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OXIDATIVE STRESS IN TYPE 2 DIABETES WITH IRON DEFICIENCY IN ASIAN INDIANS

OKSIDATIVNI STRES U DIJABETESU TIPA 2 SA NEDOSTATKOM GVOŽĐA KOD INDIJACA

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Summary: A close relationship exists between iron metabolism, diabetes and oxidative stress. Both diabetes and redox active iron are individually known to enhance oxidative stress. However, the role of iron deficiency and oxidative stress in diabetes is not clear; hence, the levels of oxidative stress in type 2 diabetes with and without iron deficiency have been compared. Two groups of 30 patients each with diabetes were selected (one group with iron deficiency and the other group with normal iron levels) and compared with 30 normal healthy controls. The anthropometric parameters, fasting blood sugar, iron profile and oxidative stress parameters (malondialdehyde levels (index of lipid peroxidation) and serum uric acid levels (antioxidant)) were measured. While the diabetes group had significantly increased serum levels of ferritin (an acute phase reactant and antioxidant) in comparison with normal controls (P=0.040), the diabetic group with iron deficiency had decreased serum levels of iron (P =0.000), ferritin (P = 0.000) and uric acid (P =0.006) and increased levels of malondialdehyde (P = 0.000) in comparison with diabetics without iron deficiency. This study shows an increase in oxidative stress in the diabetic group with iron deficiency together with reduction in antioxidant levels could further promote prooxidant levels and inflammation and in turn result in the development of complications in this high-risk Asian Indian population.

Keywords: diabetes mellitus, iron deficiency, oxidative stress

Kratak sadržaj: Metabolizam gvožđa, dijabetes i oksidativni stres blisko su povezani. Dijabetes i redoks-aktivno gvožđe zasebno pojačavaju oksidativni stres. Međutim, još nije ustanovljena uloga nedostatka gvožđa i oksidativnog stresa u dijabetesu; stoga su upoređeni nivoi oksidativnog stresa u dijabetesu tipa 2 sa i bez nedostatka gvožđa. Izabrane su dve grupe od po 30 pacijenata sa dijabetesom (jedna grupa sa nedostatkom gvožđa a druga sa normalnim nivoima gvožđa) i upoređene sa 30 zdravih kontrolnih subjekata. Izmereni su antropometrijski parametri, šećer u krvi na prazan stomak, profil gvožđa i parametri oksidativnog stresa (nivoi malondialdehida (indeks lipidne peroksidacije) i nivoi mokraćne kiseline u serumu (antioksidant)). Dok su u grupi dijabetičara nivoi feritina (reaktant akutne faze i antioksidant) u serumu bili značajno povišeni u poređenju sa zdravim kontrolnim subjektima (P = 0,040), u grupi dijabetičara s nedostatkom gvožđa bili su sniženi nivoi gvožđa (P = 0,000), feritina (P = 0,000) i mokraćne kiseline (P = 0,000) u serumu u poređenju s dijabetičarima bez nedostatka gvožđa. Ova studija pokazuje da bi porast oksidativnog stresa u grupi dijabetičara s nedostatkom gvožđa uz redukciju nivoa antioksidanata mogao dodatno povećati nivoe prooksidanata i pojačati inflamaciju, što bi za posledicu imalo razvoj komplikacija u ovoj visokorizičnoj indijskoj populaciji.

Ključne reči: šećerna bolest, nedostatak gvožđa, oksidativni stres

Introduction

A bi-directional relationship exists between iron and glucose metabolism (1). Insulin regulates the cellular uptake of micronutrients including iron; iron interferes with the function and metabolism of insulin in the liver (1). Disturbances in iron metabolism such as iron overload are known to cause diabetes, as seen in hereditary hemochromatosis (2), and diabetics are often predisposed to anaemia (3).

