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Long-term Effect of *Helicobacter pylori* Infection on Serum Pepsinogens

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Serum pepsinogen values are markers of gastric mucosal status and of gastric cancer risk. The effect of *Helicobacter pylori* infection and sibship size on change of serum pepsinogen values over a seven-year span was investigated. Data from 2584 subjects with phlebotomy were analyzed both in 1989 and in 1996. The subjects were classified by *H. pylori* serology and sibship size (1–3 vs. 4 and more). Pepsinogen I (PG I) to II (PG II) ratio in '96 minus that in '89 was defined as ΔPG I/II and compared among the groups. ΔPG I/II was lower and decrease of PG I/II was more frequent among *H. pylori*-positive subjects than among negative subjects. The difference was owing to a decrease of PG I in all subjects and owing to an increase of PG II in those not younger than 30 years in '89. In *H. pylori*-positive subjects, those with a larger sibship size showed lower ΔPG I/II and higher frequency of PG I/II decline. *H. pylori* infection exerts a reducing effect on PG I/II during the seven-year span. The effect of *H. pylori* is stronger among those with a larger sibship size, who are expected to have been infected with *H. pylori* in childhood. Inducing atrophy of gastric mucosa, which is reflected by a decline of PG I/II, may be one of the mechanisms through which *H. pylori* elevates the risk of gastric cancer.

Key words: Long-term effect — *Helicobacter pylori* — Serum pepsinogens — Inflammation — Atrophy of gastric mucosa

Serum pepsinogen is a known marker of gastric mucosal status, including mucosal atrophy.[1, 2] It is also considered a marker of inflammation. Eradication of *Helicobacter pylori* decreases the severity of gastritis and provokes a significant change in serum pepsinogen values: it reduces both pepsinogens I (PG I) and II (PG II), and elevates the PG I to II ratio (PG I/II).[3] It has been established that serum pepsinogen is related to risk of gastric cancer, and a low PG I/II ratio predicts an increased risk of gastric cancer.[4–6] *H. pylori* infection, which is closely associated with serum pepsinogen values,[7, 8] is also related to gastric cancer risk.[9–12] In our previous study, which involved the same subject group, those with a large sibship size showed a higher prevalence of *H. pylori*.[13] However, the long-term effect of *H. pylori* on serum pepsinogen remains unclear.

A low or decreasing PG I/II value is related to an increased risk of gastric cancer. The purpose of this study was to analyze the relationships between *H. pylori* status and changes of serum pepsinogen values including PG I/II. Data from 2584 subjects who underwent phlebotomy twice with a seven-year interval were analyzed.

SUBJECTS AND METHODS

The subjects were from a bureau, in which about 5000 public service workers are employed. The offices of the subjects were distributed from the east to the west coast in the middle of Honshu island. About 90% of the workers underwent a general health check program annually. Serum pepsinogen values were measured using residual sera from the health check program both in '89 and in '96. Ages of the subjects refer to their age in '89. Measurements were performed by BML Co., Ltd. (Tokyo) using the RIAbeads Pepsinogen I and II kits produced by Dainabot Co., Ltd. (Tokyo). *H. pylori* antibody was measured using residual sera from '96 by BML Co., Ltd., using Pilika plate G Helicobacter II produced by Biomerica Co., Ltd. (Newport, CA). Instead of the kit-recommended cut-off value of 20.0 units/ml, the cut-off value was defined as 16.0 units/ml, which gave optimal sensitivity and specificity against the urea breath test among 491 Japanese subjects.[14] The subjects were asked to fill out a questionnaire on food intake, past and family history including sibship size in '96.

