Prevalence of *Helicobacter pylori* infection in dyspeptic patients and its association with clinical risk factors for developing gastric adenocarcinoma

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ABSTRACT – Background – In Brazil, particularly in the underdeveloped localities, the prevalence of *Helicobacter pylori* (*H. pylori*) infections can range up to 90%. These rates are higher in older individuals and vary by country region. *H. pylori* infections are linked to the development of gastric pathologies, namely mild to moderate gastritis, gastroenteritis, peptic ulcer, intestinal metaplasia, and gastric cancer. In 1994, this organism was classified by the International Agency for Research on Cancer (IARC) as pertaining to the Group 1 carcinogen for gastric adenocarcinoma etiology. Gastric cancer represents a significant public health problem, being the fourth most common malignant tumor and the second largest cause of cancer-related deaths. Objective – To investigate the prevalence of *H. pylori* infection in dyspeptic patients and determine the link between clinical risk factors and gastric adenocarcinoma. Methods – Polymerase chain reaction (PCR) analysis was employed for molecular diagnosis of gastric tissue biopsies collected from 113 dyspeptic patients at the University Hospital of Federal University of Goiás. Molecular analyses allowed the identification of *H. pylori* infections. Furthermore, histopathological examinations were performed to determine the clinical risks of developing gastric malignancies. Results – The test results identified 69 individuals older than 44 years, from 75 (66.4%) positive *H. pylori* infection samples. The prevalence of gastric adenocarcinoma in this study was 1.3%. Among the infected patients, six (8.2%) had high risk, and 67 (91.8%) had a low risk of developing gastric cancer (*P*<0.05). Conclusion – This study shows a high prevalence of *H. pylori* infection and identifies its contribution to gastric inflammations, which in turn are manifested in high-risk clinical factors for the development of gastric adenocarcinoma.


INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is a spiral, gram-negative, microaerophilic and fastidious microorganism. This bacterium exhibits two to six helix flagella, it grants high motility and support for penetrating the human gastric mucosa[1,2].

The prevalence of infections by *H. pylori* is highly variable, owing to its geographical distribution and host’s ethnicity, age, sex and socioeconomic factors[3]. It is estimated that half of the world’s population is infected by this organism[11]. Indeed, infection rates are significantly lower in the developed countries (20%) compared with locations where sanitation conditions are underdeveloped[4].

In Brazil, the prevalence of *H. pylori* infection can range up to 90% in more impoverished areas[5]. These indices are higher in older populations and are region specific, which confirms that economic status and hygiene are risk factors for *H. pylori* infection[6].

The exact transmission mode of this bacterium remains to be understood. However, it is known that this organism colonizes the gastric mucosa, suggesting that the infection occurs through the gastro-oral, oral-oral, fecal-oral routes[7] or by zoonotic transmission. For instance, domestic flies have zoonotic potential mainly due to its contact with decomposing organic matter and contaminated feces, which supports their proliferation to subsequently infect humans, animals and plant hosts[8,9].

The risk factors for *H. pylori* infection are sex[10], age[11,12], smoking[13], alcohol consumption[14], contaminated food[12], untreated water intake[15], family size[16], bed sharing[17], poor living conditions in childhood[17], educational[13] and socioeconomic level[18], hygiene[19], and family medical history of gastric disease[11].

*H. pylori* infection is primarily observed in children and may remain asymptomatic until adulthood[20]. At this stage, the infection provokes abdominal pain[6], anorexia[21], gastrointestinal bleeding, and antral modularity[6].
The development of gastric pathologies is directly associated with \textit{H. pylori} infection, which induces mild to moderate gastritis\(^{(20)}\), gastroenteritis and peptic ulcer\(^{(20)}\). These conditions often lead to intestinal metaplasia\(^{(22)}\), and gastric cancer\(^{(23)}\). Therefore, in 1994, \textit{H. pylori} was classified as Group 1 carcinogen for Gastric Adenocarcinoma by the IARC\(^{(24)}\).