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The presence of iron in reversible oxidized and reduced forms is responsible for its metabolic function and also its potential toxicity (4). The redox active, Fe^{2+}, catalyses the generation of a powerful free radical – the hydroxyl radical – by the Fenton reaction (5), resulting in an increase in oxidative stress and damage to cellular macromolecules. Hence, in plasma there is normally limited bioavailability of free iron due to iron sequestration in transport and storage proteins. Oxidative stress is associated with diabetes and also with disturbances in iron metabolism. Diabetes alters the availability of redox active Fe^{2+} either from excessive iron stores or from alterations in the protective mechanisms which normally prevent the release of free iron (6).

In iron-deficiency anaemia, a common global nutritional disorder, although the levels of free iron in the circulation are found to be normal or low, there is enhanced oxidative stress (7). Alterations in the prooxidant/antioxidant balance are considered to be the cause of oxidative stress (7). The deficiency of iron causes tissue hypoxia and also affects the production of iron-containing antioxidant proteins which tilts the balance to the oxidative side (8). Iron deficiency also affects mitochondrial oxidative phosphorylation leading to decreased ATP production and causes loss of structural and functional integrity of the cell (9). In addition, impairment of the antioxidant defence system and decreased cellular immunity have also been reported in patients with iron deficiency anemia (8).

While both diabetes and iron-deficiency are known to be individually responsible for enhanced oxidative stress, studies on patients with both diabetes and iron deficiency are limited. Enhanced oxidative stress would increase the morbidity and mortality in these conditions, especially in populations at high risk for diabetes and having nutritional iron deficiency. Hence, the aim of this investigation is to study the levels of oxidative stress (malondialdehyde (MDA) levels (index of lipid peroxidation), uric acid and ferritin levels (antioxidants) as markers of oxidative stress) and iron profile in type 2 diabetic Asian Indians (a population prone to iron deficiency and at high risk for diabetes) to assess the prooxidant role of iron due to the cumulative effect of diabetes and iron-deficiency.

Materials and Methods

Adult subjects attending the Endocrine Clinic at the M.S. Ramaiah Hospitals, Bangalore, South India, were recruited after informed consent was obtained from them. The study protocol was approved by the Ethical Review Board of the Institution.

The subjects of either sex aged between 25 and 60 years were divided into three groups. Group I – 30 patients diagnosed with type 2 diabetes as per the WHO Guidelines (10) and having iron deficiency. Iron deficiency for Indians was defined as Hb <100 g/L for females and <110 g/L for males (11). Group II – 30 patients diagnosed with type 2 diabetes as defined above, but with normal iron levels. Group III – 30 normal healthy volunteers serving as controls.

The inclusion criteria were: subjects of either sex between the ages of 25 and 60 years diagnosed with type 2 diabetes (Groups I and II) who were receiving treatment with either insulin or oral antihyperglycemic agents. Group I subjects, in addition, had iron deficiency, while both Groups II and III were not iron deficient. Most of the subjects of Groups I and II were from a semi-urban background. The exclusion criteria were: smokers, tobacco users, alcoholics, subjects with nutritional disorders (except for iron deficiency in Group I), secondary endocrine disorders (Groups I and II), systemic disorders like hypertension, ischemic heart disease, asthma and other complications of diabetes. Subjects on other medication like vitamins, steroids and antioxidants, those with an acute illness or chronic inflammatory conditions were also excluded from the study. Anemia due to causes other than iron deficiency was also excluded (Group I).

A complete medical history including duration of the disease and the treatment details was elicited from all the patients. Anthropometric measurements including height, weight, waist and hip circumferences were taken as per standard procedures. Body mass index (BMI) and waist: hip ratio (WHR) were calculated. Clinical parameters and blood pressure (using standard procedures) were recorded.

