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Original article

Increased plasma malondialdehyde and fructosamine in iron deficiency anemia: Effect of treatment

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Abstract

Glycation and lipid peroxidation are spontaneous reactions that are believed to play a key role in the pathogenesis of many clinical disorders. Glycation of proteins is enhanced by elevated glucose concentrations. However, increased glycated hemoglobin levels have been documented in iron deficiency anemic patients without any history of diabetes. Collective evidences reveal that lipid peroxidation can modulate protein glycation. This study was undertaken to unravel the possible association of malondialdehyde and fructosamine in iron deficient anemic patients and to observe the possible alteration in malondialdehyde and fructosamine levels in these patients after one month supplementation with iron. Twenty non-diabetic anemic patients and 16 age-matched healthy subjects were enrolled for this study. Plasma lipid peroxides, fasting glucose, fructosamine, iron, ferritin and hemoglobin were analyzed in both the groups. Partial correlation analysis was performed to predict the independent association of malondialdehyde and fasting glucose on fructosamine. In anemic patients, while fructosamine and malondialdehyde levels were found to be significantly increased, hemoglobin, iron and ferritin levels decreased significantly when compared to before treatment. Fructosamine was found to have a significant positive correlation with malondialdehyde even after nullifying the effect of glucose. After one month supplementation with iron, both fructosamine and malondialdehyde levels decreased significantly when compared to before treatment. There was a significant increase in iron, ferritin and hemoglobin levels in anemic patients after one month of treatment. In conclusion, an increased level of fructosamine and malondialdehyde was found in anemic patients. These data suggest that fructosamine levels are closely associated with malondialdehyde concentrations in iron deficient anemic patients.

Keywords: Anemia; Fructosamine; Malondialdehyde; Iron; Glycation

1. Introduction

According to the World Health Organization (WHO), iron deficiency is the commonest of deficiency diseases worldwide [1]. Iron deficiency anemia (IDA) is a major public health problem in developing countries like India. Low dietary intake of poorly bioavailable iron is believed to be the principal cause of IDA in the developing countries [2]. Conditions affecting gastric acid secretion are also said to be potentially important factors in the etiology of IDA [3]. Iron deficiency anemia causes a number of biochemical abnormalities and impaired cell-mediated immunity with increased susceptibility to infection [4–6]. The associated ill-effects of IDA have been described as 'devastating' [7] and, in some contexts, as 'irreversible' [8].

One of well-studied processes known to cause pathological ill-effects in the biological system is glycation of proteins [9]. Nonenzymatic glycation of proteins has pronounced effects on the structure and function of proteins. The pathological consequences of these alterations depend on the nature of proteins involved as well as on their function and concentration in specific tissue localization [10].

The two known factors which can modulate glycation of proteins are the prevailing concentration of glucose and half

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life of the protein [11]. But evidences in the literature have documented increased glycated protein levels in some non-diabetic pathological states [12–14]. Increased glycated hemoglobin has been observed in patients with myocardial infraction, nephrotic syndrome and chronic renal failure [12–14]. Previous authors have detected elevated levels of glycated hemoglobin in anemic patients [15,16]. Some authors have also found that on supplementation with iron therapy there is a significant decrease in the levels of glycated hemoglobin [16]. Animal experiments have also indicated that copper deficiency can enhance the glycation process [17]. Evidence has accumulated which supports the hypothesis that glycation reaction apart from the traditional factors can be modulated by the levels of malondialdehyde [18]. It has been reported that MDA *per se* can be a major determinant of glycation reaction. In accordance to this report we have recently reported the role of MDA in enhancing the glycation of hemoglobin [19].

Even though much effort has been spent studying the level of glycated hemoglobin and the effect of treatment in anemic patients, yet to our knowledge no previous report exists to define the levels of plasma glycated proteins in anemic patients. If the degree of glycation of other proteins in anemic patients was similar to that of glycated hemoglobin it would have important clinical implications. Thus, the objectives of the present study were: (1) to determine whether fructosamine levels were increased among anemic patients and, if so (2) to determine the relationship between fructosamine levels and MDA concentration and (3) to delineate if iron supplementation has any beneficial effect in combating the increase in both MDA and fructosamine.

2. Patients and methods

Blood sample (3 ml) was obtained from 22 anemic patients and 16 age-matched healthy subjects. Anemic patients were recruited from the medicine outpatients department of our institute, JIPMER, Pondicherry, India. Only patients with 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 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 rpm for 10 min. The serum was separated and analyzed for lipid peroxides, fructosamine, iron, ferritin and glucose. Serum 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 serum was measured by thiobarbituric acid method [20]. Serum 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. Only patients who were having no hookworm infection and negative for benzidine test were included in the study.

All anemic patients received oral ferrous sulfate tablets 200 mg thrice a day. All the above-mentioned biochemical and hematological parameters were assayed after one month of therapy. This study was approved by the Ethics Committee of JIPMER. Informed consent was obtained from all subjects.

3. Statistical analysis

All results are presented as mean ± S.D. The statistical significance of difference between groups was evaluated by Student's *t*-test. Correlation was assessed by the partial correlation analysis. A p value of 0.05 was selected as the point of minimal statistical significance.

4. Results

All the parameters tested in both the groups are reported in Table 1. The fructosamine levels were significantly increased among anemic patients compared with controls. Levels of malondialdehyde were significantly increased in anemic patients when compared to controls. There was no difference in levels of fasting glucose levels between anemic patients and control groups. Before iron treatment, the mean hemoglobin, ferritin and iron levels were significantly low when compared to the healthy age-matched controls. In the test group, a significant correlation ($r = 0.60$, $p < 0.01$) was observed between fructosamine and MDA using partial correlation analysis controlling the blood glucose level.

In iron deficiency anemic patients, malondialdehyde levels decreased significantly after iron treatment for one month. After iron therapy, the mean hemoglobin, iron and ferritin levels were higher when compared with the basal levels. There was a significant decrease in fructosamine levels in response to iron therapy in anemic patients.

Table 1
Comparison of biochemical and hematological parameters in iron deficient anemic patients before and after treatment with control subjects

Parameters	Control	Anemic patients	
		Before treatment	After treatment
Hemoglobin (g/dl)	12.65 ± 0.74	7.36 ± 2.88 [a]	9.62 ± 2.28 [b]
Iron (μg/dl)	110.65 ± 26.57	38.80 ± 20.98 [a]	71.10 ± 25.48 [b]
Ferritin (ng/dl)	140.43 ± 30.72	29.60 ± 20.57 [a]	106.65 ± 73.37 [b,c]
Fructosamine (nmol/l)	1.95 ± 0.21	2.58 ± 0.60[a]	2.02 ± 0.44 [b,c]
MDA (mmol/l)	2.71 ± 0.40	5.52 ± 2.01[a]	3.51 ± 1.79 [b,c]
Glucose (mg/dl)	81.19 ± 9.74	85.25 ± 7.79	

[a] p Value < 0.05 between control group and anemic group before iron therapy.
[b] p Value < 0.05 in test group before and after one month iron therapy.
[c] p Value < 0.05 between control group and anemic group after one month of iron therapy.