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Effect of Smoking on Serum Pepsinogen I Level Depends on Serological Status of *Helicobacter pylori*

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Serum pepsinogen (sPG) levels are used in gastric cancer screening programs. However, modification of sPG levels by smoking habit, according to the status of *Helicobacter pylori* (*H. pylori*) infection has been little investigated. This study investigated the effects of smoking on serum levels of pepsinogen I (PG I), pepsinogen II (PG II), and gastrin by IgG titer of antibody against *H. pylori* (Hp-IgG titer) using the data from 356 current-smokers and 262 non-smokers (133 never-smokers and 129 ex-smokers) in a cross-sectional study of 618 men aged 40 to 49 years. PG I, PG II, PG I/PG II ratio and gastrin were significantly associated with Hp-IgG titer in never-smokers [Spearman's correlation coefficient (95% confidence interval): 0.23 (0.07, 0.39), 0.52 (0.41, 0.63), −0.40 (−0.54, −0.27), and 0.25 (0.10, 0.41), respectively]. However, the correlation coefficients of PG I and PG II decreased in current-smokers, 0.02 (−0.1, 0.13) and 0.32 (0.22, 0.42), respectively. In *H. pylori* seronegative and low titer cases, the mean PG I level was significantly ($P<0.01$) higher in current-smokers, compared with non-smokers. However, in high titer cases, the mean PG I level was lower in current-smokers. Mean PG II and gastrin levels, and PG I/PG II ratio did not differ according to smoking habits by Hp-IgG titer. The gastrin level was significantly correlated with PG II, but not PG I. These data indicate that current smoking influences the serum PG I level depending on Hp-IgG titer and the associations between sPGs and Hp-IgG titer. Gastrin is not involved in the modification of PG I levels by smoking.

Key words: Pepsinogen — *Helicobacter pylori* — Titer — IgG — Smoking

Chronic atrophic gastritis is a known precursor of an intestinal type of gastric cancer. *Helicobacter pylori* (*H. pylori*) has been acknowledged to be the etiologic agent of chronic atrophic gastritis.[1)] Detection for staging of chronic gastritis is particularly important to set a high-risk group for prevention of gastric cancer. The development of assays for serum pepsinogens (sPGs), together with tests for antibodies to *H. pylori*, has enabled noninvasive estimation of the presence of both chronic gastritis and severe atrophy.[2)] This has made it possible to screen large, asymptomatic populations for these lesions without the need for endoscopy.

Pepsinogen is secreted as two biochemically distinct groups of isozymes: pepsinogen I (PG I) and II (PG II). Both are secreted by chief and mucous neck cells of the gastric fundus and corpus, and PG II is also secreted by the pylori glands in the antrum and Brunner's glands in the proximal duodenum. Initially, in mild inflammation, circulating levels of both sPGs increase. Chief cells are gradually replaced by pyloric glands as gastritis progresses, the result being a decrease in PG I but maintenance of (or increased) PG II levels. As a consequence, PG I/PG II ratio is thought to be an indicator of mucosal atrophy.[3)]

H. pylori infection causes an immunological host response that is characterized mainly by the production of antibodies detectable in the circulation at high titers for a long time. Behavior of IgG antibody titer in relation to *H. pylori* is associated with the density of *H. pylori* infecting the gastric mucosa.[4)] The accumulating data show positive relationships among the qualitative serological evaluations of *H. pylori* infection, sPG levels, and gastric cancer.[2, 5–8)] However, few reports showed a relationship between quantitative status of *H. pylori* infection and sPGs in a large number of people.

There are several problems to be resolved when screening to detect a high-risk group for gastric cancer using sPGs and serology of *H. pylori*. Previously, the ratio of PG I/PG II was reported to be an indicator in association with mucosal atrophy and gastric cancer.[9–11)] However, investigations have shown that a low PG I level is associated with an increased risk of gastric cancer in men, rather than the ratio of PG I/PG II.[12)] In addition, although smoking habits are considered to influence the risk of gastric cancer,[13)] the effect of smoking on sPG levels in the people with or without *H. pylori* infection is not clear. It is very important to clarify the modification of sPG levels caused

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by smoking in order to screen a high-risk group for cancer among aged people. The present study aims to investigate the behavior of sPG levels by smoking status using IgG titer of *H. pylori* (Hp-IgG titer) in a middle-aged population. In addition, we discuss the significance of the PG I/PG II ratio.