Gastric adenocarcinoma accounts for 90\% of gastric cancer cases\(^{(23)}\). This cancer type represents a significant health issue, being the fourth most common malignant tumor and the second largest cause of cancer related deaths\(^{(23)}\).

The diagnosis of gastric adenocarcinoma is rarely detected at early stages. Consequently, most patients exhibit metastatic lesions when first diagnosed\(^{(26,27)}\). If untreated, 63\% of cases progress to incurable disease within five years\(^{(24)}\).

Gastric cancer screening consists of two phases, namely primary prevention (identifying possible risk factors) and secondary screening method (early detection)\(^{(28)}\). In Japan younger than 20 years are screened with primary prevention methods to identify and treat \textit{H. pylori} infection. On the other hand, individuals aged 50 years or more are tested with the combination of the two method through endoscopic examination. Since its implementation in 2013, this plan significantly reduced the incidence, mortality and annual cost for gastric cancer treatment in Japan\(^{(29)}\).

By determining the relevance of preventing \textit{H. pylori} infection for public health costs, this study aimed to investigate the prevalence of this organism in dyspeptic patients. Moreover, we aimed to evaluate the link between the infection and clinical risk factors for gastric cancer development. Our findings may contribute to emerging public prevention and treatment policies for cancer in Brazil.

**METHODS**

**Ethical considerations**

The study protocol was approved by the Research Ethics Committee of the University Hospital (CAAE): 83422017.7.0000.5078, published under the number 2.519.032, according to the Resolution CNS 196/96. All patients enrolled in this study signed an Informed Consent Form.

The age bar for patients enrolled in the study was above 18 years. Subjects treated with proton pump inhibitors or blocking agents (one week before endoscopy) and immunosuppressants or antibiotic (eight weeks before of endoscopy) were excluded from our study. Moreover, we also excluded individuals presenting active gastrointestinal bleeding, pregnancy and those with inability to undergo endoscopy.

**Patients**

Were investigated 113 dyspeptic patients submitted to endoscopic examination at the University Hospital. During the endoscopy three fragments of the antrum and the gastric body of each patient were collected. One fragment was submitted to molecular diagnosis (polymerase chain reaction - PCR), and the other two were submitted to histopathological examination.

**Risk criteria**

The endoscopy results were stratified according to the risk of developing gastric adenocarcinoma. Patients exhibiting gastric atrophy and intestinal metaplasia were classified as high risk for gastric cancer diagnosis. Conversely, patients with normal endoscopic result, esophagitis, duodenitis, gastritis, ulcer, and xanthelasma\(^{(30)}\) were categorized as low risk.

**DNA extraction**

DNA extraction was performed at the Laboratory of Biotechnology of Microorganisms of the Federal University of Goiás through KitQiap DNA minikit\(^{®}\) (Qiagen, Valencia, CA, United States). Ten μL aliquot of each sample were used for DNA quantification and purity analysis with NanoDrop\(^{®}\) (ND-1000 UV-Vis).

**PCR**

Genomic DNA was amplified by PCR following previously described protocols by Nevoa et al. (2017). Molecular detection of \textit{H. pylori} was performed by amplifying the 16S ribosomal gene (rRNA) using the HpX (CTGGAGARACTAAGYC-CTCC)/HpX1 (AGGGAATACCTATGCGAGCGCA) oligonucleotides. Each reaction consisted of 0.5 μL of Taq polymerase DNA (2.5 units), 5 μL of 10 × CoralLoad PCR buffer (QIAamp, Qiagen) containing MgCl\(_2\) (1.5 mM), 2 μL (2.5 mM) of dNTP (deoxyribonucleotides 5’-triphosphate-dATP, dCTP, dGTP, dTTP), 4 μL of each oligonucleotide pair (10 pmol each), 33.5 μL of ultrapure milli-Q water, and 5 μL (50 ng) of Genomic DNA, totalling 50 μL per reaction. Negative and positive controls were used with an aliquot of \textit{H. pylori} DNA provided by Dr. Lucas Trevizani Rasmussen of the University of Sagrado Coração (Bauru, SP).

