Different risk factors influence peptic ulcer disease development in a Brazilian population

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RESULTS: Of 1466 patients submitted to endoscopy, 1060 (72.3%) presented CG [male/female = 506/554; mean age (year) ± SD = 51.2 ± 17.81], 88 (6.0%) presented DU [male/female = 54/34; mean age (year) ± SD = 51.3 ± 17.12] and 75 (5.1%) presented GU [male/female = 54/21; mean age (year) ± SD = 51.3 ± 17.12] and were included in the comparative analysis. Sex and age showed no detectable effect on CG incidence (overall $\chi^2 = 30.5$, $P = 0.0058$) but not age (OR = 0.9929, $P = 0.3423$). Sex [Odds ratios (OR) = 1.8631, $P = 0.0058$] but not age (OR = 0.9929, $P = 0.2699$) was associated with DU and both parameters had a highly significant effect on GU (overall $\chi^2 = 2.1$, $P = 0.0324$). The histopathological results showed a significant contribution of ageing for both atrophy (OR = 1.0297, $P < 0.0001$) and intestinal metaplasia (OR = 1.0520, $P < 0.0001$). Presence of H. pylori was significantly associated with decreasing age (OR = 0.9827, $P < 0.0001$) and with the incidence of GU (OR = 3.6077, $P < 0.0001$). The prevalence of m1 in DU was statistically significant (OR = 2.3563, $P = 0.0018$) but not in CG (OR = 2.678, $P = 0.0863$) and GU (OR = 1.520, $P = 0.2863$).

CONCLUSION: In our population, male gender was a risk factor for PU; ageing for GU, atrophy and metapla-
Helicobacter pylori (H. pylori), a Gram-negative microaerophilic bacterium, is associated with a broad spectrum of digestive tract diseases such as chronic gastritis, gastric and duodenal ulcers, gastric cancer, and lymphoproliferative disorders. H. pylori infection prevalence and clinical outcome of the colonized patients varies according to several considerations including bacterial factors and host and environmental characteristics such as age, ethnic group, genera, geography and socioeconomic conditions.

The role of H. pylori in the interaction with the host has an impact on the pattern and severity of gastritis and its clinical outcome. The physiologic mechanisms involved in these H. pylori-induced pathological differences are still unknown; however, one of the major bacterium virulence factors, the vaculating cytotoxin A (vacA), seems to be involved. The vacA protein encoded by the polymorphic H. pylori vacA gene, is produced and secreted by all bacterium strains and induces the formation of intracellular vacuoles in epithelial cell lines in vitro. Polymorphism of the vacA gene is distributed in three principal regions: the signal (s), intermediate (i), and middle (m) regions, each being divided in two main types, numbered 1 and 2, which are differently associated with several mechanisms of pathogenicity in vitro and in vivo. The s1, i1, and m1 types have been shown to be independently associated with more severe forms of H. pylori-induced diseases.

Studies conducted in several countries have shown that type 1 and 2 alleles of vacA polymorphisms are both widespread in all populations examined, except in the Japanese, among whom type 2 alleles are rare. Thus, outside Asia, vacA-type 1 and cagA-positive H. pylori strains are more frequently associated with severe H. pylori-induced peptic ulcer diseases than vacA-type 2 cagA-negative bacterium strains. In Brazil, a country of continental dimensions, this association has been observed in children but is controversial in the adult population. Environmental and demographic data also interfere with the pathophysiology of H. pylori-associated gastric diseases. In the adult population of Brazil, H. pylori infection can range from 60%-90%. In Marilia, a city of São Paulo State, the serological prevalence of H. pylori determined in blood donors was 57% and a risk factor associated with IgG and/or IgA H. pylori antibodies was low educational level. Considering the large geographical dimensions of Brazil, with its regionally specific socioeconomic and cultural conditions reflected by the high and variable prevalence of H. pylori, there are few epidemiological studies on gastric diseases.