Biochemical Parameters

Blood samples were drawn after a 12-hour overnight fast for the determination of fasting blood glucose, haemoglobin levels, complete iron profile (serum iron, total iron binding capacity (TIBC) and ferritin levels), serum uric acid levels and malondialdehyde (MDA) levels. Fasting blood glucose was estimated by the glucose oxidase method (Biosystems, S.A. Barcelona, Spain) and hemoglobin by the cyanmethemoglobin method (Drabkins method) on a semi-automated analyser. Serum iron (Biosystems, S.A. Barcelona, Spain), TIBC (Biosystems, S.A. Barcelona, Spain) and uric acid (Biosystems, S.A. Barcelona, Spain) were assayed on a fully automated (Olympus AU400 analyzer) and ferritin was estimated by a chemiluminescent enzyme immunometric assay using ELECSYS 2010 (Roche Hitachi Electro-chemiluminescence system). The following parameters were calculated: transferrin levels = TIBC x 0.7; transferrin saturation = serum iron levels/ TIBC and unsaturated iron binding capacity (UIBC) = TIBC – serum iron. MDA levels were estimated by the thiobarbituric acid reactive substance method (12).

Statistical Analysis

The data are expressed as mean ± SD. Statistical analysis was done using the SPSS version 13 software. Since this study included a wide age group ranging from 25 to 60 years, the data were analysed by two-way ANCOVA taking age as covariate.

Differences between groups and gender and the interaction between these two factors were calculated using two-way ANCOVA. Multiple comparisons by the Bonferroni test were used to analyse the difference between groups. Pearson correlation coefficient was used to obtain the relationship between variables. 'P' values < 0.05 were considered to be statistically significant.

Results

The clinical characteristics of the three groups are shown in *Table I*. The groups were both age and sex-matched. Group I had a significantly higher mean BMI than Group II (P = 0.000), but within the normal range. However, the WHR was lower in Group I than in Group III (P = 0.016).

The fasting blood sugar levels were similar in both diabetic groups (Groups I and II). The hemoglobin levels in Group I were significantly lower than in Groups II and III (P = 0.000). While Group I subjects had iron deficiency, Groups II and III were not deficient in iron.

The iron profile of the three groups is presented in *Table II*. The serum iron levels were significantly decreased in Group I when compared to the other two groups (P=0.000). Group II had a significantly higher ferritin level when compared to Groups I (P = 0.000) and III (P=0.040). The transferrin levels, TIBC and transferrin saturation were not significantly different between the groups. Although Group I exhibited low serum iron levels, suggesting the presence of iron deficiency in this group, there was no alteration in transferrin levels or in transferrin satu-

Table I Clinical characteristics of the three groups. (Mean ± SD)

Parameter	Group I n=30	P value I vs II	Group II n=30	P value II vs III	Group III n=30	P value I vs III
Age, years	40.0 ± 8.72	–	43.57 ± 9.49	–	41.83 ± 6.70	–
Sex, M/F	11/19		11/19		12/18	
BMI, kg/m^2	22.37 ± 2.35	0.000	19.70 ± 2.22	0.004	21.56 ± 1.87	–
WHR	0.84 ± 0.051	–	0.87 ± 0.06	–	0.87 ± 0.04	0.016
Treatment: OHA/insulin	20/10	–	20/10	–	–	–
Fasting blood sugar, mmol/L	14.04 ± 1.62	–	14.09 ± 1.33	0.000	6.05 ± 1.18	0.000
Hemoglobin, g/L	79.6 ± 16.22	0.000	128.0 ± 9.25	–	130.1 ± 11.91	0.000

Group I = Diabetics with iron deficiency
Group II = Diabetics without iron deficiency
Group III = Normal subjects
n = number of subjects; BMI= body mass index; WHR = waist: hip ratio
OHA = oral antihyperglycaemic agents
Significant P values (<0.05) have been indicated.

