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Serum Gastrin and Pepsinogen I, II Concentrations in Children with *Helicobacter pylori* Infection : the Role of CagA and VacA

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Serum gastrin and pepsinogen concentrations were measured in 51 children infected with Helicobacter pylori, to investigate the clinical significance and influence of CagA and VacA on serum concentrations of these peptides. CagA + was 44/51 (86%) and VacA + was 42/51 (82%). Type I (CagA +/VacA +) included 39/51 (76%), type II (CagA −/VacA −) was 4/51 (8%), and intermediate (CagA −/VacA +, CagA +/VacA −) was 8/51 (16%). There was no significant correlation between endoscopic diagnosis and the state of CagA/VacA. Serum gastrin concentrations were not significantly correlated with the state of CagA/VacA. Serum pepsinogen I and II concentrations were significantly higher in CagA + than in CagA −, but there was no significant difference between VacA + and VacA −. Serum pepsinogen I/II ratio was not significantly correlated with the state of CagA/VacA. There was no significant difference between serum concentrations of gastrin, pepsinogen I and H. pylori phenotypes. However, pepsinogen II concentration was significantly higher in type I than type II. Pepsinogen I/II ratio was significantly lower in type I and intermediate than in type II. These findings suggest that CagA positivity and phenotype of H. pylori could play a role in the development of upper gastrointestinal diseases in children.

Key Words: *Helicobacter pylori,* gastrin, pepsinogen, CagA, VacA, phenotypes

Serologic responses to the CagA and VacA protein have been associated with the development of peptic ulcerations in *Helicobacter pylori (H. pylori)* infected persons (Marshall, 1994). The cytopathic effect of cytotoxin (vacuolating cytotoxin; 87kD) is derived from the vacuolization of the cytoplasm in gastric epithelial cells (Figura *et al.* 1989; Covacci *et al.* 1993). The vacuolating activity of the cytotoxin is neutralized by specific rabbit antiserum, while neutralizing antibodies to the cytotoxin are detectable in sera from *H. pylori*-infected persons (Cover and Blaser, 1995). It appears that the toxic effect of VacA is expressed in the presence of 128kD cytotoxin-associated antigen A (CagA) (Cover *et al.* 1990). In a previous study, we reported the high serologic response rate of CagA and VacA in peptic ulcer and chronic superficial nodular gastritis in Korean children infected with *H. pylori* (Kim and Chung, 1996).

It is well known that raised serum gastrin and pepsinogen concentrations associated with *H. pylori* infection, has been found in patients with peptic ulcer disease and gastritis (Liebman, 1980; Tam, 1987; Oderda *et al.* 1990). We have reported the high serum concentrations of gastrin and pepsinogen, and the low serum pepsinogen I/II ratio in

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Korean children infected with *H. pylori* (Kim and Chung, 1997; Kim and Chung, 1998).

Therefore, we have investigated the role CagA and VacA status on the serum gastrin and pepsinogen concentrations in children with *H. pylori* infection.

MATERIALS AND METHODS

Patients

A total of 51 children who had visited the Department of Pediatrics, Yonsei University College of Medicine from July 1992 to July 1997 for chronic recurrent abdominal pain or upper gastrointestinal bleeding and who had also been diagnosed with *H. pylori* infection, were included. All children underwent gastroduodenal endoscopy and gastric biopsies. CLO test (CLO™, Delta-West, Pty Ltd, Bently, Western Australia), Warthin-Starry silver stain with biopsy specimens, and a Western blot commercial test (Helicoblot 2.0 kit, Genelabs Diagnostics, Singapore, Singapore) were performed with patient serum. The mean age of children was 11.8±2.1 years (range: 5 to 15 years), and there were 26 females and 25 males (Table 1).

Methods

The upper gastroduodenal endoscopy and gastric biopsies were performed with GIF-XP20 and GIF-P30 (Olympus optical Co, Tokyo, Japan) for all 51 children. The endoscopic diagnoses were made by Sidney system classifications (Price, 1991; Tytgat, 1991). We classified nodular changes of antral mucosa into 'chronic superficial nodular gastritis' apart from the Sidney system. Four biopsy specimens were obtained from the antrum, near the pyloric canal and body of the stomach in each patient. The two biopsy specimens (one from the antrum and the other from the body) were applied simultaneously to the CLO test. The remaining two specimens were assessed by histological examination, including Warthin-Starry silver stain. One or more positive results in these tests were determined as having *H. pylori* infection. Serum samples were obtained from each patient on the day of the endoscopic procedure. Five milliliters of obtained blood was centrifuged at 1,500 rpm for 5 minutes, and the separated serum was frozen up to −70°C immediately.

