Correlations between the CagA Antigen and Serum Levels of Anti-\textit{Helicobacter pylori} IgG and IgA in Children

Ji-Hyun Seo,1 Chun Woo Lim,1 Ji Sook Park,1 Jung Sook Yeam,1 Jae-Young Lim,1 Jin-Su Jun,1 Hyang-Ok Woo,1 Hee-Shang Youn,1 Seung-Chul Baik,2 Woo-Kon Lee,2 Myung-Je Cho,2 and Kwang-Ho Rhee2

1Department of Pediatrics, Gyeongsang National University School of Medicine, Gyeongsang Institute of Health Science, Jinju, Korea; 2Department of Microbiology, Gyeongsang National University School of Medicine, Gyeongsang Institute of Health Science, Jinju, Korea

Received: 1 September 2015
Accepted: 10 December 2015

Address for Correspondence:
Hee-Shang Youn, MD
Department of Pediatrics, Gyeongsang National University School of Medicine, 15 Jinju-daero 816-beon-gil, Jinju 52727, Korea
E-mail: hsyoun@gnu.ac.kr

Funding: This study was supported by a grant from the National R&D Program for Cancer Control of the Ministry of Health & Welfare of the Republic of Korea (0820050).

INTRODUCTION

\textit{Helicobacter pylori} is an important etiologic factor for acute and chronic gastritis, gastric and duodenal ulcers, and gastric adenocarcinoma (1). The severity of \textit{H. pylori} infection depends on the strain virulence, host susceptibility, and environmental factors (2). The measurement of specific antibodies in serum has been used as a noninvasive method for detecting \textit{H. pylori} infection (3) and over 90% of \textit{H. pylori}-infected patients have detectable serum IgG antibodies (4). Serological tests are commercially available, which are easy to perform and inexpensive, but studies on children indicate a high sensitivity range of 50%-90% and the specificity ranges from 83% to 100% (5-8).

The number of immunoreactive bands significantly increases with age and reactions to the VacA and CagA antigens are more frequently found in older children (9). In our previous study, we found that more than 80% of the seropositive enzyme-linked immunosorbent assay (ELISA) results were CagA positive, whereas the other 20% of the seropositive results using ELISA could be attributed to its reaction with another \textit{H. pylori} antigen (10). Therefore, the use of whole-cell lysates of \textit{H. pylori} strain 51 in ELISA may increase the yield when detecting anti-\textit{H. pylori} antibodies in the Korean population.

Several studies have investigated the relationship between antibody titers and the pathogenesis of \textit{H. pylori}, but the results are inconclusive (3,11). Quantitative evaluations of anti-\textit{H. pylori} antibodies or against \textit{H. pylori} recombinant purified proteins have been performed in some human diseases where \textit{H. pylori} infections may play a role in their pathogenesis (9,12-14). However, the clinical significance of high antibody levels to \textit{H. pylori} according to quantitative ELISA has not been established and high anti-\textit{H. pylori} antibody levels have not been demonstrated to be predictive of the severity of gastroduodenal diseases or the density of \textit{H. pylori} colonization.

Thus, to help identify factors that correlate with antibody levels in children, we evaluated the correlations between the levels of anti-\textit{H. pylori} IgG and IgA antibodies and the urease test grade, presence of CagA antigen, degree of gastritis, and age.

MATERIALS AND METHODS

Study population
As a member of the National Biobank of Korea, Gyeongsang National University Hospital (GNUH) collects serum samples
from random patients and stores them at -80°C. Among the samples collected over 21 years, we examined those from 509 children who underwent upper gastroduodenoscopy at GNUH during 1991-2010. Thus, we enrolled 509 children and we reviewed the results of urease test and the histopathological findings, and tested the reserved serum. The sera were stratified into three age groups: 0-4 years (n = 132), 5-9 years (n = 274), and 10-15 years (n = 103) (Table 1).

