

# Increased Protein Carbonylation and Decreased Antioxidant Status in Anemic *H. Pylori* Infected Patients: Effect of Treatment

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## ABSTRACT

**Background/Aim:** Collective evidences suggest the causal association of *Helicobacter pylori* infection with iron deficiency anemia. Generation of free radicals against this bacterium can lead to turbulence in oxidative-antioxidative system. This study was undertaken to evaluate the marker of oxidative protein injury, protein carbonylation, and total antioxidant status in anemic *H. pylori*-infected patients and to observe the alteration in them after treatment for 1 month with oral ferrous sulfate and anti-*H. pylori* therapy. Twenty anemic *H. pylori*-infected patients were randomly divided into 2 groups. The *H. pylori*-infected patients in Group I received both iron supplementation and anti-*H. pylori* therapy, whereas patients in Group II received only the iron supplementation. Fifteen healthy volunteers served as controls. All the study parameters were estimated after 1 month of the treatment. **Materials and Methods:** Protein carbonylation and total antioxidant status were estimated using colorimetric method. Hematologic parameters were evaluated using Sysmex-K-100 automated cell counter. **Results:** In anemic *H. pylori*-infected patients, the protein carbonyls (PCOs) were significantly increased, whereas the total antioxidant status, iron, hemoglobin, and ferritin levels were significantly decreased compared with the controls. In Group I, while the PCOs level decreased significantly, there was a significant increase in the total antioxidant status, iron, hemoglobin, and ferritin levels after 1 month. No significant alterations were noted in the levels of PCOs, total antioxidant status, iron, hemoglobin, or ferritin in Group II patients after 1 month of the treatment. **Conclusions:** The findings from this study indicate that treatment for both anemia and *H. pylori* infections is required for lowering the oxidative stress markers, which synergistically bring about an appropriate correction of anemia soon in these patients.

**Key Words:** *H. pylori*, iron deficiency anemia, protein carbonyls, total antioxidant status

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*Helicobacter pylori*, a gram-negative bacillus, is the most common pathogenic bacteria in the world.<sup>[1]</sup> Approximately half of the population worldwide has *H. pylori* infection, and the prevalence is expected to be 80–90% in the developing countries and 30–50% in the developed countries.<sup>[2]</sup>

Earlier studies suggest an association between *H. pylori*-induced gastritis and iron deficiency anemia.<sup>[3-6]</sup> Epidemiologic studies have shown that persons seropositive

for *H. pylori* infections have a significant lower serum ferritin level.<sup>[3,4]</sup> It has also been found that eradication of *H. pylori* infection in iron-deficient anemic patients was found to reverse the iron deficiency status in both children and adults.<sup>[5,6]</sup>

*H. pylori* infection increases free radical generation.<sup>[7]</sup> *H. pylori*-induced gastritis is an established risk factor for gastric cancer.<sup>[1]</sup> One of the mechanisms that predisposes to cancer is the generation of free radicals due to inflammatory response against the bacterium. The generated free radicals bring about carcinogenesis by direct effect on host DNA and also by promoting the production of genotoxic products, such as Malondialdehyde (MDA).<sup>[8,9]</sup> Several studies are available that suggest the altered oxidative stress and antioxidant enzyme activities in *H. pylori*-induced gastritis and gastric cancer.<sup>[10-12]</sup> Even in iron deficiency anemia, an increased level of MDA was observed and iron treatment significantly reduced it.<sup>[13,14]</sup>

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Evidence for oxidative injury is obtained predominantly from the measurement of biochemical markers of lipid peroxidation and protein oxidation. MDA and protein carbonyls (PCOs) are byproducts of oxidation of lipids and proteins, respectively.<sup>[10]</sup> Oxidized proteins are generally more stable; hence PCOs have a major advantage over lipid peroxidation products as markers of oxidative stress. Moreover, PCOs are formed early and circulate in blood for longer periods, compared with other parameters of oxidative stress, such as glutathione disulfide and MDA.<sup>[15]</sup> The formation of PCOs is a common phenomenon during oxidation, and their quantification can be used to measure the extent of oxidative modification.

