Correlation between Helicobacter pylori Infection and Gastric Atrophy Examined in the Sera of Mongolian People

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Received: 11 December 2018; Accepted: 21 March 2019; Published: 3 April 2019

Abstract: Serum specimens obtained from 680 individuals were examined to measure the amounts of pepsinogens 1 and 2, anti-CagA antibodies, and anti-Helicobacter pylori antibodies. We examined sera obtained from 610 Mongolian individuals living in the capital city, Ulaanbaatar. Seventy serum specimens were collected from Japanese people who were health-screened: These were stored at the gastroenterology laboratory of Jichi Medical University. The sera of the Japanese people were used as a control specimen. Two enzyme-linked immunosorbent assay (ELISA) kits, an E-plate ELISA kit from Eiken Chemical Co., Ltd. (Tokyo, Japan), and a Biohit ELISA kit from Biohit Oyj (Helsinki, Finland), were used for the detection of anti-H. pylori IgG antibodies in the sera of the 610 Mongolian people. An ELISA kit EIA-4138 from DRG Instruments GmbH (Germany) was used for the detection of anti-CagA IgG antibodies in the serum specimens. Serum pepsinogens were detected by an ELISA kit from Biohit Oyj. Of the 610 serum specimens, 385 specimens tested positive for the detection of anti-H. pylori antibodies using the two ELISA kits, and 47 tested negative. For the detection of anti-H. pylori antibodies by the Biohit ELISA kit, 560 and 50 specimens were positive and negative, respectively. The ratio of serum pepsinogen 1/2 was statistically lower ($p < 0.0001$) in the H. pylori-positive (560 specimens) than in the H. pylori-negative (50 specimens) specimens. However, the levels of serum pepsinogen 1 had no statistical significance ($p = 0.465$) between the specimens of the H. pylori-positive and -negative specimens. The ratio of serum pepsinogen 1/2 was 6.74 ± 0.12 in the H. pylori-positive specimens, whereas the ratio of serum pepsinogen 1/2 was 12.69 ± 1.02 in the H. pylori-negative specimens. This study demonstrated the high prevalence of H. pylori infection in Mongolian people, including young generations, and the people infected with H. pylori possessed low pepsinogen 1/2 ratios, indicating atrophic gastritis. The serological examinations by the two ELISA kits did not consistently reflect the prevalence of H. pylori infection in Mongolian people.

Keywords: H. pylori; pepsinogen; pepsinogen ratio
1. Introduction

*Helicobacter pylori* is a Gram-negative curved rod bacterium with polar flagella as the motility organs. This pathogen colonizes the human stomach: Approximately half of the world’s population has been infected with it. A number of epidemiological studies have demonstrated a close relationship between *H. pylori* infection and the occurrence of gastric cancer. Since 1994, *H. pylori* has been recognized as a class 1 carcinogen, as published by the International Agency for Research on Cancer [1]. *H. pylori* colonizes the stomach of more than 50% of the world’s population. The majority of people colonized by *H. pylori* develop asymptomatic gastritis. However, approximately 10% of individual patients infected with this pathogen develop peptic ulcers. Moreover, about 1% of *H. pylori*-infected patients develop gastric cancer, and less than 0.1% develop gastric MALT lymphoma [2,3].

Epidemiological studies have reported a high seroprevalence of *H. pylori* infection in patients with gastric carcinoma and have established a positive correlation between *H. pylori* infection and the occurrence of gastric cancer [4,5]. Gastric cancer is the third leading cause of cancer-related deaths in the world. High-risk areas of gastric cancer include Korea, Japan, China, and Mongolia, and the age standardized rate (ASR) for the occurrence is higher than 20 per 100,000 in those countries. The Cancer Registry at the Korean National Cancer Center determined in 2002 that the incidence rate of gastric cancer of Korean people was 65.6 per 100,000 for men and 25.8 per 100,000 for women [6]. Though the age-adjusted mortality rate from gastric cancer in Japanese people in 1980 was 69.9 per 100,000 and 34.5 per 100,000 for men and women, respectively, its mortality rate in 2003 reduced to 34.5 per 100,000 and 13.2 per 100,000 for men and women, respectively [7]. Intermediate risk areas (ASR: 11–19 per 100,000) for the occurrence of gastric cancer include Malaysia, Singapore, and Taiwan, while low-risk areas (ASR: <10 per 100,000) include Australia, New Zealand, India, and Thailand [8]. Meanwhile, the incidence rate of gastric cancer in Mongolian people increased from 19.5 per 100,000 in 2003 to 23.4 per 100,000 in 2011 [9,10].