Serum gastrin concentrations were determined by radioimmunoassay with commercial kit (Double Antibody Gastrin kit, DPC, Los Angeles, CA, U.S.A). Serum pepsinogen concentrations were also determined by radioimmunoassay with commercial kit (PEPSINOGEN I/II-RIABEAD kit, Dainabot, Minato-Ku, Japan).

CagA and VacA cytotoxins in serum were analyzed with commercially made HELICO BLOT 2.0 kit (Genelabs Diagnostics, Singapore). Reactive band in 89kD (usually not as dark as the 116kD band) was interpreted as VacA positive and any reactive band in 116kD as CagA positive. As previously described, the phenotypes of *H. pylori* were grouped by the CagA and VacA positivities (type I; CagA+ and VacA+, type II; CagA− and VacA−, intermediate type; CagA− and VacA+ or vice versa) (Xiang *et al.* 1995).

Statistical analysis was made by the Student *t* test and the one-way ANOVA with post hoc test using SPSS 7.0™ for Windows. A *p*-value less than 0.05 was considered as statistically significant.

Table 1. Age and sex distribution of patients (N=51)

Age (years)	Male	Female	Total
≤5	0	0	0
5~10	6	2	8
>10	19	24	43
Total	25	26	51

Mean age: 11.8±2.1 years (5-15)

RESULTS

Results of HELICO BLOT 2.0 test

Urease (26.5kD; one single band) was positive in 51/51 (100%) children, CagA (116kD) positive in 44/51 (86%), and VacA (89kD) positive in 42/51 (82%) children (Fig. 1).

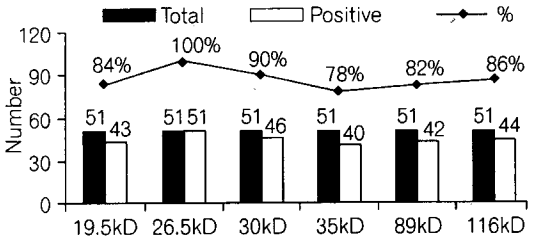

Fig. 1. Results of Helicoblot test (N=51)

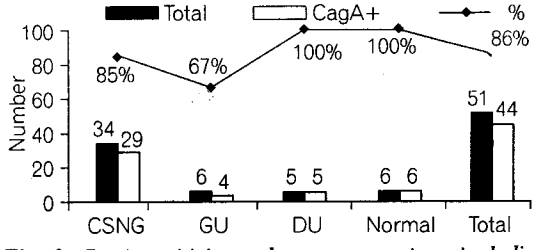

Fig. 2. CagA positivity and upper gastrointestinal diseases (N=51)

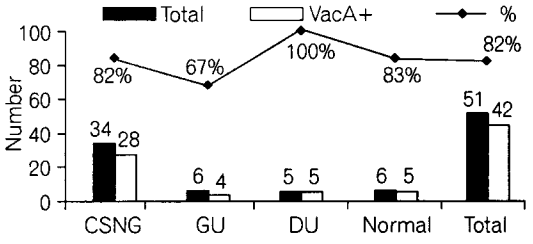

Fig. 3. VacA positivity and upper gastrointestinal diseases (N=51)

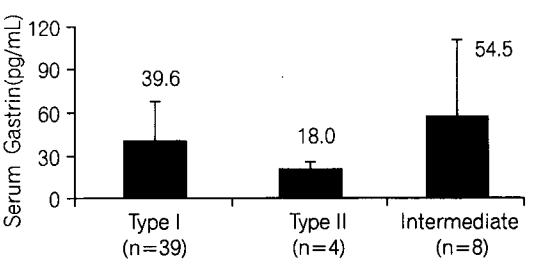

Fig. 4. Serum gastrin concentration and H. pylori phenotypes (N=51). Not significantly different among three types by ANOVA test ($p>0.05$). Statistical analysis: by one-way ANOVA post hoc test.

Upper gastrointestinal diseases and CagA/VacA state

Upper gastrointestinal diseases were diagnosed by gastroduodenal endoscopy. Of the 51 children, chronic superficial nodular gastritis was most common in 43 cases (67%), then gastric ulcer in 6 cases (12%), and duodenal ulcer in 5 cases (10%). The detection rate of CagA was 100% in children with duodenal ulcer, 85% in chronic superficial nodular gastritis, and 67% in gastric ulcer (Fig. 2). The detection rate of VacA was 100% in duodenal ulcer, 82% in chronic superficial nodular gastritis, and 67% in gastric ulcer (Fig. 3).

