive form, had been formerly attributed to biliary duodenal-gastric reflux as well as to hypomotility and hypochlorhydria, resulting in injury to the enteric nervous system caused by *T. cruzi* infection<sup>9-12</sup>. Later, some studies demonstrated a high prevalence of *H. pylori* infection in patients with Chagas disease<sup>13-16</sup>. As this small number of studies on *T. cruzi* and *H. pylori* co-infection had no control groups and/or included a low number of patients, we evaluated the prevalence of *H. pylori* infection among chagasic and non-chagasic subjects living in a region previously considered endemic for Chagas disease. We also analyzed whether *H. pylori* infection is associated with the clinical forms of chronic Chagas disease.

## METHODS

We evaluated outpatients with Chagas disease and subjects without the disease from the same geographical region living in a rural or urban area of Uberaba and other nearby cities. These cities are located in the west of the State of Minas Gerais, Brazil, in a region called Triângulo Mineiro, which was previously considered an endemic area for Chagas disease. Most of the study population was originally recruited to evaluate the prevalence of Chagas disease in the region. Therefore, the majority of the non-chagasic subjects were relatives of chagasic patients or were living near their residence. The other non-chagasic subjects were patients with dyspeptic complaints who underwent diagnostic endoscopy at the *Hospital de Clínicas* of the *Universidade Federal do Triângulo Mineiro* (UFTM). Peripheral blood (5mL) was collected from the subjects into vacuum tubes for serological tests in the period from 1995-2010. The sample was centrifuged and the serum was divided into aliquots and stored in sterile containers at -20°C before testing.

The presence of immunoglobulin (Ig)G antibodies specific to *H. pylori* was measured by using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Pyloriset EIA-GIII; Orion Diagnostica, Espoo, Finland). The antigen employed in this kit is a cell surface protein of *H. pylori* strain NCTC 11637. The assay was performed according to the manufacturer's instructions. Patients with positive serology were considered to be infected with *H. pylori* because spontaneous elimination of this bacterium without a specific eradication therapy is a rare event. The concentrations of IgG antibody in serum samples were determined by interpolation from a standard curve constructed by plotting absorbance values obtained for each of the 4 calibrator sera, which are provided by the manufacturer, against the corresponding anti-*H. pylori* concentrations in U/mL.

The diagnosis of Chagas disease was performed by means of ELISA (Chagatest ELISA-WIENER, Argentina), hemagglutination (Chagatest HAI-WIENER, Argentina), and immunofluorescence (Imuno-Con Chagas-WAMA, Brazil) tests. All chagasic patients showed positive reactions to at least 2 serological tests, while non-chagasic subjects showed negative reactions to all 3 tests. Chagasic patients were classified according to the clinical form of their disease on the basis of the results of a conventional electrocardiogram and contrast X-ray of the esophagus and colon. Clinical, epidemiological, and demographic data were collected by reviewing the patients' medical records. Only patients for who complete data were available were included in this study.

Data were analyzed using the SPSS statistical software package (version 17.0; SPSS Inc., Chicago, IL, USA). The association between *H. pylori* infection and Chagas disease was evaluated using

a logistic regression model. Variables such as gender and mean age were included in this analysis. The odds ratio (OR) as well as its 95% confidence interval (CI) were determined. The association between *H. pylori* infection and the clinical form of chronic Chagas disease was tested by the two-tailed  $\chi^2$  test with Yates correction or Fisher's exact test. To evaluate the prevalence of *H. pylori* with aging, the  $\chi^2$  test for trend was performed using Epi Info statistical software (version 3.5.1; Centers for Disease Control and Prevention, Atlanta, GA, USA). The significance level was set at  $p \leq 0.05$ .

## Ethical considerations

This study was carried out in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of UFTM, Uberaba, State of Minas Gerais, Brazil.

## RESULTS

A total of 553 subjects were included in the present study. Of these subjects, 310 (56.1%) were patients with chronic Chagas disease (142 men and 168 women; mean age  $57.7 \pm 12.6$  years, range 20-90) and 243 (43.9%) were non-chagasic subjects (100 men and 143 women; mean age  $42.4 \pm 15.9$  years, range 18-86). Among the 310 patients with Chagas disease, 106 (34.2%) had the indeterminate clinical form of disease, 102 (32.9%) had the cardiac form, 72 (23.2%) had the digestive form, and 30 (9.7%) had the cardiodigestive form.

The overall prevalence of *H. pylori* infection was significantly higher in chagasic (77.1%; 239/310) than in non-chagasic (69.1%; 168/243) subjects, even after adjustment for age and sex (OR = 1.57; 95% CI, 1.02-2.42;  $p = 0.04$ ) in multivariate analysis (Table 1).

