Determination of the frequency and ABO antigens, H, students in Catarina State or not infected by Helicobacter pylori

Abstract

The ABO blood group antigens H present in the mucus and gastric epithelia are described as the possible receptors in the epithelium of H. pylori. The goal of the research is to verify the frequency of ABO blood groups, H students or not infected H. pylori. The study was conducted at the University of Vale do Itajaí. They Were Evaluated 88 students through phenotyping tests ABO antibody titres and serological test for H. pylori. Of the 88 participants, 42.05% were blood group A; were Group 39.77%; 9.9% AB Group; were 9.9% in Group B. The frequency of active infection with H. pylori was 87.50% in group B and AB; 71.73% in the group; 70.27% in group A. In the present study, the phenotype was most frequently Group A having the same frequency to other authors, most Often infected with H. Observed was pylori in group AB and B did not show prevalence infection ratio in the Group of volunteers in agreement with other authors who used serological methods. It Can be concluded que the results of H. pylori infection rate in blood groups depend on the methodology used, the serological tests may have false positive results, and the average age of the population influence the test results. In the present study we did not obtain significant infection in the frequency of ABO blood groups, and Obtained higher percentage of infection related Symptoms of gastritis. And the average age of the population influence the test results. In the present study we did not obtain significant infection in the frequency of ABO blood groups, and Obtained higher percentage of infection related Symptoms of gastritis. The ABO blood group antigens H present in the mucus and gastric epithelia are described as the possible receptors in the epithelium of H. pylori. The goal of the research is to verify the frequency of ABO blood groups, H students or not infected H. pylori. The study was conducted at the University of Vale do Itajaí. 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Introduction

The ABO blood group antigens is the most investigated systems for all populations and, due to their ease of identifying phenotypes it has been used as a marker in genetic association studies with infectious and non infectious diseases. The phenotypic heterogeneity of the ABO blood system is due to the structural difference of the gene that synthesises the N-acetylglactosaminyltransferases glycosyltransferases or N-galactosyltransferase, which are responsible for the transfer of specific sugar residues at the H substrate, catalyzing the transglciosilação reactions between the substrate receptor and acceptor sugar, forming the antigens a, B and AB respectively. The group is the result of a functional enzyme, which does not modify the precursor substance remaining H. antigen structure. Transferases A and B have similar structures to each other, and their specificity is determined by the amino acids located near the binding site of the enzyme with its corresponding sugar. The H antigen is a carbohydrate produced by the action of alpha-2-fucosyltransferase enzyme which adds fucose precursor substance is encoded FUT1 locus on chromosome 19 q13.3 in position, and therefore genetically independent of the ABO locus. Bombay deficient phenotype was the first variant of the M gene, called Bombay phenotype or OH, this rare classical phenotype is produced by rare individuals hh/sese genotype. It is characterized serologically by the total loss of the activity of transferase A, B and H in the erythrocytes and in bodily secretions and large quantities of anti-H natural plasma, reactive to 37°C, and activators of the complement therefore of great clinical importance. This phenotype may be detected in the screening of antibodies (PAI) of type O individuals when not testing the anti-H in the sample if the result PAI is negative, it follows that the individual is because if Bombay (no H antigen) PAI should be positive, indicating the presence of anti-H plasma.

The blood group AB has the transferase activity of the two (A and B), while the Group lacks the A and B transferases, but shows the H antigen in large quantity on the surface of red blood cells. ABO antigens are not restricted to the membrane of the erythrocytes, and can also be found in a wide variety of cells such as lymphocytes, platelets, venular and arterial capillary endothelium, sinusoidal cells of the spleen, bone marrow, gastric mucosa, as well as secretions and other fluids as saliva, urine and milk.

Individuals by a specific mechanism naturally form antibodies against the antigens that do not have, and these can be detected in serum/plasma. Therefore, for the determination of the ABO phenotype is recommended to search for membrane antigens, through the use of anti-A specific antiserum, anti-B and anti-AB, then each antigen present on red cell corresponds to an antibody in the serum/plasma against the antigen specificity of the individual lacks.
Natural ABO antibodies formed against antigens that are not present in erythrocytes. The stimuli are passive, coming mainly from the bacteria begin to colonize the intestinal tract from birth because they have sugar in their cell membranes similar to sugars immunodominant antigens A and B. These bacteria, as well as other external stimuli stimulate the formation of anti-a and/or anti-B, which are now classified, therefore, as natural and regular. The anti-A and anti-B antibodies are mostly IgM and IgG.\(^{4,5}\)