| Characteristics          | No. (%) of children by age groups | P value |
|--------------------------|----------------------------------|---------|
| Number                   | 0-4 yr  5-9 yr  10-15 yr          | 0.509   |
| Sex                      | Male     Female                   | 0.999   |
| Female                   | 60 (45.5) 125 (45.6) 47 (45.6)   |         |
| Male                     | 72 (54.5) 149 (54.4) 56 (54.4)   |         |
| Histopathological findings |        |         |
| Chronic gastritis        | Normal    Mild         Moderate    | 0.021   |
|                         | 4 (3.0)  4 (1.4)  0 (0)       |         |
|                         | 94 (71.2) 211 (77.0) 64 (62.1) |         |
|                         | 28 (21.2) 46 (16.8) 28 (27.2)  |         |
|                         | 6 (4.5)  13 (4.7)  11 (10.7)  |         |
| Active gastritis         | Normal    Mild         Moderate    | 0.064   |
|                         | 103 (78.0) 214 (78.1) 65 (63.1) |         |
|                         | 20 (15.2) 44 (16.1) 27 (26.2)  |         |
|                         | 9 (6.8)  13 (4.7)  9 (8.7)    |         |
|                         | 0 (0)     3 (1.1)  2 (1.9)    |         |
| H. pylori infiltration   | Normal    Mild         Moderate    | < 0.001 |
|                         | 98 (74.2) 216 (78.8) 62 (60.2) |         |
|                         | 29 (22.0) 46 (16.8) 28 (27.2)  |         |
|                         | 2 (1.5)   11 (4.0)  12 (11.7) |         |
|                         | 3 (2.3)   1 (0.4)   1 (1.0)   |         |
| Urease test              | Negative    6-24 hr < 6 hr     | 0.003   |
|                         | 83 (62.9) 170 (62.0) 62 (60.2) |         |
|                         | 21 (15.9) 49 (17.9)  5 (4.9)    |         |
|                         | 28 (21.2) 55 (20.1) 36 (35.0)  |         |

### RESULTS

#### ELISA and western blot analysis

Anti-\(H.\ pylori\) IgG and IgA titers were measured by ELISA (10) using the coated with the prepared whole cell proteins of \(H.\ pylori\) strain 51 (10 \(\mu\)g/mL and 50 \(\mu\)L per well diluted with coating buffer). Diluted sera (IgG 1:400, and IgA 1:100) were added to antigen-coated wells (50 \(\mu\)L per well).

Anti-CagA IgG and IgA antibodies were evaluated by Western blot using whole-cell lysates of \(H.\ pylori\) strain 51 (15). The western blot patterns were assigned to four categories on the basis of CagA (pattern I), urease without CagA (pattern II), other proteins except CagA and urease (pattern III), and no band (pattern IV) (Fig. 1).

### Statistical analysis

The statistical analyses were performed using IBM SPSS Statistics 21 (IBM, Chicago, IL, USA). We tested whether the antibody titer had significant correlations with the urease test results, antigen patterns, or age. We used bivariate correlation (Spearman’s \(r_h\)), paired samples t-tests, and nonparametric tests to analyze the differences in the antibody titers between CagA-positive and-negative sera, among the three urease test grades, and in the three age groups. The numbers and sex ratios in the three age groups were different, so GLM regression analysis was used for correction. Post-hoc analysis using Scheffé’s method was applied when significant differences were detected among three groups. Statistically significant differences were accepted at \(P < 0.05\).

### Ethics statement

The study protocol was reviewed and approved by the institutional review board of GNUH (GNUHIRB-2015-08-020). Informed consent was exempted by the board.
Results of the urease test and histopathological findings
The positivity rates for the urease test were 37.1% at 0-4 years, 38.0% at 5-9 years, and 39.9% at 10-15 years (P = 0.003). The degrees of chronic gastritis (P = 0.021), active gastritis (P = 0.064), and H. pylori infiltration (P < 0.001) increased with age (Table 1).

