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Serum Iron Concentration and Lipid Profile in Type 2 Diabetes Patients

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Abstract: Recent studies have been showed important role of elevated iron levels in pathogenesis of *Type 2 Diabetes mellitus* (T2D) and insulin resistance. The aim of this study was to determine serum of free iron concentration in T2D patients and find out associations with lipid profile. The study included 51 participants (27 healthy control and 24 no treated diabetes patients), with ages from 45 to 65 45-65 ages and both gender. As expected, concentrations of serum iron were elevated in diabetic patients compare to healthy subjects while statistical significant difference were shown between iron levels in control group and group with good control of glycaemia ($p<0.05$). In addition, there was a significant positive correlation between free iron concentration and LDL cholesterol levels and negative significant correlation between iron concentrations with HDL cholesterol in diabetics ($p<0.05$). These findings suggest that increase serum of free iron concentrations may have an important role and influence in development of disease, especially in lipid metabolism and profile as well in risk of further complications of diabetes.

INTRODUCTION

Type 2 diabetes mellitus (T2D) is a chronic metabolic condition characterized by elevated glucose levels due to impaired of insulin secretion and action, or both. It is also, followed by insulin resistance (IR), increase in hepatic glucose production and elevated lipids level. The pathophysiological mechanisms of the disease are not yet fully understood despite efforts and numerous studies provided. Disturbance not only in carbohydrate pathways but also im lipid and protein metabolism was included (Swaminathan, S., Fonesca, V. A., Alam, M. G., Shah, S. V., 2007; Orban *et al*., 2014; Fernández-Real J. M., McClain, D., Manco, M., 2015).

Acute and chronic complications of this disease are caused by different metabolic abnormalities in a body oxidative stress being one of them. Oxidative stress through the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been recognized as the basic cause of the underlying mechanism for the development of IR, β-cell dysfunction, impaired glucose tolerance and at the end to T2D (Backe *et al.*, 2016; Shaaban M. A., Dawod A. E., Nasr M. A., 2016). Progression of diabetes vascular dysfunction implicated by overproduction of ROS and RNS which is the consequences of sedentary lifestyle and over nutrition. Reactive oxygen and nitrogen species initiate a chain reaction leading to reduced nitric oxide availability, augmented markers of inflammation and chemical modification of lipoproteins. Alternation of functions and contribution of macronutrient and their metabolic pathways are well known but disturbance and actions of micronutrient, although having an important roles in the diabetes are still little understood. Mineral elements as a valuable micronutrient are not only integral members of the structural components of body tissues but also, they participate in various metabolic processes (Wolide *et al.*, 2017). Recent studies investigated their roles in insulin synthesis, its release and actions, along with glucose metabolism (Stechemesser *et al.*, 2017).

Iron (Fe) as an essential trace element and is one of the most important metals for almost all living organisms. Iron is involved in various biochemical and metabolic processes, including oxygen transport, synthesis of deoxyribonucleic acid, and electron transfer as well in insulin and glucose metabolism (Abbaspour N., Hurrell R., Kelishadi R., 2014; Stechemesser *et al.*, 2017).

Iron as a transition element has significant redox activity and potential detrimental effects is presented by its binding with transport or storage proteins.
Previous studies have shown association of the T2D and iron metabolism and concluded that increased iron concentrations may predicted development of disease (Simcox J.D., McClain D.A., 2013; Arija *et al.*, 2014; Orban *et al.*, 2014). Iron and diabetes were linked by two pathological conditions: hereditary hemochromatosis and thalassemia. Also, iron has an important role in pathogenesis of the disease by mediation of β-cell dysfunction and IR, as well in regulation of energy homeostasis (Fernández-Real J. M., McClain, D., Manco, M., 2015; Podmore et al., 2016; Walace D. F., 2016; Stechemesser *et al.*, 2017).

The underlying molecular mechanisms by which iron mediates these effects are incompletely understood. They include oxidative stress, changes in adipokines and alteration in cellular signal transduction. It is well known that IR and β-cell dysfunction are risk factors in genetic predispose individuals but metabolic pathways remain largely unknown.
In its free form (released by ferritin by the action of reducers that convert Fe^{3+} to Fe^{2+}), iron promotes the oxidation of biomolecules through Haber-Weiss and Fenton reactions generating of detrimental hydroxyl radicals (Figure 1).