Serum gastrin concentrations and *H. pylori* phenotypes

Type I phenotype was most common in 39 cases, then type II in 4 cases, and intermediate type in 8 cases. The serum gastrin concentration was 39.6± 26.8 pg/mL in type I, 18.0±5.5 pg/mL in type II, and 54.5±55.3 pg/mL in intermediate. There was no significant correlation ($p>0.05$) between serum gastrin concentrations and *H. pylori* phenotypes (Fig. 4).

Serum pepsinogen I and II concentrations, pepsinogen I/II ratio, and *H. pylori* phenotypes

The serum pepsinogen I concentration was 56.4± 39.7 ng/mL in type I, 31.1±12.5 ng/mL in type II, and 39.7±12.6 ng/mL in intermediate. There was no significant correlation between serum pepsinogen I concentrations and *H. pylori* phenotypes ($p>0.05$). The serum pepsinogen II concentration was 18.3± 9.9 ng/mL in type I, 6.3±5.0 ng/mL in type II, and 13.2±5.2 ng/mL in intermediate. Statistically, serum pepsinogen II concentration was significantly higher ($p=0.04$) in type I than in type II. (Fig. 5). The serum pepsinogen I/II ratio was 3.4±1.5 in type I, 6.4±2.9 in type II, and 3.2±1.2 in intermediate. The serum pepsinogen I/II ratio was significantly lower in type I than in type II ($p=0.002$), and also lower in intermediate than in type II ($p=0.007$).

Serum gastrin concentrations and CagA/VacA state

The serum gastrin concentration was 39.7±25.5

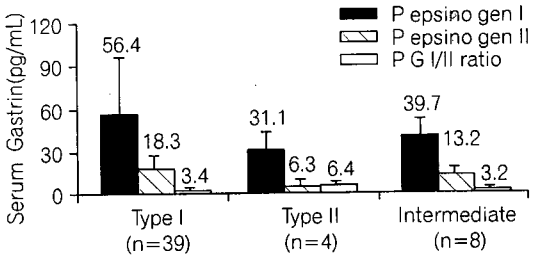

Fig. 5. Serum pepsinogen concentrations and H. pylori phenotypes (N=51). Pepsinogen 1: not significantly different among types ($p>0.05$). Pepsinogen II: type 1 > type II ($p=0.04$). Pepsinogen I/II ratio: type I < type II ($p=0.002$), intermediate < type II ($p=0.007$). Statistical analysis: by one-way ANOVA post hoc test.

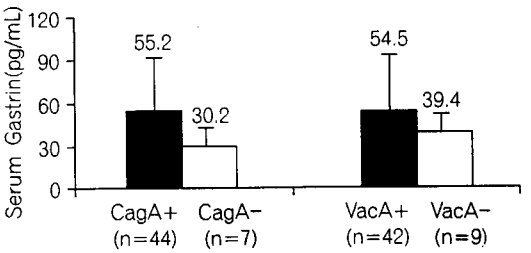

Fig. 7. Serum pepsinogen I concentrations and CagA and VacA positivity. Significantly different between CagA positive and negative ($p=0.02$). Not significantly different between VacA positive and negative ($p=0.43$). Statistical analysis: by student t-test.

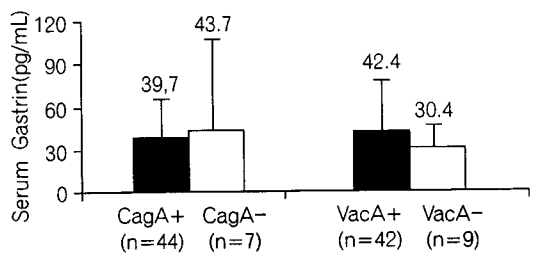

Fig. 6. Serum gastin concentrations and CagA and VacA positivity. Not significantly different between CagA positive and negative ($p=0.08$). Not significantly different between VacA positive and negative ($p=0.32$). Statistical analysis: by student-test.