Anti- H. pylori IgG and IgA titers
The median titers for anti- H. pylori IgG were 732.5 IU/mL at 0-4 years, 689.0 IU/mL at 5-9 years, and 966.0 IU/mL at 10-15 years (P < 0.001). The median titers for anti- H. pylori IgA were 61.0 IU/mL at 0-4 years, 63.5 IU/mL at 5-9 years, and 75.0 IU/mL at 10-15 years (P < 0.001). The anti- H. pylori IgG titers were higher at 10-15 years than those at 1-5 years and 6-10 years (P = 0.006), but there was no significant difference in the anti- H. pylori IgA titers among the three age groups (P = 0.454).

Western blot patterns according to age groups
The proportions of IgG positivity according to the four western blot patterns (I-IV) were 36.0%, 47.7%, 5.5%, and 10.8%, respectively (P = 0.008). The proportions of IgA positivity according to the four patterns were 17.7%, 33.8%, 23.6%, and 25.0%, respectively (P = 0.221). The CagA-positivity rates were 26.5% at 0-4 years, 36.5% at 5-9 years, and 46.6% at 10-15 years for IgG (P = 0.036, Fig. 2), and 11.4% at 0-4 years, 18.6% at 5-9 years, and 23.3% at 10-15 years for IgA (P < 0.001) (Table 2). The western blot negative (no band) rates for IgG were 13.6%, 8.8%, and 12.6% at 0-4 years, 5-9 years, and 10-15 years, respectively. Post-hoc analysis using Scheffé’s method detected no differences in the proportions of the four Western blot patterns among the three age groups for IgG (P = 0.094) and for IgA (P = 0.161).

Correlations between the urease test grade, degree of histopathological findings, western blot patterns, and antibody titers for IgG and IgA
The titers of anti- H. pylori IgG antibodies increased with the urease test grade (r = 0.527, P < 0.001), chronic gastritis (r = 0.613, P < 0.001), active gastritis (r = 0.545, P < 0.001), and the degree of H. pylori infiltration (r = 0.593, P < 0.001). The anti- H. pylori IgA titers also increased with the urease test grade (r = 0.450, P < 0.001), degree of chronic gastritis (r = 0.523, P < 0.001), active gastritis (r = 0.453, P < 0.001), and H. pylori infiltration (r = 0.480, P < 0.001). In the urease test, the anti- H. pylori IgG (Fig. 3) and IgA (Fig. 4) antibody titers were higher with grade I than the other grades (P < 0.001), regardless of age. According to the four western blot patterns, the anti- H. pylori IgG (Fig. 5) and IgA (Fig. 6) titers were higher with the CagA-positive pattern (P < 0.001), regardless of age.

Table 2. Proportions with the four western blot patterns according to age groups
| Target proteins | 0-4 yr | 5-9 yr | 10-15 yr |
|-----------------|-------|-------|---------|
| CagA IgG        | 35 (26.5) | 100 (36.5) | 48 (46.6) |
| CagA IgA        | 15 (11.4) | 51 (18.6) | 24 (23.3) |
| Urease IgG      | 72 (54.5) | 133 (48.5) | 38 (36.9) |
| Urease IgA      | 39 (29.5) | 97 (35.4) | 36 (35.0) |
| Other proteins IgG | 7 (5.3) | 17 (6.2) | 4 (3.9) |
| Other proteins IgA | 52 (39.4) | 51 (18.6) | 17 (16.5) |
| No band IgG    | 18 (13.6) | 24 (8.8) | 13 (12.6) |
| No band IgA    | 26 (19.7) | 75 (27.4) | 26 (25.2) |

Fig. 2. Proportions with the four western blot patterns according to age groups. The CagA-positivity rate was 26.5% at 0-4 years, 36.5% at 5-9 years, and 46.6% at 10-15 years for IgG (P = 0.036). Post-hoc analysis using Scheffé’s method detected no differences in the proportions of the four western blot pattern among the three age groups for IgG (P = 0.094).