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The subjects were stratified by age (20–29 or 30–56 years; that is, 27–36 or 37–63 years in '96), and the serum PG values in '89 and their changes during the seven-year span were compared between *H. pylori*-positive and negative subjects. When PG I (unit: μg/liter) and PG II (unit: μg/liter) values were compared, their natural logarithms ln(PG I) and ln(PG II), which have smaller skewness and kurtosis,[15] were used. For PG I/II, the crude value was used. To observe changes of serum pepsinogen values, Δln(PG I), Δln(PG II), or ΔPG I/II was defined as the ln(PG I), the ln(PG II), or the PG I/II value in '96 minus that in '89, respectively. Means of Δln(PG I), Δln(PG II), or ΔPG I/II and the frequencies of subjects whose PG I, PG II, or PG I/II declined during the seven-year span were calculated and compared.

For PG I/II, the relationship between the initial value and the change was observed. Subjects were stratified by PG I/II value in '89 (0.0–2.0; that is not less than 0.0 and less than 2.0, 2.0–3.0, 3.0–4.0, 4.0–5.0, 5.0–6.0, 6.0–7.0, 7.0–8.0, or 8.0+), and the means of ΔPG I/II and the frequencies of subjects with a decrease of PG I/II were compared between *H. pylori*-positive and negative subjects.

Between those with a sibship size of 1 to 3 and those with 4 and more, the means of ΔPG I/II and the frequencies of subjects with a decrease of PG I/II were also compared, for subjects stratified by age and *H. pylori* status (negative or positive). The means and frequencies were compared by use of the *t* test and χ^2 test, respectively.

Table I. Means and Standard Deviations of Serum PG I, PG II and PG I/II in '89 with Reference to *H. pylori* Status and Age

Age		*H. pylori*-negative	*H. pylori*-positive	*P* value[a]
20–29	*n*	821	503	
	ln[b](PG I[c])	3.64 (38.1)±0.30[d]	3.86 (47.7)±0.42	<0.001
	ln(PG II[c])	1.80 (6.1)±0.41	2.50 (12.2)±0.60	<0.001
	PG I/II	6.54±1.75	4.27±1.87	<0.001
30–56	*n*	602	658	
	ln(PG I)	3.71 (40.9)±0.34	3.88 (48.8)±0.47	<0.001
	ln(PG II)	1.94 (7.0)±0.47	2.70 (14.7)±0.54	<0.001
	PG I/II	6.25±2.01	3.66±1.73	<0.001

a) *P* value for means between *H. pylori*-negative and positive subjects.
b) Natural logarithm.
c) μg/liter.
d) Mean (exponential)±standard deviation.

Table II. Means and Standard Deviations of Serum PG Values in '96 Minus Those in '89 and Frequency of Declining Serum PG Values during the Seven-year Span, with Reference to *H. pylori* Status and Age

Age		*H. pylori*-negative	*H. pylori*-positive	*P* value[a]
20–29	Δln(PG I)	0.160±0.240[b]	0.096±0.347	<0.001
		154/821 (18.8%)[c]	184/503 (36.6%)	<0.001
	Δln(PG II)	0.017±0.308	0.028±0.366	0.568
		386/821 (47.0%)	281/503 (44.1%)	0.335
	ΔPG I/II	0.979±1.645	0.353±1.363	<0.001
		224/821 (27.3%)	204/503 (40.6%)	<0.001
30–56	Δln(PG I)	0.134±0.268	0.081±0.337	0.002
		142/602 (23.6%)	219/658 (33.3%)	<0.001
	Δln(PG II)	0.016±0.299	2.089±0.331	<0.001
		279/602 (46.3%)	245/658 (37.2%)	0.001
	ΔPG I/II	0.744±1.501	−0.004±1.144	<0.001
		188/602 (31.2%)	342/658 (52.0%)	<0.001

a) *P* value of χ^2 test or *t* test between *H. pylori*-negative and positive subjects.
b) Mean±standard deviation.
c) Subjects with the decline of serum PG/tested (%).

