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ABO blood groups and *Helicobacter pylori* cagA infection: evidence of an association

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ABSTRACT: Diseases resulting from *Helicobacter pylori* infection appear to be dependent on a host of genetic traits and virulence factors possessed by this microorganism. This paper aimed to investigate the association between the ABO histo-blood groups and *H. pylori* cagA infections. Genomic DNA samples (n = 110) of gastric biopsies obtained from patients with endoscopic diagnosis of peptic ulcers (n = 25) and chronic active gastritis (n = 85) were analyzed by PCR using specific primers for the cagA gene. Of the samples, 66.4% (n = 73) tested positive and 33.6% (n = 37) negative for the gene. The cagA strain was predominant in peptic ulcers (n = 21; 84.0%) compared with chronic active gastritis (n = 52; 61.2%) (p = 0.05; OR 3.332; 95% CI: 1.050-10.576). Additionally, the cagA strain was prevalent in the type O blood (48/63; 76.2%) compared with other ABO phenotypes (25/47; 53.2%) (p = 0.01; OR 2.816; 95% CI: 1.246-6.364). These results suggest that *H. pylori* cagA infection is associated with the O blood group in Brazilian patients suffering from chronic active gastritis and peptic ulcers.

KEY WORDS: ABO blood groups, *H. pylori* infection, cagA strain, chronic active gastritis, peptic ulcers.

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INTRODUCTION

Infection by *Helicobacter pylori* is linked to gastroduodenal disease and seems to be dependent on genetic factors of the host and virulence factors presented by this microorganism (1-3).

The involvement of blood group carbohydrates as facilitating factors for infection by this bacillus has previously been reported (4). Some years ago we demonstrated that infection by this Gram-negative bacillus is associated with ABO blood groups in Brazilian patients submitted to upper gastrointestinal endoscopy (5).

Previous reports have shown that *H. pylori* strains that have the *cagA* gene infect Brazilian patients suffering from various gastroduodenal diseases (6-8). The cagA virulence factor seems to be involved in the induction of proinflammatory chemokines expressed by host cells and plays a notable role in the onset and progression of gastroduodenal diseases (2, 9).

H. pylori presents a remarkable allelic diversity and genetic variability (3). In addition to readily binding to ABO blood group antigens in the South America Indian population, infective cagA-positive *H. pylori* strains have also been associated with the ABO histo-blood group in Lebanon and Iran (10-12).

Therefore, studies correlating infection by epidemiologically important microorganisms such as *H. pylori* may provide relevant biological and clinical contributions by identifying genetic risk factors of the host and the stages of virulence and pathogenesis of the infectious agent (9). The aim of this study was to investigate the association between the ABO histo-blood groups and *H. pylori* cagA infection.

MATERIALS AND METHODS

Type of Study

This cross-sectional study was carried out in a specialized gastroenterology laboratory at the Base Hospital and the Immunogenetics Laboratory, Molecular Biology Department, São José do Rio Preto Medical School (FAMERP), São José do Rio Preto, São Paulo state, Brazil. After being informed about the experimental nature of this study, written consent was obtained from all patients. The study was approved by the Research Ethics Committee of the institution.

Patient Selection

Patients with endoscopy diagnoses of peptic ulcers (PU) or chronic active gastritis (CAG) and *H. pylori* infection were candidates for this study. *H. pylori* infection was identified by routine urea breath and urease tests performed in the specialized gastroenterology laboratory. Additionally, for all cases, whether positive, negative or discordant, the presence of infection was confirmed by PCR using gastric biopsy samples as described in our previous report (5). Patients who were pregnant, aged less than 18 years, had gastrointestinal tract hemorrhages or acute gastritis, had used a proton-pump inhibitor in the previous week or had used an H_2 receptor antagonist in the previous 24 hours were excluded from the study. A total of 110 Caucasian and non-Caucasian patients was included in this study.

