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Prevalence of *Helicobacter pylori cag*A and *sab*A Genotypes in Patients with Gastric Disease

Jéssica Nunes Pereira[1], Wilson A. Orcini[1], Rita L. Peruquetti[1], Marilia A. C. Smith[2], Spencer L. M. Payão[1], Lucas T. Rasmussen[1*]

[1]Universidade do Sagrado Coração (USC), Bauru, Brasil
[2]Universidade Federal de São Paulo (UNIFESP), São Paulo, Brasil
Email: *lucasrasmussen@gmail.com

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Abstract

Gastric cancer is one of the most common types of cancer worldwide. *Helicobacter pylori* is considered one of the most important causes of this condition specially because of its virulence markers as *sab*A and *cag*A. Therefore, we aim to investigate the relation between these markers and the gastric diseases in 400 patients who underwent upper digestive endoscopy. To detect the bacteria and its genes by Polymerase Chain Reaction (PCR), the presence of *H. pylori* was significant when comparing the groups control *vs.* cancer (p value < 0.0001) OR [95% CI] 12.73 (5.45 - 29.69) and the groups control *vs.* chronic gastritis (p value < 0.0001) OR [95% CI] 12.99 (7.44 - 22.66). *cag*A was statistically significant considering its presence when comparing the chronic gastritis *vs.* cancer groups (p value = 0.0434) OR [95% CI] 2.44 (1.021 - 5.845). Associating both *sab*A and *cag*A, we found a statistically significant result (p value < 0.0001) OR [95% CI] 13.68 (3.95 - 47.33) considering the gastritis *vs.* cancer groups. *Helicobacter pylori* is directly associated to gastric diseases such as gastritis and cancer and its virulence markers: *sab*A and *cag*A increase the injury process to the gastric epithelium making the host more susceptible to cancer.

Keywords

Stomach Neoplasms, *Helicobacter pylori*, *sab*A, *cag*A

1. Introduction

Gastric cancer is one of the most common types of cancer worldwide and the third leading cause of cancer death. It is responsible for about 720,000 deaths per year around the world, and its occurrence is very frequently in Asian countries [1] [2]. The onset of cancer usually starts with lesions, which evolve into an in-

flammation that tends to be followed by chronic gastritis, gastric atrophy, and finally gastric cancer [3]. Its progression is related to genetic characteristics of the host, environmental factors, and the most important parameter: the presence of *Helicobacter pylori* infection as well as its genotype [4].

H. pylori is a Gram negative, microaerophilic bacterium that colonizes half of the world's population and whose niche is the stomach [4] [5]. Its infection is highly successful due to the interaction of this bacterium with the gastric epithelial cells, and for that reason; it is considered one of the most important causes of gastric cancer [6] [7] [8].

The sialic acid-binding adhesin (*sab*A) is an outer membrane protein that plays an important role in the first contact of *H. pylori* with the gastric tissue [9]. This marker determinates the initial and permanent colonization of the bacteria and directly influences the extent of damages by this pathogen. *sab*A acts in adhesion of *Helicobacter pylori* by binding to a specific receptor present in human stomach named sialyl-dimeric-Lewis x (Le^x) [10] [11] [12]. This binding characterizes a successful and more aggressive infection making the intense inflammation permanent in which susceptive lesions can eventually evolve into gastric cancer [13].

Another virulence marker of *H. pylori* is the *cytotoxin-associated gene* A known as *cag*A, which is related to gastric inflammation [12]. The genome of *H. pylori* includes a component called *cag*-PAI island, which encodes the type IV secretion system. This system perforates the cells of the gastric epithelium, allowing the bacteria to deposit in the host molecules, such as the *cag*A protein, which regulates the metabolism of these cells [14]. This marker is considered one of the most virulent because its presence is usually associated with the etiology of peptic ulcers and gastric cancer [10].

Considering the high occurrence of *H. pylori*, the present study aimed to investigate the relation of its genes *sab*A and *cag*A with gastric diseases and gastric cancer.

2. Material and Methods

This research was carried out over 2016-2017 at the Universidade do Sagrado Coração in Bauru—SP, Brazil, and counted with the collaboration of Hospital Estadual de Bauru and Faculdade de Medicina de Marília (FAMEMA) in Marília—SP, Brazil for the collection of samples. All the patients involved in this research signed a consent form. This study was approved by the Research Ethics Committee of the Universidade do Sagrado Coração, in Bauru, Brazil (No. 1.215.180.2.1).

3. Patients and Gastric Samples

This study analyzed 400 patients (♂173/♀227, mean age 54 years) with peptic symptoms who underwent to upper digestive endoscopy. Two gastric biopsie samples were taken from each patient, one for detection of *H. pylori* by polyme-

rase chain reaction (PCR) and the second for histopathological analyses performed according to Sydney and Lauren's classification [15] [16].