SUBJECTS AND METHODS

The subjects investigated were the participants in our cross-sectional study, which aimed to identify the causes of geographic variations in several types of cancer; the methods employed have been described in detail elsewhere.[14–17] Briefly, of 880 randomly selected men aged 40 to 49 years, 634 agreed to participate.

Blood collection was conducted for all participants, and blood samples were stored at −80°C. The PG I and PG II levels in sera were determined by the radioimmunoassay method (PG I/PG II RIABEAD, Dainabot Co., Ltd., Tokyo). Because of an insufficient quantity of serum samples, PG II was not measured in a total of 10 subjects of the 634.

Specific anti-*H. pylori* IgG antibody titers in sera (Hp-IgG titers) were measured by enzyme-linked immunosorbent assay (Helico G, Porton Cambridge, Oxford, UK). The threshold cut-off value in this kit was 10 units/ml. The sensitivity and specificity of the assay were 96% and 86%, respectively, upon comparison with gastric biopsy findings.[10] All samples were assayed in duplicate with negative and positive quality control samples on each plate. The antibody concentration was determined from the optical density reading through a calibrator using the kit. *H. pylori* antibodies were not measured in 6 subjects due to insufficient quantity of serum sample. Serum gastrin levels were measured radioimmunologically using kits from Baxter Healthcare Co.[18] Information about smoking habit was obtained by questionnaire in an interview by trained public health nurses or nutritionists.

Statistical analysis Differences among three groups were determined using one-way analysis of variance (ANOVA) and Bonferroni's test for pairwise comparisons. Mean values of PG I, PG II, PG I/PG II ratio and gastrin were adjusted for age by analysis of covariance using the GLM procedure in the SAS program package. As a measure of the association between Hp-IgG titer and PG I, PG II, PG I/PG II ratio and gastrin, Spearman's rank correlation coefficients were computed.

RESULTS

Six hundred and eighteen men from whom complete information was obtained at interview, and whose levels of PG I, PG II and Hp-IgG titer were determined, were divided into three groups based on information regarding their smoking habits; 133 never-smokers, 129 ex-smokers and 356 current-smokers. Subjects' characteristics are summarized in Table I. There were significant differences in age among the three groups. Ex-smokers were the youngest. The prevalence of low PG I (<25 ng/ml) and the low ratio of PG I/PG II (<3.0) did not differ among them. The subjects with Hp-IgG titer <10 units/ml were defined as seronegative for *H. pylori* infection. Although the prevalence of seropositive cases was not different in terms of smoking status, Hp-IgG titer was lowest in current smokers.

Table II shows the mean sPG and gastrin levels, and PG I/PG II ratio by smoking status by Hp-IgG titer with adjustment for age. In addition, the subjects with Hp-IgG titer of 10 or more were classified into two groups of equal numbers. The threshold value was 31.0 units/ml. In seronegative and low titer cases, the mean PG I level in current-smokers was significantly higher than among never- or ex-smokers. However, in high titer cases, there was no difference in PG I levels according to smoking status; in fact, the mean PG I level was lowest in current

Table I. Characteristics of Participants

			Never-smokers $n=133$	Ex-smokers $n=129$	Current smokers $n=356$	P
Age		(mean±SD)	45.1±2.9**, ##	43.8±2.8	44.2±3.0	0.002 *a)*
BMI (kg/m^2)		(mean±SD)	24.1±3.1	23.8±2.7	23.8±2.9	NS *a)*
Pepcinogens	PG I (ng/ml)	(mean±SD)	51.4±29.5	52.3±25.9	57.4±26.4	0.039 *a)*
	PG II (ng/ml)	(mean±SD)	13.2±8.0	13.7±8.6	13.4±7.5	NS *a)*
	PG I/PG II	(mean±SD)	4.6±2.3	4.4±2.0	4.8±2.3	NS *a)*
	low PG I	(%)	11.3	10.1	7.9	NS *b)*
H. pylori	prevalence	(%)	73.7	76.0	75.3	NS *b)*
	IgG titer (units/ml)	(mean±SD)	34.4±33.0	36.7±37.2	29.6±25.8	0.045 *a)*

NS: not significant.
** $P<0.01$, compared to ex-smokers; ## $P<0.01$, compared to current smokers.
a) One-way ANOVA.
b) χ^2 test.