Because the ABO and H antigens are antigens histocompatabilidades, studies show that they may serve as binders infections. ANDM in 1970, a survey found that individuals with blood group O were more susceptible to severe cholera than others.\(^{9,10}\) Since then, several case-control studies have shown that people with blood group O have higher risk of hospitalization due to Vibrio cholerae.\(^{8}\) Among the first epidemiological studies to establish associations between blood groups and disease, there were some demonstrations of high frequencies of blood group in patients suffering from peptic ulcers.\(^{10,11}\) Later, it was shown that *H. pylori* bacillus is the main etiological agent associated with gastric ulceration and is present in over 80% of patients with this disease.\(^{11,12}\)

The *H. pylori* is a gram-negative spiral bacterium and whose mobility is rapidly and shaped corkscrew provided by polar flagella. Specifically colonizes the gastric mucosa and microvilli of epithelial cells causing their destruction by producing toxic enzymes that effects in that deregulate the immune cells of stomach epithelium. The incidence and prevalence of *H. pylori* infection is entirely related to the country’s development level, including factors such as income, housing conditions, level of education and hygiene, with the main transmission means the fecal-oral and oral-oral.\(^{13,14}\) In developed countries, the rate ranges from 25% to 50%, while those under development is around 70% to 90%.\(^{15}\)

The colonization of bacteria in gastric tissue is often followed by inflammation acting as immune response of the body against the bacteria. It has slow growth, with broad activity in the synthesis of toxins it produces very active urease degrading urea, which joins these bacteria to form ammonia, leaving the pH of the alkaline environment in the vicinity where the bacteria settles allowing their survival in the gastric environment. The same has great ability to grip that is through adhesion along with some outer membrane proteins which are also recognized as virulence factors and a form of the bacteria is not eliminated by peristalsis can cause gastric mucosal inflammation (gastritis) and gastric metaplasia.\(^{16,17}\)

With this observation, the authors tried to establish links between the association between ABO blood group and secretory phenotypes ABH antigens with peptic ulcers that had been observed in previous decades.\(^{16,18}\) It is described in the literature that human enantiomers Group A antigens present in the gastric mucosa.\(^{5}\) Stowell et al.,\(^{20}\) have demonstrated *in vitro* that bacteria such as *Escherichia coli* O86 have the carbohydrate systems ABO, Lewis M and on its surface. *Escherichia coli* some species of *Salmonella* and *Arizona* also have almost all ABO carbohydrate in their plasma membranes: D-galactose, L-fucose and N-acetyl-D-galactosamine (excluding N-acetyl-D-glucosamine).\(^{20}\) However, not enough to be the monosaccharide used to stimulate the development of antibodies to the ABO system antigens, but it should be an active carbohydrate, since only one of the enantiomers of the monosaccharide is immunogenic.\(^{21}\)

Thus, this study has with the main objective determining the distribution of phenotypes and ABO Blood Group M or not on infected individuals *Helicobacter pylori*.

**Sampling**

After approval by the Research Ethics Committee in Human Beings UNIVALI under Opinion No. 1,016,880, 90 students of the courses of Biomedicine and Pharmacy, University of the Valley Itajaí (UNIVALI) showed that ages 18 to 65 years. Students signed the Consent Esclarecgone and the questionnaire that had intended to capture data such as age of the participants, the absence or presence and frequency of stomach pains, he had carried out treatment for *H. pylori* and had some medical condition that weakened immunity.

Then, blood collection was performed using as anticoagulant 1mg/ml of ethylene diamine tetraacetic acid disodium salt (EDTA) to identify the phenotype ABO red blood cells. As questionnaire answers previously applied to the collection, were excluded students diagnosed with the presence of *H. pylori* who had undergone treatment for this infection, and is also excluded were those taking antibiotics and students with weakened immune systems (HIV seropositivity and other disorders related to the immune system in relatas questionnaire for collecting the blood sample that could influence the detection of antibodies anti-ABO). Furthermore, during the tests they were excluded from subjects who had inconclusive results for the serological markers for anti-*H. pylori*.

