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ROLE OF *HELICOBACTER PYLORI* INFECTION AND LIFESTYLE HABITS IN THE DEVELOPMENT OF GASTRODUODENAL DISEASES IN A POPULATION FROM THE BRAZILIAN AMAZON

Ruth Maria Dias Ferreira **VINAGRE**[1], Adenielson **VILAR-e-SILVA**[2], Amanda Alves **FECURY**[2] and Luisa Caricio **MARTINS**[2]

ABSTRACT – ***Context*** – Although more than half of the world's population is colonized with *Helicobacter pylori*, it remains unknown why this organism is able to produce severe disease in some hosts and be innocuous in others. The clinical outcome of infection is determined by several factors, including differences in the host response to bacterial stimulation, specific virulence factors of the organism and environmental influences, or a combination of these factors. ***Objectives*** – This study compared the prevalence of *H. pylori* infection and risk factors (infection with CagA+ strains, excessive alcohol consumption, smoking, and inadequate eating habits) between patients with different gastrointestinal disorders and associated these risk factors with the histopathological findings. ***Methods*** – In a prospective study, samples were collected from 442 patients and a standardized questionnaire regarding lifestyle habits (excessive alcohol consumption, smoking, and eating habits) was applied. The presence of *H. pylori* and of the cagA gene was investigated by polymerase chain reaction (PCR). Gastric biopsies were obtained for histological assessment. ***Results*** – The frequency of alcohol consumption, smoking, inadequate diet and infection with CagA+ *H. pylori* was higher among patients with peptic ulcer and adenocarcinoma when compared to those with gastritis. Gastric inflammation was more pronounced in patients infected with CagA+ strains. ***Conclusion*** – We conclude that infection with CagA+ *H. pylori* strains, excessive alcohol consumption, smoking and inadequate eating habits increase the risk of developing peptic ulcer and gastric carcinoma.

HEADINGS - *Helicobacter pylori*. Gastrointestinal diseases. Adenocarcinoma. Peptic ulcer. Amazonian ecosystem.

INTRODUCTION

Helicobacter pylori colonizes the human stomach mucosa, causing chronic gastritis, and is an important risk factor for the development of peptic ulcers and gastric carcinoma[21]. However, only some infected individuals develop severe diseases[16]. Various factors, particularly bacterial virulence factors, have been studied in an attempt to explain this clinical diversity in *H. pylori* infection[11]. One virulence factor is cytotoxin-associated gene A (CagA), an immunodominant protein with a high molecular weight (140-160 kDa) which is encoded by the cagA gene present on the cag pathogenicity island. The CagA protein is injected into gastric epithelial cells by a type IV secretion system and induces rearrangements of the actin cytoskeleton that alter the morphology and function of epithelial cells[20]. Studies have demonstrated a high frequency of anti-CagA IgG antibodies in patients with atrophic gastritis, peptic ulcers, and gastric carcinoma[4, 27].

The combined presence of adverse environmental factors, such as unhealthy lifestyle habits of the patients, and *H. pylori* infection has been associated with progression to more severe gastroduodenal disease. Excessive alcohol consumption, smoking and inadequate eating habits, such as excess salt, fats and canned products, have been related to the development of peptic ulcers and stomach cancer[12, 18, 24].

The objective of the present study was to compare the prevalence of *H. pylori* infection and risk factors (bacterial virulence factors and lifestyle habits) between patients with different gastrointestinal diseases and to associate these risk factors with the histopathological findings.

METHODS

Patients

Gastric biopsy samples were collected from 442 patients with different gastrointestinal disorders seen

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[1] Departamento de Clínica Médica, Hospital Ophir Loyola, Belém, PA, Brasil; [2] Laboratório de Clínica e Patologia de Doenças Tropicais da Universidade do Pará, Belém, PA, Brasil. Correspondence: Luisa Caricio Martins. Trav. Mauriti, 3269, ap. 704 B, Bairro: Marco - 66095-360 – Belém, PA, Brasil. E-mail: luisacaricio@uol.com.br

at the Endoscopy Department of the Ofir Loiola Hospital between 2010 and 2011.

Four biopsy fragments were obtained from the stomach of each patient during endoscopy: two from the gastric lesion for histological analysis and two from the antrum for analysis by molecular methods. In cases suggestive of malignancy, biopsies were obtained from the cancer lesion and the adjacent area (perilesion) for histological analysis. None of the patients had received antimicrobial drugs, H2-receptor antagonists, acid pump inhibitors, nonsteroidal anti-inflammatory drugs, or any medication for at least 60 days prior to endoscopy.