The gastric mucosa biopsy samples were separated into groups according to histopathological analysis: 120 control patients, with normal gastric tissue (♂40/♀80), 241 patients with chronic gastritis (♂111/♀130), and 39 with gastric cancer (♂22/♀17). The control and chronic gastritis samples were obtained from the Departments of Gastroenterology of the State Hospital of Bauru and of Marília Medical School, and the gastric cancer samples were obtained in the Federal University of São Paulo. Patients were excluded from this study who had undergone antimicrobial therapy treatment, received treatment via proton pump inhibitors, or used NSAIDs in the three months prior to the endoscopy.

3.1. DNA Extraction and *Helicobacter pylori* Diagnostic

DNA extraction was performed using QIAamp® tissue kit (Qiagen, Germany) according to the manufacturer's instructions. For diagnostic of the *H. pylori*, PCR assays were performed using one pair of oligonucleotides Hpx1: 5'-*CTGGAGARACTAAGYCCTCC*-3' and Hpx2: 5'-*GAGGAATACTCATTGCGAAGGCGA*-3' that amplifies a 150 bp fragment corresponding to 16S-rRNA from *H. pylori*. The reaction conditions were the same as described by Scholte *et al.* (1997) [17] and Pereira *et al.*, 2014 [18]: 40 cycles: 1 min, 94˚C; 1 min, 59˚C and 1 min, 72˚C. In each experiment, positive (strain 26695) and negative (water) controls were included.

3.2. *sab*A and *cag*A Detection

The presence of target genes *cag*A and *sab*A was also analyzed through PCR, using one pair of oligonucleotides for each gene fragment. To detect the *sab*A gene, we used the primers *Fm* 5'-*CCGCTAGTGTCCAGGGTAAC*-3' and *Rm* 5'-*CACCGCGTATTGCGTTGGGTA*-3' to amplified a fragment of 400 bp, and the reaction conditions described by Shao *et al.* [19] were optimized—35 cycles: 30 sec, 94˚C; 30 sec, 50˚C;30 sec, 72˚C. The detection of a 232 bp fragment *cag*A gene was performed according to Rasmussen *et al.* (2012) [20] and van Doorn *et al.* (1998) [21]: we used the primers *Cag*1 5'-*ATGACTAACGAAACTATTGATC*-3' and *Cag*2 5'-*CAGGATTTTTGATCGCTTTATT*-3' and the conditions 40 cycles: 1 min, 94˚C; 1 min, 53˚C; 1 min, 72˚C.

3.3. Statistical Analysis

Statistical analysis was performed using the two-tailed Chi-square test with Yates' correction and/or Fischer's exact test. Differences were considered significant when p value was less than 0.05. All statistical analyses were performed with software GraphPad Prism 5.0.

3.4. Results

In the 400 analyzed gastric mucosa samples, *Helicobacter pylori* was detected in

222 (55.5%) (Table 1). The result was significant for the presence of *H. pylori* when compared the control *vs.* cancer groups ($p < 0.0001$) OR (95% CI) 12.73 (5.45 - 29.69) and the control *vs.* chronic gastritis groups ($p < 0.0001$) OR (95% CI) 12.99 (7.44 - 22.66). These results found a significant relation between *H. pylori* and gastric diseases considering the effects of this bacterium in gastric mucosa.

The virulence marker *sab*A was detected in 129 (58.10%) *H. pylori* positive samples of which 11 were from control group, 106 from chronic gastritis group, and 12 from gastric cancer group (Table 1). Comparing the groups control *vs.* gastritis ($p = 0.63$) OR (95% CI) 0.78 (0.30 - 1.99), control *vs.* cancer ($p = 0.55$) OR (95% CI) 1.63 (0.51 - 5.17), and gastritis *vs.* cancer ($p = 0.097$) OR (95% CI) 2.07 (0.92 - 4.66), we found no significant results in any of the analysis.

The *cag*A gene was detected in 102 (45.94%) *H. pylori* positive samples also divided in the control, chronic gastritis, and cancer groups, with 8, 86, and 8 samples, respectively (Table 1). This virulence marker was statistically significant considering its presence when comparing the chronic gastritis *vs.* cancer groups ($p = 0.0434$) OR (95% CI) 2.44 (1.021 - 5.84). Table 1 showed the results of *H. pylori* and virulence markers in each group of patients.

4. Associations between *cag*A and *sab*A

Analysis of the association between *cag*A and *sab*A found no significant results (Table 2). However, the combination of both markers with the groups of patients produced a statistically significant result ($p < 0.0001$) OR (95% CI) 13.68 (3.95 - 47.33), considering the gastritis *vs.* cancer groups (Table 3). This result suggests that when these markers act together they potentiate the action of *H. pylori* by increasing the inflammatory process that can evolve from chronic gastritis into cancer.

Table 1. Distribution of the *Helicobacter pylori*, *sab*A and *cag*A genotypes, and the risk of developing chronic gastritis and gastric cancer.

