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# Serum Pepsinogens as Markers of Response to Therapy for *Helicobacter pylori* Gastritis

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*We have investigated the effect of therapy for* Helicobacter pylori *gastritis on serum concentrations of pepsinogen I and II in 43 patients. In the 22 patients in whom therapy resulted in dramatic decrease in gastritis scores and in clearance of the bacteria, there was a highly significant* (P = *0.0001) fall in mean serum pepsinogen II from 13.3 ± 0.8 to 7.9 ± 0.7 μg/liter, and a less pronounced fall in pepsinogen I from 89.0 ± 5.9 to 78.5 ± 0.4 μg/liter* (P = *0.01). These changes resulted in a significant* (P = *0.01) increase in the pepsinogen I/II ratio. In contrast, nonsignificant declines of 3.5% and 11.6% were observed in mean pepsinogen I and II levels in the 21 patients whose gastritis failed to resolve histologically and whose infection did not clear. These findings suggest that serum pepsinogen levels, especially pepsinogen II, are a new tool that may be found to be clinically useful in evaluation of treatment outcome in patients with* H. pylori*-associated gastritis.*

**KEY WORDS:** *Helicobacter pylori*; gastritis; pepsinogens.

It has been reported previously that nonatrophic gastritis is associated with increased levels of serum pepsinogen I (PG I, also called PGA) and pepsinogen II (PG II, also called PGC) and with a decrease in the PG I/PG II ratio (1); that *H. pylori* infection is a major cause of chronic gastritis; and that successful treatment of *H. pylori* infection results in rapid improvement of the gastritis (2). This study examines the hypothesis that successful treatment of *H. pylori* infection of the gastric mucosa is accompanied by a prompt decrease in serum concentrations of PG I and PG II and by an increase in the PG I/PG II ratio due to a proportionally greater fall in PG II than in PG I.

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## MATERIALS AND METHODS

Patients referred for upper gastrointestinal endoscopy at Charity Hospital, New Orleans, Louisiana, were evaluated for *H. pylori* infection. The overall prevalence of *H. pylori* in this patient population is about 80%. A minimum of three antral (lesser curvature, greater curvature, and posterior wall) and one corporal (posterior wall) biopsies were obtained and evaluated with the following tests: rapid urease (3), Gram's stain, culture, and the Steiner modification of the Warthin Starry stain (4). Patients who were positive on rapid urease were invited to enter the study. All patients except one were positive on all four tests. One patient was positive on rapid urease and confirmed by Warthin Starry but not culture and Gram's



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stain. Of the 65 patients who volunteered, 43 (10 males and 33 females ranging in age between 19 and 64) were good compliers, completed treatment, and returned for follow-up at the time of this report. The endoscopic diagnosis for these patients included "gastritis" and nonulcer dyspepsia ($N = 22$), duodenal ulcer ($N = 8$), and peptic ulcer ($N = 13$). All patients had chronic gastritis by histopathologic criteria at the time of entry. The focus of the study was the histopathology of the gastric mucosa and the presence or absence of *H. pylori.*

The treatment regimen consisted of bismuth subsalicylate, 640 mg/day for 28 days with nitrofurantoin, 400 mg/day, on days 8–17 and metronidazole, 2 g/day, on days 18–28. Medication was given to patients in packages containing tablets for each day of treatment. Patients were instructed to leave tablets not ingested in the package. Compliance was evaluated on return of these packages. Patients who did not take medication for more than two consecutive days were dropped from the study as noncompliers.

Endoscopy and biopsies were repeated immediately at the end of treatment and, in 28 patients, at six months as part of a longitudinal two-year follow-up study. The rapid urease test, Gram's stain, culture, and the Steiner modification of the Warthin Starry stain were repeated at each visit. Patients were considered to have cleared *H. pylori* if all four tests were negative.

Gastric histopathology was evaluated without knowledge of treatment outcome and was classified according to previously published criteria (5). A detailed protocol was used to evaluate polymorphonuclear infiltrate, mononuclear infiltrate, and mucus depletion in the antrum and in the corpus. Each parameter was independently and blindly evaluated by two pathologists (P.C. and B.R.) using four categories of severity for each parameter (normal, mild, moderate, severe). Disagreements were resolved by consensus using a double-headed microscope.