So far, all comparative Brazilian studies on gastric disease epidemiology, H. pylori prevalence and vacA gene mosaicism have generally been carried out on small populations and on H. pylori strains isolated in culture. Therefore, considering that gastric and duodenal ulcer diseases depend on different physiological trigger mechanisms, we comparatively investigated the role of age, gender, histopathologic antral gastric alterations and the H. pylori status, including the vacA m region mosaicism detected directly in biopsies, as risk factors for gastric ulcer (GU), duodenal ulcer (DU) and chronic gastritis (CG) in patients consecutively attending at Hospital das Clínicas of Marilia, São Paulo, Brazil, during a period of four years.

MATERIALS AND METHODS

Patients

Adult patients (n = 1466) resident in Marilia city, São Paulo State, Brazil, aged 19 to 91 years, underwent esophagogastroduodenoscopy (EGD) for upper abdominal pain or dyspeptic symptoms from January 2003 through December 2006 at the Gastroenterology Outpatient Clinic of the Hospital das Clínicas of Marilia Medical School. All who presented GU, DU (by endoscopy) and/or CG (by histology), were enrolled in this study.

Endoscopy and biopsies

The EGD was accomplished by fibroendoscope (GIF-XP20, GIF-QX20) or video-endoscope (GIF-100), both from Olympus Medical Systems, Shinjuku-ku, Tokyo, Japan. Gastric or duodenal ulcer diagnosis was defined directly in biopsies, as risk factors for gastric ulcer (GU), duodenal ulcer (DU) and chronic gastritis (CG) in patients consecutively attending at Hospital das Clínicas of Marilia, São Paulo, Brazil, during a period of four years.

Histology

One antral specimen was fixed in 40 g/L of formaldehyde and embedded in paraffin. Sections were Giemsa stained for H. pylori evaluation and were stained with he-
Suzuki RB et al. Peptic ulcer disease epidemiology in Brazil

**Statistical analysis**

Incidences of CG, DU, and GU in patients submitted to endoscopy were investigated using age (in years) and sex as independent factors. Only for patients who developed at least one of these diseases, the role of age, sex, CG, GU, and DU were verified as independent factors to evaluate the presence of atrophy, intestinal metaplasia, *H. pylori* and *vacA* m1 and m2 genotypes, each assessed individually. Incidences were coded as “1” and absences (of evidence) as “0” (males were also coded as “1”, females as “0”). Cases without the complete records needed for each analysis were discarded. Multivariable screenings were performed by additive logistic regression models for detection of significant effects of the independent factors on each response variable. New models were made after discarding irrelevant variables and significant parameters of these last models were used to describe the relationship among factors and responses by means of logit functions. Critical P-values were considered after a Bonferroni correction based on the number of similar tests. All logistic regression analyses were performed using a free on-line device for Logistic Regression calculation provided by Pezzulo (2012).\(^{[6]}\)

**RESULTS**

Among 1466 patients submitted to endoscopy, 1060 (72.3%) presented CG [male/female = 506/554; mean age (year) ± SD = 51.2 ± 17.81], 88 (6.0%) presented DU [male/female = 54/34; mean age (year) ± SD = 51.4 ± 17.14], and 75 (5.1%) presented GU [male/female = 54/21; mean age (year) ± SD = 51.3 ± 17.12], and were included in the comparative analysis. More than one of these diseases was presented by 120 (8.2%) individuals and 369 (25.2%) patients were free of them (Figure 1). Most of the other endoscopic and histopathologic alterations were related to gastroesophageal tract diseases (data not shown). Mean age and gender of the included CG, GU and GC patients are summarized.

Distribution of atrophy, intestinal metaplasia, *H. pylori* histological diagnosis and *vacA* in region mosaicism in patients presenting CG, DU and GU are summarized in Figure 1.

Atrophy and intestinal metaplasia were investigated among antral gastric biopsies of 1020 and 1094 patients, respectively, with 243 (23.8%) positive for atrophy and 140 (12.8%) for intestinal metaplasia.