The amplified products were analyzed by electrophoresis on 1.6% agarose gel stained with ethidium bromide (10 mg/mL). Fragments with 150 base pairs were considered positive for the HpX/HpX1 PCR primers.

**Data analysis**

The biopsy results and PCR amplifications were analyzed by descriptive statistics, Chi-square test ($\chi^2$), and Pearson Contingency test ($P<0.05$ and 95\% Confidence Interval). The analysis was performed through IBM SPSS (Statistical Package for the Social Sciences) version 25.0.

**RESULTS**

From the 113 investigated dyspeptic patients, 89 (78.8\%) were women, and 24 (21.2\%) were men. According to histopathological examination, the patients were often diagnosed with multiple outcomes in the endoscopy report ($n=155$). From the 113 subjects, 77 patients had only one outcome and the remaining 36 exhibited two to four outcomes. We found that 83 (53.5\%) patients had gastritis, 16 (10.3\%) patients had esophagitis, 16 (10.3\%) had duodenitis, and 13 (4.8\%) presented gastric atrophy. An additional observation was that intestinal metaplasia, gastric or duodenal ulcer, gastric adenocarcinoma and xanthelasma were found in 2.6\%, 1.9\%, 1.2\%, and 0.6\% of patients, respectively. Lastly, 17 (12.4\%) patients presented with normal biopsy results. (FIGURE 1). Excluding the two diagnosed cases of cancer, 96 (86.5\%) patients were classified as having low risk and 15 (13.5\%) as high risk for developing gastric adenocarcinoma ($n=111$) ($P<0.0001$) (TABLE 1).
Borges SS, Ramos AFPL, Moraes Filho AV, Braga CASB, Cameiro LC, Barbosa MS. Prevalence of Helicobacter pylori infection in dyspeptic patients and its association with clinical risk factors for developing gastric adenocarcinoma

Through PCR, we detected H. pylori infections in 75 (66.4%) of the investigated patients. On gender basis, 59 (78.7%) were women and 16 (21.3%) were men. The data also depicted that 92% of infected patients were older than 44 years, confirming this age group, was the most prone to infections (61.1%). Despite these numbers, we did not detect a statistically significant relationship between H. pylori infection and gender, age, educational level, and endoscopy diagnosis (TABLE 2).

Lastly, we verified the associations between H. pylori status determined by PCR and the risk for developing gastric adenocarcinoma in 111 patients. From the 73 (65.77%) infected patients, 6 (8.2%) had a high risk and 67 (91.8%) had a low risk of developing gastric cancer. This difference in developing gastric adenocarcinoma between infected and non-infected patients was statistically significant (P<0.05) (FIGURE 2).

DISCUSSION

An increasing prevalence of H. pylori infection has been observed in developing countries, such as Brazil[3]. The infection prevalence in dyspeptic patients examined at the University Hospital was 61.1%, which is in keeping with the infection rate data from other Brazilian states[31,32].

The inflammatory process resulting from durable bacteria exposure in the gastric mucosa is directly related to the development of gastric cancer[20]. In this study, the presence of the bacteria was detected mainly in patients older than 44 years, showing that growing trend with age as a potential risk factor.

TABLE 1. Distribution by risk criteria for developing gastric adenocarcinoma according to histopathological diagnosis in 111 dyspeptic patients.