	Control (%)	Chronic Gastritis (%)	Gastric Cancer (%)	TOTAL
***H. pylori*+**	20 (16.7)	174 (72.20)*	28 (71.80)*	222
***H. pylori*–**	100 (83.3)	67 (27.80)	11 (28.20)	178
TOTAL	120 (100)	241	39	400
***sab*A+**	11 (55)	106 (60.92)	12 (42.86)	129
***sab*A–**	9 (45)	68 (39.08)	16 (57.14)	93
TOTAL	20	174	28	222
***cag*A+**	8 (40)	86 (49.43)▲	8 (28.57)▲	102
***cag*A–**	12 (60)	88 (50.57)	20 (71.43)	120
TOTAL	20	174	28	222

*Results statistically significant when compared *control vs. cancer group* ($p< 0.0001$) and *control vs. chronic gastritis group* ($p < 0.0001$). ▲Results statistically significant when compared *chronic gastritis group vs. cancer group* ($p = 0.0434$).

Table 2. Association between the *cag*A and *sab*A genes in the studded groups.

	Control		Chronic Gastritis		Gastric Cancer		All groups		Total
	*sab*A+	*sab*A−	*sab*A+	*sab*A−	*sab*A+	*sab*A−	*sab*A+	*sab*A−	
***cag*A+**	3	5	57	29	5	3	65	37	102
***cag*A−**	8	4	49	39	7	13	64	56	120
Total	11	9	106	68	12	16	129	93	

Table 3. Distribution of the combination between *cag*A, and *sab*A genotypes and the risk of gastric disease. Comparisons among: control *vs* chronic gastritis[(a)]; control *vs* gastric cancer[(b)]; and chronic gastritis *vs* gastric cancer group[(c)].

	Control	Chronic Gastritis	Gastric Cancer
***cag*A+ *sab*A+**	0	57	5
***cag*A− *sab*A −**	1	10	12
OR (95% CI), p		[a]0.06 (0.002 - 1.6) p = ns	[b]0.75 (0.02 - 21.70) p = ns
		[c]13.68 (3.95 - 47.33) $p < 0.0001$*	

*Statistically significant association.

5. Discussion

Our results show that *Helicobacter pylori* is significantly related to gastric diseases, such as gastritis, and increase the risk of gastric cancer. Considering that this bacterium is capable of establishing an intense inflammatory process in the human gastric epithelium, these results were expected, because since the discovery of *H. pylori*, other works have concluded the sam [22] [23] [24] [25].

The outer membrane protein *sab*A, which interacts with a specific receptor present in gastric epithelium, can be responsible for facilitating binding between the bacteria and the host [26]. This gene has been identified in more than a half of the *H. pylori* positive studied population; however, no statistically significant results were found in this work. Our results are in the line with those published in Pakbaz *et al.* (2013) [13], but not in agreement with Oleastro *et al.* (2013) [27] who found association between this adhesin and gastric cancer in western population.

Although we did not find any significant results for the *sab*A gene, it is still recognized as a virulence marker for its ability to promote intense recruitment of neutrophils and establishment of a persistent colonization [28] [29].

One of the most aggressive virulence markers expressed for *Helicobacter pylori* and probably the most studied is *cag*A [4] [10]. This gene confers to the bacteria the ability to modulate the cell metabolism of the host [30], and its presence is related to development of ulcer and gastric cancer [31]. Our data indicate that *cag*A gene is a risk factor for the onset of gastric cancer in patients who have previously injured gastric epithelium. This result is similar to the results found by Yamaoka *et al.* (2006) [32] and Oldani *et al.* (2009) [31].

It is known that *H. pylori* depends on a strong initial bond to the gastric tissue

to conclude a successful and permanent infection, and it has developed mechanisms that greatly help this process [27]. Once the infection is established, *H. pylori* starts to release its toxins that regulate the host cells and assist in its adaption to the stomach niche [12] [33].

Both *sab*A and *cag*A are important virulence markers for *Helicobacter pylori* [11] as they participate in the processes mentioned above. Our results indicate that these genes taken together make the infection process more aggressive and increase the lesion of the gastric epithelium making the host more susceptible to cancer. Our data about this association is consistent with the data presented by Backert *et al.* (2011) [33].

We were expecting to have positive results correlating *sab*A only and the gastric cancer. We believe that we haven't got this result because of the sample size and the target population of this work. In addition, we had difficulty finding in the literature works that approached the questions of this article in a similar way to ours and, therefore, there were few comparisons of results that we were able to accomplish.

6. Conclusion

Taken together, our data confirm that *Helicobacter pylori* is directly related to the emergence and evolution of gastric diseases, especially chronic gastritis and cancer. Its virulence markers *sab*A and *cag*A are responsible for a persistent and very aggressive infection that moves the patient's situation from an initial injury of the gastric epithelium to a greater susceptibility for gastric cancer.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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