Although separate reports are available suggesting altered oxidative stress in *H. pylori* infection and iron deficiency anemia, to the best of our knowledge, the levels of protein carbonylation and antioxidant status in anemic *H. pylori* infection and the effects of treatment still remain unexplored.

Therefore, the major aims of this study were (a) to compare the levels of PCOs and total antioxidant status in anemic *H. pylori*-infected individuals with normal age-matched controls and, (b) to determine the effect of treatment on the levels of these parameters.

## MATERIALS AND METHODS

Three milliliters of blood samples were obtained from 20 anemic patients with *H. pylori* infection (12 females and 8 males) and 16 age-matched apparently healthy subjects. Anemic patients were recruited from the outpatient department of our institute, JIPMER, Puducherry, India. Only 13 years or older patients were enrolled for this study. The patients were aged between 30 and 49 years. Anemic patients were selected based on the hemoglobin levels (Hb < 11 g/dL) and peripheral blood smear suggesting iron deficiency anemia. One milliliter of the whole blood in EDTA vials was collected and used for the analysis of hemoglobin and red cell indices using Sysmex-K-100 automated cell counter (Sysmex Singapore Pvt. Ltd, Singapore). The rest of the sample was centrifuged at 3000g for 10 min and stored at -20°C until use. Plasma ferritin level was determined by ELISA using human ferritin enzyme immunoassay test kit (IBL Immunobiological Laboratories, Hamburg, Germany). Plasma iron was measured by fully automated ferrozine method in Ciba Corning 550 Express Plus. All patients who were found to have iron deficiency by the above parameters underwent stool examination on 3 consecutive days for the presence of hookworm ova on microscopy and for occult blood by benzidine test. After informed consent, upper gastrointestinal endoscopy was done and multiple biopsy specimens were obtained from the antral mucosa for rapid urease test and histology. Tissue sections were stained for *H.*

*pylori* with Geimsa. *H. pylori* infection was defined as a visible organism seen under microscopy and a positive rapid urease test. Among the 20 *H. pylori* patients, 7 had antral gastritis, 5 had atrophic gastritis, and 2 had pangastritis; in the remaining 6 patients, endoscopy revealed normal mucosa. Patients with a history of consumption of nonsteroidal anti-inflammatory drugs (NSAIDs), anticoagulants, or corticosteroids; those with hematologic disorders or stool samples positive for occult blood or hookworm ova; those diagnosed with duodenal or gastric ulcers or carcinoma stomach at endoscopy were excluded from the study. Those positive for *H. pylori* infection detected by rapid urease test and histology were randomly placed into 2 groups (groups I and II) by creating block sizes of 6 or 8 and linking to 5-digit random numbers (Rand Corporation; New York: The Free Press, 1955). Patients in Group I received oral ferrous sulfate tablets 200 mg thrice daily for 1 month, and a 14-day course of anti-*H. pylori* therapy consisting of clarithromycin 250 mg BD, lansoprazole 30 mg BD, and tinidazole 500 mg BD. Those in Group II received only oral ferrous sulfate tablets as mentioned above. All the above-mentioned biochemical and hematologic parameters were assayed before and after 1 month of therapy. The *H. pylori* infection in the control group was excluded by the rapid urease test. Patients with this study were approved by the Human Ethics Committee and Institute Research Committee of JIPMER. Informed consent was obtained from all subjects.

### Assay of carbonylation of plasma protein

The assay was done according to the method of Levine *et al.*<sup>[16]</sup> The millimolar extinction coefficient of the colored product is 22.01 mmol<sup>-1</sup>cm<sup>-1</sup> at 366 nm. The carbonylated protein reacts with 2,4-dinitrophenylhydrazine to form a colored adduct of 2,4-dinitrophenylhydrazone. Plasma total protein was estimated by biuret method using fully automated autoanalyzer 550 express plus (Bayers Diagnostics Ciba, Corning, NY, USA).

### Total plasma antioxidant status estimation

Total plasma antioxidant status (TAS) in plasma was estimated by the ferric reducing antioxidant assay.<sup>[17]</sup> The method is based on the principle that at low pH, reduction of ferric-tripyridyltriazine (Fe III-TPTZ) complex to the ferrous form, which has an intense blue color can be monitored by measuring the absorbance. The absorbance is directly related to the combined or "total" reducing power of the electron-donating antioxidants present in the reaction mixture.