Table 2 shows that the prevalence of *H. pylori* infection increased with age ( $p = 0.007$ ,  $\chi^2$  for trend) from 61.9% (18-29-years age group) to 81.6% (50-59-years age group) in the non-chagasic group, but not in the chagasic group ( $p = 0.15$ ,  $\chi^2$  for trend). The rate of *H. pylori* infection decreased after 60 years of age among the non-chagasic subjects.

Figure 1 shows the prevalence of *H. pylori* infection according to the clinical forms of chronic Chagas disease. The rates of *H. pylori* positivity were 79.2% (84/106) in the group of patients with the cardiac form of the disease, 77.5% (79/102) in those with the indeterminate form, 76.7% (23/30) in those with the cardiodigestive form, and 73.6% (53/72) in those with the digestive form. There was no significant association between *H. pylori* infection and the clinical forms of Chagas disease ( $p = 0.27$ ).

There was no significant difference ( $p = 0.34$ ) in the concentrations of anti-*H. pylori* IgG for the chagasic (mean, 260.1U/mL) and non-chagasic (mean, 274.1U/mL) subjects. The mean concentration of anti-*H. pylori* IgG was also similar ( $p = 0.54$ ) among the subgroups of patients with Chagas disease: digestive (278.1U/mL), indeterminate (mean, 267.3U/mL), cardiac (247.5U/mL), and cardiodigestive (239.5U/mL).

TABLE 1 - Variables associated with *Helicobacter pylori* infection in univariate and multivariate analysis.

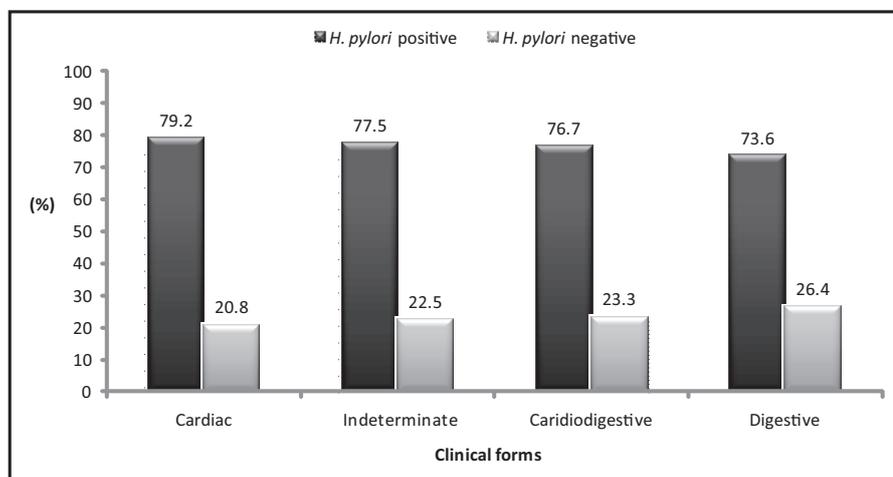
Variables	Univariate analysis p value	Multivariate analysis	
		OR (95% CI)	p value
Age	0.57	-	-
Gender	0.57	-	-
Chagas disease	0.03	1.57 (1.02-2.42)	0.04

OR: Odds ratio; CI: confidence interval.

**TABLE 2 - Prevalence of *Helicobacter pylori* infection in chagasic and non-chagasic patients according to age group.**

Patients	Number (%) of <i>H. pylori</i> -positive individuals according to age group (years)					$\chi^2$ for trend p value
	18-29	30-39	40-49	50-59	$\geq 60^a$	
Chagasic	5 (83.3)	19 (82.6)	37 (86.0)	64 (72.7)	114 (76.0)	0.15
Non-chagasic	39 (61.9)	35 (63.6)	41 (82.0)	31 (81.6)	22 (59.5)	0.007

<sup>a</sup>This age group was not included in the  $\chi^2$  for trend analysis.



**FIGURE 1 - Prevalence of *Helicobacter pylori* infection according to the clinical forms of chronic Chagas disease.**

## DISCUSSION

Approximately 50% of the world's population is estimated to be infected with *H. pylori*, but individuals living in countries with low socioeconomic conditions have high prevalence rates of *H. pylori* acquired at an early age<sup>4,17</sup>. In developing countries, individuals are also more susceptible to acquiring chronic concurrent infections with other pathogens; however, the co-infection prevalence is still largely unknown as are the effects of each other in the natural history of the infections.