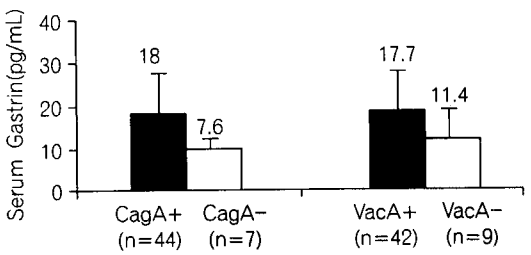

Fig. 8. Serum pepsinogen II concentrations and CagA and VacA positivity. Significantly different between CagA positive and negative ($p=0.003$). Not significantly different between VacA positive and negative ($p=0.08$). Statistical analysis: by student t-test.

pg/mL in CagA positive children, and 43.7±63.7 pg/mL in CagA negative. There was no significant correlation between serum gastrin concentrations and CagA positivity (p=0.08). The serum gastrin concentration was 42.4±34.7 pg/mL in VacA positive children, and 30.4±15.4 pg/mL in VacA negative. There also was no significant correlation (p=0.32) between serum gastrin concentrations and VacA positivity (Fig. 6).

Serum pepsinogen I concentrations and CagA/VacA state

The serum pepsinogen I concentration was 55.2±37.5 pg/mL in CagA positive children, and 30.2±11.5 pg/mL in CagA negative. Serum pepsinogen I concentrations were significantly higher in CagA positive than CagA negative children (p=0.02). The serum pepsinogen I concentration was 54.5±39.0 pg/mL in VacA positive children, and 39.4±12.3 pg/mL in VacA negative. And so there was no significant correlation (p=0.43) between serum pepsinogen I concentration and VacA positivity (Fig. 7).

Serum pepsinogen II concentrations and CagA/VacA state

The serum pepsinogen II concentration was 18.0±9.5 pg/mL in CagA positive children, and 7.6±4.0 pg/mL in CagA negative. Serum pepsinogen II concentrations were significantly higher in CagA positive than CagA negative children (p=0.003). The serum pepsinogen II concentration was 17.7±9.9 pg/mL in VacA positive children, and 11.4±6.8 pg/mL in VacA negative. And so there was no

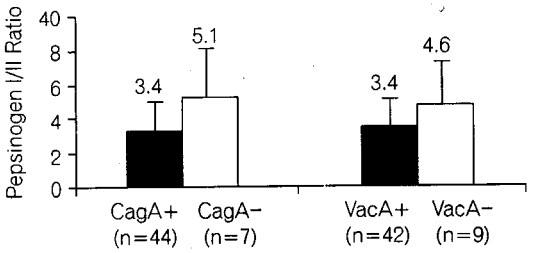

Fig. 9. Serum pepsinogen I/II rations and CagA and VacA positivity. Not significantly different between CagA positive and negative (p=0.08). Not significantly different between VacA positive and negative (p=0.17). Statistical analysis: by student t-test.

significant correlation (p=0.08) between serum pepsinogen I concentration and VacA positivity (Fig. 8).

Serum pepsinogen I/II ratio and CagA/VacA state

The pepsinogen I/II ratio was 3.4±1.5 in CagA positive children, and 5.1±1.9 in CagA negative. There was no significant correlation between the serum pepsinogen I/II ratio and CagA positivity (p =0.08). The serum pepsinogen I/II ratio was 3.4± 1.5 in VacA positive children, and 4.6±2.5 in VacA negative. There was no significant correlation (p= 0.17) between the serum pepsinogen I/II ratio and VacA positivity (Fig. 9).

DISCUSSION

Gastrin, the peptide produced by G cells located in gastric antral mucosa, has the main action of stimulating gastric acid secretion from parietal cells and it also has the action of secreting pepsin from gastric chief cells (Michael and Andrew, 1988).

Although the precise mechanisms of hypergastrinemia in *H. pylori*-associated gastritis have not been clearly determined yet, it is known that the hypergastrinemia in *H. pylori* infection has been related to peptic ulcer development (Levi *et al.* 1989). Furthermore, McColl *et al.* reported that the eradication of *H. pylori* was followed by a remarkable decrease in serum gastrin concentration (McColl *et al.* 1991). In our previous study (Kim and Chung, 1997), serum gastrin concentrations were investigated in 166 Korean children with *H. pylori* infection who had had upper gastroduodenal endoscopy performed for chronic recurrent abdominal pain or upper gastrointestinal bleeding. The serum gastrin concentrations were significantly higher (P< 0.001) in *H. pylori* positive (40.1±13.7 pg/mL) than in *H. pylori* negative children (29.5±7.5 pg/mL). As well, serum gastrin concentrations were highest in duodenal ulcer (42.5±16.3 pg/mL), followed in descending order by gastric ulcer (36.9±17.9 pg/mL), chronic superficial nodular gastritis (34.4± 9.9 pg/mL), and superficial gastritis patients (30.2± 8.0 pg/mL) as compared with endoscopically normal children (29.8±8.4 pg/mL).