The criteria for the classification of symptoms of pain related to infection *H. pylori* was taken into consideration symptoms temporality as described as follows: much Frequency - Every day; little Frequency (2 to 3 times a week); rare Frequency (1 to 2 times per month) and Absent- Individuals who have never had symptoms. This temporality was established once the *H. pylori* infection is often asymptomatic and when symptoms occur, they represent nonspecific forms of stomach discomfort.\(^{22}\) Therefore, this study was established as criterion of pain stomach discomfort related to infection by *H. pylori*.

**ABO phenotyping**

The blood sample collected in EDTA was used to perform the phenotyping ABO as prescribed in existing legislation.\(^{21}\) The phenotyping ABO was completed by performing the Direct Support in the respective pipe A and B was added 50µl of RBC suspension 3% saline solution held in the sample and with a drop of serum monoclonal anti-a, anti-B (BioRad®). The tubes were centrifuged for 15 seconds at 2326g, Continuing to agglutination reading. To perform the reverse grouping the tubes A and B, respectively was added 50µl of serum and erythrocyte reagents A and B at 3% (BioRad®). The tubes were centrifuged for 15 seconds at 2326g, Continuing to agglutination reading. The tube technique followed the standard reaction intensity reading as girello & Kuhn.\(^{4}\)

**Determination of titers of anti-A and anti-B**

The determination of titer of anti-A and anti-B antibodies was performed according to Judd et al.,\(^{24}\) by serial titration of sera of individuals. For determination of antibody titer (IgM) were made serial dilution to 1/512. The tubes were homogenized and centrifuged at 2326g for 15 seconds to reading the reaction temperature (RT) verifying the presence or absence of agglutination. If it has been verified agglutination at a dilution of 1/512, it was expanded.\(^{24}\)

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Irregular antibody screening

We used two tubes respectively identified with the numbers I and II, all added to two drops of plasma, HI tube I had a RBC suspension drop (Triacel®). The tube II RBC one drop of suspension OII (Triacel®). The tubes were homogenized and centrifuged for 15 seconds at 2326g. The reading was performed by visualizing the presence or absence of agglutination. The intensity of reaction was noted crosses (0 to 4+). In all tubes enhancer reagent was added BioPeG® 15% (Fresenius Kabé®) and then homogenized. All tubes were incubated in a water bath at 37°C for 15 minutes, the samples were washed 3 times with 0.9% NaCl solution. Subsequently were added two drops of Coombs serum anti-mouse IgG (Bio-Rad®), and we are centrifuged for 15 seconds at 2326g. After centrifugation was reading done viewing the presence or absence of agglutination in both the intensity of crosses was recorded (0 to 4+). In the absence of agglutination was added a drop of red blood cells to Coombs Control (Bio-Rad®). In the tubes, which were centrifuged at 2326g for 15 seconds, and the presence of double red blood cell population.

Implementation of serological ELISA test for Helicobacter pylori IgA/IgG/IgM

*H. pylori* antigen adhered to a solid support (Serion ELISA plate) were incubated with sera from individuals in the search for antibodies against the antigen. In the case of a positive sample, i.e. serum antibody formation occurs patient with an antigen-antibody binding, detected later by the addition of antibody directed against immunoglobulin linked to peroxidase. This antibody bound to the enzyme is called conjugate and adding to this product a suitable substrate, the wells where there was antigen-antibody reaction have a color. The reading was performed from the analysis of Optical density (OD) at 405nm against the substrate blank; reference wavelength between 620 nm and 690nm.

From the results, the following parameters were identified: active infection (marking reagents for IgM, IgA and IgG) and prior contact (Marker reagent for IgG and/or IgA).

Data analysis

Through the results data analysis was conducted using the Excel spreadsheet. Were averaged and standard deviation of age of the subjects was determined as well as the prevalence of female and male and frequency of ABO blood group system. In the spreadsheet it was carried out the percentage of individuals with active infection and prior contact. These were sectioned according to the frequency of the symptoms described in the questionnaire thus establishing the prevalence of symptoms according to the ABO Group.

Results

Among the students surveyed none were excluded due to positive PAI. Thus, all individuals classified as Group C were individuals who had voted H antigen, since Bombay phenotypes exhibit anti-H natural antibodies which would result in positive outcomes in the PAI. However, two subjects were excluded from this study because they had inconclusive results for serological detection of antibodesanti-*H. pylori*. This, so the number of participants was 88 individuals. Among the 88 students average age was 23±6 years. The percentage of female students was 85.23% (75/88) and Male 14.77% (13/88). Among the individuals surveyed 72.73% (64/88) were Biomedicine course and 27.27% (24/88) were Pharmacy course. Of the 88 participants, 42.05% (37/88) were blood group A; 39.77% (35/88) were Group; 9.09% (8/88) Group B were 9.09% (8/88) were the AB Group.