Fig. 3. Anti- H. pylori IgG antibody titers according to age groups and the urease test grade. The anti- H. pylori IgG antibody titers were higher with grade I (positive within 6 hours) than the other grades (P < 0.001) in all age groups.

http://dx.doi.org/10.3346/jkms.2016.31.3.417  http://jkms.org 419
DISCUSSION

In the present study, we found that the presence of the CagA antigen was the major factor related to high levels of anti-\textit{H. pylori} IgG and IgA antibodies, regardless of age. CagA is known to be an important virulence factor in \textit{H. pylori} (14) and antibodies against CagA have been observed in gastritis, gastroduodenal ulcer, and gastric cancer patients (13,16,17). In the early 2000s, 80%-100% of \textit{H. pylori} strains possessed the \textit{cagA} gene in East Asia (18,19) and 94% were \textit{cagA}-positive in \textit{H. pylori} DNA extracts from 33 Korean children (20). In Japan, the CagA was the most reactive antigen recognized by all the \textit{H. pylori} positive sera even from children under the age of 3 years (21). Therefore, the regional CagA antigens for serodiagnosis of \textit{H. pylori} would be important, which could affect the rate of seropositivity (21). Thus, a positive test result for anti-CagA antibody was regarded as an \textit{H. pylori} infection in Korean studies (15,22). In the present study, the highest positivity rate for CagA was 46.6% among the 10-15 years group, although this CagA-positivity rate is lower than the seroprevalence (59.6%) of \textit{H. pylori} infections in a recent study of the general population in Korea (23), as well as the seroprevalence rate (68.0% CagA-positive) in children aged 6-15 years using the same immunoblot analysis during 1998-1999 (17). Recent seroprevalence studies of \textit{H. pylori} infection suggest that the decrease in the seroprevalence of \textit{H. pylori} may be related to the improved socioeconomic status of Koreans (23,24).

Young children may have a different immune response to \textit{H. pylori}, with preferences for specific antigens, as well as lower titers than adults (8). A lower sensitivity has been reported based on serological \textit{H. pylori} tests in children compared with adults (25). Using commercial ELISA kits, false-negative results were found more often in children aged younger than 5 years (8,26). In our previous study, we showed that the Genedia IgG ELISA kit, which uses \textit{H. pylori} antigen obtained from a Korean \textit{H. pylori} strain, achieved a higher seropositivity rate than other ELISA kits (e.g., GAP IgG, HM-CAP, and Pyloriset EIA-G obtained from USA and Finland) (17). There are differences in the antigenicity of multiple \textit{H. pylori} strains and even among different
antigens in the same strain (9). In the present study, the median levels of anti-\textit{H. pylori} IgG and IgA antibody also increased with age but the antibody titers were higher in the CagA-positive cases than those in the CagA-negative cases, even in children aged under 5 years.

Age is strongly related to \textit{H. pylori} infections (27). In the present study, the degree of chronic gastritis, active gastritis, and \textit{H. pylori} infiltration and the positive urease test rate increased significantly with age. The CagA-positivity rate increased with age but not significantly. Previously, the grades of active and chronic inflammation, atrophy, lymphoid follicles, and \textit{H. pylori} density were correlated with the IgG antibody levels in the antral mucosa (3,11,28). The serum antibody response to \textit{H. pylori} also depends on the severity of \textit{H. pylori}-associated diseases (29). In the present study, the anti-\textit{H. pylori} IgG and IgA antibody levels were correlated with the degree of chronic gastritis and \textit{H. pylori} infiltration. The degrees of the histopathological findings and the anti-\textit{H. pylori} IgG and IgA antibody titers were also correlated with the ages of the children.

A positive urease test within 6 hours was another factor that affected the anti-\textit{H. pylori} IgG and IgA antibody levels regardless of age. Buffered urease tests require at least 1,000 organisms to generate a positive reaction (30) and a higher degree of \textit{H. pylori} infiltration is correlated with a faster positive reaction in the urease test (31,32). Therefore, a rapid reaction in the urease test may be related to a high density of bacteria. In the present study, there was no significant correlation between the presence of anti-urease antibody, the titer of anti-\textit{H. pylori} antibodies, and the urease test grade. The change in \textit{H. pylori} strains from CagA-positive to CagA-negative in Korea should be considered when evaluating the seroepidemiology of \textit{H. pylori} infections.

