**Helicobacter pylori gastritis, a presequeale to coronary plaque**

Shrikant C. Raut,1 Vinayak W. Patil,1 Shubhangi M. Dalvi,1 Girish D. Bakhshi2
1Department of Biochemistry; 2Department of Surgery, Grant Government Medical College and Sir JJ Group of Hospitals, Byculla, Mumbai, India

**Abstract**

*Helicobacter pylori* are considered the most common human pathogen colonizing gastric mucosa. Gastritis with or without *H. pylori* infection is associated with increase in levels of homocysteine and high-sensitivity C-reactive protein (hs-CRP) but a more pronounced increase is noted in gastritis with *H. pylori* infection. Increasing level of homocysteine, due to decreased absorption of vitamin B12, and folic acid, together with increased CRP levels in gastritis with *H. pylori* infection may be the earliest event in the process of atherosclerosis and plaque formation. Retrospective study conducted at tertiary care hospital in Mumbai by Department of Biochemistry in association with Department of Surgery. Eighty patients who underwent gastroscopy in view of gastritis were subjected to rapid urease test for diagnosis of *H. pylori* infection. Vitamin B12, folic acid, homocysteine and hs-CRP were analyzed using chemiluminescence immuno assay. Student’s t-test, Pearson’s correlation and linear regression used for statistical analysis.

Patients with *H. pylori* gastritis had significantly lower levels of vitamin B12 (271.6±101.3 vs 390.6±176.7 pg/mL; *P*=0.0005), as well as higher levels of homocysteine (17.4±7.4 vs 13.8±7.8 μmol/L; *P*=0.037) and hs-CRP (2.5±2.9 vs 1.2±1.1 mg/L; *P*=0.017), than in patients without *H. pylori* gastritis. However, folic acid showed (8.9±3.2 vs 10.0±3.6 ng/mL; *P*=0.171) no significant difference.

Elevated homocysteine and hs-CRP in *H. pylori* gastritis may independently induce endothelial dysfunction, leading to cardiovascular pathology.

**Introduction**

*Helicobacter pylori* are gram-negative, spiral shaped bacteria, that commonly and efficiently colonize the human gastric mucosa. They are associated with wide spectrum of gastrointestinal disorders like, chronic active gastritis (predominantly antral gastritis), peptic ulcer disease, gastric mucosa associated lymphoid tissue lymphoma and gastric adenocarcinoma.

Recent studies indicate a possible correlation between *H. pylori* infection and coronary heart disease. If *H. pylori* infection may affect atherosclerotic processes via increased serum homocysteine levels. Accumulation of homocysteine has been found toxic to endothelial cells and a risk factor for atherosclerosis. Hyperhomocysteinemia secondary to impaired absorption of folic acid and vitamin B12 might be the link between *H. pylori* infection and coronary heart disease.

C-reactive protein (CRP) is an acute-phase reactant, identified as a marker of inflammation as well as an independent risk factor for cardiovascular diseases. Assay of serum levels of CRP using high-sensitivity assay (hs-CRP) can detect subclinical inflammatory status, which may reflect vascular inflammation.

Seroepidemiologic studies have demonstrated that atherosclerosis is associated with several infectious pathogens, including cytomegalovirus, *H. pylori,* and *C. pneumonia.* If *H. pylori* has effects on the function of vascular endothelial cells, apart from homocysteine, serum CRP could be the other molecule to connect both. The pro-inflammatory cytokines produced due to stimulation by *H. pylori* infection regulate the production of CRP, which may create a pro-coagulant environment in the vascular endothelium thus forming a presequeale to coronary plaque.

The present study was conducted to compare the serum levels of vitamin B12, folic acid, homocysteine and hs-CRP in gastritis with and without *H. pylori*, irrespective of staging of gastritis or presence or absence of gastric atrophy, so as to evaluate the cardiovascular risk imposed by the mere presence or absence of *H. pylori* infection in patients suffering from gastritis.

**Materials and Methods**

This study was conducted at Department of Biochemistry in collaboration with Department of Surgery, at a tertiary care hospital in Mumbai. Ethical committee approval was taken from the Institutional Review Board of the hospital and patients enrolled in the study consented for the same. The SPSS software (IBM Corp., Armonk, NY, USA) was used, t-test applied, Pearson’s correlation and linear regression analysis was used to study correlation between the biomarkers, P-value less than 0.05 were considered to be statistically significant. Patients in the age group 20-60 years of either sex, suspected of gastritis having one or more symptoms like, epigastric pain or burning, abdominal bloating, regurgitation, altered bowel habits, who attended the surgery clinic in our hospital between January to March 2013 were selected. On confirmation of gastritis by endoscopy, biopsy was taken from the gastric antrum to diagnose the presence of *H. pylori* infection with rapid urease test. Patients with renal or liver diseases, with a recent history of *H. pylori* eradication therapy (six months prior to the study), taking vitamin B12 and folic acid supplements or drugs that affect their serum levels, with history or presence of other causes of vitamin malabsorption and pregnant women were excluded. A total of 80 patients (40 with *H. pylori* positive gastritis and 40 with *H. pylori* negative gastritis), who met these criteria were selected and subjected to blood investigations on fully automated enzyme amplified chemiluminescent immuno assay based Immulite 1000 analyzer by using commercial kits from Siemens Medical Solutions Diagnostics (Los Angeles, CA, USA).