Detection of *H. pylori* was performed directly from biopsy specimens by two different tests: histology and RUT. Histology is the gold standard *H. pylori* diagnostic test employed in our clinical routine which together with histopathological analysis is used to decide for *H. pylori* eradication therapy. RUT showed a very low positive predictive value for *H. pylori*-associated gastric diseases and a high discrepancy when compared to histology; consequently these data were excluded from the study (data not shown). Among 1045 patients investigated for *H. pylori* by histology, 539 (51.6%) were positive. Among 668 biopsies of patients positive for RUT in-

![Figure 1](image_url)  
**Figure 1** Incidence of chronic gastritis, duodenal ulcer and gastric ulcer disease and distribution of the investigated independent variables. A: Incidence of gastric diseases in patients who underwent endoscopy (n = 1466); B: Distribution of the histopathological parameters of atrophy (n = 1020); C: Intestinal metaplasia (n = 1094); D: Positive histological diagnosis of *Helicobacter pylori* (*H. pylori*) (n = 1045); E: *H. pylori* vacA m1 genotypes (n = 668); F: *H. pylori* vacA m2 genotypes (n = 668). CG: Chronic gastritis; DU: Duodenal ulcer; GU: Gastric ulcer; vacA: Vacuolating cytotoxin A. Values outside circles are negative tests.
investigated for vacA m region mosaicism by PCR directly on biopsy specimens, 484 were positive, with 251 (51.8%) of m1 and 233 (48.2%) of m2 genotypes.

Results of the additive logistic regression models to evaluate the contribution of age and sex to the incidence of CG, DU and GU among patients who underwent gastroscopy are summarized in Table 1. Sex and age showed no detectable effect on CG incidence (overall \( \chi^2 = 2.1, P = 0.3423 \)). Sex (OR = 1.8631, \( P = 0.0058 \)) but not age (OR = 0.9929, \( P = 0.2699 \)) affected DU, and both parameters had a highly significant association with GU (overall \( \chi^2 = 30.5, P < 0.0001 \)), with contributions of both coefficients (age OR = 1.0233, \( P = 0.0017 \); sex OR = 3.0790, \( P < 0.0001 \)). A posterior test for the age/sex interaction showed it to be nonsignificant (OR = 0.9888, \( P = 0.4844 \)). The incidence of GU increased with age and further, at a given age, males had a higher probability of developing GU (Figure 2).

Additive logistic regression models were constructed to investigate the contribution of age, sex, CG, DU and GU to atrophy, intestinal metaplasia, \( H. pylori \) presence and vacA m1 and m2 mosaicism (Table 2).

The results for histopathological parameters showed a significant contribution of age for both atrophy (OR = 1.0297, \( P < 0.0001 \)) and intestinal metaplasia (OR = 1.0520, \( P < 0.0001 \)). Atrophy incidence increased with age (Figure 3) and a new model with only age as the independent factor also had a highly significant fit (overall \( \chi^2 = 35.2, P < 0.0001 \); age OR = 1.0273, \( P < 0.0001 \)) though more parsimonious (Table 2). In the case of intestinal metaplasia, it was necessary to check for the contribution of CG, which was discarded after the Bonferroni correction. A new model with only age as the independent factor also had a highly significant fit (overall \( \chi^2 = 71.7, P < 0.0001 \); age OR = 1.0509, \( P < 0.0001 \)) though more parsimonious (Table 2). Intestinal metaplasia increased with age, but at a lower level than atrophy for a given age (Figure 3).

Presence of \( H. pylori \) was significantly associated with age (OR = 0.9827, \( P < 0.0001 \)) and occurrence of DU (OR = 3.6077, \( P < 0.0001 \)) (Table 3). A new model with only age and DU as independent factors also had a highly significant fit (overall \( \chi^2 = 47.8, P < 0.0001 \); age OR = 0.9826, \( P < 0.0001 \); DU OR = 3.5063, \( P < 0.0001 \)) but was more parsimonious (Table 3). An additional model showed that age/DU had no effect on the presence of \( H. pylori \) (OR = 0.0085, \( P = 0.6142 \); DU OR = 1.0085, \( P = 0.9758 \); DU+ = 1.0423). \( H. pylori \) incidence decreased with age and DU incidence contributed to higher probabilities in developing detectable levels of the bacterium, despite individual age (Figure 4).