Serum samples were obtained before starting therapy and at each visit and were stored at −20°C. These were coded and shipped in Dry Ice to Los Angeles for determination of serum PG I and PG II levels by radioimmunoassay (6). The coefficient of variation of the assays is 5.5% for PG I and 7.2% for PG II. All patients gave written informed consent and the study was approved by the Louisiana State University Medical School Institutional Review Board.

The paired $t$ test was used to evaluate the null hypothesis that there were no differences in PG I and II concentrations and the PG I/II ratio before and after treatment. Wilcoxon rank test was used for nonnormally distributed data. The operational characteristics of serum pepsinogen concentrations as predictors of response to therapy were determined using both absolute values and mean percentage changes before and after treatment.

## RESULTS

Mean (± SEM) serum concentrations of PG I, PG II, and the PG I/PG II ratio before and after therapy according to treatment outcome are shown in Table 1. After one month of treatment, no evidence of *H. pylori* infection was found in 22 of the 43 patients. Mean pretreatment levels of serum PG I and PG II and the mean PG I/PG II ratio did not differ significantly between subjects who later cleared or did not clear the infection (Table 1). In the 22 patients who cleared, mean serum levels of PG I and PG II at the end of one month's therapy were significantly lower than before therapy. The decrease in serum PG II was proportionally greater than the decrease in serum PG I (40% vs 12%). This resulted in a significant increase in the PG I/PG II ratio. By contrast, in the patients who failed to clear their infection, there were no significant changes in mean levels of serum PG I and PG II or the PG I/PG II ratio. The distributions of values for PG II and the PG I/PG II ratio are shown in Figures 1 and 2. Using the mean percent changes in PG II and I/II ratio as cut-points for predicting response to therapy, a decrease greater than 25% in PG II was observed in 18 (82%) who cleared *H. pylori* bacteria vs only 8 (38%) who did not clear. Thus, a decrease >25% in PG II after treatment had a sensitivity of 82% and a specificity of 62% as an indicator of *H. pylori* clearance. For the PG I/PG II ratio, the specificity was 81% and the sensitivity was 64%. The two sets of results therefore complement each other. A decrease of PG II was a more sensitive indicator, whereas an increase in the ratio was a more specific marker of clearance.

TABLE 1. MEAN (± SEM) SERUM PEPSINOGEN I, II (μG/LITER) AND MEAN PG I/PG II RATIO BY *H. pylori* STATUS BEFORE AND AFTER 1 MONTH OF TREATMENT

| *Pepsinogen* | H. pylori | | *Months* 0 | *Months* 1 | *Change (%)* |
|---|---|---|---|---|---|
| PGI | + | +* | 92.7 ± 11.8 | 89.5 ± 10.8 | −3.5 |
| | + | −† | 89.0 ± 5.9 | 78.5 ± 0.4‡ | −11.8 |
| PGII | + | + | 15.4 ± 1.9 | 13.6 ± 1.7 | −11.6 |
| | + | − | 13.3 ± 0.8 | 7.9 ± 0.7§ | −40.6 |
| PGI/PGII | + | + | 6.4 ± 0.5 | 7.0 ± 0.5 | +4.9 |
| | + | − | 7.2 ± 0.6 | 11.0 ± 0.9§ | +52.8 |

*H.p. + at 0 and 1 month.
†H.p. + at 0 and H.p. − at 1 month.
‡$P < 0.05$.
§$P < 0.001$.

Twenty-eight patients returned for repeat evaluation six months after the initiation of treatment. None had received therapy for *H. pylori* after the first month. Their PG II data are shown in Table 2. Of 13 patients who had cleared their infection at one month, 10 remained negative for all tests of *H. pylori* infection. In the subjects who remained negative for *H. pylori,* PG II levels decreased to an

