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Study on the serum levels of soluble intercellular adhesion molecule-1 (sICAM-1) in patients with *Helicobacter pylori* Infection

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Abstract: Objective: To evaluate the interaction between serum levels of soluble intercellular adhesion molecule-1 (sICAM-1) and *Helicobacter pylori* (*H. pylori*) infection in patients with chronic gastritis and peptic ulcer. Methods: The serum levels of sICAM-1 in 205 patients with chronic gastric diseases were detected by ELISA method and the status of *H. pylori* was determined by histologic examination, RUT, ^{14}C-UBT, and serology. The sera obtained from 18 healthy volunteers served as controls. Results: The serum levels of sICAM-1 were significantly higher in patients with *H. pylori* positive than those of H. pylori negative (889.43±32.52 ng/ml vs. 747.07±30.45 ng/ml, $P<0.05$). The serum levels of sICAM-1 in patients with mild, moderate and severe infection of *H. pylori* were 841.68±72.36 ng/ml, 905.43±37.59 ng/ml and 1012.54±49.34 ng/ml, respectively ($P<0.05$). The serum levels of sICAM-1 proved to be significantly correlated with the density of *H. pylori* colonization in gastric mucosa ($r_s=0.316$, $P<0.001$). The serum levels of sICAM-1 in patients with chronic gastritis and peptic ulcer were significantly higher than those in healthy controls ($P<0.05$). Conclusions: These results indicated that *H. pylori* infection up-regulates the expression of sICAM-1.

Key words: *Helicobacter pylori*, sICAM-1, Serum, Enzyme-linked immunosorbent assay (ELISA)
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INTRODUCTION

Since the first successful isolation of *H. pylori* in 1982, *H. pylori* infection has been found to be associated with various diseases including chronic gastritis, peptic ulcer diseases (Dooley et al., 1989; Graham et al., 1992), and gastric neoplasms (Bayerdorffer et al., 1995). It was suggested that *H. pylori* stimulates and activates neutrophils and other inflammatory cells, and increases neutrophil chemotactic activity, with the activated leukocytes causing tissue injury (Kozol et al., 1991). Adhesion molecules of both leukocytes and endothelial cell are important in the migration of leukocytes into the extravascular space and are involved in cytokine-mediated tissue injuries (Mulligan et al., 1993). Intercellular adhesion molecule-1 (ICAM-1, also called CD54) has a potential role in immunoregulation by mediating immune cell infiltration into the tissue (Wagrowska-Danilewicz et al., 1998).

ICAM-1 is a member of the immunoglobulin supergene family of adhesion proteins, and serves as the counter-receptor for lymphocyte function-associated antigen-1 (LFA-1, also referred to as CD11a/CD18), and macrophage differentiation antigen (MAC-1, CD11b/CD18). Adhesion molecules can be detected in soluble forms in the circulation, and raised levels have been reported under conditions, such as viral (Yang et al., 1996) or bacterial infections (Nakae et al., 1996, Iwagaki et al., 1997), connective tissue diseases (Egerer et al., 2000), parasitic disease (Afifi et al., 2000), etc. However, the expression of sICAM-1 molecules in chronic gastritis and peptic ulcer associated with *H. pylori* infec-

tion remains largely unknown. In this study, we analyzed serum levels of sICAM-1 in patients with upper gastro-intestinal diseases to clarify the clinical significance of serum levels of sICAM-1 and the correlation between serum levels of sICAM-1and the density of *H. pylori* infection.

METHODS

Patients

In this study, 205 patients (132 males and 73 females) aged 21 to 70 years (45.43 ± 12.24 years) with upper gastrointestinal symptoms were included. The sera obtained from 18 age- and sex-matched healthy blood donators, including 10 men and 8 women, served as controls.

Informed consent was obtained from each patient before enrollment in the study. Their medical histories were recorded in detail. The exclusion criteria included previous surgery, gastric malignancy, upper gastrointestinal bleeding, pregnancy, breast feeding, autoimmune disease, any immunosuppressive therapy or on corticosteroids, liver dysfunction, inability to give informed consent, use of antibiotics, proton pump inhibitors, and compounds containing bismuth, up to four weeks before the study.