Table II. Age-adjusted Mean sPG Levels, PG I/PG II Ratio and Gastrin Level by Smoking Habits According to IgG Titer of *H. pylori* (Hp-IgG titer)

	Hp-IgG titer (units/ml)	Never-smokers n=133 (mean±SE)	Ex-smokers n=129 (mean±SE)	Current-smokers n=356 (mean±SE)	P [a)]
PG I (ng/ml)	<10	42.3±4.5**	45.2±4.8*	60.3±2.8	0.001
	10–30.9	45.7±3.3*	45.4±3.3*	56.0±1.8	0.002
	31.0–	61.1±4.3	62.1±4.2	57.3±2.7	NS
PG II (ng/ml)	<10	8.8±1.3	10.1±1.4	11.7±0.8	NS
	10–30.9	11.7±0.9	12.9±0.9	12.6±0.5	NS
	31.0–	17.1±1.2	16.5±1.2	15.7±0.8	NS
PG I/PG II	<10	5.7±0.4	5.3±0.5	5.9±0.4	NS
	10–30.9	4.7±0.3	4.1±0.3	4.9±0.2	NS
	31–	3.7±0.2	4.2±0.2	4.1±0.2	NS
Gastrin (pg/ml)	<10	88.1±5.3	86.7±5.7	87.0±3.4	NS
	10–30.9	109.1±7.3	87.3±7.4	94.1±4.1	NS
	31.0–	95.2±4.9	102.9±4.7	102.6±3.1	NS

NS: not significant.
** P<0.01, * P<0.05, compared to current smokers.
a) ANCOVA.

smokers. There was no difference in PG I levels between never- and ex-smokers. Behavior of mean PG II and gastrin levels and PG I/PG II ratio according to Hp-IgG titer showed no difference in terms of smoking status.

Table III presents Spearman's correlation coefficient between Hp-IgG titer and PG I, PG II, PG I/PG II ratio, and gastrin by smoking status. PG I showed a moderate positive correlation in non-smokers, but not in current-smokers. PG II showed a strong correlation in never-smokers, but it decreased in ex- and current smokers. PG I/PG II ratio showed a significantly negative association in never- and current smokers, but not in ex-smokers.

To assess the effects of smoking on the PG I level and Hp-IgG titer, the number of cigarettes smoked per day was classified into 3 levels; 1–19, 20–39, and 40 or more (40–) cigarettes smoked/day. In addition, pack-years were calculated and classified into 3 grades; 1–19, 20–39, and 40–. There was no significant difference in PG I level in terms of the number of cigarettes smoked/day or pack-years. However, the mean (±SE) PG I level (ng/ml) in seronegative subjects increased in parallel with pack-years (56.9±7.8, 61.0±4.8, and 64.3±6.8, respectively), whereas in high titer cases, they decreased in parallel with pack-years (63.9±5.7, 57.8±3.2, and 55.0±4.9, respectively).

Hp-IgG titer was lowest in current smokers. We examined the dose-response relation between Hp-IgG titer and number of cigarettes smoked/day and pack-years. The mean values (±SE) (units/ml) of Hp-IgG titer by the number smoked (1–19, 20–39, and 40–) were 66.5±5.4, 59.3±2.5, and 57.4±5.8 (P=0.337). The mean (±SE) of Hp-IgG titer by pack-years (1–19, 20–39, and 40–) was 58.5±4.9, 56.5±2.7, and 58.0±4.0 (P=0.912). There was no significant difference among the number of cigarettes smoked/day or pack-years. However, the mean Hp-IgG titer tended to decrease in parallel with the number of cigarettes smoked.

Table III. Correlation Coefficients between IgG Titer of *H. pylori* and sPG Levels, PG I/PG II Ratio and Gastrin Level

	Never-smokers n=133	Ex-smokers n=129	Current-smokers n=356
PG I	0.23	0.29	0.02
	(0.07, 0.39)	(0.13, 0.45)	(−0.08, 0.13)
PG II	0.52	0.32	0.32
	(0.41, 0.63)	(0.16, 0.48)	(0.22, 0.42)
PG I/PG II	−0.40	−0.13	−0.31
	(−0.54, −0.27)	(−0.29, 0.04)	(−0.41, −0.21)
Gastrin	0.25	0.21	0.17
	(0.10, 0.41)	(0.05, 0.38)	(0.07, 0.27)

95% confidence interval.