Table II Iron profile of the three groups. (Mean ± SD)

Parameter	Group I n=30	P value I vs II	Group II n=30	P value II vs III	Group III n=30	P value I vs III
S. Iron, μmol/L	7.73 ± 3.73	0.000	14.75 ± 4.67	–	13.00 ± 4.28	0.000
S. Ferritin, μg/L	62.03 ± 46.25	0.000	132.47 ±67.44	0.040	96.28 ± 50.79	–
TIBC, mmol/L	63.95 ± 22.24	–	65.45 ± 15.00	–	69.37 ± 14.50	–
UIBC, mmol/L	59.82 ± 22.85	–	49.51 ± 15.07	–	59.00 ± 13.97	–
Transferin saturation, %	20.70 ± 1.44	–	20.50 ± 0.77	–	20.87 ± 1.17	–
S.Transferrin, g/L	1.24 ± 0.43	–	1.26 ± 0.30	–	1.33 ± 0.24	–

Group I = Diabetics with iron deficiency
Group II = Diabetics without iron deficiency
Group III = Normal subjects
S. iron = serum iron; S. Ferritin = serum ferritin; TIBC = total iron binding capacity; UIBC = unsaturated iron binding capacity;
S. transferrin = serum transferrin
Significant P values (<0.05) have been indicated.

Table III Oxidative stress parameters in the three groups. (Mean ± SD)

Parameter	Group I n=30	P value I vs II	Group II n=30	P value II vs III	Group III n=30	P value I vs III
MDA, nmol/L	454.25 ± 88.47	0.000	212.80 ± 93.80	–	165.67 ± 85.03	0.000
S.Uric acid, μmol/L	211.22 ± 69.03	0.006	281.25 ± 102.58	–	300.13 ± 81.22	0.000

Group I = Diabetics with iron deficiency.
Group II = Diabetics without iron deficiency.
Group III = Normal subjects.
MDA = malondialdehyde; S. uric acid = serum uric acid.
Significant P values (<0.05) have been indicated

ration which is commonly observed in iron deficiency anemia.

The serum MDA levels (index of lipid peroxidation) were significantly higher in Group I than in Groups II or III (P=0.000) (*Table III*). However, although the MDA level in Group II was higher than in Group III, it was not significant. Plasma total antioxidant capacity, which is commonly used to measure antioxidant status, is predominantly determined by uric acid. Hence, in this study only serum uric acid levels were measured as an index of antioxidant capacity. Uric acid levels were significantly decreased in Group I when compared with Groups II (P =0.006) or III (P = 0.000); however, the levels in the three groups were within the normal range. Serum ferritin levels, also an antioxidant, were decreased in Group I and increased in Group II.

The two-way ANCOVA analysis taking age as a covariate showed that there was a significant difference between the genders with respect to only BMI (P=0.001) and WHR (P=0.000). However, there was a significant difference between the groups with respect to BMI, WHR, fasting blood sugar, hemoglobin, serum iron, serum ferritin (P=0.000), serum uric acid and MDA (P=0.001).

MDA levels negatively correlate with haemoglobin (r = –0.726, P = 0.000), serum iron (r = –0.435, P = 0.000), ferritin (r = –0.323, P = 0.002) and uric acid (r = –0.414, P = 0.000) levels and positively correlate with fasting blood sugar (r = 0.463, P = 0.000) in the entire group of subjects (Groups I+II+III). Also, ferritin correlates with haemoglobin (r = 0.344, P = 0.001), serum iron (r = 0.345, P = 0.001) and uric acid (r = 0.227, P =0.031) levels. Such correlations were not observed when the groups were considered individually.

Discussion

Asian Indians are a high-risk population for the development of diabetes and have a high prevalence of nutritional deficiencies, especially among the lower socio-economic groups. Nutritional disorders, like iron overload (e.g. hereditary hemochromatosis), predominant in the developed countries, promote oxidative stress, tissue damage and the development of diabetes (5). In this study, the haemoglobin levels were normal in both Groups II and III; hence, the diabetes seen in Group II cannot be attributed to iron overload.

Ferritin levels have been associated with central body fat (1). In diabetic patients (Group II) there was an increase in the ferritin levels, but the BMI was reduced and WHR was unaltered in comparison to Group III. In Group I, there was a slight increase in BMI and decrease in both WHR and serum ferritin levels when compared with Group III. However, there was no correlation between BMI, WHR and ferritin levels in any of the groups.