Blood Sampling and ABO Blood Phenotyping

Five milliliters of whole blood was drawn from each patient and placed in vacuum tubes with EDTA. The ABO blood group phenotypes were identified by hemagglutination using commercial anti-A, anti-B and anti-A,B sera for forward typing and standard A_1 and B red blood cells for reverse typing (Fresenius Kabi, Brazil). A drop of a red blood cell suspension in 5% sterile saline solution (0.9% NaCl) prepared for each sample was mixed with a drop of each of the anti-A, anti-B and anti-A,B sera to define the erythrocyte antigens. Two drops of blood plasma from each sample were mixed with one drop of each of the standard 5% A_1 and B red blood cell suspensions to identify the anti-A and anti-B antibodies. The tubes were centrifuged at 3400 rpm for 1.5 minutes with the interpretation of the results being based on the presence or absence of hemagglutination. The recommendations of the manufacturer of all reagents were strictly followed.

***H. pylori* cagA Genotyping**

Genomic DNA samples were extracted from gastric biopsies obtained during endoscopy examinations. The identification of the *H. pylori* strain, based on the detection of the *cagA* gene, was achieved by PCR according to the previously published protocol (13). PCR reactions were carried out in a final volume of 25 μL containing Tris-HCl 10 mM (pH 8.3); $MgCl_2$ 1.5 mM; KCl 50 mM; 5 mM of each dNTP (dATP, dTTP, dCTP, dGTP), 0.5 mM of each *primer* (forward: 5'-GGGCGATCGGTTAGCCCTGA-3'; reverse: 5'–TACCTTACTGAGATC ATCAA-3');

0.5 U of Taq polymerase and 10 ng of genomic DNA. The amplification conditions were: 35 denaturation cycles at 94°C for one minute, annealing at 55°C for two minutes and extension at 72ºC for two minutes. Specific amplified fragments containing 102 base pairs corresponding to the *cagA* gene were separated by electrophoresis on a 2% agarose gel in TBE 1X and visualized using ultraviolet light after ethidium bromide staining.

Statistical Analysis

The two-tailed Fisher's exact test and odds ratio with a 95% confidence interval were used to identify associations.

RESULTS

Of the 110 patients with *H. pylori* infection, 65.4% (n = 72) were Caucasians and 43.6% (n = 48) were non-Caucasians. There was a slight predominance of female (n = 61; 55.4%) over male (n = 49; 44.6%) patients. Based on endoscopy results, there were three times more patients suffering from CAG (n = 85; 77.3%) than those with PU (n = 25; 22.7%). The O blood group (n = 63; 57.3%) was prevalent over non-O blood groups (A: n = 33, 30.0%; B: n = 12, 10.9%; AB: n = 2, 1.8%).

The rate of *H. pylori* cagA infections was high (66.4%) but no statistically significant difference was observed when cagA (+) and cagA (–) patients were compared by gender and ethnic background. Table 1 summarizes the results in relation to cagA-positive and -negative *H. pylori* strains.

Table 1. Frequencies of O and non-O blood groups and *H. pylori* infection among 110 patients

Characteristics	*cagA* (+)		*cagA* (–)		OR	95% CI	P*
	N	%	N	%			
Gender							
Male (n = 49)	33	67.3	16	32.7	1.083	0.4878-2.403	1.0
Female (n = 61)	40	65.6	21	34.4			
Endoscopy							
PU (n = 25)	21	84.0	4	16.0	3.332	1.050-10.576	0.05
CAG (n = 85)	52	61.2	33	38.8			
ABO blood groups							
O (n = 63)	48	76.2	15	23.8	2.816	1.246-6.364	0.01
Non-O (n = 47)	25	53.2	22	46.8			
Total (n = 110)	73	66.4	37	33.6			

*Calculated by Fisher's exact test

DISCUSSION

Previous reports that suggesting that infections by the *H. pylori* bacillus may be influenced by genetic traits of the host prompted us to test the hypothesis of an association between the ABO histo-blood groups and cagA infections.

It is believed that studies on the involvement of this aggressive *H. pylori* strain in the onset and progression of gastroduodenal diseases may provide information central to the development of control and preventive measures against infection (9).