To elucidate the behavior of sPGs, we examined the relationship between the levels of sPGs and gastrin, according to smoking habits. The serum gastrin level was significantly correlated with PG II, but not PG I, and the correlation coefficients between gastrin and PG I did not differ, according to smoking habit (Table IV).

DISCUSSION

The present study clearly demonstrated that Hp-IgG titer antibody and current smoking modified the behavior of serum pepsinogen levels.

Table IV. Correlation Coefficients between Serum Gastrin Level and sPG Levels, and PG I/PG II Ratio

	Never-smokers n=133	Ex-smokers n=129	Current-smokers n=356
PG I	0.06	0.13	0.09
	(−0.12, 0.25)	(−0.56, 0.31)	(−0.02, 0.20)
PG II	0.43	0.37	0.22
	(0.29, 0.57)	(0.22, 0.52)	(0.12, 0.33)
PG I/PG II	−0.41	−0.39	−0.18
	(−0.56, −0.26)	(−0.54, −0.24)	(−0.29, −0.07)

95% confidence interval.

sPG levels are determined by the biosynthesis rate of pepsinogens per cell and the number of producing cells. They depend on the gene expression rate and histological appearance. Biochemically, the biosynthesis of pepsinogens is regulated by several compounds, including gastrin and secretin, but not much is known about the regulatory mechanism of pepsinogen gene expression.[19, 20] Takahashi *et al.*[21] demonstrated that the 5′-flanking region of the *PG I* gene is different from that of *PG II* gene, suggesting that *PG I* gene and *PG II* gene are differently regulated. PG I is secreted mainly by chief and mucous neck cells of the gastric fundus and corpus and PG II is also secreted by the pylori glands in the antrum and Brunner's glands in the proximal duodenum. Therefore, the PG I level depends on the number of cells producing PG I, and it is indicative of the histological status of glandular cells of the stomach. On the other hand, the PG II level might depend on the rate of gene expression, rather than the number of producing cells. In this study, we found a strong correlation between serum gastrin and PG II levels, but none between serum gastrin and PG I levels. These results underscore the above discussion.

Hp-IgG titer is a sensitive marker indicating the histological grade of inflammation due to *H. pylori* infection.[4] In this study, both PG I and PG II were significantly correlated with Hp-IgG titer in never-smokers, suggesting that inflammation of the gastric mucosa elevates serum PG levels. Our data and those of other reports demonstrated a strong correlation between inflammation in the gastric mucosa and the PG II level, rather than the PG I level. Histological grades of inflammation in the fundus and antrum reportedly have a great influence on the PG II level.[4] Moreover, recent studies have shown marked reductions in PG II and minor declines in PG I after eradication therapy for *H. pylori*.[22] The immediate reduction after the clearance of *H. pylori* suggests that inflammation up-regulates *PG II* gene expression and has little effect on *PG I* gene expression. *H. pylori* infection causes inflammation in the gastric mucosa, mediating several inflammatory cytokines, and the inflammation-associated nuclear factor, NF-kB, enhancing gene transcription.[23] However, a computer search of the nucleotide sequence in the promoter region of *PG I* and *PG II* gene revealed no region corresponding to an NF-kB binding sequence (unpublished data). Gisbert *et al.*[24] showed that inflammation evoked by *H. pylori* causes an elevation of the serum gastrin level. In our study, the serum gastrin level was significantly correlated with Hp-IgG titer. In particular, PG II was strongly correlated with the gastrin level. These data suggest that those inflammatory mediators indirectly up-regulate sPGs, via a mediator such as gastrin, but do not directly enhance gene expression.

In subjects seronegative for *H. pylori*, the mean serum level of PG I in current smokers was significantly higher than in no smoking subjects (never-smokers and ex-smokers). The PG II level was likely to be elevated in current smokers, but not significantly. Previous studies have reported a dose-dependent positive association between smoking and the PG I level, but not PG II, in a large-scale population.[25] The present data and previous data showed that the PG I level in current smokers was higher than that in ex-smokers, suggesting that current smoking elevates the PG I level.