Environmental factors, especially water supply and diet, contribute to the infection rate of *H. pylori* in people in developing and developed countries [11,12]. The prevalence rate of *H. pylori* infection varies from 20% to 50% in people in industrialized countries, whereas it is over 90% of people in developing countries, such as Ethiopia, Gambia, Libya, Egypt, Nigeria, and Bangladesh. In addition, the prevalence rate of *H. pylori* infection reaches about 82–88% of people in India, China, Siberia, Brazil, and Iran. In the group investigated in India, the prevalence rates of *H. pylori* infection were 88% in adults greater than 21 years old and 22% in children aged 0–4. In China, the prevalence of *H. pylori* infection is 88% in adults and 68% in children aged 3–12. In Kazakhstan, the *H. pylori*-seropositive rate is 86% in adults and 64% in children. The *H. pylori*-seropositive rate is 77% in people below 20 years old and 100% in people over 60 years old.

Cytotoxin associated gene A (CagA) is the most important pathogenic factor of *H. pylori* and plays a crucial role in the occurrence of gastric cancer [13]. Earlier studies by other groups have reported that CagA-positive *H. pylori* strains were isolated from almost all patients with gastric cancer [14–17].

Pepsinogens are the proenzymes of the digestive enzyme pepsin, with two immunologically distinguishable isoforms (pepsinogens 1 and 2) [18]. Pepsinogen 1 is secreted from fundic mucosa cells and gastric chief cells, and pepsinogen 2 is secreted from similar cells in the pyloric glands in the antrum [19]. The levels of pepsinogen 1 decrease in atrophic stomachs of patients infected with *H. pylori*, thereby reducing the ratios of pepsinogen 1/2. Therefore, the detection of serum pepsinogen levels in patients infected with *H. pylori* is important in assessing the grade of atrophic gastritis and predicting the gastric cancer that will be caused by long-term colonization by this pathogen [20].

However, earlier investigations have provided negligible information as to the prevalence of *H. pylori* infection in Mongolian people. Therefore, we examined the levels of serum anti-*H. pylori* antibodies in Mongolian people using two ELISA kits and the levels of serum pepsinogen in people infected with *H. pylori*.
2. Results

2.1. Detection of Anti-\textit{H. pylori} Antibodies in the Sera of Mongolian People by Two Different ELISA Kits

As described earlier, 43 and 27 serum specimens from the 70 Japanese people tested negative and positive, respectively, during the examination for anti-\textit{H. pylori} IgG antibodies using a Japanese ELISA kit (an E-plate ELISA kit). However, 41 and 29 serum specimens from the same 70 people tested negative and positive, respectively, during the examination for anti-\textit{H. pylori} IgG antibodies by a Finnish ELISA kit (a Biohit ELISA kit) (Table 1). In summary, no statistical significance was observed in the detection of anti-\textit{H. pylori} antibodies in sera between the two ELISA kits via nonparametric tests of the 70 serum specimens (Wilcoxon, \(p = 0.157\); McNemar’s test, \(p = 0.5\)).

