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**Original Article**

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# Comparison of Four Commercial ELISA Kits and In-House Immunoblotting for Diagnosis of *Helicobacter pylori* Infection

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**Purpose:** Commercial enzyme-linked immunosorbent assay (ELISA) kits have been considered less reliable for children than for adults. The aim of this study was to compare four ELISA kits and in-house immunoblotting based on the analysis of anti-*H. pylori* -IgG antibody reactivity.

**Methods:** A total of 399 serum samples were collected at the GNU Hospital during 1998–1999. All sera were tested using ELISA and immunoblotting. Statistically significant differences were determined by the $\chi^2$ test.

**Results:** The overall seropositivity rates using GAP IgG, Genedia IgG, HM-CAP, Pyloriset EIA-G, and immunoblotting were 13.0%, 25.1%, 18.3%, 15.8%, and 62.9%, respectively. Immunoblotting showed a higher seropositivity rate than did all four ELISA kits in all age groups. Genedia IgG had the highest seropositivity among the ELISA kits. The seropositivity rate for children aged 13 to 18 months was lowest, and that of children aged 15 years was highest (90.0%). The seropositivity rate for children aged 7 months to 5 years was significantly lower than that for children aged 6 to 15 years among the four ELISA kits ($p < 0.0001$) and immunoblotting ($p=0.02$).

**Conclusion:** Immunoblotting is the most sensitive test for detection of anti-*Helicobacter pylori* IgG antibodies among the serological tests in this study. These results emphasize the need for standardization when commercial ELISA tests are used in different nations or in young age groups. Immunoblotting could be a suitable noninvasive assay for serodiagnosis and seroepidemiologic study of *H. pylori* infection in Korean children. **(Pediatr Gastroenterol Hepatol Nutr 2012; 15: 85～90)**

**Key Words:** ELISA, Immunoblot, *Helicobacter pylori* infection, Children

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## INTRODUCTION

Diagnostic tests for *Helicobacter pylori* infection can be divided into two categories in terms of method: invasive or noninvasive [1-3]. The invasive methods, such as culture, histopathologic examination, and urease tests, require endoscopic biopsies. Noninvasive tests include the urea breath test and serologic tests. Serologic testing is an easy, noninvasive, and commercially available method to detect anti-*H. pylori* IgG. Commercial enzyme-linked immunosorbent assay (ELISA) kits could be advantageous in screening children for anti-*H. pylori* IgG. *Helicobacter* strains that prevail in Asia may exhibit antigenic properties that differ from those of Western countries where the ELISA kits are developed [4]. The ELISA for anti-*H. pylori* IgG in children showed controversial results with various sensitivities and specificities [5,6], and serological testing was considered to be a less reliable test for children than for adults in Europe [7,8]. The immunoblot assay has been an alternative serologic assay available to diagnose *H. pylori* infection in children [9].

The aim of this study was to compare the diagnostic values of four commercial ELISA kits and an in-house immunoblot assay in children in Jinju city, South Korea.

## MATERIALS AND METHODS

### Serum collection

A total of 399 serum samples from patients without gastrointestinal disease were collected at the Gyeongsang National University Hospital (GNUH) from 1998 to 1999 and stored at −20°C until analysis. Patient age ranged from 0 to 15 years, and the patients were divided into 20 groups according to age (Table 1). All sera were provided by the GNUH, a member of the National Biobank of Korea, after the permission from the hospital ethics committee (GNUHIRB-2012-003).