H. pylori status

During endoscopy, three or four biopsy specimens were taken from the gastric antrum 1 – 5 cm near the pylorus. One biopsy specimen was detected for *H. pylori* with RUT (rapid urease test, Sanqiang co. Fujian, China). The rest were fixed in 10% buffered formalin and embedded in paraffin for histological examination. All biopsy specimens were stained with methylene blue (MB) and read by an experienced histopathologist who was unaware of patients' clinical, history, endoscopic findings, and results of other tests. Histomorphological characteristics of biopsy specimens were classified according to the Sydney classification. For serology, the serum *H. pylori* IgG antibodies were detected with the routine method of enzyme-linked immunosorbent assay (ELISA). For 14C-urea breath test(14C-UBT, Yanghe co. Shenzhen, China), participants were asked to take a standard 37.0 kBq of 14C-urea in capsule during the fasting status. Breath samples were collected before and 20 min after administration of ^{14}C-urea.

Because no single test suffices as a criterion standard (Cutler et al., 1995), *H. pylori* status was defined as positive when at least two of the four tests (histology, RUT, ^{14}C-UBT and serology) were positive. Patients with negative results on all four tests were assessed as non-infected. Patients who could not be categorized according to this standard for determination were judged as "others" and excluded from data analysis.

The density of *H. pylori* colonization was graded semiquantitatively using the terms "absent", "mild", "moderate", and "severe", in accordance with The Sydney System revisited: The Houston International Gastritis Workshop (Genta et al., 1995).

Assay of circulating sICAM-1.

Venous blood samples were drawn from each patient before endoscopy and sera were stored at -20℃ until assayed. Serum levels of sICAM-1 were measured with commercial enzyme-linked immunosorbent assay (ELISA) (Coulter, French) according to the manufacturer's instructions. The concentration of each serum sample was determined by calculating the concentration of sICAM-1 corresponding to the mean absorbance from the standard curve using the sICAM-1 control. No cross-reactivity was found with human IgG, soluble vascular cell adhesion molecule-1.

Statistical analysis

Data are expressed as the mean ± SE. The correlation between the sICAM-1 level and age was assessed by Spearman's rank correlation coefficient test. We used the unpaired t-test to compare the sICAM-1 level between the two groups. Differences with P values less than 0.05 were considered to be statistically significant.

RESULTS

Serum level of sICAM-1 in healthy controls

The mean ± SE of the serum level of sICAM-1 in 18 healthy controls was 557.81 ± 27.46 ng/ml. There was no correlation between serum level of sICAM-1 and age or gender of healthy controls as examined by Spearman's

rank correlation coefficient test ($r_s = 0.044$, $p = 0.515$). No significant difference was observed in serum level of sICAM-1 between 10 healthy males and 8 healthy females.

Impact of *H. pylori* infection on serum level of sICAM-1

Serum level of sICAM-1 of patients with *H. pylori* positive was significantly higher than that of patients with *H. pylori* negative ($P < 0.05$). Of the 205 patients, 127 were *H. pylori* positive and 78 were *H. pylori* negative. *H. pylori* positive group and *H. pylori* negative group did not differ in age and gender. There was no significant difference between the serum level of sICAM-1 in patients with *H. pylori* negative infection and those of healthy controls (Table 1).

Table 1 Impact of *H. pylori* infection on serum level of sICAM-1

	No. of cases	sICAM-1(ng/ml)[a]
Ⅰ *H. pylori* positive	127	889.43±32.52*
Ⅱ *H. pylori* negative	78	747.07±30.45**
Ⅲ Healthy controls	18	557.81±27.46

[a] values are expressed as mean±SE.
*Ⅰ to Ⅲ $P<0.005$; Ⅰ to Ⅱ $P<0.05$; **Ⅱ to Ⅲ $P>0.05$

Correlations between serum level of sICAM-1 in patients and the density of *H. pylori*

The serum levels of sICAM-1 proved to be closely and significantly correlated with the density of *H. pylori* colonization in gastric mucosa ($r_s = 0.316$, $P<0.000$). The serum levels of sICAM-1 were significantly higher in "moderate" and "severe" *H. pylori* infection patients than in "absent" ones, the same trend was also found in those with "severe" to "mild" *H. pylori* infection ($P<0.05$). There was no correlation between the serum level of sICAM-1 and the age or gender of patients with *H. pylori* infections (Table 2).

Serum level of sICAM-1 in patients with chronic gastric disease.

The serum levels of sICAM-1 in patients with chronic superficial gastritis (CSG), chronic atrophic gastritis (CAG), and peptic ulcer (PU) were significantly higher than those of healthy controls ($P < 0.05$). The serum levels of sICAM-1 in patients with PU were higher than those with CSG and CAG, but no statistically significant difference existed ($P>0.05$). However the prevalence of *H. pylori* infection in PU was significantly higher than that in CSG and CAG (Table 3).