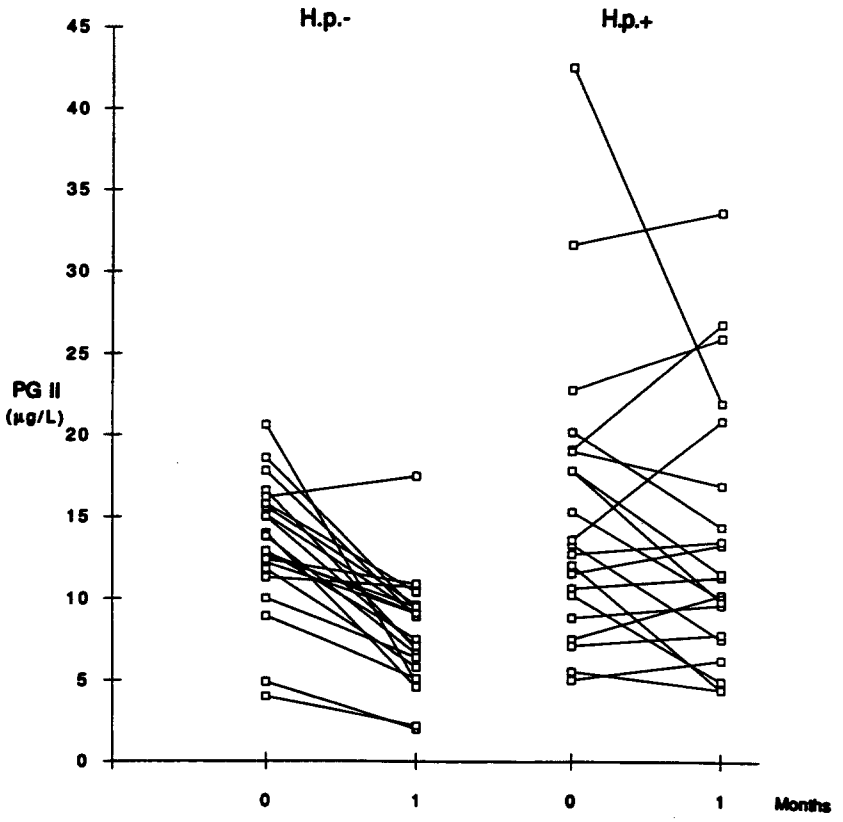


**Fig 1.** PG II levels (µg/liter) by *H. pylori* status before (time 0) and immediately after treatment (1 month).

average of 5.8 ng/ml, slightly lower than immediately after treatment. Each of the three patients who were positive for *H. pylori* at six months had a rise in serum PG II.

With clearing of *H. pylori,* there was a decrease in the percentage of biopsies with moderate and severe changes for each histologic parameter. The changes associated with successful treatment were most immediate for polymorphonuclear infiltrate (Figure 3). Each histologic parameter correlates well with PG II concentrations and the PG I/PG II ratio (not shown). The correlation with PG I was less clear.

## DISCUSSION

The objective of the study was not to find the best therapy of *H. pylori* gastritis but to explore the natural history of chronic gastritis in terms of histopathologic, bacteriologic, and serologic parameters. This trial was designed several years ago when knowledge about optimal treatment for *H. pylori* gastritis was not available. However, the relatively poor therapeutic outcome served the study purposes well by providing equivalent numbers of patients with favorable and unfavorable treatment outcomes. The demographic composition of our population, which consisted mainly of socioeconomically disadvantaged black patients, is different from other available series and may have contributed to the poor therapeutic outcome.

A marked rise in blood pepsinogen levels in patients with severe active gastritis was first reported by Spiro and Schwartz in 1958 (7). They speculated that mucosal inflammation stimulated the release of pepsinogens into the circulation. Since then, it has been shown that there are two immunologically distinct types of pepsinogen, PG I and PG II, and that these differ partially in their cellular distribution (8). It is also now well recognized that *H. pylori* is a major cause of active chronic gastritis (2).

Increasing severity of gastritis has been associated with characteristic changes in serum concentrations of PG I and PG II and in the PG I/PG II ratio (9). In comparison to subjects with normal gastric mucosa, patients with chronic gastritis show an elevation of both PG I and PG II levels as well as a