### ELISA

Four commercial ELISA tests were chosen in this study to detect serum IgG against *H. pylori*: GAP IgG (Bio-Rad, Hercules, CA, USA) against *H. pylori* outer-membrane antigen, Genedia IgG (MBRIHP-2, Green Cross Co., Seoul, South Korea) using whole-cell sonicates of *H. pylori* strains isolated from Korean patients with chronic gastritis, HM-CAP (Enteric Products, Stony Brook, NY, USA) against high-molecular-weight cell-associated proteins of *H. pylori*, and Pyloriset EIA-G (Orion Diagnostica, Espoo, Finland) against acid glycine-extracted surface antigens of *H. pylori*. All tests were performed as a single batch according to the manufacturers' instructions. The optical density of each sample was translated into an ELISA value as suggested. A positive test was obtained when the ELISA value exceeded a specific level as defined by the manufacturers (GAP IgG=20 antibody titer; Genedia IgG=mean negative control's OD+0.4; HM-CAP=2.2; Pyloriset EIA- G=300).

### In-house IgG immunoblotting

*Helicobacter pylori* strain 51 isolated from patients at

**Table 1.** Number of Normal, Healthy Children without Gastroduodenal Symptoms Examined in this Study by Age and Sex in 1998–1999

| Age | Number | | |
|---|---|---|---|
| | Male | Female | Total |
| 0-3 months | 10 | 10 | 20 |
| 4-6 months | 10 | 10 | 20 |
| 7-9 months | 10 | 9 | 19 |
| 10-12 months | 10 | 10 | 20 |
| 13-18 months | 10 | 10 | 20 |
| 19-24 months | 10 | 10 | 20 |
| 2 years | 10 | 10 | 20 |
| 3 years | 10 | 10 | 20 |
| 4 years | 10 | 10 | 20 |
| 5 years | 10 | 10 | 20 |
| 6 years | 10 | 10 | 20 |
| 7 years | 10 | 10 | 20 |
| 8 years | 10 | 10 | 20 |
| 9 years | 10 | 10 | 20 |
| 10 years | 10 | 10 | 20 |
| 11 years | 10 | 10 | 20 |
| 12 years | 10 | 10 | 20 |
| 13 years | 10 | 10 | 20 |
| 14 years | 10 | 10 | 20 |
| 15 years | 10 | 10 | 20 |
| Total | 200 | 199 | 399 |

GNUH was cultivated overnight at 38°C in 10% $CO_2$ and a 100% humidity atmosphere. For preparation of whole-cell proteins, cells were washed with a washing solution (0.1 M phosphate buffer solution [PBS], pH 7.4) and resuspended with PBS, and phenylmethylsulfonyl fluoride (PMSF) was then added. The cells were disrupted with an ultrasonicator (Ultrasonics W-380) to a protein concentration of 20 $\mu$g/mL by the Lowry method.

The whole-cell lysate of *H. pylori* strain 51 was separated by 10%- to 20%-gradient SDS-PAGE and then transferred onto a nitrocellulose filter. The blot was incubated with a 1:5 dilution of serum and subsequently labeled with alkaline phosphatase-conjugated goat anti-human IgG (Promega) before staining with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. The presence of a 120,000-MW protein (120-kDa antigen [CagA]) band was considered seropositive [10].

Responses to antigens in the immunoblot results were divided into three patterns (Fig. 1). Pattern I was defined as CagA antigen positive. Pattern II was defined as antigen positive, but without CagA antigen. Pattern III was defined as no positive reactivity. A serum sample was considered to have tested positive by immunoblot analysis if it reacted with the 120-kDa band (Pattern I).

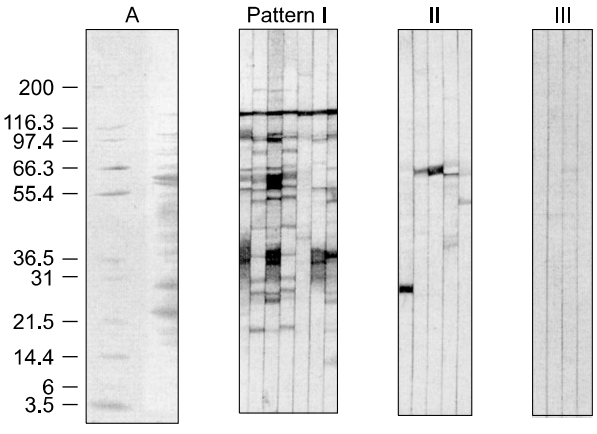


**Fig. 1.** Immunoblot assay results were classified into three patterns based on immunoreactive bands. Only Pattern I, which shows reactivities against 120-kDa antigens as well as other antigens of *H. pylori*, was considered to be a specific marker of *H. pylori* infection in this study. Panel A shows a Ponceau S-stained nitrocellulose membrane onto which marker proteins and separated *H. pylori* antigen were transferred.