We next measured anti-\textit{H. pylori} IgG antibodies in the 610 serum specimens from Mongolian people using either the E-plate ELISA kit or the Biohit ELISA kit to examine the \textit{H. pylori}-positive rate. Of the 610 serum specimens, 385 aliquots tested \textit{H. pylori}-positive and 47 aliquots tested \textit{H. pylori}-negative in both ELISA kits. However, 175 serum aliquots tested \textit{H. pylori}-positive using the Biohit ELISA kit, despite testing \textit{H. pylori}-negative in the E-plate ELISA kit. In addition, 3 serum aliquots tested \textit{H. pylori}-negative using the Biohit ELISA kit, but tested \textit{H. pylori}-positive in the E-plate ELISA kit. The regression line, based on the serum concentrations of anti-\textit{H. pylori} IgG antibodies detected in the 610 specimens using each ELISA kit, had a low correlation (\(R = 0.496\)) (Figure 1).

As summarized in Table 2, the positive ratios of anti-\textit{H. pylori} IgG antibodies in the 610 serum specimens measured by the Biohit ELISA kit were higher in all age groups than those of anti-\textit{H. pylori} IgG antibodies in the same serum specimens measured by the E-plate ELISA kit. Anti-\textit{H. pylori} IgG antibodies (positive ratio: 91.5%) were detected in 333 of the 364 serum specimens from Mongolian females investigated by the Biohit ELISA kit. Similarly, anti-\textit{H. pylori} IgG antibodies (positive ratio: 92.3%) were detected in 227 of the 246 serum specimens from Mongolian males investigated by the same ELISA kit (data not shown). On this basis, no statistical significance was observed in the positive ratio of anti-\textit{H. pylori} antibodies between males and females in Mongolia using the Biohit ELISA.

Table 1. Status of age and gender of the 610 sera collected from Mongolian people.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Gender</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>10–15</td>
<td>137</td>
<td>102</td>
</tr>
<tr>
<td>16–19</td>
<td>29</td>
<td>27</td>
</tr>
<tr>
<td>20–29</td>
<td>100</td>
<td>55</td>
</tr>
<tr>
<td>30–39</td>
<td>54</td>
<td>33</td>
</tr>
<tr>
<td>40–49</td>
<td>38</td>
<td>24</td>
</tr>
<tr>
<td>50–59</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Over 60</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>364</td>
<td>246</td>
</tr>
</tbody>
</table>

Mean age: 22.9

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Anti-\textit{H. pylori} IgG Biohit</th>
<th>Anti-\textit{H. pylori} IgG E-plate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>10–19</td>
<td>34</td>
<td>261</td>
</tr>
<tr>
<td>20–39</td>
<td>13</td>
<td>229</td>
</tr>
<tr>
<td>Over 40</td>
<td>3</td>
<td>70</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>560</td>
</tr>
</tbody>
</table>
2.2. Detection of Anti-CagA Antibodies in the Sera of Mongolian People

A DRG ELISA kit (for the detection of anti-CagA IgG antibodies) judged that 42 and 28 serum aliquots of the 70 serum specimens from Japanese people were anti-CagA IgG antibody-negative and -positive, respectively (Table 3). Meanwhile, 41 of the 42 antibody-negative serum specimens retained neither anti-\textit{H. pylori} IgG antibodies nor anti-CagA IgG antibodies in the Biohit ELISA and DRG ELISA. However, of the 42 serum specimens, only one aliquot, in which the anti-CagA IgG antibodies were below the limits of detection, tested IgG antibody-positive against \textit{H. pylori} via the Biohit ELISA. Of the same 70 serum specimens, 27 aliquots, in which anti-\textit{H. pylori} IgG antibodies were detected by the E-plate ELISA, turned out to include anti-CagA IgG antibodies in the DRG ELISA. The 42 aliquots in which the anti-\textit{H. pylori} IgG antibodies were below the limits of detection in the E-plate ELISA did not include anti-CagA IgG antibodies. However, only one aliquot in which anti-CagA IgG antibodies were detected anti-\textit{H. pylori} IgG antibody-negative via the E-plate ELISA.

Table 3. Detection of anti-cytotoxin associated gene A (\textit{CagA}) antibody status and cross-tabulation of the two anti-\textit{H. pylori} IgG ELISA kits.