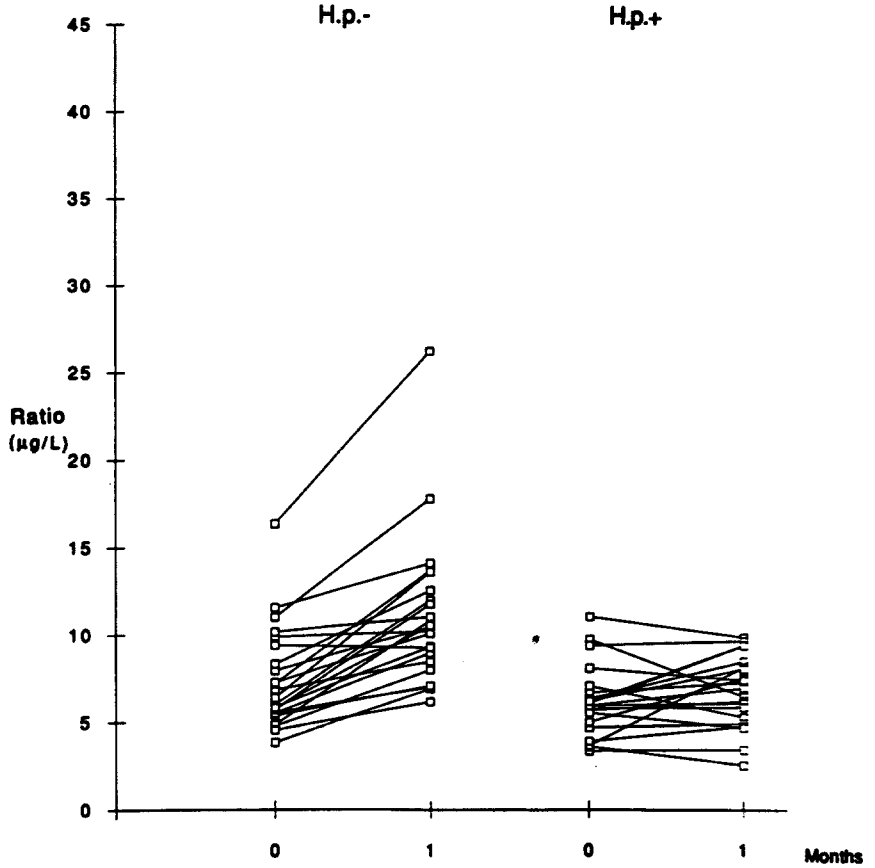


**Fig 2.** Serum pepsinogen (PG) I/II ratio by *H. pylori* status before (time 0) and immediately after treatment (1 month).

lower PG I/PG II ratio, due to a proportionally greater increase in PG II than PG I. The overall increase in pepsinogen levels has been attributed to an increased rate of entry into the circulation, and it was speculated that the proportionally greater rise in PG II was related to the fact that pyloric glands in the gastric antrum synthesize PG II but not PG I (10). Oderda et al have reported that the mean serum PG I level in children with *H. pylori* infection is significantly higher than in noninfected controls (11) and that successful eradication of the organism is accompanied by a significant fall in PG I (12). The observation that sonicated preparations of *H. pylori* increase pepsinogen secretion from isolated rabbit gastric glands (13) may relate to the elevated blood pepsinogens observed in humans.

TABLE 2. MEAN (+ SEM) SERUM PEPSINOGEN II LEVELS IN 28 PATIENTS FOLLOWED FOR 6 MONTHS ACCORDING TO TREATMENT OUTCOME

| *Treatment outcome* | *No.* | *Months* | | |
|---|---|---|---|---|
| | | *0* | *1* | *6* |
| Cured* | 10 | 12.6 + 1.7 | 6.9 + 0.9§ | 5.8 + 0.9¶ |
| Relapsed† | 3 | 15.9 + 1.1 | 7.9 + 1.7 | 13.8 + 3.4 |
| No response‡ | 15 | 14.4 + 2.3 | 11.9 + 1.7 | 16.4 + 3.1 |

**H. pylori*-negative at 1 month and at 6 months.
†*H. pylori*-negative at 1 month and *H. pylori*-positive at 6 months.
‡*H. pylori*-positive at 1 month and at 6 months.
§Significant difference between month 1 and baseline ($P < 0.01$).
¶Significant difference between month 1 and month 6 ($P < 0.05$: Wilcoxon rank test).