The current study had some limitations as follows. We conducted the current study with a retrospective design. We simply analyzed the results of urease tests and histopathological findings in this study, and we did not evaluate the clinical histories of the children.

In summary, we found that the anti-\textit{H. pylori} IgG and IgA antibody titers were higher in the CagA-positive sera of children regardless of age, while higher IgG and IgA titers were observed with a higher degree of active gastritis and \textit{H. pylori} infiltration, higher urease test grade, and greater age. In conclusion, the presence of CagA antigen is the main factor that affects the levels of anti-\textit{H. pylori} IgG and IgA antibodies regardless of age. The ELISA test is a valuable diagnostic tool for diagnosing CagA-positive \textit{H. pylori} infections in children. However, further studies are needed of CagA-negative strain infections and the levels of anti-\textit{H. pylori} IgG and IgA antibodies.

**ACKNOWLEDGMENT**

The serum samples used in this study were provided by the Gyeongsang National University Hospital, which is a member of the National Biobank of Korea, which is supported by the Ministry of Health, Welfare and Family Affairs. All samples derived from the National Biobank of Korea were obtained with informed consent under institutional review board approved protocols.

**DISCLOSURE**

The authors have no potential conflicts of interest to disclose.

**AUTHOR CONTRIBUTION**

Research conception & design: Seo JH, Youn HS, Cho MJ, Rhee KH. Performing the experiments: Lim CW, Jun JS, Baik SC, Lee WK. Data acquisition: Seo JH, Park JS, Yeom JS, Lim JW, Woo HO, Youn HS. Data analysis and interpretation: Seo JH, Cho MJ. Statistical analysis: Seo JH, Youn HS. Drafting of the manuscript: Seo JH, Lim CW, Jun JS, Youn HS. Critical revision of the manuscript: Seo JH, Woo HO, Youn HS. Receiving grant: Youn HS. Approval of final manuscript: all authors.

**ORCID**

Ji-Hyun Seo http://orcid.org/0000-0002-0691-3957
Chun Woo Lim http://orcid.org/0000-0003-4146-4349
Ji Sook Park http://orcid.org/0000-0002-4704-2246
Jung Sook Yeom http://orcid.org/0000-0003-0688-7493
Jae-Young Lim http://orcid.org/0000-0001-5205-202X
Jin-Su Jun http://orcid.org/0000-0002-6382-6286
Hyang-Ok Woo http://orcid.org/0000-0001-8849-9341
Hee-Shang Youn http://orcid.org/0000-0002-5498-838X
Seung-Chul Baik http://orcid.org/0000-0001-6033-4078
Woo-Kon Lee http://orcid.org/0000-0003-3913-2265
Myung-Je Cho http://orcid.org/0000-0002-4958-9827
Kwang-Ho Rhee http://orcid.org/0000-0002-4422-4992

**REFERENCES**

1. McColl KE. Clinical practice. Helicobacter pylori infection. N Engl J Med 2010; 362: 1597-604.
2. Malaty HM. Epidemiology of Helicobacter pylori infection. Best Pract Res Clin Gastroenterol 2007; 21: 205-14.
3. Chen TS, Li FY, Chang FY, Lee SD. Immunoglobulin G antibody against Helicobacter pylori: clinical implications of levels found in serum. Clin Diagn Lab Immunol 2002; 9: 1044-8.
4. Schumann C, Triantafillou K, Rasche FM, Möricker A, Vogt K, Triantafillou M, Hahn P, Schneider EM, Lepper PM. Serum antibody positivity for distinct Helicobacter pylori antigens in benign and malignant gastroduodenal disease. Int J Med Microbiol 2006; 296: 223-8.
5. de Oliveira AM, Rocha GA, Queiroz DM, Mendes EN, de Carvalho AS, Ferrari TC, Nogueira AM. Evaluation of enzyme-linked immunosorbent as-

http://dx.doi.org/10.3346/jkms.2016.31.3.417
say for the diagnosis of Helicobacter pylori infection in children from different age groups with and without duodenal ulcer. *J Pediatr Gastroenterol Nutr* 1999; 28: 157-61.