Auto oxidation of Fe^{2+}	$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet-}$
Fenton reaction	$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$
Haber-Weiss reaction	$H_2O_2 + O_2^{\bullet-} \xrightarrow{Fe^{3+}} O_2 + OH^- + OH^{\bullet}$

Figure 1: Formation of free radicals catalyzing by ferric form of iron in Fenton and Haber-Weiss reactions.

These free radicals are strong pro-oxidants which cause lipid peroxidation of cellular membrane, damage the configuration of proteins and damage nucleic acids in genes. These catalytic activities of free iron lead to IR through suppressed insulin release and action followed by glucose derangements upon iron-related pathways. Oxidative stress–triggered inflammatory cytokines e.g. C reactive protein (CRP) and interleukin 6 (IL-6) may amplify and potentiate the initiated events and cause deterioration of diabetes vascular chronic complications mediate by Fe (Fernández-Real, J. M., McClain, D., Manco, M., 2015).

Inconsistent results have been reported for iron in last years and its role in pathogenesis of *diabetes mellitus* and insulin resistance. Authors emphasize that a prominent role of free iron depend of race and ethnicity of study populations as well as their nutrition habits and diet. Clinical trials are warranted to clarify the impact of dietary or pharmacological iron reduction on the development of this metabolic disorder and therefore, suppress or diminish its complications (Swaminathan, S., Fonesca, V. A., Alam, M. G., Shah, S. V., 2007; Rajpathak, S. N., Crandall, J. P., Wylie-Rosett, J., Kabat, G. C., Rohan, T. E., Hu, F. B., 2009; Sanjeevi, N., Freeland-Graves, J., Beretvas, S. N., Sachdev, P. K., 2018).
The aim of this work was to found association of free iron concentrations and lipid profile in selected, non-treated type 2 diabetes population both gender in Bosnian and Herzegovina.

EXPERIMENTAL

Subjects

This study included 51 subjects out of them 24 patients having nontreated Type 2 *diabetes mellitus* with good glycemic control (11 participants) and poor glycemic control (13 participants) and 27 normal healthy control were selected. *Diabetes mellitus* was diagnosed according to The Expert Committee on the Diagnosis and Classification of Diabetes of ADA (American Diabetes Association, 2004).
Inclusion criteria: All participants divided into four groups: Group 1-Control group (27) consisted of healthy subjects; they were free from any illness which could affect the parameters under study. Also, they were taken from general population and they were not on any medication.
Group 2 as untreated Type 2 diabetics (24); they were not on antidiabetics drugs and much older than controls, and this group further was divided on the two groups Group 2a and Group 2b as patients with good control and poor control of glycaemia, respectively. Group 2a consisted of patients (11) with good control of glycaemia i.e. glycated hemoglobin levels less than 6.5% and they were free from clinical evidence of any complication of *diabetes mellitus*.
Group 2b participants with poor control of glycaemia (13) represent by patients with glycated hemoglobin level more than 7.0% and associated with one or more diabetic complications.
Exclusion criteria: hepatitis B and C or virus infection as well patients who received antidiabetic drugs.
Study was performed in accordance to Ethical Committee and Declaration of Helsinki and informed written consent of all subjects included in the study was obtained and participation in this study was voluntary.
Diabetic patients and healthy subjects were recruited at the Clinic for Endocrinology and Diabetes, University Clinical Centre of Sarajevo, Department of Endocrinology and Internal Medicine in Sarajevo. The investigations were carried out from January to September.

Sample collection

After overnight fasting, a 5 ml of venous blood was drawn from each volunteer and serum or plasma separated within 30 min and stored at 2-8^{0}C temperature till analysis was done.

Analysis of samples

Fasting glucose was estimated by glucose oxidase-peroxidase (GODP) enzymatic method, glycated hemoglobin level measured by immunoturbidimetric method in whole blood, while lipid profile (i.e. total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides) analyses was done according standard clinical laboratory protocols and IFCC recommendations. Serum free iron concentration done by Ferrozine method and all these determinations performed by Autoanalyzator Dimension® RXL clinical-chemical system (Siemens, Germany).