All patients included in the study were of the same socioeconomic level, had similar cultural habits, were born in the state of Pará, and had the same ethnic background (approximately 50% Portuguese, 40% Amerindian, and 10% African)[3]. The study was approved by the Ethics Committee of the Nucleus of Tropical Medicine, Federal University of Pará.

Evaluation of lifestyle habits

A standardized questionnaire regarding lifestyle habits (excessive alcohol consumption, smoking, and eating habits) was used. Average drinking levels were evaluated to calculate the amount of alcohol consumed: sugarcane rum = 40%; beer = 5%; wine = 12%; other distilled beverages = 55%. The threshold of alcohol consumption was standardized by calculating average daily intake in grams, with an acceptable dose of 70 g alcohol per week for women and 140 g for men[7].

Smoking intensity was defined as the total number of cigarettes consumed per day, with each hand-rolled cigarette being equivalent to five manufactured cigarettes[17]. Subjects smoking more than five cigarettes per day were classified as smokers and those smoking fewer than five cigarettes per day or did not smoke at all were classified as non-smokers. The patients were also divided into two groups according to age (>50 years and <50 years).

Dietary habits were classified as inadequate when the subjects reported to consume large amounts of salty, fatty and canned foods and low amounts of fruits and vegetable, and as adequate when the subjects reported to frequently consume fruits and vegetables and to rarely consume fatty, salty and canned foods.

Extraction of DNA and PCR

Gastric fragments were collected from the antrum for histopathological analysis and extraction of bacterial DNA[14]. Total DNA was extracted from frozen gastric biopsy specimens by adding 10 µl proteinase K and 300 µL lysis buffer (200 mM Tris-HCl, 25 mM EDTA, 300 mM NaCl, 1.2% sodium dodecyl sulfate) and incubating the mixture for 12 h at 55°C. The lysate was extracted with an equal volume of phenol-chloroform, precipitated with isopropanol, and washed with 70% ethanol. The pellet was dried and resuspended in 200 µL sterile distilled water. Extracted DNA was stored at - 20°C until the time for analysis.

A set of primers (p1 and p2)[10] amplifying a fragment of 298 bp of the urease gene present in all *H. pylori* strains was used to detect bacterial DNA. Only positive samples were used for amplification of the cagA gene. The protocol and primers described by Tumuru et al.[25] were used for amplification of the cagA gene. Negative and positive controls were included in all reactions. The PCR products were submitted to electrophoresis on 2% agarose gel stained with ethidium bromide and visualized under a UV transilluminator.

Histopathological analysis

The gastric biopsies were stained with hematoxylin-eosin for histological analysis. The histopathological parameters were scored from 0 to 3 according to the Sydney classification system[6] for the analysis of polymorphonuclear and mononuclear cell infiltration.

Statistical analysis

Odds ratios and G-tests were used to evaluate the proportion of risk factors in the control and patient groups. The level of significance was set at 95%. Statistical analysis was performed using the Biostat 5.0 program[2].

RESULTS

Of the 442 patients with different gastrointestinal diseases studied, 45% (198/442) had gastritis, 17% (78/442) had gastric ulcer, 22% (96/442) had duodenal ulcer, and 16% (70/442) gastric adenocarcinoma (intestinal type). Among patients with gastritis, all had superficial gastritis based on the endoscopy report and a second histopathological analysis revealed chronic gastritis without atrophy in all patients.

Excessive alcohol consumption, smoking and inadequate eating habits were more frequent among patients with peptic ulcers and gastric adenocarcinoma compared to those with gastritis (Table 1). Male patients and those older than 50 years predominated in the group with gastric adenocarcinoma (Table 1).

Investigation of the presence of *H. pylori* infection showed a higher prevalence of the bacterium and of CagA-positive strains in patients with gastric ulcers, duodenal ulcers and gastric adenocarcinoma when compared to patients with gastritis. However, no difference in prevalence was observed between patients with peptic ulcers and gastric adenocarcinoma (Table 1).

Table 2 shows the association between histopathological findings and lifestyle risk factors in the patients studied. Excessive and prolonged smoking and inadequate dietary habits were associated with a high degree of inflammation and intense neutrophilia. No association was observed with excessive alcohol consumption.

The degree of inflammation and neutrophil activity was higher in patients infected with *H. pylori* than in uninfected subjects (Table 3). Similarly, patients infected with CagA positive strains presented a higher degree of inflammation and neutrophil activity than patients infected with CagA negative *H. pylori* strains (Table 3).