<table>
<thead>
<tr>
<th>Endoscopic diagnosis</th>
<th>Low</th>
<th>%</th>
<th>High</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenite</td>
<td>14</td>
<td>12.6</td>
<td>0</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Esophagitis</td>
<td>4</td>
<td>3.6</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Gastric atrophy</td>
<td>0</td>
<td>0.0</td>
<td>11</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>2</td>
<td>1.8</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Gastritis</td>
<td>58</td>
<td>52.3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>0</td>
<td>0.0</td>
<td>4</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>Normal exam</td>
<td>17</td>
<td>15.3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2. Distribution by age, sex, educational level, endoscopic and molecular diagnoses of 113 dyspeptic patients.

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>%</th>
<th>Positive</th>
<th>%</th>
<th>Negative</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-24</td>
<td>10</td>
<td>8.8</td>
<td>8</td>
<td>80.0</td>
<td>2</td>
<td>20.0</td>
<td>0.159</td>
</tr>
<tr>
<td>24-34</td>
<td>15</td>
<td>13.3</td>
<td>12</td>
<td>80.0</td>
<td>3</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>&gt;44</td>
<td>69</td>
<td>61.1</td>
<td>41</td>
<td>59.4</td>
<td>28</td>
<td>40.6</td>
<td></td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Sex</th>
<th>n</th>
<th>%</th>
<th>Positive</th>
<th>%</th>
<th>Negative</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>89</td>
<td>78.8</td>
<td>56</td>
<td>66.3</td>
<td>30</td>
<td>33.7</td>
<td>0.973</td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
<td>21.2</td>
<td>16</td>
<td>66.7</td>
<td>8</td>
<td>33.3</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Educational level</th>
<th>n</th>
<th>%</th>
<th>Positive</th>
<th>%</th>
<th>Negative</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>66</td>
<td>58.5</td>
<td>46</td>
<td>69.7</td>
<td>20</td>
<td>30.3</td>
<td>0.306</td>
</tr>
<tr>
<td>Medium</td>
<td>37</td>
<td>32.7</td>
<td>24</td>
<td>64.9</td>
<td>13</td>
<td>35.1</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>8.8</td>
<td>9</td>
<td>90.0</td>
<td>1</td>
<td>10.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>n</th>
<th>%</th>
<th>Positive</th>
<th>%</th>
<th>Negative</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenite</td>
<td>16</td>
<td>10.3</td>
<td>13</td>
<td>81.3</td>
<td>3</td>
<td>18.8</td>
<td></td>
</tr>
<tr>
<td>Esophagitis</td>
<td>16</td>
<td>10.3</td>
<td>11</td>
<td>68.8</td>
<td>5</td>
<td>31.3</td>
<td></td>
</tr>
<tr>
<td>Gastric adenocarcinoma</td>
<td>2</td>
<td>1.3</td>
<td>2</td>
<td>100.0</td>
<td>0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Gastric atrophy</td>
<td>13</td>
<td>8.4</td>
<td>5</td>
<td>38.5</td>
<td>8</td>
<td>61.5</td>
<td></td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>3</td>
<td>1.9</td>
<td>2</td>
<td>66.7</td>
<td>1</td>
<td>33.3</td>
<td>0.339</td>
</tr>
<tr>
<td>Gastritis</td>
<td>83</td>
<td>53.5</td>
<td>56</td>
<td>67.5</td>
<td>27</td>
<td>32.5</td>
<td></td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>4</td>
<td>2.6</td>
<td>2</td>
<td>50.0</td>
<td>2</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>Normal exam</td>
<td>17</td>
<td>11.0</td>
<td>13</td>
<td>76.5</td>
<td>4</td>
<td>23.5</td>
<td></td>
</tr>
<tr>
<td>Xanthelasma</td>
<td>1</td>
<td>0.6</td>
<td>0</td>
<td>100.0</td>
<td>0</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>
This observation suggests that long-lasting periods of exposure to \textit{H. pylori} may provoke tissue damage. An association of infection with the development of gastric adenocarcinoma was identified in this study\cite{33}. However, no significant associations were found regarding sex, age, endoscopy diagnosis\cite{34} and educational levels\cite{35}. Our study cohort was primarily composed of patients with low levels of education since the population served by public health system exhibit lower socioeconomic conditions\cite{36}.