RESULTS

There were 2584 subjects: 1180 males and 144 females aged 20–29 years, and 1156 males and 104 females aged 30–56. PG I, PG II, and PG I/II values in '89 are shown in Table I. *H. pylori*-positive subjects showed significantly higher PG I, PG II, and lower PG I/II values than negative subjects. During the seven-year span, PG I, PG II and PG

Table III. Increase of PG I/II during a Seven-year Span with Reference to *H. pylori* Status and PG I/II in '89

PG I/II ('89)	*H. pylori*-negative	*H. pylori*-positive	*P* value
0–2.0[a)]	3.02±2.72[b)]	0.53±1.00	<0.001
	3/20 (15.0%)[c)]	35/114 (30.7%)	0.243
2.0–3.0	1.44±2.23	0.23±0.87	<0.001
	16/47 (34.0%)	107/261 (41.0%)	0.463
3.0–4.0	1.25±1.58	0.06±0.97	<0.001
	15/60 (25.0%)	175/339 (51.6%)	<0.001
4.0–5.0	1.21±1.35	0.13±1.43	<0.001
	21/137 (15.3%)	105/186 (56.5%)	<0.001
5.0–6.0	1.07±1.28	0.12±1.49	<0.001
	63/305 (20.6%)	50/106 (47.2%)	<0.001
6.0–7.0	1.05±1.46	0.35±1.66	<0.001
	83/342 (24.2%)	31/77 (40.3%)	0.007
7.0–8.0	0.63±1.43	−0.42±1.78	<0.001
	95/274 (34.7%)	24/41 (58.5%)	0.006
8.0+	0.10±1.77	−0.32±2.44	0.320
	116/238 (48.7%)	19/37 (51.4%)	0.905

a) Not less than left figure and less than right figure.
b) Mean±standard deviation of PG I/II ('96) minus PG I/II ('89).
c) Subjects with the decline of PG I/II/tested (%).

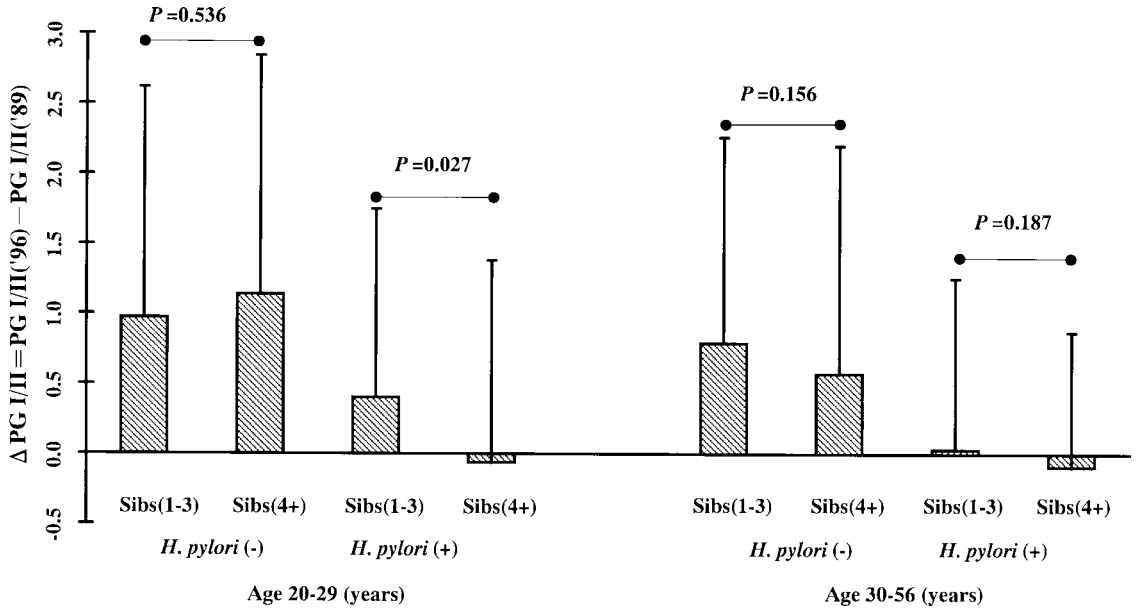

Fig. 1. Increase of PG I/II during a seven-year span with reference to *H. pylori* status and sibship size (Sibs). Means and standard deviations of PG I/II in '96 minus that in '89 are shown. They were compared between those with sibship size 1 to 3 and those with 4 or more, being stratified by *H. pylori* status and age.

