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RELATIONSHIP BETWEEN FREE IRON AND GLYCATED HEMOGLOBIN IN UNCONTROLLED TYPE 2 DIABETES PATIENTS ASSOCIATED WITH COMPLICATIONS

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ABSTRACT

Free iron in serum has been found in several disease conditions including diabetes. In the present work, we studied the relationship between free iron, fasting blood glucose (FBG) and glycated haemoglobin (HbA_{1c}). Study was carried out on 50 type 2 diabetes cases under poor glycemic control associated with complications, 53 type 2 diabetes cases under good glycemic control and 40 healthy controls. We estimated free iron, both ferrous (Fe^{+2}) and ferric (Fe^{+3}) form, protein thiols, lipid hydroperoxides, FBG, HbA_{1c} and serum ferritin levels in serum. There was a significant increase in free iron in Fe^{+3} state ($p < 0.01$), HbA_{1c} ($p<0.01$), serum ferritin ($p<0.01$), lipid hydroperoxides ($p<0.01$) and significant decrease in protein thiols (<0.01) in diabetes cases under poor glycemic control compared to diabetes cases under good glycemic control and healthy controls. Free iron correlated positively with HbA_{1c} ($p<0.01$). Poor glycemic control and increase in glycation of haemoglobin is contributing to the increase in free iron pool which is known to increase oxidant generation.

KEY WORDS

Free Iron, Glycated Hemoglobin, Ferritin.

INTRODUCTION

There is considerable current interest in the relationship between insulin and iron pool in the body. Insulin influences the iron uptake and storage by increasing the cell surface transferrin receptors (1), reciprocally iron influences the insulin activity by interfering with glucose uptake and utilization (2). Iron causes hyperinsulinaemia by decreasing the insulin uptake and metabolism by hepatocytes (3). Iron in its free form i.e., in non-transferrin bound form is known to induce oxidation of biomolecules through Heber-Weiss and Fenton reactions by producing harmful hydroxyl radicals (4). This non-transferrin bound iron or free iron has been found in various disease conditions like hemochromatosis (5), thalasemias (6) and patients on supplemental iron therapy (7). In our previous work we also found such free iron in hemodialysis patients not on any supplemental iron (8).

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Previous studies have demonstrated the release of such free iron from ferritin (9), transferrin (10), and heme (11) under certain conditions. Most of the free iron estimated in different disease conditions was found to be in its ferric state (8). Free iron in its Fe^{3+} state will be reduced to Fe^{2+} state by various agents like superoxide and ascorbate (12) and glucose induced reduction by NADPH dependent reactions (13). Existence of oxidative stress in diabetes is well proved by numerous studies (14). Previous studies have proved that poor glycemic control causes glycation of proteins and increased oxidative damage of proteins which are responsible for the diabetes related complications (15). In this context, the presence of oxidative stress, hyperglycemia, hyperinsulinaemia, iron overload, and protein glycation will all lead to insulin resistance and poor glycemic control that causes early appearance of complications associated with diabetes (3).

In the current study, we estimated free iron along with glycated hemoglobin (HbA_{1C}) and oxidative stress parameters in uncontrolled type 2 diabetes cases associated with complications, type 2 diabetes cases under control and compared both with healthy non diabetic controls.

MATERIALS AND METHODS

The study was carried out on 50 type 2 diabetic patients with poor glycaemic control (Group I), 53 diabetic patients with good glycemic control (Group II) and 40 healthy non diabetic controls. Group I diabetic patients with poor glycemic control (HbA_{1c} > 7 %, mean of three determinations, once in three months during follow-up) were associated with complications like nephropathy (12 cases), neuropathy (13 cases), retinopathy (5 cases), ischemic heart diseases (15 cases) and non-healing ulcers of foot (5 cases). The mean duration of diabetes was 10 years and the patients were on insulin (32 cases), oral hypoglycemic drugs (12 cases) and combination of both (6 cases). Group II diabetic patients with good glycemic control (HbA_{1c} < 7%, mean of three determinations, once in three months during follow-up) were free from any clinical evidence of retinopathy, nephropathy or neuropathy and were on oral hypoglycemics (43 cases) and life style modification (10 cases) and mean duration of diabetes was 5 years. None of the patients was known to suffer from any acute illness or chronic inflammatory condition at the time of study. The healthy non-diabetic controls were not on any medication and were free from any acute or chronic illness. Other demographic and clinical characteristics are reported in Table I. Informed consent was obtained from all subjects involved in the study.

All patient samples collected from our clinical laboratory were sent for routine analysis. Blood samples (5ml) were drawn into plain vacutainers from the anticubital veins of healthy controls. The collected blood was allowed to clot for 30 minutes, and then centrifuged at 2000 g for 15 minutes for clear separation of serum. All assays were performed immediately after serum was separated.