*H. pylori* infection was diagnosed by PyloDry test (manufactured and marketed by Halifax Research Laboratories, Kolkata, India) which is a rapid urease test.

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**Key words:** Helicobacter pylori, cardiovascular disease, homocysteine, C-reactive protein, vitamin B12, chemiluminescence.

**Correspondence:** Shrikant C. Raut, 5/501 Mangalmurti Annexe, S.K. Bole Road, Agar Bazar, Dadar(W), Mumbai-400028, Maharashtra, India. Tel.: 9737109466. Email: dr.shrikantraut@yahoo.in

**Key phrases:** Helicobacter pylori, Cardiovascular disease, Homocysteine, C-reactive protein, Vitamin B12, Chemiluminescence.
Procedure for rapid urease test

The procedure for rapid urease test was performed by following these steps:

- Patients subjected to upper gastrointestinal endoscopy (nil by mouth for six to eight hours) were sprayed with lidocaine topical aerosol (LOX 10% spray) for local anesthesia and a flexible, fiber-optic, endoscope [PENTAX EG – 2770K (2.8)] was maneuvered into the stomach.
- A gastric mucosal biopsy was taken from the pyloric antrum after confirmation of gastritis. The biopsy specimen was transferred from the pyloric antrum after confirmation of gastritis.
- The biopsy forceps onto the exposed yellow media of the Pylo-dry test kit, after pulling the sticker of the kit.
- One drop of distilled water was added onto the yellow media containing the biopsy specimen and covering sticker was placed back as before.
- Urease enzyme of *H. pylori*, if present, reacts with urea of the media and changes the color from yellow to red or pink altering the pH to make it alkaline.
- The color change from yellow to red or pink was observed at 15 min interval for one hour and 30 min interval for the next hour.13
- The change in the color of the media from yellow to red or pink was taken as a positive test. Patients were then categorized into *H. pylori* positive gastritis and *H. pylori* negative gastritis.
- The patients who were found rapid urease test positive were prescribed anti *H. pylori* treatment.

Observations and Results

The mean age of patients with gastritis was 39.56±10.29 years (range 20-60 years), whereas, the mean age of patients with *H. pylori* positive gastritis was 39.12±10.89 years and that of *H. pylori* negative gastritis was 40.73±9.77 years. There was no significant difference in gender between *H. pylori* positive and negative gastritis (Table 1).

Serum vitamin B12 levels were significantly lower and serum homocysteine and hs-CRP levels were significantly higher in patients with *H. pylori* positive gastritis than in those with *H. pylori* negative gastritis, whereas, there was no significant difference between mean serum levels of folic acid in *H. pylori* positive gastritis and *H. pylori* negative gastritis (Table 2).

Serum homocysteine was found to have a significant negative correlation with serum vitamin B12 (Figure 1), and it showed a negative but not a significant correlation with folic acid (Figure 2) in *H. pylori* positive gastritis.

Table 1. Sex wise distribution of patients with type of gastritis.

| Patient group | Sex       | Total | P value |
|---------------|-----------|-------|---------|
|               | Male      | Female|         |
| *H. pylori* positive gastritis n=40 (%) | 29 (72.5) | 11 (27.5) | 40 (100) | 0.469 |
| *H. pylori* negative gastritis n=40 (%) | 26 (55)  | 14 (35)  | 40 (100) |         |
| Total         | 55 (68.8) | 25 (31.2) | 80 (100) |         |

Pearson Chi-Square value=0.524.

Table 2. Vitamin B12, folic acid, homocysteine and high-sensitivity C-reactive protein levels in *Helicobacter pylori* positive and *H. pylori* negative gastritis.

| Parameters          | *H. pylori* positive gastritis | *H. pylori* negative gastritis | P value |
|---------------------|-------------------------------|-------------------------------|---------|
| Vitamin B12 (pg/mL) | 271.6±101.3                   | 390.6±176.7                  | 0.005** |
| Folic acid (ng/mL)  | 9.0±3.2                       | 10±3.6                       | 0.172   |
| Homocysteine (μmol/L) | 17.4±7.4                     | 13.8±7.8                     | 0.057*  |
| hs-CRP (mg/L)       | 2.5±2.9                       | 1.2±1.1                      | 0.017*  |

**P<0.001, highly significant; *P<0.05, significant, values in mean±standard deviation. hs-CRP, high-sensitivity C-reactive protein.