<table>
<thead>
<tr>
<th></th>
<th>Japanese Anti-CagA Antibody</th>
<th>Mongolians Anti-CagA Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Biohit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>41</td>
</tr>
<tr>
<td>E-plate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>42</td>
</tr>
</tbody>
</table>

Next, we examined the anti-CagA IgG antibodies in sera obtained from Mongolian people using the same ELISA kit. Of the 560 anti-\textit{H. pylori} IgG antibody-positive serum specimens (Biohit ELISA), 500 serum specimens tested positive for the detection of anti-CagA antibodies (Table 3). Six serum specimens tested positive for the detection of anti-\textit{H. pylori} IgG-negative antibodies in the Biohit ELISA, but also tested negative for the detection of anti-CagA antibodies. Meanwhile, the positive ratio of anti-CagA IgG antibodies was 94.6% in the 388 serum specimens that tested positive for the detection of anti-\textit{H. pylori} IgG antibodies using the E-plate ELISA. Intriguingly, of the 222 serum specimens, of which anti-\textit{H. pylori} IgG antibodies were below the limits of detection in 139 during the examination using the E-plate ELISA kit, 139 aliquots (62.6%) included anti-CagA IgG antibodies.

2.3. Relationship between the Ratio of Pepsinogen 1/2 and \textit{H. pylori} Infection

Figure 1 shows the ratio of pepsinogen 1/2 in the serum specimens from the 70 Japanese people, with 29 and 41 aliquots being positive and negative, respectively, in the examination for anti-\textit{H. pylori} antibodies using the Biohit ELISA kit. The ratio of pepsinogen 1/2 was obviously lower in the \textit{H. pylori}-positive serum specimens than in the \textit{H. pylori}-negative serum specimens. The mean values of the ratio of pepsinogen 1/2 were 4.1 ± 0.41 and 14.55 ± 1.04 in the \textit{H. pylori}-positive and -negative serum specimens, respectively.

We next examined the ratio of pepsinogen 1/2 in the serum specimens from the 610 Mongolian people, with 560 and 50 aliquots testing positive and negative, respectively, in the examination for anti-\textit{H. pylori} antibodies using the Biohit ELISA kit. The ratio of pepsinogen 1/2 was statistically lower \((p < 0.0001)\) in the \textit{H. pylori}-positive serum specimens than in the \textit{H. pylori}-negative serum specimens (Figure 2). However, the levels of serum pepsinogen 1 in the \textit{H. pylori}-positive specimens were comparable to those of pepsinogen 1 in the \textit{H. pylori}-negative specimens \((p = 0.465)\). The mean values of the ratio of pepsinogen 1/2 were 6.74 ± 0.12 and 12.69 ± 1.02 in the \textit{H. pylori}-positive and -negative serum specimens, respectively.
Figure 1. Pepsinogen 1/2 ratio in the H. pylori-negative and -positive groups: (A) 610 Mongolian people; (B) 70 Japanese people.

Anti-H. pylori IgG antibody in Mongolian people (Finland Biohit kit)

Anti-H. pylori IgG antibody in Japanese people (Finland Biohit kit)
The ratio of pepsinogen 1/2 were 6.74 ± 0.12 and 12.69 ± 1.02 in the H. pylori-positive and -negative serum specimens, respectively.

Table 4 shows the ratios of pepsinogen 1/2 in each age group of Mongolian people. The lowest values (below 3 or 5) of the pepsinogen 1/2 ratio were observed in the 204 serum specimens with positive antibodies against H. pylori, as detected by the Biohit ELISA kit, and the serum specimens were assumed to be associated with atrophic gastritis.

Table 4. Cross-tabulation of the pepsinogen 1/2 ratio in each age group.