### Statistical analysis

Data were analyzed using the statistical program SPSS for Windows, version 13 (SPSS, USA). Results are given as mean ± standard deviation (SD). Independent Student's *t* test was used to determine the statistically significant

difference between cases and controls. Paired Student's *t* test was used for comparison of the parameters studied before and after treatment. *P* values less than 0.05 were considered statistically significant.

## RESULTS

Hematologic and biochemical parameters in *H. pylori*-infected anemic group are given in Table 1. Levels of PCOs were significantly increased in the test group than the healthy age-matched controls ( $P < 0.001$ ). Total antioxidant status was significantly lower in the cases than in the controls ( $P < 0.001$ ). The baseline levels of hematologic and biochemical parameters studied were comparable between the 2 groups [Table 2]. Hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte count, serum iron, and ferritin levels were significantly reduced in *H. pylori*-infected patients when compared with controls.

### Response to therapy

The mean increase in hemoglobin levels (2.97 vs 0.71 g/L), serum iron levels (47.8 vs 16.3 mg/dL), and ferritin levels (78 vs 26.6 ng/mL) were more marked in Group I than in Group II. Patients in Group I had a greater decrease in mean PCOs (1.24 nmol/mg protein) after 1 month of treatment than those in Group II (0.612 nmol/mg protein). Increase in the mean TAS (375 nmol/L) after treatment was also higher in Group I in comparison with Group II (129 nmol/L). After therapy, the mean PCO levels were significantly lower and TAS higher when compared with the basal levels in Group I and no significant difference was found in Group II [Table 2]. After treatment, the levels of PCO and TAS improved and no significant difference was found in Group I when compared with controls, whereas significant difference was seen in Group II.

**Table 1: Comparison of hematologic and biochemical parameters in anemic *Helicobacter pylori*-infected patients and controls**

Parameters	Control group (n=16)	Test group (n=20)
Hemoglobin(g/dL)	12.70±0.75	6.81±2.23*
Mean corpuscular volume (fl)	79.29±4.42	72.79±4.03*
Mean corpuscular hemoglobin (pg)	28.13±2.42	23.99±2.29*
Mean corpuscular hemoglobin Concentration (g/dL)	33.57±2.24	28.66±1.72*
Reticulocyte count (%)	0.63±0.35	1.23±0.70*
Serum iron (µg/dL)	109.0±27.02	30.7±15.77*
Serum ferritin (ng/dL)	140.49±31.79	29.55±16.45*
Protein carbonyls (nmol/mg protein)	1.23±0.28	2.62±0.92*
Total antioxidant status µmol/L (ferric reducing antioxidant assay)	1158±97	703±194*

\* $P < 0.05$  vs control group

## DISCUSSION

Free radical generation is an important phenomenon that is known to contribute in a great variety of deleterious reactions in the aerobic cells.<sup>[18,19]</sup> It is now generally accepted that oxidative stress plays an important role in the pathogenesis of various forms of tissue injuries.<sup>[20-23]</sup> Oxidative damage caused by free radicals that are generated against the *H. pylori* plays a crucial role in the pathogenesis of gastric cancer.<sup>[24,25]</sup> Increased amounts of oxidative markers of lipid injury have been found in patients with *H. pylori* infection.<sup>[11,12]</sup> However, to the best of our knowledge, this is the first study attempted to elucidate whether there is any change in the protein carbonylation and total antioxidant status in anemic *H. pylori*-infected patients and effects of treatment on them.