Table 1 shows that the highest frequency of subjects with active infection had Groups B and AB having 87.50%. In Group O, students with active infection were 71.43% and 70.27% showed the phenotype. Of individuals who had previous contact 29.73% had the phenotype, 29.57% and 12.50% had the phenotype B and AB.

<table>
<thead>
<tr>
<th>Results</th>
<th>%THE</th>
<th>%B</th>
<th>AB%</th>
<th>%O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active infection</td>
<td>70.27</td>
<td>87.5</td>
<td>87.5</td>
<td>71.73</td>
</tr>
<tr>
<td>Contact Early</td>
<td>29.73</td>
<td>12.5</td>
<td>12.5</td>
<td>28.57</td>
</tr>
<tr>
<td>Absolute number of individuals of Blood Groups</td>
<td>37</td>
<td>8</td>
<td>8</td>
<td>35</td>
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</tbody>
</table>

Table 1 shows the frequency of active infection parameters classifying the frequency of symptoms reported by patients with ABO phenotype. The same analysis was conducted for individuals who have had contact prior. A Table 1 show that is the highest percentage of individuals who demonstrated pain often are present in individuals have the B antigen (Group B and AB). However, it is also possible to observe that 12.50% of these groups showed no symptom of pain. In individuals who have just had previous contact (marking reagents for IgG and/or IgA), the group that reported infrequently symptoms, had the highest percentage for the phenotype THE it is 16.22%.

Discussion

Knowing the frequency of ABO antigens vary in different populations, the most common blood group among the students evaluated in the research was the blood group A, with the same prevalence that Schmitt evaluated in the research was the blood group A, with the same prevalence than Schmidt that of 166 volunteers, 71 (42.77%) subjects belonged to blood group A, 67 (40.36%) belonged to the group, 22 (13.23%) of the blood group B and 6 (3.61%) blood group AB. Oliveira evaluated the frequency of antibody titters in the ABO blood groups and their study noted that the frequency of the Group titters of anti-B antibodies ranged from 4 to 128, most often having as title 64. For antibody anti-A was observed title variation from 4 to 64 and the most frequent title was 36. Já in Group B of the title anti-a varied from 4 to 32 and had the most frequent title 16. However, in Group a anti-B antibody titter ranged from 2 to 128 and had the most frequent title 64. These results are consistent with the results found in this study although the selection of individuals has occurred randomly.

Studies have assessed the frequency of *H. pylori* in the ABO blood groups using serological tests and ELISA. Loffeld serological tests used book IgG antibodies against *H. pylori* in serum of healthy blood donors with a mean age of 42 years. It was observed that 64.5% were seronegative donors showed 35% seropositivity, and 176 (43.8%) of the Group, 179 (44.5%) Were A. Blood of blood group

B and AB groups occurred in 34 (8.4%) and 13 (3.3%) subjects, respectively. 179 (44.5%) were in Group A, 34 (8.4%) in Group B, and 13 (3.3%) were group AB. The authors concluded that there was no significant difference in the distribution of blood groups among seropositive and seronegative individuals.\(^{18}\)

Another study determined the infection serology and reported a significant increase in the incidence of seropositivity until the age of 31 years and over this age a significant decrease.\(^{27}\) These results corroborate the results of the present study since it was observed high rate of infection and the average age of the subjects was 23.6 years. In the same study autodemonstraram significant association between blood group and infection caused by \textit{H. Pylori}.\(^{29}\) However, this study was not found in the prevalence of infection ABO blood groups, with group AB and group B with a higher percentage of infected.

In addition to the assessments of the ABO and \textit{H. Pylori} system, the authors studied the prevalence of seropositivity for \textit{H. Pylori} infection which was 64.8% in symptomatic patients.\(^{29}\) This result is consistent with our study and the authors of this study believe that this gap may be primarily due to the characteristics used for population studies that were performed in healthy individuals, rather than symptomatic.\(^{29}\)

Tadesse et al.,\(^{30}\) reviewed 408 consecutive patients with abdominal complaints serological tests using anti-Hpylori antibody (IgG) using two different ELISA. \textit{H. pylori} (IgG). The distribution of \textit{H. pylori} infection among participants with different blood types Indicated That Those with type AB blood, A, O and B had 88.9, 84.2, 83.7 and 80.9% rate of infection, respectively. (Table 2) among participants with different blood types indicated that AB type was 88.9%, 84.2%, O 83.7% B and 80.9% infection rate\(^{30}\) being equivalent results with this study that also had high prevalence of infection and symptoms in blood groups studied.

