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RAPID COMMUNICATION

Increased plasma malondialdehyde and fructosamine in anemic *H pylori* infected patients: Effect of treatment

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Abstract

AIM: To unravel the possible association of malondialdehyde (MDA) and fructosamine in anemic *H pylori* infected patients and to observe the alteration in MDA and fructosamine levels in these patients after treatment for one month.

METHODS: Fructosamine, MDA and glucose were estimated in 22 anemic *H pylori* infected patients and 16 healthy controls. Hematological parameters were also evaluated in both the groups using Sysmex-K-100 automated cell counter. The *H pylori* infected patients were randomly divided into two groups. *H pylori* infected patients in Group Ⅰ received both iron supplementation and anti-*H pylori* therapy, while patients in Group Ⅱ received only iron supplementation. All the biochemical and hematological parameters were estimated after one month of treatment.

RESULTS: In anemic *H pylori* infected patients, while MDA (5.41 ± 2.16 *vs* 2.26 ± 0.50; $P < 0.05$) and fructosamine (2.64 ± 0.93 *vs* 1.60 ± 0.35; $P < 0.05$) were significantly increased, iron (32.72 ± 14.93 *vs* 110.25 ± 26.58; $P < 0.05$), hemoglobin (6.9 ± 2.6 *vs* 12.66 ± 0.74; $P < 0.05$) and ferritin (28.82 ± 16.27 *vs* 140.43 ± 30.72; $P < 0.05$) levels were significantly decreased compared with the controls. With partial correlation analysis, fructosamine was found to have a significant positive correlation with MDA. In Group Ⅰ, while MDA level decreased significantly (3.11 ± 1.73 *vs* 5.50 ± 2.46; $P < 0.05$), there was a significant increase in iron (84.09 ± 29.51 *vs* 36.09 ± 17.81; $P < 0.05$), hemoglobin (10.40 ± 1.11 *vs* 7.42 ± 1.90; $P < 0.05$) and ferritin (116.91 ± 63.34 *vs* 30.46 ± 17.81; $P < 0.05$) levels after one month. There was no significant change in the levels of fructosamine in group Ⅰ after treatment. Similarly, no significant alterations were noted in the levels of MDA, fructosamine, hemoglobin or ferritin in Group Ⅱ patients after one month of treatment.

CONCLUSION: An increased level of fructosamine and MDA was found in anemic *H pylori* infected patients. Present data supports the premise that lipid peroxides *per se* do play a role in the glycation of plasma proteins. Furthermore, the findings from this study indicate that treatment for both anemia and *H pylori* infections is required for lowering the levels of lipid peroxides in these patients.

Key words: *H pylori*; Anemia; Fructosamine; Malondialdehyde; Iron; Glycation

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INTRODUCTION

H pylori, a gram negative bacillus is the most common pathogenic bacteria in the world[1]. Even though approximately half of the population in the world has *H pylori* infection, the prevalence and severity vary greatly among countries and population groups within the same countries. The overall prevalence of *H pylori* is strongly correlated with socio-economic conditions[1]. The prevalence among middle aged adults is over 80% in many developing countries as compared with 20%-50% in industrialized countries[2].

Accumulating evidences suggest an association between gastric *H pylori* infection and low iron stores and anemia[3-6]. Epidemiological studies have demonstrated a close relationship between serum ferritin and presence of anti-*H pylori* IgG[3,4]. A fall in serum ferritin reflects declining body iron stores and is an accepted marker of iron deficiency. 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[5,6].

Spontaneous nonenzymatic modifications of protein are commonly reported in tissues with slow turnover and they are considered by several authors as a possible common mechanism involved in the progression of many pathological conditions[6]. Among the nonenzymatic processes, oxidative stress and glycation have aroused a particular interest in recent years[7,8].