Some years ago we reported an association between the O blood group and *H. pylori* infections in patients who were submitted to gastric endoscopy with our data being subsequently verified by two studies involving adult and infant patients from different regions of Brazil (5, 14, 15). Observations that emerged from these studies have been supported by several publications over the last few years (1-3, 9, 16).

In this paper we demonstrate that in Brazil infection by *H. pylori* carrying the cagA virulence factor is associated with the O blood group. The rate of infection by this strain reported herein is similar to previous Brazilian studies of adult and infant populations published in recent years (6-8).

The relation between infection by cagA-positive *H. pylori* strains and ABO histo-blood groups has been investigated in different regions. A significant association between this strain and the development of peptic ulcers was observed among Taiwanese patients belonging to the O blood group (17). These authors found that more than

85% of patients suffering from PU and more than 60% of those with CAG were infected by cagA-positive *H. pylori* strains.

Our study is in agreement with the Taiwanese study which revealed high rates of infection by this strain among patients belonging to the O blood group with a borderline significance level probably due to the small number of patients suffering from PU.

Two recent reports evaluated the association between ABO histo-blood groups and infection by cagA-positive *H. pylori* strains. One of them reported a significant relationship in Lebanon among three factors, namely, infection by this strain, the A blood group and the risk of gastric malignancy (11). The other one demonstrated that the anti-cagA antibody was also slightly more prevalent among infected children with A and O blood groups in Iran (12).

Our results are not concordant with the Lebanese study, but agree, at least in part, with the Iranian study (11, 12). It is possible that additional differences in genetic variability of some *H. pylori* strains infecting patients in the Middle East accounted for the differences between these studies. The basis for this association is not totally understood but the ability of *H. pylori* to bind to carbohydrate structures found in receptors, especially those related to histo-blood groups, is attractive (4).

There is no evidence that cagA-positive *H. pylori* strains are able to directly recognize the H antigen, expressed in the gastrointestinal tract of O blood group individuals, as a receptor by using the cytotoxin associated antigen A (cagA) as ligand. However, the co-expression of other virulence factors such as blood group adhesin binding A2 (BabA2), which binds to ABO blood group antigens, could explain, at least in part, the association reported in this paper.

H. pylori infections by strains carrying the *cagA*, *vacA*, *iceA* and *babA2* genes were observed among Brazilian adults and children suffering from gastroduodenal diseases (6-8, 18). Unfortunately these studies did not analyze the correlation between ABO blood groups and infection by these strains.

It has been shown that some *H. pylori* strains infecting Amerindians present a certain degree of adaptation that coincides with the high prevalence of the O blood group among native South Americans. More than 95% of the BabA-positive *H. pylori* strains are able to bind to ABO antigens and 60% of them present a specialized binding pattern to O blood group antigens (10).

The ABO blood group antigens are carbohydrate structures present in the gastrointestinal tract epithelium while the H antigen is a fucosylated antigen expressed by the majority of individuals belonging to the O blood group. This antigen is not modified by glycosyltransferases coded by A or B genes of the ABO locus responsible for the expression of A and B antigens while its level of expression is quantitatively higher than that detected in non-O blood group individuals (19, 20). Thus, these quantitative differences may contribute to the high prevalence of *H. pylori* infections among Brazilian patients carrying the *babA2* and *cagA* genes and suffering from CAG and PU.

It is presumed that *H. pylori* co-evolved with its human host but it is not clear whether its introduction into South America was due to waves of native Amerindians migrating across the Central American isthmus or due to colonization by Europeans. The suggestion that ancestral *H. pylori* was present in Peruvian Amerindians prior to the arrival of European colonizers 500 years ago is attractive by virtue of the fact that possible competition between Amerindian and European strains could contribute to the diversification and high genetic variability in the genome of this bacillus in South America (21). Therefore, the consequent acquisition of *babA2* and *cagA* genes would allow the *H. pylori* bacillus to adapt based on the prevalent ABO blood groups in South America (10).

In conclusion, this paper presents evidence of an association between the O blood group and cagA-positive *H. pylori* infections among Brazilian patients suffering from PU and CAG. This association seems to be an important event which could elucidate the epidemiological basis of the interaction between humans and *H. pylori*.

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