The five independent factors studied for the \( H. pylori \) vacA m1 and m2 genotypes resulted in nonsignificant ad-
Table 1 Additive logistic regression models to evaluate the contribution of age and sex among patients who underwent gastroscopy (n_total = 1466)

| Factor | CG | DU | GU |
|--------|----|----|----|
| Overall | n (+) | 1060 | 88 | 75 |
| χ² (v = 2) | 2.14 | 8.99 | 30.51 |
| P | 0.3425 | 0.0112 | < 0.0001¹ |
| β | 0.738 | -2.724 | -4.836 |
| β | 0.003 | -0.007 | 0.023 |
| SE | 0.0034 | 0.0065 | 0.0073 |
| P | 0.3511 | 0.2699 | 0.0017² |
| OR | 1.003 | 0.993 | 1.023 |
| CI- | 0.997 | 0.980 | 1.009 |
| CI+ | 1.010 | 1.006 | 1.038 |
| β | 0.1124 | 0.622 | 1.125 |
| SE | 0.1102 | 0.2256 | 0.2636 |
| P | 0.3598 | 0.0038 | < 0.0001¹ |
| OR | 1.132 | 1.863 | 3.079 |
| CI- | 0.912 | 1.197 | 1.837 |
| CI+ | 1.405 | 2.899 | 5.162 |

¹Significant effects. n_total: Number of investigated cases; n (+): Number of positive cases; CG: Chronic gastritis; DU: Duodenal ulcer; GU: Gastric ulcer; OR: Odds ratios; CI: Confidence intervals. Critical P-values were adopted after a Bonferroni correction for the number of similar tests: P = 0.05/3 = 0.0167 for overall models; and P = 0.05/2 = 0.025 for each independent factor.

Table 2 Logistic regression models to evaluate the contribution of age, sex, chronic gastritis, duodenal ulcer

| Factor | Atrophy | Metaplasia | H. pylori | m1 | m2 |
|--------|---------|-----------|-----------|----|----|
| Overall | n (+) | 1020 | 1094 | 1045 | 668 | 668 |
| χ² | 35.18 | 71.67 | 47.83 | 9.88 | 5.85 |
| v | 1 | 1 | 2 | 1.00 | 1 |
| P | < 0.0001¹ | < 0.0001¹ | < 0.0001¹ | 0.0017 | 0.0156 |
| β | -2.614 | -4.733 | 0.881 | -0.589 | -0.038 |
| β | 0.027 | 0.050 | -0.038 | 0.3511 | 2.065 |
| SE | 0.0047 | 0.0063 | 0.0039 | - | 0.0048 |
| P | < 0.0001¹ | < 0.0001¹ | < 0.0001¹ | - | 0.0162 |
| OR | 1.027 | 1.051 | 0.983 | - | 0.989 |
| CI- | 1.018 | 1.038 | 0.975 | - | 0.979 |
| CI+ | 1.037 | 1.064 | 0.990 | - | 0.998 |
| DU | β | - | - | 1.255 | 0.857 |
| SE | - | - | 2.072 | 0.279 |
| P | - | - | < 0.0001¹ | 0.0018 |
| OR | - | - | 3.506 | 2.356 |
| CI- | - | - | 2.065 | 1.377 |
| CI+ | - | - | 5.954 | 4.031 |

¹Significant effects after Bonferroni correction. n_total: Number of investigated cases; n (+): Number of positive cases; CG: Chronic gastritis; DU: Duodenal ulcer; OR: Odds ratios; CI: Confidence intervals; H. pylori: Helicobacter pylori.

DISCUSSION

In order to verify the differential contribution of age, gender, histopathological outcome, H. pylori and vacA m region mosaicism with incidence of PU and CG, we investigated comparatively all cases of DU (88), GU (75) and CG (1060) found after analysis of 1466 patients consecutively submitted to gastroscopy in Hospital das Clinicas of Marilia, São Paulo, Brazil, over four years.