Glycation is a non-enzymatic condensation reaction between reducing sugars and free amino groups at NH_2-terminus or susceptible ε-amino groups of lysine residues of proteins. The reaction is initiated by the reversible formation of a schiff base, which undergoes a rearrangement to form a relatively stable Amadori product[9]. The pathological consequences of these alterations very much depend on the nature of proteins involved as well as on their function and concentration in a particular organ[9]. The rate of formation of glycated protein is considered to depend on the ambient concentration of glucose and half life of the protein[9]. However, there is convincing evidence that concentrations of nonenzymatically glycated protein are increased in many non-diabetic pathological states[10-13]. Elevated concentrations of glycated hemoglobin have been found in myocardial infarction, chronic renal failure, and nephrotic syndrome patients with normal blood glucose concentrations[10-12]. Similarly high concentrations of fructosamine are reported in non-diabetic chronic renal failure and rheumatic arthritis patients[12,13]. Increased levels of glycated hemoglobin (HbA_{1C}) have also been documented in iron deficiency anemic patients without any history of diabetes[14-16].

We have recently demonstrated that lipid peroxides *per se* can enhance the process of protein glycation[17]. This result was in agreement with the findings of Jain *et al*[18]. We have also demonstrated that the process of lipid peroxidation and glycation are closely associated in patients with chronic renal failure, hyperthyroid, asthma, nephrotic syndrome and patients with rheumatoid arthritis[19-24].

Even though there are substantial reports demonstrating the presence of excess lipid peroxides in patients with *H pylori* infection, there is a dearth of information regarding the levels of glycated proteins in these patients with anemia. Given the importance of glycated protein in causing pathological complication in various diseases, it was deemed pertinent to investigate the levels of glycated plasma protein and the possible association with the levels of lipid peroxides in *H pylori* infected patients who were anemic. Furthermore, we explored the effect of treatment on the levels of these parameters. This study is the first to describe an association between lipid peroxides and glycated protein in anemic *H pylori* infected patients.

MATERIALS AND METHODS

Blood sample (3 mL) was obtained from 22 anemic patients with *H pylori* infection and 16 age matched healthy subjects. Anemic patients were recruited from the outpatient department of our institute, JIPMER, Pondicherry, India. Only patients of 13 years of age or older were enrolled for this study. Anemic patients were selected based on the hemoglobin levels (Hb < 11 g/dL) and peripheral blood smear suggesting iron deficiency anemia. Selected patients underwent detailed physical examination and laboratory evaluation.

One milliliter of the whole blood in EDTA was 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 3000 r/min for 10 min. The plasma was separated and analyzed for lipid peroxides, fructosamine, iron, ferritin and glucose. Plasma ferritin level was determined by ELISA using human ferritin enzyme immunoassay test kit (IBL Immunobiological Laboratories, Hamburg, Germany). Fructosamine was measured by p-indonitrotetrazolium violet kinetic method using the Raichem Kits (Haemagen Diagnostics, San Diego, CA) adapted to 550 Express Plus Analyzer (Ciba Corning Diag., Oberlin, OH). The concentration of lipid peroxides in plasma was measured by thiobarbituric acid method[25]. Plasma iron and glucose were measured by fully automated ferrozine and glucose oxidase methods respectively in Ciba Corning 550 Express Plus. All patients who were found to have iron deficiency by the above parameters underwent stool examination on three 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. Patients with histories of consumption of nonsteroidal anti-inflammatory drugs (NSAIDs), anticoagulants or corticosteroids, those with hematological disorders or stool samples positive for occult blood or hookworm ova, and those with duodenal or gastric ulcers or carcinoma stomach at endoscopy were excluded from the study.

Patients positive for *H pylori* infection by rapid urease test and on histology were randomly assigned to two groups (Group I and Ⅱ) by creating block sizes of six or eight and linking to five-digit random numbers (Rand Corporation; New York: The Free Press, 1955). Patients in Group I received oral ferrous sulfate tablets 200 mg thrice a day for 1 mo, and a 14-d course of anti-*H pylori* therapy consisting of clarithromycin 250 mg bid, lansoprazole 30 mg bid and tinidazole 500 mg bid. Those in Group Ⅱ received only oral ferrous sulfate tablets as above. All the above-mentioned biochemical and hematological parameters were assayed after 1 mo therapy. This study was approved by the ethics committee of JIPMER. Informed consent was obtained from all subjects.

Statistical analysis

Student's *t* test was used to estimate the differences between the groups. Correlation was assessed by the partial correlation analysis. All results are presented as mean ± SD. $P < 0.05$ was considered statistically significant.