Protein carbonylation is one of the reactions set into motion as a consequence of the formation of these radicals in cells and tissues.<sup>[26]</sup> PCOs are formed early and circulate in the blood for longer periods, compared with the other parameters of oxidative stress, such as glutathione disulfide and MDA.<sup>[15]</sup> Quantification of PCOs could be used to measure the extent of oxidative modification. Our results indicate increased PCOs in *H. pylori*-infected anemic patients. Previous reports have also reported high levels of lipid peroxidation in patients infected with *H. pylori*.<sup>[11,12]</sup>

There was a significant decrease in the PCO levels in *H. pylori*-infected patients after 1 month of treatment with both ferrous sulfate and anti-*H. pylori* therapy. Previous reports have indicated that the levels of lipid peroxides decrease significantly after treatment of *H. pylori* infection.<sup>[11,12]</sup> Similarly, in iron deficiency anemia it has been found that supplementation with iron reduces the levels of MDA.<sup>[14]</sup> In this study, there was no significant decrease in PCO levels in the test group treated with only oral iron. This clearly shows that in *H. pylori*-infected individuals, anti-*H. pylori* therapy is needed along with iron for early reduction of oxidative stress. This finding also reveals that the contribution of oxidative stress by *H. pylori* infection might be greater than anemia per se in anemic *H. pylori* infection. Also, except for the reticulocyte count, there was no significant improvement in hemoglobin, serum iron, ferritin levels, and hematologic parameters in Group II, whereas Group I showed good improvement. These results support the conclusion that anti-*H. pylori* treatment is needed for early correction of iron deficiency anemia in these patients. Both improvement in the iron absorption and reduction of oxidative stress with anti-*H. pylori* therapy might have contributed for early correction of anemia in these patients.

A number of researches have been carried out to study the alterations in vitamin C levels in both gastric juice and

**Table 2: Changes in protein carbonyls and total antioxidant status in test groups after treatment**

Parameters	Group I ( <i>Helicobacter pylori</i> treatment + oral iron therapy) (n=10)	Group II (oral iron therapy) (n=10)
Hemoglobin(g/dL)		
Baseline	7.40±2.08	6.22±2.23
1 Month after therapy	10.37±1.22*	6.93±2.12
Mean corpuscular volume (fl)		
Baseline	72.79±4.39	72.78±3.84
1 Month after therapy	77.93±3.51*	75.24±3.77
Mean corpuscular hemoglobin (pg)		
Baseline	24.18±3.04	23.80±1.31
1 Month after therapy	29.29±2.94*	24.76±2.30
Mean corpuscular hemoglobin concentration (g/dL)		
Baseline	28.66±2.15	28.76±1.31
1 Month after therapy	31.67±2.31*	30.32±2.01
Reticulocyte count		
Baseline	1.3±0.86	1.15±0.53
1 Month after therapy	2.23±0.84*	1.70±0.42*
Serum iron(µg/dL)		
Baseline	32.50±20.69	28.90±9.46
1 Month after therapy	80.30±31.08*	45.20±22.51
Serum ferritin (ng/dL)		
Baseline	31.50±17.49	27.6±16.02
1 Month after therapy	109.50±62.85*	54.2±40.88
Protein carbonyls (nmol/mg protein)		
Baseline	2.79±1.11	2.46±0.72
1 Month after therapy	1.52±0.57*	1.85±1.07
Total antioxidant status µmol/L (ferric reducing antioxidant assay)		
Baseline	737±248	670±126
1 Month after therapy	1120±153*	809±242

\*P&lt;0.05 vs baseline value before treatment

plasma of patients with *H. pylori* infection. A significant decrease in the levels of vitamin C has been reported and successful eradication of *H. pylori* infection restored the gastric juice and plasma levels of ascorbic acid.<sup>[11,27-29]</sup>

Our study indicates a significant reduction in the total antioxidant status in *H. pylori*-infected patients. The mean rise in total antioxidant status of the blood samples from *H. pylori*-infected subjects was significant after *H. pylori* treatment. Eradication of *H. pylori* thus appears to inhibit the activation of circulating mononuclear cells in patients. Therefore, oxidative stress not only in the gastric mucosa but also in the circulation of patients with *H. pylori* infection is significantly decreased after eradication of this pathogen.

We conclude that increased levels of PCOs and decreased total antioxidant status were found in anemic *H. pylori*-infected patients. Also this study indicates that treatment for both anemia and *H. pylori* infections is required for decreasing the oxidative stress, which synergistically brings

about an appropriate correction of anemia soon in these patients.

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