Table IV. Effect of Sibship Size on Frequency of Declining PG I/II during a Seven-year Span, with Reference to Age and *H. pylori* Status

Age/*H. pylori* status	Sibship size 1−3	Sibship size 4+	*P* value[a)]
20−29 years			
H. pylori-negative	206/755 (27.3%)[b)]	11/40 (27.5%)	1.000
H. pylori-positive	172/446 (38.6%)	27/47 (57.4%)	0.019
30−56 years			
H. pylori-negative	134/460 (29.1%)	49/123 (39.8%)	0.031
H. pylori-positive	234/474 (49.4%)	95/167 (56.9%)	0.114

a) *P* value of χ^2 test between those with sibship size of 1−3 and 4+.
b) Subjects with the decline of PG I/II tested (%).

I/II increased or did not change in 1885 (72.9%), 1452 (56.2%) and 1626 (62.9%) subjects, respectively. The means (±SD) of Δln(PG I), Δln(PG II), and ΔPG I/II were 0.12 (±0.30), 0.04 (±0.33), and 0.55 (±1.49), respectively.

Table II shows the means of Δln(PG I), Δln(PG II), and ΔPG I/II by *H. pylori* status. *H. pylori*-positive subjects showed significantly lower Δln(PG I) and ΔPG I/II than negative subjects both in younger (20−29) and in older (30−56) subjects. *H. pylori*-positive subjects showed higher Δln(PG II), which was significant only in the older subjects. The frequencies of subjects with a decrease of PG I, PG II or PG I/II is also shown in Table II. The results completely paralleled those for the means.

When stratified with PG I/II values in '89 (Table III), *H. pylori*-positive subjects showed lower ΔPG I/II and higher frequency of PG I/II decline than negative subjects, which were significant in strata less than 8.0 and in strata between 3.0 and 8.0, respectively.

Those with a large sibship size showed a significantly lower ΔPG I/II and a significantly higher frequency of negative ΔPG I/II than those with a small sibship size in the *H. pylori*-positive younger subjects (Fig. 1 and Table IV). No other significant differences were noted for sibship size in the other subjects. In the *H. pylori*-negative older subjects, sibship size showed a significant effect on the frequency of negative ΔPG I/II, but the difference in means of ΔPG I/II had a *P* value of 0.16.

DISCUSSION

Cut-off value of *H. pylori* serology In this study, a cut-off value different from the recommended one was used. When the *H. pylori* serological test kit was tested against the urea breath test among 491 Japanese subjects, optimal sensitivity and specificity were obtained when the cut-off value was 16.0 units/ml.[14)]

Infection during the seven-year span *H. pylori* status was not determined in '89. However, in developed countries, infection in adulthood is known to be rare.[16−18)] Frequency of positive to negative change (seroreversion) is also rare.[17)] It is well-known that serum PG I and II are elevated and PG I/II is reduced in *H. pylori*-positive subjects.[7, 8)] Serum PG values in '89 with respect to *H. pylori* status in '96 were consistent with that. When *H. pylori* is successfully eradicated, PG I and PG II decline and PG I/II increases.[19)] If new infections or seroreversion had occurred frequently during the seven-year span, *H. pylori*-positive subjects, whose *H. pylori* status was determined in '96, would have shown higher Δln(PG I) than negative subjects. However, the *H. pylori*-positive subjects showed significantly lower Δln(PG I) than the negative subjects. Therefore in the present study, the effect of new infection or seroreversion during the seven-year span was considered negligible.

Mean and frequency of PG I, PG II, and PG I/II Analyses of the mean values of serum pepsinogens and of the frequency of negative Δln(PG I), Δln(PG II) and ΔPG I/II gave similar results, reinforcing the reliability of the results.