TABLE 1. Distribution of variables analyzed in patients with different gastrointestinal diseases

Variable	Gastritis (n = 198) (%)	Duodenal ulcer (n = 96) (%)	Gastric ulcer (n = 78) (%)	Gastric cancer (n = 70) (%)
Sex				
Male	89 (45)	60 (62.5)	48 (62)	50 (71)
Female	109 (55)	36 (37.5)	30 (38)	20 (29)
*P**		ns	ns	<0.05
Age				
>50 years	60 (30)	35 (36)	30 (38)	42 (60)
<50 years	138 (70)	61 (64)	48 (62)	28 (40)
*P**		ns	ns	<0.05
Alcohol consumption				
Yes	109 (55)	67 (70)	65 (83)	45 (64)
No	89 (45)	29 (30)	13 (17)	25 (36)
*P**		<0.05	<0.05	<0.05
Smoking				
Yes	81 (41)	72 (75)	55 (71)	45 (64)
No	117 (59)	24 (25)	23 (29)	25 (36)
*P**		<0.05	<0.05	<0.05
Diet				
Adequate	83 (42)	7 (7)	2 (3)	5 (7)
Inadequate	115 (58)	89 (93)	76 (97)	65 (93)
*P**	-	<0.05	<0.05	<0.05
H. pylori				
Positive	168 (85)	90 (94)	76 (97)	67 (96)
Negative	30 (15)	6 (6)	2 (3)	3 (4)
*P**	-	<0.05	<0.05	<0.05
CagA (*H. pylori* +)				
Positive	117 (59)	86 (89)	69 (88)	60 (86)
Negative	51 (26)	4 (6)	7 (9)	7 (10)
*P**	-	<0.05	<0.05	<0.05

G-test. **P* value. ns: not significant

TABLE 2. Association of the degree of inflammation and neutrophil activity with risk factors analyzed in patients with gastric diseases

Risk factor	Degree of inflammation		OR (95% CI)	Neutrophil activity		OR (95% CI)
	Low	High	*P* value	Low	High	*P* value
Smoking						
Yes	68 (27%)	185 (73%)	2.69 (1.80-4.00)	72 (17%)	181 (41%)	2.82 (1.90-4.19)
No	95 (50%)	94 (50%)	0.00	89 (20%)	100 (22%)	0.00
Alcohol consumption						
Yes						
No	106 (37%)	180 (63%)	1.02 (0.68-1.53)	99 (35%)	187 (65%)	0.76 (0.50-1.13)
	57 (36%)	99 (64%)	0.99	64 (41%)	92 (59%)	0.22
Diet						
Adequate	59 (60%)	38 (40%)	3.59 (2.25-5.74)	56 (57%)	41 (43%)	3.03 (1.91-4.82)
Inadequate	104 (30%)	241 (70%)	0.00	107 (31%)	238 (69%)	0.00

OR: odds ratio, 95% CI: 95% confidence interval

TABLE 3. Association of the degree of inflammation and neutrophil activity with the presence of *Helicobacter pylori* DNA and CagA in the group of patients with gastric diseases

Risk factor	Degree of inflammation		OR (95% CI)	Neutrophil activity		OR (95% CI)
	Low	High	*P* value	Low	High	*P* value
H. pylori						
Positive	136 (34%)	265 (66%)	3.75 (1.90-7.40)	138 (34%)	263 (66%)	2.42 (1.27-4.66)
Negative	27 (65%)	14 (35%)	0.00	23 (56%)	18 (44%)	0.00
CagA						
Positive	82 (25%)	250 (75%)	13.13 (6.80-25.23)	85 (25%)	247 (75%)	10.46 (5.61-19.50)
Negative	56 (81%)	13 (19%)	0.00	54 (78%)	15 (22%)	0.00

OR: odds ratio, 95% CI: 95% confidence interval

DISCUSSION

The clinical manifestations of *H. pylori* infection can vary from one region to another as a result of differences in the geographic distribution of *H. pylori* genotypes[26]. CagA, a cytotoxin produced by *H. pylori*, is a marker of bacterial virulence and is associated with gastric disorders such as peptic ulcer and gastric carcinoma in western countries[20, 22]. A high incidence of gastric carcinoma and peptic ulcer, as well as a high prevalence of *H. pylori* infection, is observed in Brazil, particularly in the state of Pará[1, 13, 27].