<table>
<thead>
<tr>
<th>Table 2 Frequency of individuals with active infection and Prior Contact with ABO phenotype according to the frequency symptoms</th>
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<tbody>
<tr>
<td><strong>Active infection</strong></td>
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<tr>
<td>Symptoms</td>
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<tr>
<td>Much Frequency</td>
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<tr>
<td>Little Frequency</td>
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<tr>
<td>Rare Frequency</td>
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<td>Absent</td>
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Based on the authors cited in the text, one can evaluate the frequency of blood groups infected with \textit{H. pylori} is on the average age of the study population, since \textit{H. pylori} infection rate is active in youth and chronicity or cure in adulthood. However, since discrepancies using the same methodology could be also related to the specificity and sensitivity of serological testing.

In studies using endoscopy, gastric biopsy urease obtained and frequency of infection in certain ABO blood groups. Allkout et al.,\(^{22}\) showed that the blood group had large susceptibility to peptic ulcers and with the H antigen had an influence on \textit{H. pylori} infection.\(^{32}\) Lin et al.\(^{33}\) demonstrated a high frequency of infection by these bacteria in 90.3% of the group of patients suffering from gastroduodenal disease.\(^{23}\) Boren et al.\(^{34}\) reported that this bacillus has a tropism for the Group, which is consists of fucose. This same carbohydrate is expressed on the surface of epithelial cells of the gastric mucosa acting as a receptor for \textit{H. pylori}.\(^{34}\) These investigators believed that this feature justifies the observation made during the past decades that people with blood group O have a greater tendency to peptic ulcers, compared with those blood groups A, B and AB. However, in this study we observed a higher tropism for individuals containing antigen B.

Another important factor when comparing the sensitivity and specificity of the serological tests and invasive methods for \textit{H. pylori} detection verifies that the invasive methods have a lower rate of false positive results, which is no longer present in the serological study because it has a higher false positive rate. On the other hand, the study by invasive methods may show false negative results due to non-homogeneous distribution of the bacteria in the stomach.\(^{35,36}\)

In this study, the sensitivity and specificity rates for IgG antibody was 93.8% sensitivity and 94.1% specificity because IgM was at 100% sensitivity and 77.8% specificity and 79 had IgA 8% sensitivity and 93.2% specificity. Sudraba\(^{37}\) compared various diagnostic methods for \textit{H. pylori}, having parameters as sensitivity and specificity, the rapid urease test sensitivity was 96% and 100% specificity, however, serology the sensitivity was 96% and the specificity 50%, with large numbers of false positives.\(^{37}\)

**Conclusion**

Currently the treatments are not specific for \textit{H. pylori}, and end up destroying the bacterial microbiota of the patient experiencing an imbalance that can lead to a decrease in immunity individual. Pode be concluded that the results of \textit{H. pylori} infection rate in blood group depend on the methodology used, as serological tests may have false positive results. In addition, the average age of the population influence the test results. In the present study we did not obtain significant rate of infection in the ABO blood groups, but higher percentage of infection related symptoms of gastritis. Thus, serological tests do not possess sufficient reliability to be used as a method of diagnosis and effective treatment of gastritis caused by H. confirmation pylori due to its high tendency to false positive results. Knowing that an in vivo study variant has the result according to the diagnostic method employed, it would be interesting to carry out a study in vitro using cell culture techniques to determine the frequency of infection and binding of \textit{H. pylori} to gastric epithelial cells with expression of different antigens. Another important factor would be the study of the connection of the isomers of \textit{H. pylori} in epithelial cells. Thus, drugs can be designed to have a mechanism to block binding of \textit{H. pylori} to immunodominant antigens with carbohydrate (fucose) were .Tratamentos further directed to preventing recontamination during the study and not only to death \textit{H. Pylori}. Nevertheless, only preventing the binding of \textit{H. Pylori}.

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Conflict of interest

Author declares that there is no conflict of interest.

References