Serum iron assay

The method applied was a modification of direct iron assays, using the chromophore Ferene (Smith et al., 1984). This method is a direct iron measurement using a surfactant to prevent protein precipitation. A serum blank was used to correct for differences in specimen turbidity while potential copper interference was minimized by addition of thiourea. In the serum, presence of hemolysed cells had no effect on serum iron levels and measurement. Principle of assay: Under acidic conditions (pH 4.5), iron bound to the protein transferrin is released in the presence of the reducing agent, ascorbic acid. The resulting product, Fe^{2+} (ferrous ions) forms a blue complex with 3-(2- pyridyl)-5,6-bis-2-(5-furyl sulfonic acid)-1,2,4-triazine, disodium salt (Ferene). The absorbance of the complex, measured using a dichromatic (600, 700 nm), endpoint technique, is proportional to the concentration of transferrin-bound iron in the serum. Normal concentration and reference range for iron in serum samples is 35-150 µg/dl (6-27 µmol/L).

Statistical analysis

Statistical analyses were carried out using SPSS software version 17.0 (Chicago, IL, USA). Statistical significance was set a *P* value of <0.05. Data are expressed as medians (lower–upper quartile) and nonparametric Mann-Whitney U test used for unpaired samples to estimated differences of iron, glycated hemoglobin and other biochemical variables between groups. Independent sample *t* tests were used to compare the mean values of biochemical parameters between the diabetic and control groups while Spearman's correlation coefficient was calculated to analyze the relationships between the study variables.

RESULTS AND DISCUSSION

The characteristics of type 2 diabetes patients and control subjects are presented in Table I. Values of all the measured traits were significantly different between cases and controls. The iron concentrations were significantly higher in diabetic patients compared to healthy subjects.

Table 1: Biochemical parameters in study population

Parameters	NT-T2D	Healthy control	*P* *value
Number	24	27	-
Gender (M/F)	14/10	14/13	-
Age (years)	64 (60-68)	48 (42-54)	0.000
Glucose(mmol/L)	8.20 (7.59-8.81)	5.37 (5.24-5.49)	0.000
Total cholesterol (mmol/L)	5.34 (5.02-5.65)	4.53 (4.12-4.94)	0.001
HDL cholesterol (mmol/L)	1.17 (0.89-1.44)	1.27 (1.16-1.38)	0.021
LDL cholesterol (mmol/L)	3.45 (3.11-3.78)	2.47 (2.08-2.86)	0.000
Triglygerides (mmol/L)	2.08 (1.77-2.40)	1.68 (1.28-2.08)	0.021
Glycated hemoglobin HbA1c, (%)	7.16 (6.88-7.44)	5.74 (5.57-5.90)	0.000
hs-CRP (mg/L)	3.29 (2.41-4.17)	2.07 (1.19-2.95)	0.013
Iron (µmol/L)	23.38 (14.44-32.33)	19.40 (19.52-9.95)	0.000

*NT-T2D, no treated Type 2 diabetes; HDL cholesterol, high density lipoprotein; LDL cholesterol, low density
lipoprotein; hs-CRP high-sensitive C reactive protein. Data represent medians (upper and lower values).
*Significance of difference in Mann–Whitney test.

These findings are in line with data obtained from other investigation and research studies. Numbers of studies were showed significant increase of iron concentrations in diabetic patients compared to non-diabetic subjects regardless of racial or ethnic origin. Elevated iron levels and iron overload not only increases risks for insulin resistance and Type 2 diabetes, but also, causes cardiovascular disorder (CVD) in both, non-diabetic and diabetic individuals. Recent data point out the importance of determination of iron and ferritin concentrations in diabetics, because they can be used as markers for severe hepatic IR, higher risk for vascular complications and their progression (Fernández-Real, J. M., McClain, D., Manco, M., 2015).

Earlier studies have shown a gender differences in iron concentrations of T2D patients but no significant differences were found in this study (Aregbesola et al., 2017).