The frequency of the cagA gene observed in the patients studied here was higher than that reported in other studies conducted in different Brazilian states[4, 23]. Furthermore, the results showed an association between the presence of CagA and the development of peptic ulcer and gastric carcinoma. This finding agrees with other studies carried out in Brazil[14, 19, 27] and in other countries[8, 9, 16]. Noqueira et al.[15], studying Portuguese patients, found a significant association between the presence of the cagA gene and a higher degree of inflammation and neutrophil activity in gastric mucosa. In the present study, histopathological analysis also demonstrated an association between the presence of CagA and a high degree of inflammation and neutrophil activity in gastric mucosa.

The present study analyzed the presence of *H. pylori* infection and other risk factors in patients with gastroduodenal diseases. Smoking, alcohol consumption and inadequate dietary habits were the most frequent risk factors. Heavy and prolonged smoking and inadequate dietary habits were associated with a higher degree of inflammation and intense neutrophilia. However, no association was observed with excessive alcohol consumption. Epidemiological studies suggest that alcohol plays a fundamental role in the development of gastrointestinal diseases such as peptic ulcer and gastric cancer since it causes gastric mucosal injuries and stimulates parietal cells to produce gastric acid, in addition to its synergistic action with smoking[5, 29]. Inadequate dietary habits such as excess intake of salt, preservatives and smoked produced, as well as diets poor in vegetable and fruits, are also strongly associated with the development of gastric cancer[24, 28].

Food diaries have been used in studies investigating the association between diet and cancer in different populations in order to obtain nutritional information. However, this tool is limited since it is based on the information provided by the interviewed person and on the skills of the interviewer[24]. Careful analysis is therefore needed before attributing a carcinogenic or anticarcinogenic role to one or more dietary factors and studies on risk factors and cancer are potentially prone to errors. Nevertheless, despite the errors inherent to surveys of habitual food intake, these tools are important since they provide knowledge of dietary patterns and their temporal variation in different populations worldwide. In this respect, evidence indicates the negative influence of the modern western diet (high in fat and processed foods and low in fibers) on the development of various types of cancer in developed and developing countries[28].

The present results and those of other studies led us to conclude that infection with CagA-positive *H. pylori* strains, excessive alcohol consumption, smoking and inadequate eating habits increase the risk of developing peptic ulcer and gastric carcinoma.

Vinagre RMDF, Vilar e Silva A, Fecury AA, Martins LC. Papel da infecção por *Helicobacter pylori* e hábitos de vida no desenvolvimento das doenças gastroduodenais em uma população da amazônia brasileira. Arq Gastroenterol. 2013,50(3):170-4.

RESUMO – ***Contexto*** - Apesar de o *H. pylori* colonizar o estômago de aproximadamente metade da população mundial, ainda se desconhece por quê esse organismo é capaz de causar doença severa em certos hospedeiros e ser inofensivo em outros. As manifestações clínicas da infecção são determinadas por vários fatores, tais como: diferentes respostas do hospedeiro ao estímulo bacteriano, fatores de virulência específicos do organismo e influências ambientais ou a combinação desses fatores. ***Objetivos*** – Esse estudo tem como objetivo comparar a prevalência e a associação dos fatores de risco, tais como: infecção por cepas *H. pylori* CagA+, consumo excessivo de bebidas alcoólicas, uso de tabaco e hábitos inadequados de alimentação entre pacientes com diferentes doenças gastrointestinais e a associação deles com achados histopatológicos. ***Métodos*** – Em estudo prospectivo, foram coletadas amostras de 442 pacientes submetidos a um questionário padronizado com perguntas sobre hábitos de vida (uso excessivo de álcool, tabaco e, hábitos alimentares). A presença do *H. pylori* e do gene cagA foi detectada utilizando a Reação em Cadeia da Polimerase (PCR). As biópsias gástricas foram avaliadas histologicamente. ***Resultados*** – O consumo de bebida alcoólica, uso de tabaco, dieta inadequada e infecção por cepas *H. pylori* $CagA^+$ foram maiores entre pacientes com úlcera péptica e adenocarcinoma do que em pacientes com gastrite. Os pacientes infectados por cepas *H. pylori* $CagA^+$ apresentaram inflamação gástrica de maior intensidade. ***Conclusão*** – A presença de infecção por cepas *H. pylori* CagA+, o consumo excessivo de álcool, de tabaco e hábitos de alimentação inadequados aumentam o risco de desenvolvimento de úlcera péptica e carcinoma gástrico.

DESCRITORES - *Helicobacter pylori.* Gastroenteropatias. Adenocarcinoma. Úlcera péptica. Ecossistema amazônico.

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