The results of our study indicate that eradication of *H. pylori* infection is accompanied by a significant decrease in levels of PG I and PG II and a significant rise in the PG I/II ratio. The latter is due to a proportionally greater decrease in PG II than in PG I. The greater fall in PG II was anticipated because *H. pylori* gastritis is mainly antral and because only PG II is produced by pyloric glands in the gastric antrum (10). It has been reported recently that successful treatment of *H. pylori* was accompanied by a fall in serum PG I and PG II levels (14). However, in that study the magnitude of

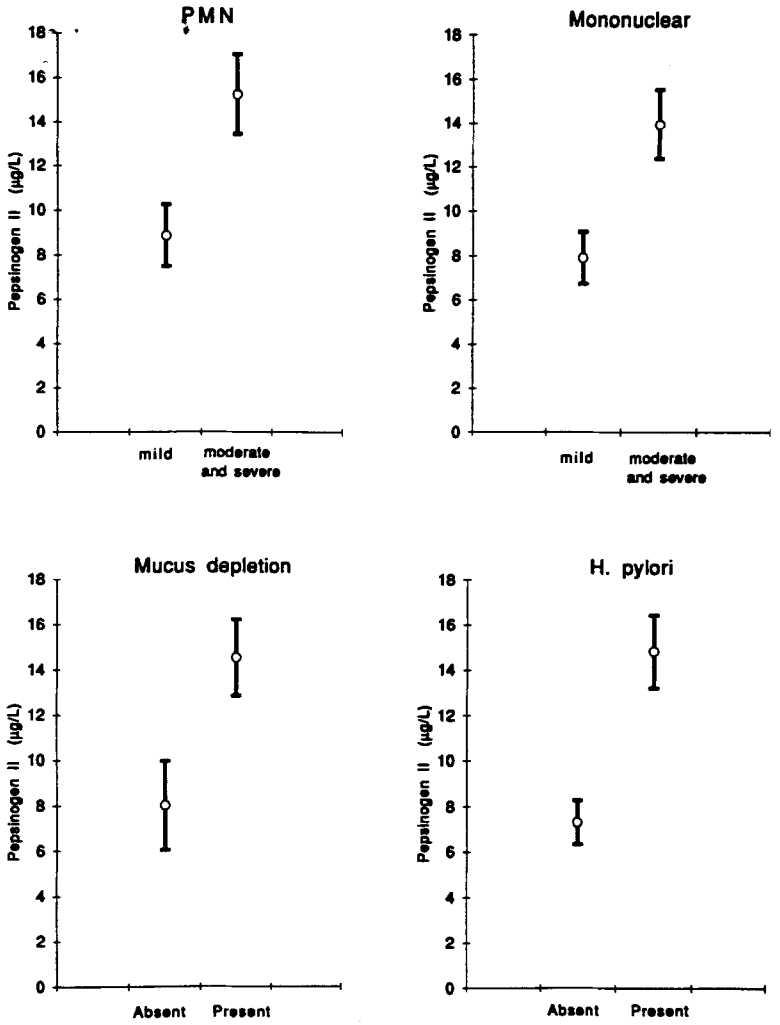


**Fig 3.** Correlation between posttreatment histologic parameters and blood levels of pepsinogen II (µg/liter).

the decrease was similar for the two zymogens and only the fall in serum PG I was statistically significant. Several recent abstracts have reported significant lowering of both pepsinogen groups after triple therapy for *H. pylori* infection (15–18).

Numerous studies have found that successful treatment of *H. pylori* infection is accompanied by an approximately 20% fall in the titer of *H. pylori* antibody (19, 20). In a recent report, a 20% IgG reduction occurred in 65% of successfully treated patients three months posttreatment and 89% by six months posttreatment (20). By comparison, we found a 25% decrease in serum PG II immediately after completion of a one-month course of therapy in 82% of subjects cleared of the bacteria. The changes in serum pepsinogen concentrations immediately after a course of therapy suggests that the assay of these zymogens may also be clinically useful as biomarkers of response to therapy. Other clinical tests, such as the breath test with isotopes of urea, are also very good but require more involved and costly procedures, which currently preclude their use in general gastroenterology practice. The optimal time for obtaining a blood sample after therapy remains to be determined. It is possible that the fall in PG I and PG II levels in some patients who did not clear their *H. pylori* infection was due to a nonspecific effect of bismuth salts on gastric mucosal integrity or to a temporary reduction in bacterial load or bacterial activity. It is possible that a delay of several weeks between the cessation of therapy and the collection of a posttreatment serum sample would enhance the operational characteristics of the test.

Our findings clearly demonstrate an inverse relationship between pepsinogen levels and histopathological parameters of gastritis. Relapse in three patients was associated with an elevation of pepsinogen levels and a worsening of histopathologic parameters. This correlation between histologic parameters and pepsinogen levels confirms earlier predictions of the value of serum PG I and PG II concentrations as "serological biopsies" (1) of the gastric mucosa.

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