<table>
<thead>
<tr>
<th>H. pylori</th>
<th>Pepsinogen Ratio</th>
<th>Total</th>
<th>Total Positive Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10–19</td>
<td>20–39</td>
</tr>
<tr>
<td>Positive</td>
<td>&lt;3</td>
<td>8</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>&lt;5</td>
<td>28</td>
<td>91</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt;3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&lt;5</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

Of the 560 serum specimens in which the anti-H. pylori IgG antibodies were positive in the examination using the Biohit ELISA, 47 aliquots and 157 aliquots were below 3 and 5, respectively, for the values of the pepsinogen 1/2 ratio. The difference in the pepsinogen 1/2 ratio between the H. pylori-positive and -negative serum specimens was greater in the group aged 10 to 19 than in the other age groups (Figure 2). Of the 50 serum specimens in which the anti-H. pylori IgG antibodies were negative in the examination using the Biohit ELISA, 4 aliquots from the groups aged 20 to 39 and over 40 were less than 3 (for the pepsinogen 1/2 ratio). In addition, the ratio of pepsinogen 1/2 was statistically lower \((p < 0.0001)\) in the anti-CagA IgG-positive serum specimens than in the anti-CagA IgG-negative serum specimens, all of which were positive in the examination for anti-H. pylori IgG antibodies by the Biohit ELISA (Figure 3). On the contrary, no difference in the levels of serum
pepsinogens 1 and 2 was observed between the anti-\textit{H. pylori} IgG-positive and -negative specimens which judged by the E-plate ELISA kit, and therefore the ratio of pepsinogen 1/2 in the anti-\textit{H. pylori} IgG-positive serum specimens was comparable (no statistical significance) to that of pepsinogen 1/2 in the anti-\textit{H. pylori} IgG-negative serum specimens.

![Figure 3. Pepsinogen 1/2 ratio in the anti-CagA antibody-negative and -positive cases (\(p = 0.000063\)).](image)

3. Discussion

Several studies have investigated the prevalence of \textit{H. pylori} infection in Mongolia. In a study of \textit{H. pylori}, HPV, HCV, HBV, and JC virus infection, anti-\textit{H. pylori} antibodies were detected by the two antigens (NapA and GroEL) in 1022 dry-blood spot samples that were collected from Mongolian people. The prevalence of \textit{H. pylori} infection in the samples was 45.5\% in the detection by the NapA antigen and 86.0\% in the detection by the GroEL antigen [21]. In addition, the detection of \textit{H. pylori} genes through immunohistochemistry (IHC) analysis has suggested \textit{H. pylori} infection in 76\% of biopsy specimens obtained from 736 Mongolian people [22]. There are a number of methods for diagnosing an \textit{H. pylori} infection. Among those methods, a serological examination is less accurate than a breath test, stool antigen test, and rapid urease test. However, serological examinations have still been reliable and are used widely in prevalence studies because other tests have difficulty examining \textit{H. pylori} infections in various peoples within a relatively short time [23]. In the studies mentioned above, \textit{H. pylori} infections were detected with unusual methods. Here, we present the prevalence of \textit{H. pylori} infection in Mongolian people using a serological examination.

We performed this study using sera collected from Mongolian people who were over 11 years old, because it has been reported that some sera collected from children under 10 years old indicate a false-negative in serological examinations used to detect the anti-\textit{H. pylori} IgG antibody.

In terms of sensitivity and specificity for the diagnosis of \textit{H. pylori} infection, it has been reported that ELISA kits show some differences within a country. It is believed that an ELISA kit using a domestic representative strain as an antigen should be used in each country or area. In Japan, an ELISA kit that has been developed by a Japanese company is used for the diagnosis of \textit{H. pylori} infection.
Japanese ELISA kits such as the E-plate are higher in terms of sensitivity and specificity for the diagnosis of *H. pylori* infection in Japanese people than other ELISA kits that have been developed by other countries’ companies [24]. The Eiken E-plate ELISA kit is frequently used in Japan and is the most reliable in the detection of Japanese *H. pylori* strains. The E-plate ELISA kit is one of the most representative ELISA kits used worldwide [25] and adopts an HM-CAP antigen derived from a U.S. strain. ELISAs using the HM-CAP antigen have been reported to be 95% to 100% valid in the examination of *H. pylori* infection in Western countries [24,26,27]. Meanwhile, the Biohit ELISA kit is frequently used in European countries, especially Finland, Italy, and the United Kingdom. In sum, we used two ELISA kits that adopt different antigens in order to detect anti-*H. pylori* IgG antibodies: One kit is from Japan, and the other kit is from Finland.