### Statistical analysis

The data were analyzed using PASW Statistics 18. Statistically significant differences in the seropositivity rate among age groups were determined using the $\chi^2$ test. Nonparametric correlation (Spearman's rho test) was used to analyze the correlations among the seropositivity rates of the four ELISA kits and immunoblotting. $p$-values $< 0.05$ were considered to be statistically significant.

## RESULTS

Fig. 2 shows the seropositivity rates of anti-*H. pylori* IgG of the four commercial ELISA kits and immunoblotting according to the age. The overall seropositivity rates of GAP IgG, Genedia IgG, HM-CAP, Pyloriset EIA-G, and immunoblotting were 13.0%, 25.1%, 18.3%, 15.8%, and 62.9%, respectively. The seropositivity rates for immunoblotting were higher than those for the four ELISA kits in all age groups. The overall seropositivity rate for Genedia IgG was

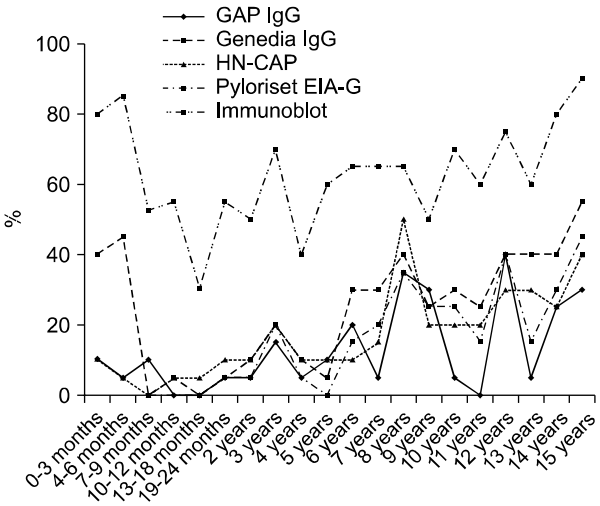


**Fig. 2.** Seropositivity rates of the four commercial ELISA kits and immunoblotting according to age. The seropositivity rates increased with age. The seropositivity rates of immunoblotting were higher than those of the ELISA kits, and the discrepancy in the seropositivity rates of anti-*H. pylori* IgG antibody was highest in 0- to 6-month-old infants.

the highest among all ELISA kits. The seropositivity rate in the 13- to 18-month-old group was lowest among all age groups (Fig. 2).

The seropositivity rate increased with age and was highest in the 15-year-old group (90.0% on immunoblotting) (Fig. 2). There was no statistical difference between males and females according to ELISA and immunoblotting (Table 2). The seropositivity rate of children aged 7 months to 5 years was significantly lower than that of children aged 6 to 15 years for the four ELISA kits ($p<0.0001$) and immunoblotting ($p=0.02$) (Fig. 3).

In 0- to 3-month-old infants, the seropositivity rate for GAP IgG, Genedia IgG, HM-CAP, Pyloriset EIA-G, and immunoblotting was 10%, 40%, 10%, 10%, and 80%, respectively. In 4- to 6-month-old infants, the rate was 5%, 45%, 5%, 5%, and 85%, respectively. The discrepancy in seropositivity rates between the ELISA kits and immunoblotting was highest in these two age groups. The high seropositivity rates of immunoblotting in these age groups might be related to the level of maternal anti-*H. pylori* IgG transported to the fetus via the placenta.