ΔPG I/II was lower among the *H. pylori*-positive subjects than among the negative subjects. In younger subjects, this is mainly because *H. pylori*-positive subjects had a lower Δln(PG I). In older subjects, this is because *H. pylori* positive subjects had a lower Δln(PG I) and a higher Δln(PG II). The effect of sustained *H. pylori* infection on PG II was different between the younger and the older subjects. This effect may depend on either age or duration of sustained infection.

Among PG I, PG II and PG I/II, PG I/II is known to have the strongest association with risk of gastric cancer.[4)] Therefore, PG I/II was used in further analyses. PG I/II increased in about two-thirds of all the subjects during the seven-year span. This may be due to a relatively large percentage of the subjects being young. Decline of PG I/II was more frequent in older subjects. If the subjects had been older, perhaps the mean PG I/II would have declined during the seven-year span.

Relationship between initial value and change of PG I/II Sustained *H. pylori* infection seemed to reduce PG I/II or to prevent it from increasing irrespective of initial PG I/

II value, although the effect was vague when initial PG I/II values were not in the range of 3.0–8.0. It did not seem to be necessary to consider the initial value of PG I/II, when discussing the effect of sustained *H. pylori* infection.

***H. pylori* and sibship size** Among *H. pylori*-positive subjects, those with a large sibship size showed a frequent decline of PG I/II and reduced ΔPG I/II. In our previous study, subjects with a large sibship size showed an increased risk of *H. pylori* positivity.[13] The association between high *H. pylori* prevalence and large sibship size must be due to infection from close contact of siblings, i.e., in childhood. It is expected that among *H. pylori*-positive subjects, those with a large sibship size have been infected for a longer duration than those with a small sibship size. The results of the present study indicate that *H. pylori* more significantly decreases PG I/II or more strongly prevents it from increasing when the infection has occurred in childhood or when the infection has continued over a long period of time. The difference in the decline of PG I/II associated with sibship size was greater in younger subjects than it was in older subjects. In the older subjects, factors other than *H. pylori*, such as smoking and food intake, are expected to have been strong and may mask the effect of sibship size.

PG I/II and chronic gastritis *H. pylori*-positive subjects had a lower ΔPG I/II and a higher frequency of PG I/II decline. Sustained infection with *H. pylori* during the seven-year span is thought to have reduced PG I/II or to have prevented it from increasing. PG I/II is known to reflect both atrophy and inflammation of the gastric mucosa. As discussed above, most *H. pylori*-positive subjects in the current study were *H. pylori*-positive at the time of phlebotomy in '89. The effect of inflammation on PG I/II in '89 is expected to be similar to that in '96. Thus, ΔPG I/II is thought to reflect mainly the advance of atrophy.

$$\text{PG I/II('89)} = \text{Atrophy('89)} + \text{Inflammation('89)} + \text{Constant}$$
$$\text{PG I/II('96)} = \text{Atrophy('96)} + \text{Inflammation('96)} + \text{Constant}$$
$$\text{Inflammation('96)} \fallingdotseq \text{Inflammation('89)}$$
$$\Delta\text{PG I/II} = \text{PG I/II('96)} - \text{PG I/II('89)}$$
$$\fallingdotseq \text{Atrophy('96)} - \text{Atrophy('89)}$$

The results were consistent with the fact that *H. pylori* causes chronic gastritis and atrophy of the gastric mucosa.[20]

Although no study has specifically analyzed the relationship between the decline of PG I/II and the risk of gastric cancer, it is known that a decline of PG I/II is related to an increased risk.[4–6] Consistent with the results of the present study, an earlier study involving Japanese American subjects in Hawaii demonstrated that a large sibship size is associated with a higher risk of gastric cancer among *H. pylori*-positive subjects.[21] Causing atrophy of gastric mucosa, which is reflected by a decline of PG I/II, may be one of the mechanisms though which *H. pylori* elevates the risk of gastric cancer.

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