In this study, we found a significant difference in the detection of serum anti-*H. pylori* antibodies between the two ELISA kits. Serum anti-*H. pylori* IgG antibodies were positive in 91.8% of 610 Mongolian people examined by the Finnish ELISA kit, whereas serum anti-*H. pylori* IgG antibodies were positive in 63.6% of the same Mongolian people examined by the Japanese ELISA kit. We found only a weak correlation between titers of the two ELISA kits. The antigens adopted by the two ELISA kits were qualitatively different, although we cannot confirm the antigens used in the Japanese ELISA kit (E-plate) and the Finnish ELISA kit (Biohit) in detail. The Japanese ELISA kit was incapable of detecting anti-*H. pylori* antibodies in the serum specimens obtained from 139 Mongolian people in spite of them being positive for anti-CagA antibodies. Meanwhile, the Finnish ELISA kit was capable of detecting anti-*H. pylori* antibodies in 133 serum specimens of the 139 serum specimens that were positive for anti-CagA antibodies. A statistical significance was observed in the pepsinogen 1/2 ratio between the positive and negative anti-*H. pylori* IgG antibodies detected by the Finnish ELISA kit, whereas no statistical significance was observed in the pepsinogen 1/2 ratio between the positive and negative anti-*H. pylori* IgG antibodies detected by the Japanese ELISA kit. These results suggest that the Finnish ELISA kit could be more reliable in the detection of serum anti-*H. pylori* antibodies in Mongolian people than the Japanese ELISA kit.

The detection rate of anti-*H. pylori* IgG antibodies by the Finnish ELISA kit was 91.8% and 88.5% in the total of all age groups and in the age group of 10–19, respectively. This means that *H. pylori* infection was prevalent in a young generation of Mongolians. These results are similar to the prevalence of *H. pylori* infection in people in developing countries.

In earlier studies, the clinical isolation ratio of cagA-positive *H. pylori* strains in total *H. pylori* strains has been 67% in the Netherlands [13], 94% in Brazil [14], 97% in Korea [15], 79% in Ethiopia [16], and 97.6% in Japan [17]. In this study, anti-CagA IgG antibodies were positive in 89.3% of all the *H. pylori*-positive sera of the Mongolian people examined. In sum, almost all *H. pylori* strains prevalent in Mongolia are suggested to have a cagA gene. In a previous study, seropositive CagA was significantly higher in patients with fundic atrophy than in healthy people without gastritis and gastric atrophy when the atrophic gastritis was assessed through the detection of serum pepsinogen and a histological examination [28]. In an epidemiological study, chronic atrophic gastritis was significantly associated with seropositive *H. pylori*, seropositive CagA, and serum pepsinogen levels [29]. This study showed that the pepsinogen 1/2 ratio was lower in the anti-CagA antibody-positive sera than in the anti-CagA antibody-negative sera. Further investigations will be necessary to identify the types of cagA genes in *H. pylori* strains in Mongolia and to clarify the role of CagA in the pathogenicity of Mongolian *H. pylori* strains.

