Association of Lewis and Secretor gene polymorphisms and Helicobacter pylori seropositivity among Japanese-Brazilians

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Background. Secretor (Se) and Lewis (Le) genes are involved in the synthesis of Lewis b (Leb) and type I antigens throughout the body, especially in the epithelial cells of gastric mucosa. Helicobacter pylori can attach to the gastric epithelial cells with the blood group antigen-binding adhesin, which binds to Leb or H type I carbohydrate structures. In a previous study, a marked association between H. pylori seropositivity and polymorphism of the Se and Le genes was observed among Japanese outpatients of a gastroenterology clinic. The present work aims to investigate the associations between Se and Le gene polymorphisms and H. pylori infection among Japanese-Brazilians. Methods. The subjects consisted of 942 healthy volunteer Japanese-Brazilians, who were tested for the presence of anti-H. pylori IgG antibodies and genotyped for Se and Le polymorphisms. Results. The sex-age-adjusted odds ratios (aORs) for H. pylori seropositivity were 0.99 for the Sese genotype relative to the SeSe genotype (95% confidence interval [CI], 0.73–1.33), and 1.03 for sese relative to SeSe (95% CI, 0.71–1.48). On the other hand, the aOR for the subjects with the le allele (Lele or lele) relative to the LeLe genotype was 1.48 (95% CI, 1.07–1.79). When the Se and Le genotypes were analyzed in combination according to risk group, no statistically significant association was observed. Conclusions. These results are inconsistent with previous work and may have been modulated by an external factor or some other unidentified factor. Japanese-Brazilians are genotypically the same as Japanese, but their lifestyle is adapted to that of Brazil. Further investigations are necessary to clarify this influence on susceptibility to H. pylori infection.

Key words: Secretor, Lewis, Helicobacter pylori, polymorphism

Introduction

Chronic infection with Helicobacter pylori is commonly associated with gastroduodenal diseases in humans, including stomach cancer.1–2 H. pylori infection depends largely on poor sanitary conditions, and children acquire the bacterium as infants, mainly from family.3–4 The bacteria persist in the gastric mucus layer, leading to chronic atrophic gastritis and gastric cancer.5–6 The infection activates the immune system, and the antibody serum titer persists during the course of infection. However, some individuals do not develop persistent infection even under the same poor sanitary conditions, suggesting that host factors do play an important role in the infection and its maintenance. For example, H. pylori infection studies have revealed that the bacteria attach to the gastric mucosa with the blood group antigen-binding adhesin, BabA.7–8 BabA binds to both Lewis9 and H type I carbohydrate structures, leading to a series of pathogenic processes in the gastric epithelial cells.9 Blood group antigens (Lewis and ABH antigens) are carbohydrate structures widely expressed in many tissues throughout the body, especially in the epithelial cells of gastric mucosa. The secretor status is defined by the presence of ABH antigens in body fluids and secretions such as saliva and gastric juice. Lewis (Lewisb, Leb, and Lewisb, Leb) and ABH antigens are closely interrelated; they are produced from a common precursor antigen (type I precursor) by the action of the products of Lewis (Le) and secretor (Se) genes. Type I precursor is converted in H type I antigen by the Se enzyme, and then converted to Leb by the action of the Le enzyme. The Le enzyme also metabolizes the type I precursor to Leb antigen.10 Low activity of the Se enzyme and high
activity of the Le enzyme is considered to prevent the synthesis of H type I and Le\(^b\), which may result in a reduced chance of being infected by \(H.\) \(pylori\).

The \(Se\) gene and the \(Le\) gene have many different polymorphisms (already described) and, in this way, they have different alleles. So, different alleles may lead to functional or nonfunctional genes. In individuals who have functional \(Le\) and \(Se\) genes, all the type I precursor is transformed in H type I antigen, and they express \(Le^b\) and ABH antigens in the foveolar epithelium and in the gastric juice as well. On the other hand, an individual who has nonfunctional allele of the \(Le\) and \(Se\) genes (\(se\) and \(le\)) fails to produce \(Le^b\) antigen and ABH blood antigens. Finally, individuals who have the nonfunctional \(Le\) gene do not express \(Le^a\) or \(Le^b\), but they express ABH blood antigens only if they have the \(Se\) gene.