Type 2 diabetes is associated with oxidative stress and inflammation (6). Serum ferritin levels have been reported to correlate with body iron stores in healthy individuals (13) but are elevated in diabetic patients due to abnormal indices of glucose homeostasis (14). The presence of reducing agents like ascorbic acid and superoxide ion releases free iron from ferritin which can be the potential source of peroxidation reactions in diabetes (15). Ferritin, an acute phase reactant, has been reported to increase in chronic low grade inflammation and in type 2 diabetes (16). The presence of free iron, insulin, oxidative stress and inflammation promotes the synthesis of ferritin (17). Ferritin can also function as an antioxidant because of its ability to sequester the potentially toxic iron. The elevation in serum ferritin levels in poorly controlled type 1 and type 2 diabetes probably reflects increased oxidative stress and chronic inflammation in type 2 diabetes mellitus (1).

Alteration in serum iron levels together with concomitant changes in ferritin levels are seen in both diabetic groups. In Group I, there is a significant decrease in ferritin levels which is associated with the low serum iron levels characteristic of iron deficiency. The decrease in ferritin levels may be due to decreased translation of ferritin mRNA consequent to low levels of iron. This would enhance both inflammation and oxidative stress. However, in Group II, the ferritin levels were significantly increased although there was only a slight increase in iron levels in comparison to Group III. This increase in ferritin levels may reflect other roles of ferritin in diabetes – a marker of inflammation or as an antioxidant (1).

Oxidative stress results from an imbalance between the formation of prooxidants and neutralization by antioxidants (7). MDA level (index of lipid peroxidation and marker of oxidative stress) was significantly increased in Group I in comparison to Groups II and III. In Group II also there was an increase in the MDA levels, but not significant, when compared with Group III. A similar observation in MDA levels in type 1 and 2 diabetics in comparison with normal controls has been reported by Campenhout et al. (6). Baccin et al. (18) observed significantly elevated production of MDA in the serum of iron deficient patients as an indicator of increased levels of auto-oxidizable lipids in oxidative stress, and also oxidative protein damage in plasma.

Diabetes is a state of lower antioxidant defences (6); and, in iron deficiency, the enzymes involved in the antioxidant defence system will be functionally defective (9). In Group I, there was a significant decrease in uric acid levels in comparison to Groups II and III; however, the levels were within the normal range. In iron deficiency anemia there is increased DNA damage (19) and, therefore, a concomitant increase in uric acid levels may be expected together with co-morbid conditions like diabetes (20). However, since xanthine oxidase is a non-heme iron protein, iron deficiency will lower the uric acid levels. Uric acid is an important part of the antioxidant defence system of the body, which scavenges free radicals, chelates iron and stabilizes ascorbate by inhibition of its iron-catalysed oxidation (21). While hyperuricemia has been associated with cardiovascular risk in diabetes (22), low urate levels have been associated with enhanced oxidative stress and mortality risk (20). Further, the low iron levels will decrease the levels of iron-containing enzymes like catalase and peroxidase which function in free radical scavenging (8), thus decreasing the antioxidant levels. This enhances the role of urate as an antioxidant which, in turn, may further decrease the levels of plasma urate. Low serum urate levels have been reported to increase mortality in patients on hemodialysis (also a state of severe stress) (20).

In spite of iron being potentially toxic at high concentrations, this study on type 2 diabetic patients shows that iron deficiency enhances oxidative stress and together with inflammation can promote the development of complications. The imbalance between prooxidants and antioxidants, due to decreased levels of antioxidants, promotes oxidative stress in type 2 diabetes to a greater extent when accompanied by iron deficiency.

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Author's contributions

SRM and MD conceived and designed this research project; SG and SRM performed the research; SG and SRM analyzed and interpreted the data; SG, MD and SRM wrote the paper.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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