Pepsinogens, secreted from the stomach, have been known to be a risk factor for the development of peptic ulcer diseases. Biasco *et al.* suggested that the increase of serum pepsinogen concentrations and a decrease in the pepsinogen I/II ratio were found in patients with *H. pylori* infection (Biasco *et al.* 1993). He also suggested that the increase in pepsinogen II, rather than the increase in pepsinogen I, resulted in the decrease in the pepsinogen I/II ratio. Samloff *et al.* reported that the serum pepsinogen concentration has been predictive of the histological status of the gastric mucosa (Samloff *et al.* 1982). In children, *H. pylori* as well as serum pepsinogen concentrations have also been associated with antral gastritis, and that serum pepsinogen concentrations can be used as an index of the severity of gastritis in *H. pylori* positive children (Oderda *et al.* 1990). In our previous study (Kim and Chung, 1998), serum pepsinogen I concentrations were significantly higher (p<0.01) in children with *H. pylori* infection (47.3±16.2 ng/mL) than in children without *H. pylori* infection (38.2±14.3ng/mL). Serum pepsinogen II concentrations were significantly higher (p<0.001) in children with *H. pylori* infection (14.6±8.3 ng/mL) than in children without *H. pylori* infection (6.2±4.5 ng/mL). The pepsinogen I/II ratio was significantly lower (p<0.001) in children with *H. pylori* infection (3.9±1.7) than in children without *H. pylori* infection (7.2±2.4). As well, the serum pepsinogen I and II concentrations were significantly increased (P<0.01), and the pepsinogen I/II ratio was significantly decreased (P <0.001) in children with chronic superficial nodular

gastritis and duodenal ulcer than in children without.

VacA produced by *H. pylori* induces acidic vacuoles in the cytoplasm of eukaryotic cells (Cover *et al.* 1992). Figura *et al.* reported positive rates of cytotoxin expression in patients with duodenal ulcer of up to 66% (Figura *et al.* 1989). These findings strongly support the role of VacA in the pathogenesis of peptic ulcer diseases. Tummuru *et al.* reported the high positive rates of *cagA* in duodenal ulcer patients infected with *H. pylori*, and the potential usefulness of CagA in clinical fields (Tummuru *et al.* 1994).

Xiang *et al.* analyzed the expression of *cagA* and *vacA* virulence factors in 43 strains of *H. pylori* and divided clinical isolates into two major types, suggesting that *cagA* is not necessary for the expression of *vacA* (Xiang *et al.* 1995). In his report, type I (*cagA*+ and *vacA*+) was the most prevalent strain with 51% and type II (*cagA*− and *vacA*−) strain was 16%. The remaining 28% was intermediate strain. Kim *et al.* reported the high positive rates of type I strain in 100% of adult patients with peptic ulcer disease and in 71% of adult patients with chronic gastritis (Kim *et al.* 1997). As well, the pepsinogen II concentrations were increased and the pepsinogen I/II ratio was decreased significantly in patients infected with type I strain. In our previous report (Kim and Chung, 1996), we analyzed the relationship between CagA/VacA positivity and upper gastrointestinal diseases diagnosed by endoscopy in 30 cases of children infected with *H. pylori*. In nodular gastritis, positive rates of CagA and VacA were 78% and 87% respectively, and in peptic ulcer the rates were 80% in each. Type I and II strains were found in 74% and 26% respectively in children with nodular gastritis; 80% and 20% in peptic ulcer.

In reviewing the literature, we hardly found any reports showing any relationship between serum gastrin, pepsinogen concentrations and CagA/VacA positivity. Chan *et al.* reported that VacA has the effect of secreting pepsinogen from gastric epithelial cells (Chan *et al.* 1996). Kim *et al.* reported that there was no relationship between serum gastrin concentrations and CagA/VacA positivity, but that serum pepsinogen I and II concentrations were increased significantly in VacA positive patients, especially those infected with type I strain (Kim *et al.* 1997). However, our previous data was not consistent with the data reported by Kim *et al.* (1997). In this study, there was no significant relationship between the serum gastrin concentrations and CagA/VacA positivity, but the serum pepsinogen concentrations were significantly increased in children infected with CagA positive strains. The serum pepsinogen II concentration was significantly higher in type I than in type II, and the serum pepsinogen I/II ratio was significantly lower in type I and intermediate type than in type II.

In conclusion, our data suggest that increased serum pepsinogen concentrations in association with the detection of CagA cytotoxin could play a role in the development of peptic ulcer diseases. Further study will be needed to clarify the pathophysiologic mechanism in the development of peptic ulcer diseases in association with the detection of VacA and CagA cytotoxin.

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