The most prevalent gastric disease found in our consecutive dyspeptic patients was CG (72.3%) followed by gastroesophageal alterations not included in this study. Peptic ulcer disease was prevalent in 11.1% of the patients, with 6.0% of GU and 5.1% of DU. Male gender had a statistically significant association with both PU incidences. Our results are in accordance with research done in the southern region of Brazil where DU and GU had a similar prevalence and were associated with male gender ³⁴. However, in a recent large scale PU epidemiological study carried out in a tertiary care hospital in another city of São Paulo State, Brazil, the prevalence of DU was four times higher than GU³⁷ and in this population there was a significant predominance of woman in the PU group³⁸. These regional differences reinforce the specificity of risk factors associated with severe gastric diseases and the need to perform local investigations to improve health care strategies.

There are differences in the gastrointestinal physiological modifications that lead to gastric and duodenal ulcers, whose causes are multifactorial and also related to population characteristics and to specific gastroduodenal alterations due to association of H. pylori with host mucosa³⁹. In our study, increasing age was a risk factor for the development of GU. The mean age of GU and DU patients in the Brazilian ulcer study performed in a hospital in São Paulo also differed significantly, being higher in GU³⁹. Thus, more epidemiological studies have to be done in order to identify the risk factors associated with age and the onset of GU in the Marilia population.

The eradication of H. pylori infection cured both gastric and duodenal ulcers, and the cure rates are similar, suggesting that H. pylori is the key factor in peptic ulcer diseases independent of the ulcer site⁴⁰. However, a number of studies have shown the participation of other risk factors such as smoking, alcohol intake, and nonsteroidal antiinflammatory drug (NSAID) use in the etiology of PU, which are principally associated with GU⁴¹,⁴². In our study the prevalence of H. pylori infection observed by histology was significantly higher in DU than in GU and CG (P < 0.0001). Also, in the Danish epidemiologic PU study, IgG against H. pylori was higher in DU (87.2%) when compared to GU (60%) patients. In another city of São Paulo, there was also
β increased in adult old-age groups, even when growth conditions.

β m1 genotype are more frequently associated with the development of gastric cancer, suggesting that m1 strains of H. pylori are more pathogenic. In Brazil, the involvement of vacA gene mosaicism with gastric diseases in adults is controversial [51,52]; in all these studies the size of investigated patient populations has been small and bacterium strains were isolated before genotyping which can cause a bias due to selection pressure by in vitro growth conditions. Our work was performed during a period of four years and the investigation of vacA m region mosaicism was done directly on biopsy specimens by PCR. Our results are the first in Brazil to find association of vacA m1 allele specific to adult DU patients when compared to GU and CG (P < 0.0018), indicating that in our region high prevalence of H. pylori and the strains harboring the vacA m1 genotype are more frequently associated with the development of DU.

Nowadays, PU remains the cause of significant morbidity, especially in older age groups, representing an important world health problem [53]. Its etiology in either the stomach (GU) or duodenum (DU) is multifactorial and depends on the interplay of a gastritis phenotype and of physiological gastroduodenal alterations as a result of infection with Helicobacter pylori [54].

Polymorphisms in the m region of the H. pylori vacA gene affect the cell tropism of the toxin [55]; the m1 type of vacA shows toxicity toward a broader range of cells than the m2 type [56]. In Asia, where there is a high predominance of s1 allele in the s region polymorphism of the vacA gene, the vacA m region mosaicism shows a variation within East Asia [57], with m1 strains being more prevalent in regions where there is a higher prevalence of gastric cancer, suggesting that m1 strains of H. pylori are more pathogenic. In Brazil, the involvement of vacA gene mosaicism with gastric diseases in adults is controversial [51-53]; in all these studies the size of investigated patient populations has been small and bacterium strains were isolated before genotyping which can cause a bias due to selection pressure by in vitro growth conditions. Our work was performed during a period of four years and the investigation of vacA m region mosaicism was done directly on biopsy specimens by PCR. Our results are the first in Brazil to find association of vacA m1 allele specific to adult DU patients when compared to GU and CG (P < 0.0018), indicating that in our region high prevalence of H. pylori and the strains harboring the vacA m1 genotype are more frequently associated with the development of DU.