\textit{H. pylori} positive patients showed a higher frequency of gastritis (67.5\%) compared with uninfected patients (32.5\%). Depending on the severity and distribution of gastritis, the infection may result in hypo or hyperchlorhydria. Therefore, \textit{H. pylori} infections are linked premature development of gastric and duodenal ulcers, which may progress to gastric adenocarcinoma\cite{37}.

According to the risk criteria, 52.3\% of patients were classified as having high risk developing gastric adenocarcinoma; of these, 8.2\% were infected by \textit{H. pylori}. Other risk factors, such as smoking and alcohol consumption are facilitators of \textit{H. pylori} infection and consequently decrease the carcinogenesis threshold\cite{38}. Thus, it is inconclusive whether the results for risk stratification may determine the likeliness of a patient developing gastric cancer, even if this infection is a determinant factor in 60 to 70\% of cases\cite{39}.

The prevalence of gastric adenocarcinoma in this study (1.3\%) is in accordance with the low percentages found in other institutions in Brazil. In Teresina (Piauí State), Campelo e Lima (2012) observed a prevalence of 3.8\% (about 3.7 cases per year). In Santa Maria (Rio Grande do Sul State), Ramppazzo et. al. (2012) mainly in Japan and China. In Brazil, gastric cancer is the third most common cancer in males and the fifth most common cancer in females. Rio Grande do Sul state, in Southern Brazil, has similar figures. The main histological type of gastric cancer is adenocarcinoma. OBJECTIVE: To assess the trends of this cancer over 25 years in a reference center in central Rio Grande do Sul. METHODS: We reviewed the records of upper gastrointestinal endoscopies performed at the University Hospital of Santa Maria, RS, between 1986 and 2010. We evaluated the incidence, age and gender distribution, anatomical subsite and histological subtype of gastric cancer throughout this 25-year period. RESULTS: We identified histologically confirmed primary gastric adenocarcinoma in 335 (1.6\%) identified that 1.6\% of patients had gastric cancer. Although the cases of gastric tumors are increasing and are linked to \textit{H. pylori} infections, there is a lack of screening policies and secondary prevention approaches in Brazil. In contrast, developed countries such as Japan have public health policies aiming (within 50 years) to eradicate \textit{H. pylori} infections and thereby, eliminate one of the risk factors for gastric adenocarcinoma development\cite{37}.

\textbf{CONCLUSION}

This study identified high rates of \textit{H. pylori} infection in a cohort from Brazil. The findings suggest that these infections are of clinical relevance for gastric adenocarcinoma development. In this scenario, there is an urgent need for implementing public policies and effective clinical prevention practices aiming at (a) controlling the transmission of \textit{H. pylori} (b) avoiding long-term risk gastric malignancy development and (c) reducing public health system costs.

\textbf{Authors’ contributions}

Borges SS: made all the statistical analyzes and wrote the scientific work. Ramos AFPL: made the DNA extraction. Moraes Filho AV and Braga CASB: helped in scientific writing. Carneiro LC: helped in DNA amplification. Barbosa MS: oriented in the statistical analyses and guided in the article writing.

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Borges SS, Ramos AFPL, Moraes Filho AV, Braga CASB, Carneiro LC, Barbosa MS. Prevalência do adenocarcinoma gástrico nesse estudo foi de 1,3% e dentre os pacientes positivos para a infecção bacteriana seis (8,2%) possuem alto risco e 67 (91,8%) baixo risco de desenvolver esse tipo de câncer (P<0,05).

Conclusão – Esse estudo mostra uma alta prevalência da infecção por H. pylori na população estudada e identifica sua intrínseca contribuição para infeções gástricas, que a longo prazo se manifestam em pacientes com alto risco para o desenvolvimento de adenocarcinoma gástrico.


REFERENCES