After the discovery of *H. pylori* in 1982, Barbosa et al.<sup>13</sup> were the first to investigate the presence of bacteria in the gastric mucosa of patients with Chagas disease, followed by Oliveira et al., who evaluated patients with the digestive and indeterminate clinical forms of the disease, respectively. In those studies, the prevalence of *H. pylori* infection was very high (~95%). It is worth noting that both were uncontrolled studies with a small number of patients. Later, Nascimento et al. found a significantly higher prevalence of *H. pylori* in chagasic patients aged 21-50 years compared with non-chagasic subjects living in the same urban or rural area. More recently, Pinazo et al. evaluated a short series of high-risk Latin American immigrants that lived in Barcelona, confirming the frequent coexistence of *H. pylori* and *T. cruzi* infections.

In the present study, in which a larger number of patients with chronic Chagas disease were evaluated, a significantly higher prevalence of *H. pylori* infection was found in the chagasic group compared to the non-chagasic group, even after adjustment for confounding factors such as age and gender in multivariate analysis. Considering that *H. pylori*, even with its multiple mechanisms for acid adaptation, is susceptible to acid and can only survive for a few minutes at the low pH of the stomach lumen<sup>8,18</sup>, we might speculate

that the hypochlorhydria described in the digestive form of Chagas disease could contribute to the higher prevalence of *H. pylori* among these patients. However, no significant difference between patients with the digestive and non-digestive forms was observed. Although low gastric acid secretion has been demonstrated only in patients with the digestive form, to date, it is known that patients presenting with one of the clinical forms of chronic Chagas disease can also present with lesions in other systems due to the criteria currently used. For instance, severe impairment of the autonomic control of the heart may occur in patients apparently having only digestive tract involvement<sup>19</sup>. In addition, a variable number of chronic chagasic patients with the indeterminate form show structural or functional abnormalities of the heart and digestive tract when more accurate diagnostic tests are employed<sup>20-22</sup>. Taken together, these findings could explain why we found a higher prevalence of *H. pylori* infection in chagasic patients, but without differences among the different forms of the disease.

Conversely, as it is known that chronic *H. pylori* infection exerts profound and diverse effects on gastric acid secretion, one should consider the possibility that some gastric physiological alterations such as hypergastrinemia and hypochlorhydria that were originally attributed to Chagas disease<sup>9,23,24</sup> may actually be exacerbated by concurrent *H. pylori* infection. The effects of *H. pylori* infection on the secretion of gastric acid depend on the degree and localization of the gastritis and are related to the disease outcome<sup>25</sup>. Briefly, *H. pylori* infection confined to the antrum results in antral-predominant gastritis with the hypersecretion of acid and is associated with duodenal ulceration, whereas body infection may result in atrophic gastritis and abnormally low gastric acid secretion.

In the present study, the prevalence of *H. pylori* infection increased with age among non-chagasic subjects aged <60 years of age, but not among chagasic patients, who showed relatively constant

rates of infection regardless of age. Multiple epidemiological studies have shown that the prevalence of *H. pylori* increases with age, which is best explained by a cohort phenomenon with diminished acquisition during childhood as socioeconomic development has occurred, because the risk of acquiring the infection is considered to be low in adulthood<sup>4</sup>. Our data might indicate that patients with Chagas disease have a high risk for acquiring an *H. pylori* infection in childhood as well as in adult life. Moreover, as reported in other studies, we observed a decrease in the prevalence of infection among the oldest individuals, which has been explained by the fall in the specific serologic response among older individuals due to the decrease in their general immunity<sup>17,26-28</sup>.

Considering that the transmission of *T. cruzi* and *H. pylori* is associated with poor socioeconomic conditions, the high prevalence of co-infection with these pathogens found in our study (~77%) is not surprising. In Brazil, the overall prevalence of *H. pylori* is high when compared to that of developed countries, although the rates found vary according to the geographical region studied. In the south of Brazil, the rate of *H. pylori* prevalence in adults is ~63%, while in the poorest regions such as the northeast and mid-west it is ~90%<sup>27-29</sup>. Regarding the State of Minas Gerais, where the population evaluated in the present study lives, rates of *H. pylori* infection ranging from 62% to 86% have been reported among adults<sup>17,30</sup>. The study population of chagasic and non-chagasic subjects may be considered quite homogeneous since the majority of individuals were adults living in the same geographic region and, presumably, have similar socioeconomic conditions and risk factors for acquiring an *H. pylori* infection.

In conclusion, our findings demonstrate that chagasic patients have a high prevalence of *H. pylori* compared to non-chagasic subjects in the study population. Notably, *H. pylori* infections were not associated with the digestive or other clinical forms of Chagas disease. Therefore, the factors contributing to this frequent co-infection with *H. pylori* and *T. cruzi* as well as the effects on the clinical outcomes deserve further study that will certainly contribute to a better understanding of the gastric physiological alterations induced by both of these important agents of chronic diseases.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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