The findings are consistent with reports that atrophic gastritis prevalence in the world is about 7% [30,31]. A definitive atrophy diagnosis through endoscopic and histological examinations requires invasive clinical intervention, although a histological examination using a gastric biopsy is one of the gold standards for the detection of atrophic gastritis and other diseases. In addition, an endoscopic examination for the detection of atrophic gastritis is invasive and uncomfortable, and it is unsuitable for various prevalence studies. Meanwhile, an examination of serum pepsinogen levels is non-invasive in the diagnosis of atrophic gastritis and is often carried out in European countries [32]. The test
has a sensitivity of 82% (CI 95%, 70–93%) and a specificity of 98% (CI 95%, 97–99%) in the diagnosis of moderate or severe atrophic gastritis compared to the gold standard method, histopathological biopsy microscopy [33,34]. In this study, the pepsinogen 1/2 ratio was statistically lower in the \textit{H. pylori}-positive sera than in the \textit{H. pylori}-negative sera. When the pepsinogen 1/2 ratio was low (<3), the Gastrosoft (Biohit manufacturer) interpretation was “severe atrophic corpus gastritis” [35]. In a study by Nasrollahzadeh, D., et al., the advanced cutoff values in a gastric atrophy assessment were calculated by the pepsinogen 1/2 ratio (<5) (sensitivity: 75.0%, specificity: 91.0%), and this was more suitable for the diagnosis of atrophic gastritis than the manufacturer interpretation (pepsinogen 1/2 ratio: <3) [28]. In the present study, almost all of the Mongolian people were suspected of atrophic gastritis, according to that advanced cutoff value. Because most of the study population was under 40 years old, elderly people with an \textit{H. pylori} infection could indicate a lower pepsinogen 1/2 ratio. The difference in the pepsinogen 1/2 ratio between \textit{H. pylori}-positive and -negative groups was greater in the age group of 10–19 than in the other age groups. In the age group of 10–19, some people with serum anti-\textit{H. pylori} positive antibodies clearly had gastric atrophy. These results suggest that an \textit{H. pylori} infection could induce serious damage to the gastric mucosa of Mongolian people. The pepsinogen 1/2 ratio obviously decreased with an increase in age, regardless of the presence or absence of anti-\textit{H. pylori} antibodies. Some risk factors, excluding an \textit{H. pylori} infection, may have affected gastric atrophy. In addition, the pepsinogen 1/2 ratio in ages 10 to 39 was lower in the anti-\textit{H. pylori} antibody-positive groups than in the anti-\textit{H. pylori} antibody-negative groups. Meanwhile, the pepsinogen 1/2 ratio in ages over 40 was lower in the anti-\textit{H. pylori} antibody-negative groups than in the anti-\textit{H. pylori} antibody-positive groups. This may indicate that some other risk factors exist with the possibility of influencing gastric atrophy, such as alcohol consumption and high salt usage, at over 40 years of age. In addition, research limitations may have influenced the results of this study by our group, as only three people examined by the Finnish ELISA kit were negative for an \textit{H. pylori} infection.

A study by another research group has suggested that the prediction of a high-risk status for gastric atrophy is defined as less than 3.1 for the pepsinogen 1/2 ratio, and that less than 2.2 for the pepsinogen 1/2 ratio and 28 ng/mL of pepsinogen 1 is defined as the best prediction of gastric cancers [36]. This study was mainly focused on the prediction of gastric cancers through the measurement of serum pepsinogen levels, but the prediction of gastric cancers could not be defined. In the present study, we demonstrated that an infection of \textit{H. pylori} with positive CagA is a high risk for gastric atrophy in Mongolian people.

The limitation of this study was that it was impossible to compare the ELISA with a rapid urease test or an \textit{H. pylori} culture using a gastric biopsy at the same time. It was difficult to involve relatively healthy individuals in the study because they refused involvement due to the invasive procedures of a gastric biopsy.