RESULTS

All the parameters tested in both the *H pylori* infected

Table 1 Comparison of biochemical and hematological parameters in *H pylori* infected anemic patients and controls

Parameters	Control group (n = 16)	Test group (n = 22)
Hemoglobin (g/dL)	12.66 ± 0.74	6.9 ± 2.6[a]
Iron (μg/dL)	110.25 ± 26.58	32.72 ± 14.93[a]
Ferritin (ng/mL)	140.43 ± 30.72	28.82 ± 16.27[a]
Fructosamine (nmol/L)	1.60 ± 0.35	2.64 ± 0.93[a]
MDA (mmol/L)	2.26 ± 0.50	5.41 ± 2.16[a]
Glucose (mg/dL)	81.19 ± 9.74	83.50 ± 8.27

[a]$P < 0.05$ *vs* control group.

group and healthy controls are given in Tables 1 and 2. Fructosamine levels were significantly higher in *H pylori* infected patients compared to controls. Levels of lipid peroxides were significantly increased in the test group than the healthy age-matched controls. In the test group, a significant correlation ($r = 0.50$, $P = 0.02$) was observed between fructosamine and MDA using partial correlation analysis controlling for blood glucose level. As previously reported, hemoglobin, serum iron and ferritin levels were significantly reduced in *H pylori* infected patients when compared with controls.

Response to therapy

Patients in group I had a greater increase in mean hemoglobin level (2.98 g/dL) after one mo than those in Group Ⅱ (1.07 g/dL). The increases in mean serum iron (48 μg /dL *vs* 15.91 μg/dL) and ferritin levels (86.45 ng/mL *vs* 25.28 ng/mL) after one mo of treatment were also more marked in Group I than in Group Ⅱ. There was also a significantly decreased MDA levels in Group I when compared with Group Ⅱ after one mo of treatment. However, there was no significant alteration in fructosamine levels in both the groups after one mo of treatment.

DISCUSSION

Free radicals and other reactive oxygen species (ROS) are generated by all aerobic cells and are known to participate in a great variety of deleterious reactions[26]. The oxidative damage caused by free radicals is believed to play a pivotal role in the pathogenesis of *H pylori* infection[27-29]. Lipid peroxidation is one of the reactions set into motion as a consequence of the formation of these radicals in cells and tissues[26]. The initiation of lipid peroxidation has been considered the proximal cause of cell membrane destruction and cell damage[26]. Increased amounts of malondialdehyde (MDA) have been found in patients infected with *H pylori*[30-32]. Our results also indicate an increased lipid peroxidation in *H pylori* infected patients.

Lipid peroxidation can damage proteins, lipids, carbohydrates and nucleic acids. Plasma membranes are the critical targets of lipid peroxides[26]. Apart from participating in these deleterious reactions, lipid peroxides *in vitro* have been found to enhance the glycation of proteins[18]. We have also recently reported that MDA can enhance the glycation of hemoglobin *per se*[17].

Table 2 Changes in hematological and biochemical parameters of groups after treatment

Parameters	Group Ⅰ (*H pylori* treatment + oral iron therapy) (n = 11)	Group Ⅱ (Oral iron therapy) (n = 11)
Hemoglobin (g/dL)		
Baseline	7.42 ± 1.90	6.38 ± 2.37
1 mo after therapy	10.40 ± 1.11[a]	7.45 ± 1.94
Iron (μg/dL)		
Baseline	36.09 ± 18.97	29.36 ± 9.10
1 mo after therapy	84.09 ± 29.51[a]	45.27 ± 21.36[a]
Ferritin (ng/mL)		
Baseline	30.46 ± 17.81	27.18 ± 15.26
1 mo after therapy	116.91 ± 63.34[a]	52.46 ± 39.21
Fructosamine (nmol/L)		
Baseline	2.28 ± 0.62	2.99 ± 1.06
1 mo after therapy	2.13 ± 0.63	2.27 ± 0.97
MDA (mmol/L)		
Baseline	5.50 ± 2.46	5.32 ± 1.94
1 mo after therapy	3.11 ± 1.73[a]	3.81 ± 1.61

[a]$P < 0.05$ *vs* the baseline value before treatment.