The seropositivity rate for Genedia IgG and Pyloriset EIA-G was moderately correlated with that for immunoblotting (Genedia IgG, R=0.396; Pyloriset EIA-G, R=0.318; both $p<0.0001$). The seropositivity rates for GAP IgG and HM-CAP were also mildly correlated with those for immunoblotting (GAP IgG, R=0.159; HM-CAP, R=0.229; both $p<0.0001$). Immunoblotting results were divided into the three patterns previously described: Pattern I comprised 62.9%, Pattern II comprised 33.3%, and Pattern III comprised 3.8% of immunoblotting results. Only Pattern I was considered positive. In cases of seropositivity using GAP IgG, Genedia IgG, HM-CAP, and Pyloriset EIA-G, Pattern I comprised 82.7%, 96.0%, 86.3%, and 98.4% of immunoblotting results, respectively (Fig. 4), and pattern II comprised 17.3%, 4.0%, 13.7%, and 1.6%, respectively. No results showed Pattern III.

**Table 2.** Seropositivity Rates of the Four ELISA Kits and Immunoblotting According to Sex

| | Female (%) | Male (%) | *p*-value (two-tailed, Fisher) |
|---|---|---|---|
| GAP IgG | 25/199 (12.6) | 27/200 (13.5) | 0.882 |
| Genedia IgG | 47/199 (23.6) | 53/200 (26.5) | 0.564 |
| HM-CAP | 42/199 (21.1) | 31/169 (15.5) | 0.156 |
| Pyloriset | 36/199 (18.1) | 27/200 (13.5) | 0.219 |
| Immunoblotting | 118/199 (59.3) | 133/200 (66.5) | 0.300 |

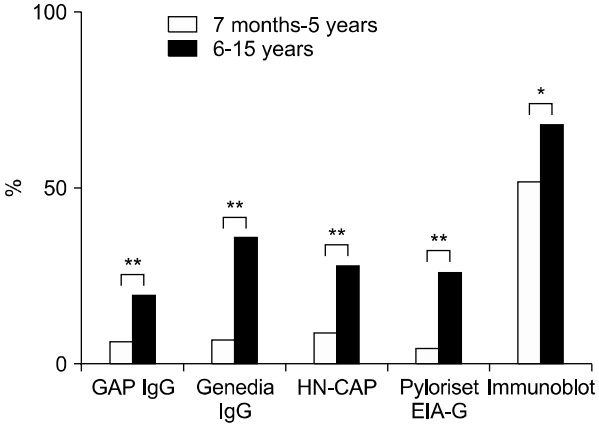


**Fig. 3.** Comparisons of seropositivity rates for GAP IgG, Genedia IgG, HM-CAP, Pyloriset EIA-G, and immunoblotting in children 7 months to 5 years of age and 6 to 15 years of age. The seropositivity rates in children aged 6 years and older were higher than those of children aged 7 months to 5 years of age in all ELISA kits and immunoblotting. *$p<0.0001$, **$p=0.02$.

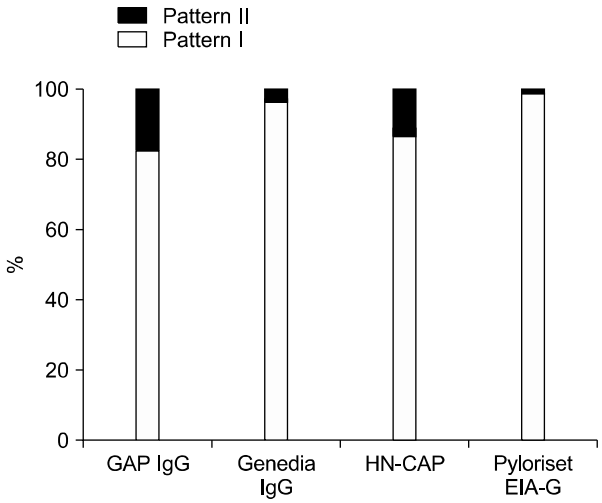


**Fig. 4.** Proportion of immunoblot patterns in the seropositive cases of the four ELISA kits. More than 80% were Pattern I. There were no results showing Pattern III.