Table I : Demographic characteristics of serum free iron, HbA_{1c} and oxidative markers in patients and healthy controls (Mean±SD)

	Healthy controls	Group I cases	Group II cases
Number	40	50	53
Age (years)	55±8	55±10	50±5
Sex (M/F)	29/11	38/12	40/13
Duration of diabetes (years)	—-	8±2	5±2
FBG (mg/dL)	90±10	195±15	110±10
Free iron (µmoles/L)	0.5±0.2	34.2±27.5*	0.8±0.5
HbA_{1c} (%)	6±1	11±2*	7±1
Protein thiols (µmoles/L)	312±48	164±10*	282±16
Lipid hydroperoxides (µmoles/L)	0.4±0.2	2.4±0.6*	0.8±0.3

*p<0.01; compared to group II cases and healthy controls

All glassware and other apparatus used for the experiment were thoroughly washed initially with detergent solution, then acid washed using 1 mol/L concentrated nitric acid. After acid wash, they were rinsed four times in double distilled deionized water. All possible precautionary measures were taken to prevent trace metal ion contamination particularly iron, during all stages of the procedure. All spectrometric readings were done using GENESYS 10UV spectrophotometer.

Special chemicals like bathophenanthroline disulphonate (BPS), dithionitrobenzoic acid (DTNB), Xylenol orange (XO), butylated hydroxytoluene (BHT), triphenylphoshine (TPP) were obtained from Sigma chemicals co. (St Louis, MO, USA). All other reagents used were of analytical grade.

Serum free iron was estimated by using bathophenthroline disulphonate (BPS) assay (16). Serum protein thiols were measured by a spectrophotometric method using 5 5' dithio-bis nitrobenzoic acid (17). The lipid hydroperoxide content of whole serum was determined with the FOX version II assay for lipid hydroperoxides (FOX_2) (18, 19). Serum ferritin, FBG and HbA_{1c} levels were estimated by electro-chemiluminescence immunoassay (20) glucose oxidase method (21) and affinity chromatography method (22) respectively.

All the values are expressed as Mean±SEM and p value of <0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS/PC; SPSS, Chicago, III, USA). One way analysis of variance was used to compare mean values in the three groups, followed by multiple comparison post-hoc tests. Pearson correlation was applied to correlate between the parameters.

RESULTS AND DISCUSSION

In our study, we found significant increase in free iron majorly in Fe^{3+} state in group I cases (p<0.01). However we did not observe increase in free iron in group II cases who were in good glycemic control (The means of the parameters in all the groups are presented in Table I). According to previous studies, poor glycemic control leads to increase in the levels of lipid hydroperoxides and decrease in total thiols (15). In line with previous studies we found significant decrease in protein bound thiols (p<0.01), which may be due to increased oxidative degradation of proteins especially albumin or increased consumption of this antioxidant in stress environment. We also found significant increase in lipid peroxidation measured as lipid hydroperoxides (p<0.01).

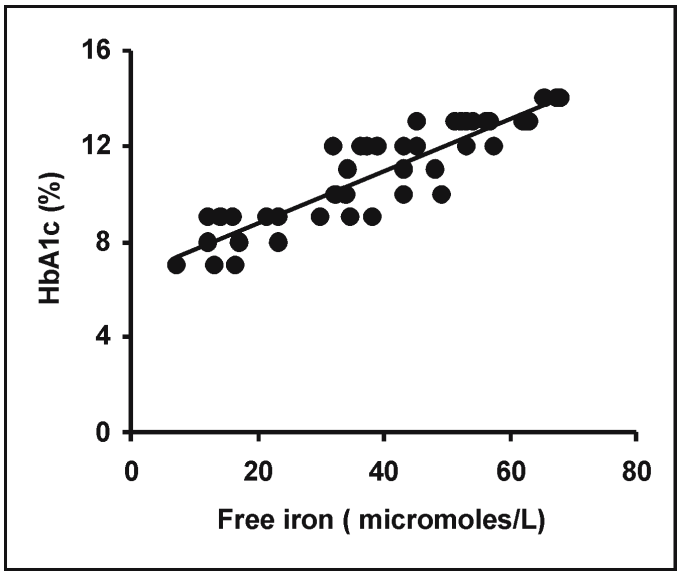

Figure I : Correlation between free iron (Fe^{3+}) and Glycated hemoglobin (HbA_{1c}) in group I cases

There was a significant correlation between free iron and HbA_{1c} ($r = + 0.436$; $p<0.01$) (figure I). The FBG which was significantly higher in group I cases ($p<0.01$) was correlated with increased HbA_{1c} ($r = + 0.513$; $p<0.01$). Previous studies have proved that poor glycemic control causes increased glycation of proteins, especially haemoglobin, which releases the iron in its free state (3). Hence increased presence of free iron in its Fe^{3+} state in association with hyperglycemia might have caused decreased in the levels of protein bound thiols and increase in lipid hydroperoxides. Studies have shown linear relationship between free iron and glycated hemoglobin in in vitro experiments (11). To our knowledge this is the first study to prove the positive correlation between glycated haemoglobin and free iron in type 2 diabetes patients associated with complications. Positive correlation between FBG and HbA_{1c} as well as free iron and HbA_{1c}, indicates hyperglycemia causing increased glycation of haemoglobin and increased release of free iron from glycated proteins like haemoglobin. This makes a vicious cycle of hyperglycemia, glycation of haemoglobin and increase in levels of free iron. This increased presence of free iron pool will enhance oxidant generation leading damage to biomolecules. However, at present the exact nature of free iron pool in vivo is not clearly known (23), it needs further studies in this population with various study designs to know the catalytic action of free iron and its relation to glucose.

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