4. Materials and Methods

4.1. Study Population

Serum specimens from 680 individuals were examined to measure the amounts of pepsinogens 1 and 2, anti-CagA antibodies, and anti-\textit{H. pylori} antibodies. The specimens were collected from 610 Mongolian people living in the capital city, Ulaanbaatar, and were stored in the virology laboratory at Jichi Medical University in Japan. Here, 295 aliquots of the serum specimens were provided from blood donors over 20 years old. Another 315 aliquots were obtained from people (ages 10 to 19) who underwent a medical checkup. Seventy serum specimens were collected from Japanese people over 40 years old who took a health screening: They were stored in the gastroenterology laboratory at Jichi Medical University. The sera of the Japanese people were used as the control specimens. Of the 70 serum specimens, 43 aliquots were negative in the examination for anti-\textit{H. pylori} antibodies by an E-plate ELISA kit (Eiken Chemical Co., Ltd., Tokyo, Japan). The other 27 aliquots were positive in the examination for these antibodies by the same ELISA kit.
We divided the serum specimens of the Mongolian people into the following three groups: A group of ages 10 to 19, ages 20 to 39, and over 40 (Table 1): 295 serum specimens were from the group aged 10 to 19, 242 serum specimens were from the group aged 20 to 39, and 73 serum specimens were from the over-40 age group. Those serum specimens included 364 serum specimens from females and 246 serum specimens from males. The mean age of the 610 Mongolian people (ranging from 11 to 66 years old) was 22.9 ± 11.5 years old (the median value: 20 years old).

4.2. Detection of Antibodies against H. pylori, CagA, and Pepsinogens in Serum Specimens

The two ELISA kits, an E-plate ELISA kit (Eiken Chemical Co., Ltd., Tokyo, Japan) and a Biohit ELISA kit (Biohit Oyj, Helsinki, Finland), were used to determine the concentration of anti-H. pylori IgG antibodies in the sera of the 610 Mongolian people. The protocol of the E-plate ELISA kit defines serum specimens as positive for anti-H. pylori IgG antibodies when antibodies are detected at a concentration greater than 10 U/mL. Meanwhile, the protocol of the Biohit ELISA kit defines serum specimens as positive for anti-H. pylori IgG antibodies when antibodies are detected at greater than 30 EIU.

An ELISA kit EIA-4138 from DRG Instruments GmbH (Marburg, Germany) was used to detect anti-CagA IgG antibodies in the serum specimens. The protocol of the ELISA kit defines the limits of detection of anti-CagA antibodies as below a concentration of 10 RU/mL.

Pepsinogens 1 and 2 in the serum specimens were measured using an ELISA kit (pepsinogen I and II, Biohit Oyj), and the ratios of pepsinogen 1/2 were calculated. Based on the instructions in the ELISA kit, gastric atrophic grades were assessed from the serum levels of the pepsinogens and the ratios of pepsinogen 1/2.

4.3. Ethics

The local ethics committees of both the Mongolian National University of Medical Sciences and Jichi Medical University in Japan approved the use of the above-mentioned sera in the investigations of this study. There is no information about patients written in this article. See ethical application ref: 2014/D/2014-11 (approved date: 6 November 2014).

4.4. Statistical Analysis

A computer program, SPSS version 20.0, was used to calculate statistical significances, regression line types, and mean values ± standard errors at a 95% confidence interval. Statistical evaluations comparing the groups were performed via an independent Student’s t-test and a regression line correlation analysis. A statistical comparison of nonparametric tests between the groups was evaluated via a Wilcoxon test and McNemar’s test.

5. Conclusions

In this study, we demonstrated the high retention rates of anti-H. pylori antibodies and the low pepsinogen 1/2 ratio in Mongolian people, indicating the prevalence of atrophic gastritis in Mongolian people. Furthermore, eradication therapy with triple drugs could be applied actively to people infected with H. pylori.

In the future, we will need to validate the gold standard for the diagnosis of H. pylori infection in Mongolia. The limitation of our study was that both serological test kits were not validated by comparing them with the gold standard in Mongolia, so we will need to determine the validity of these two kits in the future.

Author Contributions: Study concept and design, H.S., Y.H., and A.A.; acquisition of data, A.A.; analysis and interpretation of data, A.A.; drafting of manuscript, A.A. and H.S.; critical revision of the manuscript for important intellectual content and approval of the final version, A.A., T-O.B., K.S., H.O., H.S., and Y.H.; supervision, K.S., H.O., and Y.H.

Funding: This research received no external funding.
Conflicts of Interest: The authors declare no conflicts of interest.

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