Discussion

It is well known that *H. pylori* infection is commonly associated with chronic active gastritis and peptic ulcer disease. The infection is
widespread in developing nations, where prevalence is believed to be more than 80% among middle-aged adults, whereas it is considerably lower in industrialized countries, with 20% to 50% of the population infected. The prevalence of *H. pylori* infection in India is up to 80%.16

In the present study, serum vitamin B<sub>12</sub> levels were significantly lower in patients with *H. pylori* positive gastritis as compared to those without. Whereas, folic acid levels showed no significant difference between the two groups. A similar study on 132 patients with functional dyspepsia also found an insignificant difference in folate levels between *H. pylori* positive and negative gastritis patients. 17 It is an established fact that chronic *H. pylori* infection produces atrophic gastritis.18 Tamura et al.19 conducted a study on 93 patients who underwent coronary arteriography and suggested that chronic atrophic gastritis due to *H. pylori* infection decreases plasma vitamin B<sub>12</sub> and folic acid level there by increasing the circulating homocysteine levels.

Vitamin B<sub>12</sub> and folic acid malabsorption in gastric mucosal atrophy due to *H. pylori* infection may be due to, hypochlorhydria failing to split vitamin B<sub>12</sub> from food binders and its subsequent transfer to R-binder (haptocorrin) in the stomach,20 or decreased secretion of ascorbic acid and secretory dysfunction of the intrinsic factor.21,22 A study on 145 dyspeptic patients also provided a strong evidence that, even in absence of gastric mucosal atrophy in *H. pylori* positive group, the cobalamin deficiency as a consequence of food cobalamin malabsorption is due to consumption of vitamin itself by *H. pylori* or effects of the infection (inflammation and related factors).23

Present study demonstrates significantly higher circulating homocysteine levels in *H. pylori* positive group as compared to the negative group. It also depicts a good negative and significant correlation between homocysteine and vitamin B<sub>12</sub> and negative and insignificant correlation between homocysteine and folic acid in *H. pylori* positive group. These results to some extent support the hypothesis that *H. pylori* gastritis leads to decreased circulating vitamin B<sub>12</sub>, thereby increasing circulating homocysteine levels. Homocysteine has been shown to contribute to the development of coronary artery disease24-26 by causing direct endothelial damage,27-29 affecting platelet function, coagulation factors30,31 and promoting oxidation of low density lipoproteins,32 thereby, responsible for the development of atherosclerosis in the setting of chronic *H. pylori* infection.

A large number of studies have shown the pivotal role of inflammation in progression of atherosclerosis, hs-CRP is one such marker which may also play a role in formation and worsening the plaque by directly and indirectly activating inflammation and cytotoxicity.33,34 Several mechanisms by which CRP can promote a pro-atherogenic environment in endothelial cells have been suggested.40

Significant association between *H. pylori* infection and serum CRP has been noted.12,41 Our study not only demonstrated significantly higher levels of hs-CRP in gastritis with *H. pylori* as compared to that without, but also noted that, 55% of patients with *H. pylori* positive gastritis had serum hs-CRP levels in *H. pylori* positive patients with intermediate to high cardiovascular risk range as compared to only 35% in *H. pylori* negative gastritis.

A pro-atherogenic, pro-coagulant and pro-inflammatory environment is thus created both by elevated homocysteine and hs-CRP levels, suggesting a possible presequale to coronary plaque in *H. pylori* gastritis.

### Conclusions

Our study, *H. pylori* gastritis not only depicts elevated serum homocysteine levels due to reduced serum vitamin B<sub>12</sub> levels, but also, an elevated serum hs-CRP level. Homocysteine and hs-CRP are said to exert toxic effects on vascular endothelial cells via independent mechanisms. They could thus be forming the possible link between *H. pylori* gastritis and coronary heart disease.

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Table 3. Cardiovascular risk stratification by high-sensitivity C-reactive protein value.

| hs-CRP (mg/L) | Cardiovascular risk |
|--------------|---------------------|
| < 1          | Low                 |
| 1-3          | Intermediate/average|
| >3           | High                |

Table 4. Cardiovascular risk stratification of patients with *Helicobacter pylori* positive and negative gastritis with serum high-sensitivity C-reactive protein levels.

| hs-CRP levels | *H. pylori* positive gastritis n=40 (%) | *H. pylori* negative gastritis n=40 (%) |
|---------------|----------------------------------------|----------------------------------------|
| <1 mg/L (low CVS risk) | 18 (45) | 26 (65) |
| 1-3 mg/L (intermediate CVS risk) | 11 (27.5) | 07 (17.5) |
| >3 mg/L (high CVS risk) | 11 (27.5) | 07 (17.5) |
| Total         | 40 (100) | 40 (100) |

*hs-CRP*, high-sensitivity C-reactive protein; CVS, cardiovascular.
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