Epidemiological and experimental studies have demonstrated a close association between smoking and peptic ulcer disease in humans and animals. Smoking accelerates gastric ulceration and delays ulcer healing. However, the exact mechanisms by which smoking causes mucosal damage are unknown. Reduction of prostaglandin levels, neutrophil accumulation in the gastric mucosa, enhancement of coagulation by activation of platelets and so on, are thought to play key roles in mucosal damage from smoking.[26, 27] These changes may disturb the microcirculation, thereby triggering inflammation in the gastric mucosa. However, smoking was associated with PG I, but not PG II, which was more associated with inflammation. From these observations, we speculate that some compounds present in cigarettes directly enhance *PG I* gene expression.

In subjects seropositive for *H. pylori* infection, the relationship between behavior of sPGs and smoking was complex. In the subjects with low IgG titer, the relationship between smoking habits and the PG I level was similar to that of subjects seronegative for *H. pylori*. However, the subjects with high titer showed no association between smoking and the PG I level. In fact, the mean PG I level in current smokers was lower than that in non-smokers. These data are not consistent with our speculation that some compounds in cigarettes might stimulate *PG I* gene expression. The fact that the mean PG I level was decreased in the subjects with high titer and/or with heavy smoking is likely due to histological atrophic change and/or functional disturbance of the chief cell and mucus neck cells producing PG I. Our examined population was aged

40 to 49 years. Data from another of our cohort studies with the mean age of 59 years showed that the PG I level in current smokers with high IgG titer was significantly lower than that in non-smokers (unpublished data). On the other hand, a dose-dependent increase of PG I by cigarette dose was observed in a younger population, 20–29 yr,[25] having a low rate of atrophic change in the gastric mucosa. These observations suggest that, in older persons, chief cells and mucus neck cells may readily tend to be functionally disturbed by smoking and/or by severe inflammation induced by *H. pylori*. On the other hand, in younger men, the PG I-producing function of these cells may be maintained, and the gene expression may be enhanced by smoking.

The relationship between Hp-IgG titer and sPGs levels was different between PG I and PG II. Epidemiologically, the low PG I level in man is associated with an increased risk of gastric cancer.[12] The PG I level is indicative for the histological state of the gastric mucosa; in particular, a low level of serum PG I has been identified as a reliable indicator of chronic corpus atrophy.[3] However, the mean PG I level in the seronegative subjects was significantly lower than in the seropositive subjects. Therefore, a combination with other markers, together with the PG I level, is needed to estimate the risk for gastric cancer, and the ratio of PG I/PG II may well do this. However, given our data, we doubt whether the ratio of PG I/PG II is indicative for gastric atrophy. Our data showed that this ratio was lower in seropositive as opposed to seronegative subjects. Previously, this phenomenon has been explained by the decrease in PG I through the atrophic change caused by *H. pylori*, whereas the PG II level remained unchanged. However, in this study, the negative correlation between the PG I/PG II ratio and Hp-IgG titer was due to a greater increase in the PG II level than PG I level, according to Hp-IgG titer, but not to any decrease in PG I. This suggests that the PG I/PG II ratio is not available as a marker for mucosal atrophy, whereas a low PG I/PG II ratio is indicative for *H. pylori* infection. Thus, it is reasonable that a combination of low PG I and low PG I/PG II ratio has been reported to be a marker of mucosal atrophy caused by *H. pylori* infection.

In the present study, the mean Hp-IgG titer was lower in current smokers than non-smokers, although not significantly so, when the multiple comparison method was used. These data suggest the possibility that smoking decreases the IgG titer by immunological action, or, since it hampers survival of *H. pylori*, the number of those infected became fewer and so the IgG titer could not increase. This is a phenomenon of great interest for further study.

In conclusion, the serum PG I levels obviously varied due to smoking, and this result depended on Hp-IgG titers. This means that smoking habits or Hp-IgG titers are key factors in speculating as to the histological status of the gastric mucosa on basis of the pepsinogen level. Therefore, when using sPGs clinically and for mass screening, the cut-off value must be determined in relation to both smoking habits and IgG titers.

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