## DISCUSSION

The present comparison of four commercially available ELISA kits and in-house immunoblotting analysis for the serodiagnosis of *H. pylori* infection in 399 Korean children without gastrointestinal diseases revealed a large discrepancy in seropositivity rates between the ELISA kits and immunoblotting. Determination of antibody levels is affected by the antigen preparations used in the assay. However, most commercial assays are generally limited by the use of antigens that are sensitive and specific only for populations in which the assays were validated [11]. Despite the high accuracy reported in Western countries, the commercial serological tests in some studies were unsatisfactory when used in Chinese [12] and Korean people [13,14]. *H. pylori* antigen obtained from Korean *H. pylori* strains was used in the Genedia kit. The seropositivity rate of Genedia IgG was higher than that of the other three ELISA kits. In 1998, the seroprevalence of *H. pylori* infection in asymptomatic Korean children (n=2,336) was 17.2% using this ELISA test [15]. The difference between our results and those of previous studies might be related to the sensitivity and specificity of the Genedia IgG ELISA (80% and 84.8% in children) [16].

The accuracy of ELISA is lower in children than in adults because of the differences in the immune response [17]. Immunoblotting was more sensitive (100%) and specific (88%) than ELISA in the evaluation of *H. pylori* infection in children [9]. In the present study, the accuracy of commercial assays was also greatly reduced when serum from younger patients, especially those younger than 5 years, was evaluated.

Most strains of *H. pylori* isolated in Korea and Japan are CagA-positive strains; we considered positive as immunoreactivity against a 120-kDa band (immunoblot Pattern I) [18]. The diagnostic sensitivity and specificity of commercially available immunoblot assays indicated that they were accurate, but evaluation of CagA status and VacA was poor [19]. The CagA-positive rate in our study was 62.9% by in-house immunoblot analysis, and this result was higher compared with the CagA-positivity rate in French children (43.1%) by the immunoblot technique [9]. More than 80% of seropositive ELISA results were CagA positive in our study. This is consistent with the finding that 94% of Korean children with recurrent abdominal pain and *H. pylori* gastritis had a cagA-positive genotype [20].

In the present study, Pattern II showed that many antigens without CagA antigen were immunoreactive. The proportion of Pattern II was 33.3% in this study. Pattern II involved immunoreactive antigens other than CagA. This result might be related to the fact that the immunoreactive rates of the 25-, 30-, and 19.5-kDa antigens were higher than that of the 120-kDa antigen in children [6]. Further study is needed to clarify the geographical differences in immune reactions according to age and strain.

There were several limitations to the present study, including the fact that the study population comprised asymptomatic children, no endoscopic study was performed, the specificity and sensitivity of in-house immunoblotting was not proven, the study population was small in each age group, and Pattern II may be positive in other studies [9]. The CagA-positive *H. pylori* strain was predominant in Korea, and we considered that only Pattern I was positive. Positivity of antibody against *H. pylori* could not differentiate the recent and past infections and not suitable for follow-up tests after chemotherapy of *H. pylori* [21,22].

In summary, the seropositivity rate for in-house immunoblotting was higher than that for commercially available ELISA kits. The seropositivity rate of children 7 months to 5 years of age was lower than that of children 6 to 15 years of age.

In conclusion, in-house immunoblotting is the most sensitive test with which to detect anti-*H. pylori* IgG antibodies among the serological tests in this study. These results emphasize the need for standardization when commercial ELISA tests are used in different nations or in young age groups. In-house immunoblotting could be a suitable noninvasive assay for serodiagnosis and seroepidemiologic study of *H. pylori* infection in Korean children.

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