6. Raymond J, Kalach N, Bergeret M, Barbet JP, Benhamou PH, Gendrel D, Dupont C. Evaluation of a serological test for diagnosis of Helicobacter pylori infection in children. *Eur J Clin Microbiol Infect Dis* 1996; 15: 415-7.

7. Raymond J, Sarvestre C, Kalach N, Bergeret M, Dupont C. Immunoblotting and serology for diagnosis of Helicobacter pylori infection in children. *Pediatr Infect Dis J* 2000; 19: 118-21.

8. Kindermann A, Konstantopoulou N, Lenn N, Demmelmaier H, Koletzko S. Evaluation of two commercial enzyme immunoassays, testing immunoglobulin G (IgG) and IgA responses, for diagnosis of Helicobacter pylori infection in children. *J Clin Microbiol* 2001; 39: 3591-6.

9. Rocha GA, Oliveira AM, Queiroz DM, Carvalho AS, Nogueira AM. Immunoblot analysis of humoral immune response to Helicobacter pylori in children with and without duodenal ulcer. *J Clin Microbiol* 2000; 38: 1777-81.

10. Seo JH, Jun JS, Youn HS, Yeom JS, Park JS, Park CH, Woo HO, Lee WK, Cho MJ, Rhee KH. Development of an ELISA for quantitative detection of immunoglobulin G (IgG) and IgA antibodies to Helicobacter pylori for use in Korean pediatric patients with H. pylori-associated diseases. *Gut Liver* 2013; 7: 437-42.

11. Sheu BS, Shih SC, Yang HB, Su IJ, Chen CY, Lin XZ. Implications of Helicobacter pylori serological titer for the histological severity of antral gastritis. *Endoscopy* 1997; 29: 27-30.

12. Yokota S, Amano K, Fuji T, Yokoi T. Comparison of serum antibody titers to Helicobacter pylori lipopolysaccharides, CagA, VacA and partially purified cellular extracts in a Japanese population. *FEMS Microbiol Lett* 2000; 183: 193-8.

13. Gao L, Michel A, Weck MN, Arndt V, Pawlita M, Brenner H. Helicobacter pylori infection and gastric cancer risk: evaluation of 15 H. pylori proteins determined by novel multiplex serology. *Cancer Res* 2009; 69: 6164-70.

14. Satomi S, Yamakawa A, Matsunaga S, Masaki R, Inagaki T, Okada T, Suto H, Ito Y, Yamazaki Y, Kuriyama M, et al. Relationship between the diversity of the cagA gene of Helicobacter pylori and gastric cancer in Okinawa, Japan. *J Gastroenterol* 2006; 41: 668-73.

15. Jeong HL, Jung YS, Jun JS, Yeom JS, Park JS, Seo JH, Lim JY, Park CH, Woo HO, Youn HS, et al. Comparison of four commercial ELISA kits and in-house immunoblotting for diagnosis of Helicobacter pylori infection. *Pediatr Gastroenterol Hepatol Nutr* 2012; 15: 85-90.

16. Holttmann G, Talley NJ, Mitchell H, Hazell S. Antibody response to specific H. pylori antigens in functional dyspepsia, duodenal ulcer disease, and health. *Am J Gastroenterol* 1998; 93: 1222-7.

17. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyow PH, Stemmermann GN, Nomura A. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995; 55: 2111-5.

18. Yamazaki S, Yamakawa A, Okada T, Ohtani M, Suto H, Ita Y, Yamazaki Y, Keida Y, Higashi H, Hatakeyama M, et al. Distinct diversity of vacA, cagA, and cagE genes of Helicobacter pylori associated with peptic ulcer in Japan. *J Clin Microbiol* 2005; 43: 3906-16.