In the Japanese population, \(sej\) and \(se5\) alleles of the \(Se\) gene and \(le1\) and \(le2\) alleles of the \(Le\) gene products are described to have low or no activity.\(^{11-13}\)

Ikehara et al.\(^{14}\) reported an association of low seropositivity of anti-\(H.\) \(pylori\) IgG antibody and low expression of \(Se\) alleles and high expression of \(Le\) alleles, indicating that \(Se\) and \(Le\) genotypes affect susceptibility to \(H.\) \(pylori\) infection. However, in a recent report, Hamajima et al.\(^{15}\) described no consistency in the association of \(H.\) \(pylori\) infection and \(Se\) and \(Le\) polymorphisms, suggesting that even in the same ethnic group (Japanese) different subject sources lead to different results that may be the result of an unidentified effect modification.

Today, \(H.\) \(pylori\) still infects, chronically, over half of the world's population, in part because of the development of a unique set of virulence factors, including adhesin Bab1.\(^9\) On the other hand, with more sanitary living conditions that come with increasing socioeconomic status, \(H.\) \(pylori\) infection prevalence decreases. In Japan, the prevalence of \(H.\) \(pylori\) infection in individuals aged more than 40 years is as high as that in developing countries (over 70%)\(^{16,17}\) and it is associated with the development of severe atrophic and metaplastic gastritis and, probably, with the high incidence and mortality of gastric cancer.\(^{18}\) The prevalence rates of \(H.\) \(pylori\) infection among Japanese-Brazilians are similar to those in residents of Japan.\(^{19,20}\) In our previous work, we investigated \(H.\) \(pylori\) seropositivity and lifestyle factors among 963 Japanese-Brazilians, and we observed an inverse association between infection and length of education, while fruit intake was positively associated with \(H.\) \(pylori\) infection.\(^{20}\)

The present study aimed to correlate \(H.\) \(pylori\) infection and \(Le\) and \(Se\) genotypes in Japanese-Brazilians. As Japanese migrants or their descendants belong to the same ethnic group, of Japanese, the genotype results may be comparable. When living in a different country, Japanese migrants acquire a different lifestyle, with the intake of different foods that may influence the development of some diseases. We found that the \(Le\) genotype was associated with \(H.\) \(pylori\) infection, but polymorphism of the \(Se\) genotype was not. These results are inconsistent with previous work, and may have been influenced by external factors or by some other unidentified factor. Japanese-Brazilians have a lifestyle adapted to that of Brazil, which may influence \(H.\) \(pylori\) infection susceptibility.

**Methods**

**Study subjects**

The subjects of our study were apparently healthy adult Japanese Brazilian volunteers from four different cities, São Paulo, Curitiba, Mogi das Cruzes, and Mirandopolis, who were enrolled from March to May 2001.\(^{20}\) Those with a history of disease such as ulcer and stomach cancer were not excluded. Japanese migrants and their descendants try to keep Japanese traditions through Japanese cooperative societies, country clubs, and other non-profit Japanese associations. After a first contact with these associations, 12 in São Paulo, 4 in Curitiba, 1 in Mogi das Cruzes, and 1 in Mirandopolis responded to the first call within a predetermined period. With the approval of the directors of the associations, the members were invited, through a standardized letter informing them of the study objectives, the procedures, and confidentiality, to take part in the study. The total number of applicants was 967; the individuals were aged 33 to 69 years, and comprised first to fourth generations. Six applicants younger than 33 years or older than 69 years were excluded.

**DNA extraction and anti-\(H.\) \(pylori\) antibody test**

A 10-ml peripheral blood sample was obtained from each participant. Plasma samples were separated after centrifugation and frozen at \(-20^\circ C\), following the same protocol as that in our previous study.\(^{20}\) For the identification of \(H.\) \(pylori\)-infected participants, an anti-\(H.\) \(pylori\) IgG antibody test, high molecular-weight \(Campylobacter\)-associated-protein (HM-CAP) enzyme-linked immunosorbent assay (ELISA; Detaminor \(H.\) \(pylori\) antibody; Enteric Products, Westbury, NY, USA) was performed.\(^{21}\) The test was conducted at SRL (Tokyo, Japan), where routine measurements of IgG antibody have been established. A value of 2.3 EV (ELISA value) or over was regarded as positive for \(H.\) \(pylori\) infection.

DNAs were extracted from the blood by a salting-out method\(^{22}\) and utilized for polymorphism analysis.