Table 2 Correlations between serum level of sICAM-1 in patients and the density of *H. pylori*

The density of *H. pylori*	No. of cases	sICAM-1(ng/ml)[a]
Ⅰ mild	26	841.68± 72.36△
Ⅱ moderate	39	905.43± 37.59*
Ⅲ severe	62	1012.54±49.34**
Ⅳ absent	78	747.07±30.45

[a] values are expressed as mean±SE.
*Ⅱ to Ⅳ $P<0.05$, **Ⅲ to Ⅳ $P<0.001$, △Ⅰ to Ⅲ $P<0.05$

Table 3 Serum levels (ng/m) of sICAM-1 in patients with chronic gastric diseases

	No. of cases	sICAM-1(ng/ml)[a]	No. of *H. pylori*
Ⅰ CSG	54	855.03±40.10	33(61%)
Ⅱ CAG	109	844.85±32.36	60(55%)
Ⅲ PU	42	994.33±63.22	34(81%)**
Ⅳ Controls	18	557.81±27.46*	

[a] values are expressed as mean±SE.
*Ⅰ,Ⅱ,Ⅲ to Ⅳ $P<0.05$; **Ⅰ、Ⅱ to Ⅲ $P<0.05$.

DISCUSSION

In the present study, we assessed the serum levels of sICAM-1 in patients with *H. pylori* infection, without *H. pylori* infection and in healthy controls. The data indicated a significantly higher expression of sICAM-1 in patients with *H. pylori* infection. sICAM-1 is an adhesion molecule of the immunoglobulin in superfamily which plays a role in cell migration from peripheral blood to tissues and in immune-competent cell-cell interactions. We found this molecule expressed at various levels according to the three densities of *H. pylori* colonization at gastric mucosa, which suggested a close relationship between the density of *H. pylori* infection and sICAM-1 expression. The serum levels of sICAM-1 in patients with PU were higher than

those in patients with CSG and CAG, probably because the prevalence of *H. pylori* infection in PU is significantly higher than that in CSG and CAG. This result agrees with those of Archimandritis et al., who demonstrated that ICAM-1 expression did not correlate with gastritis parameters (Archimandritis et al., 2000).

H. pylori is now recognised to be the major cause of antral gastritis and a risk factor for further development of gastric cancer. The inflammatory response in *H. pylori*-associated gastritis (HAG) is characterized by an intense infiltrate of granulocytes and lymphocytes. The indensity of *H. pylori* infection and severity of the mucosal injury are directly correlated with the extent of neutrophil infiltration (El Kaissouni et al., 1998). In the context of *H. pylori* infection, the production of chemoattractive cytokines and cell adhesion molecules could provide a means of recruiting and retaining inflammatory cells within the gastric epithelial layer, contributing to *H. pylori*-mediated tissue injury (Dooley et al., 1989).

ICAM-1 is an inducible cell surface glycoprotein expressed at a low level on a wide variety of cells, including leukocytes, antigen-presenting cells, vascular endothelium, fibroblasts, endothelial cells and certain epithelial cells. The increased expression of ICAM-1 is linked with massive infiltration of inflammatory cells that express LFA-1 and Mac-1, and also with antigen-presenting cells (APCs) that express HLA-DR (Human leucocyte antigen-D-related), suggesting that ICAM-1 exerts a key role in immuno-inflammatory responses in gastric mucosa of patients with *H. pylori*-associated gastritis (Higuchi et al., 1997). Archimandritis A et al demonstrated that ICAM-1 was expressed by gastric epithelial cells in 80% of *H. pylori* positive patients.

Soluble ICAM-1 (sICAM-1) is a circulating substance can bind with LFA-1 of leukocytes, thus, making leukocytes less available for binding with cell surface ICAM-1 on target cells (Rothlein et al., 1991). The sera levels of sICAM-1 were shown to be elevated in inflammation, infection, and cancer, indicating that sICAM-1 may be a useful parameter for diagnosis and evaluation of these pathologic condition (Gearing et al., 1993). sICAM-1 is up regulated by interferon gamma and TNF-α as well as IL-1, IL-2 (Fonsatti et al., 1997). Cytokines such as TNF-α that are increased during infection with *H. pylori* could augment the expression of sICAM-1 or other adhesion molecules and thereby, contribute to epithelial cell injury caused by the attachment of inflammatory cells. These data support the theory that *H. pylori* infection plays an active role in initiating the increased expression of sICAM-1 in chronic gastritis and peptic ulcer. The overexpression of sICAM-1 is considered to be involved in the inflammatory responses induced by *H. pylori* infection. In the present study we demonstrated the overexpression of sICAM-1 correlated significantly with the density of *H. pylori* colonization.

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