19. Wong BC, Yin Y, Berg DE, Xia HH, Zhang JZ, Wang WH, Wong WM, Huang XR, Tang VS, Lam SK. Distribution of distinct vacA, cagA and iceA alleles in Helicobacter pylori in Hong Kong. *Helicobacter* 2001; 6: 317-24.

20. Ko JS, Kim KM, Oh YL, Seo JK. cagA, vacA, and iceA genotypes of Helicobacter pylori in Korean children. *Pediatr Int* 2008; 50: 628-31.

21. Akada J, Okada M, Hiramoto N, Kitagawa T, Zhang X, Kamei S, Ito A, Nakamura M, Uchida T, Higatani T, et al. Proteomic characterization of Helicobacter pylori CagA antigen recognized by child serum antibodies and its epitope mapping by peptide array. *PLoS One* 2014; 9: e104611.

22. Kim EA, Kim YO, Lim JY, Jung YS, Park CH, Woo HO, Youn HS, Ko GH, Baik SC, Lee WK, et al. Antibody response of infants to Helicobacter pylori infection. *Korean J Gastroenterol* 2000; 35: 704-15.

23. Yin JY, Kim N, Choi SH, Kim YS, Cho KR, Kim SS, Seo GS, Kim HH, Baik GH, Sin CS, et al. Seroprevalence of Helicobacter pylori in South Korea. *Helicobacter* 2007; 12: 333-40.

24. Kim HY, Kim N, Kim SM, Seo JH, Park EH, Lee DH. Seroprevalence of Helicobacter pylori infection in Korean health personnel. *Gut Liver* 2013; 7: 648-54.

25. Crabtree JE, Mahony MJ, Taylor JD,Heatley RV, Littlewood JM, Tompkins DS. Immune responses to Helicobacter pylori in children with recurrent abdominal pain. *J Clin Pathol* 1991; 44: 768-71.

26. Corvaglia L, Bontemps P, Devaster JM, Heimann P, Glupczynski Y, Keppens E, Cadranel S. Accuracy of serology and 13C-urea breath test for detection of Helicobacter pylori in children. *Pediatr Infect Dis J* 1999; 18: 976-9.

27. Pounder RE, Ng D. The prevalence of Helicobacter pylori infection in different countries. *Aliment Pharmacol Ther* 1995; 9 Suppl 2: 33-9.

28. Hsu PI, Lai KH, Tseng HH, Liu YC, Yen MY, Lin CK, Lo GH, Huang RL, Huang JS, Cheng JS, et al. Correlation of serum immunoglobulin G Helicobacter pylori antibody titers with histologic and endoscopic findings in patients with dyspepsia. *J Clin Gastroenterol* 1997; 25: 387-91.

29. Chomvarin C, Ottiowet O, Hahnvajjana Wong C, Intapun PM, Wongwajana S. Seroreactivity to specific antigens of Helicobacter pylori infection is associated with an increased risk of the dyspeptic gastrointestinal diseases. *Int J Infect Dis* 2009; 13: 647-54.

30. Graham DY. Helicobacter pylori and the endoscopist: whether to diagnose. *Gastrotest Endosc* 1991; 37: 577-9.

31. Seo JH, Youn HS, Park JI, Yeom JS, Park JS, Jun JS, Lim JY, Park CH, Woo HO, Ko GH, et al. Influencing factors to results of the urease test: age, sampling site, histopathologic findings, and density of Helicobacter pylori. *Pediatr Gastroenterol Hepatol Nutr* 2013; 16: 34-40.

32. Seo JH, Park JS, Yeom JS, Lim JY, Park CH, Woo HO, Baik SC, Lee WK, Cho MJ, Rhee KH, et al. Correlation between positive rate and number of biopsy samples on urease test in childhood Helicobacter pylori infection. *J Korean Med Sci* 2014; 29: 106-9.