We have found that lipid peroxides are closely associated with glycated hemoglobin in hyperthyroid, chronic renal failure and asthma patients[20-22]. We observed a significant association between MDA and fructosamine in non-diabetic asthma, nephrotic syndrome, rheumatoid arthritis and chronic renal failure patients[19,22-24].

In the present study, the levels of fructosamine were increased significantly in anemic patients infected with *H pylori* when compared with healthy controls. Among the various methods proposed for the measurement of glycated serum proteins, fructosamine is the method of choice for the clinicians[9]. As albumin is the most abundant protein in serum and contains multiple lysine residues, measurement of fructosamine is mainly the determination of glycated albumin[9].

In anemic patients, the concentrations of HbA_{1C} have been reported to be increased[14-16]. Several hypotheses have been formulated to explain the increase in glycated hemoglobin concentrations in these patients. It has been proposed that in iron deficiency the quarternary structure of the hemoglobin molecule may be altered, thus glycation of the β-globin chains occur more readily[14]. According to some investigators, the increase in glycated hemoglobin in non-diabetic anemic patients is mainly attributed to the decrease in hemoglobin levels in these patients[16].

To our knowledge, no study to date has attempted to clarify whether there is any increase in glycated protein levels in anemic, *H pylori* infected patients and whether this increase is the consequence of an increased lipid peroxidation among these patients.

To verify this hypothesis, we tested a well-defined group of anemic, *H pylori* infected patients before and after one month of treatment. In our study, the increased MDA was found to be significantly associated with increased fructosamine concentrations ($r = 0.50$, $P = 0.02$) before treatment, even when the proposed effect of

glucose on the concentrations of fructosamine was refuted by partial correlation analysis. The mechanism by which MDA enhances the glycation process has not been clearly elucidated. MDA is thought to enhance the process of protein glycation by acting as an anchor between sugar and hemoglobin moieties[18]. It has also been suggested that oxidative stress can facilitate the autoxidation of glucose to dicarbonyl intermediates, an early step in the Maillard reaction[33].

There was a significant decrease in MDA levels in *H pylori* infected patients after one 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 for *H pylori* infection[30,32]. Similarly, in iron deficiency anemia it has been found that supplementation with iron reduces the levels of glycated hemoglobin[14]. There was no significant decrease in MDA levels in the test group treated with only oral iron. In both the test groups, there was no significant reduction in fructosamine levels after one month of treatment. There also existed no significant association in the anemic *H pylori* infected group between fructosamine and MDA after one month of treatment. One reason for these observed results can be attributed to the half-life of glycated proteins.

Recent studies have uncovered a myriad of pathological events induced by glycated albumin. These include increasing the expression of extracellular matrix protein, activation of protein kinase C, and stimulating the expression of transforming growth factor β_1 and its primary signaling receptor, the TGF-β type Ⅱ receptor. Apart from these alterations, glycated albumin has been found to alter the levels of NF-κB. These data support the hypothesis that glycated albumin is a sufficient stimulus to set into motion pathogenic signaling pathways. Several investigators have also reported that *H pylori* can activate NF-κB in human gastric mucosa *in vivo* and cultured gastric epithelial cells *in vitro*, thus the pathogenesis may be mediated through NF-κB[34-36]. Studies have shown that these alterations in molecular pathways play an essential role in the *H pylori* induced inflammation and associated complications.

In conclusion, this study gives a snapshot of an increased glycated serum protein and lipid peroxide levels in *H pylori* infected patients who were anemic as well. These data from the present study also supports the notion that alteration in the levels of MDA in anemic *H pylori* infected patients may be the basis for enhanced levels of fructosamine. Furthermore, the findings from our study indicate that treatment for both anemia and *H pylori* are required for reducing the levels of lipid peroxides in these patients.

It would be interesting to investigate the levels of fructosamine in non-anemic *H pylori* infected patients and it is worthwhile to investigate if additional supplementation of antioxidants would potentiate any further attenuation of protein glycation in *H pylori* infected patients when compared with the regular therapy. A longer follow-up investigation after treatment would shed more light into the above-mentioned alterations in anemic *H pylori* infected subjects.

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