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Table 3 Additive logistic regression models to evaluate the contribution of age, sex, chronic gastritis, duodenal ulcer and gastric ulcer disease

| Factor  | Atrophy | MetaPlasia | H. pylori |
|---------|---------|-----------|-----------|
| Overall | Overall | Overall   | Overall   |
| Age     | Age     | Age       | Age       |
| Sex     | Sex     | Sex       | Sex       |
|CG       | CG      | CG        | CG        |
|DU       | DU      | DU        | DU        |
|GU       | GU      | GU        | GU        |

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| Age     | Age     | Age       | Age       |
| Sex     | Sex     | Sex       | Sex       |
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|GU       | GU      | GU        | GU        |

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| Age     | Age     | Age       | Age       |
| Sex     | Sex     | Sex       | Sex       |
|CG       | CG      | CG        | CG        |
|DU       | DU      | DU        | DU        |
|GU       | GU      | GU        | GU        |

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| Factor  | Atrophy | MetaPlasia | H. pylori |
|---------|---------|-----------|-----------|
| Overall | Overall | Overall   | Overall   |
| Age     | Age     | Age       | Age       |
| Sex     | Sex     | Sex       | Sex       |
|CG       | CG      | CG        | CG        |
|DU       | DU      | DU        | DU        |
|GU       | GU      | GU        | GU        |

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| Factor  | Atrophy | MetaPlasia | H. pylori |
|---------|---------|-----------|-----------|
| Overall | Overall | Overall   | Overall   |
| Age     | Age     | Age       | Age       |
| Sex     | Sex     | Sex       | Sex       |
|CG       | CG      | CG        | CG        |
|DU       | DU      | DU        | DU        |
|GU       | GU      | GU        | GU        |

Significant effects. n(+) Number of investigated cases; n (*): Number of positive cases; CG: Chronic gastritis; DU: Duodenal ulcer; GU: Gastric ulcer; OR: Odds ratios; CI: Confidence intervals; H. pylori: Helicobacter pylori. Critical P-values were adopted after a Bonferroni correction for the number of similar tests: P = 0.05/5 = 0.01 for overall model and for each independent factor.

found to be a higher prevalence of H. pylori in DU (64%) than in GU (57%) patients [17]. These results suggest the participation of a major number of risk factors not associated with H. pylori involved in the development of GU rather than DU. Moreover, a very important and large scale follow-up study of ulcer patients performed in Europe [18] showed that GU can be a risk factor in the development of gastric cancer, while in DU this relationship was inversely observed, which suggests that GU and gastric cancer have etiologic factors in common that are not found in DU. As a consequence of H. pylori gastric mucosa colonization, gastric acid secretion is altered with it being induced or impaired in response to the release of factors produced or induced by the bacterium, resulting in different topographic phenotypes of gastritis and the presence of atrophy. Gastritis associated with atrophy in the corpus is accompanied by hypochlorhydria and carries the highest risk for GU and cancer, whereas hyperchlorhydria produced by gastritis in the antrum predisposes to DU [19,44]. In our patients, antrum atrophy was more prevalent in DU than in GU, corroborating the hypothesis that DU can be a result of antral gastric atrophy. However, increasing age and not H. pylori presence was significantly related to the occurrence of atrophy and intestinal metaplasia. These results are in agreement with an epidemiological study performed in a rural population of Korea [45] and also in previous gastric physiological studies which have demonstrated the association of atrophic gastritis with increasing age [46,47].

In Northern Peninsular Malaysia, the prevalence of H. pylori increased in adult old-age groups, even when old and geriatric adults were compared [48]. In our investigated population, H. pylori incidence decreased significantly with age (Figure 4). These results can be indicative of a populational specific characteristic associated with the clearing of H. pylori during the course of a chronic infection, or can be related to the diagnosis of H. pylori in antrum biopsies since the expression of gastritis in the antrum, but not in the cardia or corpus, seems to decrease with age [49]. New research is necessary to answer these questions.
were associated with DU while older age at disease commitment was associated with GU. Thus, in spite of the few large scale Brazilian studies on epidemiological characteristics of gastric diseases with stratification of PU in GU and DU, we find high regional variation, indicating that local population investigation has to be carried out in order to improve treatment and prevention of severe gastric diseases.

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Suzuki RB et al. Peptic ulcer disease epidemiology in Brazil

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