A main fraction of cellular iron is bonded with proteins in the form of heme, a protoporphyrin IX and Fe^{2+} ion. Chemical activity of iron is derived from a variety of coordination complexes with organic ligands, and electron transition of reduced iron form, Fe^{2+} and oxidized form Fe^{3+} ions. The efficiency of electron transfer between two iron forms is a basic feature for many biochemical reactions and iron action as an essential element and nutrient. The major form of glycated haemoglobin in diabetic patients is haemoglobin A1c (HbA1c). In non-diabetic subjects, the HbA1c levels with 3.0% to 6.5% of the haemoglobin represent optimal glucose control while the HbA1c fraction with ≥ 6.5% is abnormally elevated haemoglobin in patients with chronic hyperglycaemia and correlates positively with glycaemic control (American Diabetes Association., 2004; International Diabetes Federation., 2006; Fernández-Real, J. M., McClain, D., Manco, M., 2015). Control of glycaemia based on glucose and glycated haemoglobin levels, shown significant differences only between controls and diabetics with good control of glycaemia (Table II).

Table 2: Mean concentration of serum iron and glycemic control in study population.

Group of participant	Number	Mean of Fe(µmol/L)	*P* value
Group 1	27	19.40	0.002
Group 2a	11	26.00	
Group 1	27	19.40	0.125
Group 2b	13	21.15	
Group 1	27	19.40	0.375
Group 2	24	26.00	
Group 2a	11	26.00	0.146
Group 2b	13	21.15	

*Group 1, healthy control subjects
Group 2, no treated diabetics
Group 2a, good control of glycaemia
Group 2b, poor control of glycaemia

Similar results of the association of serum free iron levels with glycated haemoglobin reported in studies of others (Gohel M., Sirajwala H. B., Chacko A., 2013; Misra et al., 2016).

Lipid profile referred as high levels of low-density lipoprotein cholesterol and triglycerides and low high-density lipoprotein cholesterol level correspond to dyslipidaemia. Dyslipidaemia is involved in glycaemic control and plasma lipid elevation. It is known that diabetic patients with dyslipidaemia have higher risk for macro-vascular and microvascular complications and atherosclerosis (Stechemesser et al., 2017; Wolide et al., 2017).

Correlations between serum free iron concentrations and lipid profile, presented in Table III and Table IV for control subjects and cases, respectively. In healthy subjects, there were not demonstrated significant association while in diabetic patients, positive significant correlation was showed between iron and LDL levels and negatively correlation between iron and HDL levels.

These results are in line with previously reported data. Fernández-Real and collegues found a proportional relationship between serum iron stores (ferritin) and serum glucose concentration, diastolic blood pressure, HDL cholesterol, and insulin resistance. In study of Victoria Arija and co-works demonstrated that excess of body Fe store and lipid abnormalities associated with an increased risk of T2D, CVD and other diabetic complications.

Table 3: Spearman´s correlation coefficient (rho) between serum free iron concentrations (µmol/L) and HbA1c, fasting plasma glucose, lipid profile (total cholesterol, HDL, LDL, TGs) in controls.

Parameters	Sample size	rho	*p* value
HbA1c	27	0.068	0.735
Glucose	27	0.137	0.495
TC	27	-0.081	0.688
HDL	27	0.292	0.139
LDL	27	-0.097	0.632
TGs	27	-0.252	0.205

* TC, total cholesterol
HDL cholesterol, high density lipoprotein
LDL cholesterol, low density lipoprotein
TGs, triglycerides

Table 4: Spearman´s correlation coefficient (rho) between serum free iron concentrations (µmol/L) and HbA1c, fasting plasma glucose, lipid profile (total cholesterol, HDL, LDL, TGs) in no treated Type 2 diabetics.

Parameters	Sample size	rho	*p* value
HbA1c	24	0.303	0.150
Glucose	24	0.039	0.856
TC	24	0.250	0.239
HDL	24	-0.414	0.044
LDL	24	0.438	0.037
TGs	24	-0.133	0.536

*TC, total cholesterol
HDL cholesterol, high density lipoprotein
LDL cholesterol, low density lipoprotein
TGs, triglycerides

The present work has some limitations which should be mentioned. First, a major limitation of this study is related to the relatively small number of populations' cohort, and future analysis must be done on large number of participants. Secondly, the diabetic patients were considerably older than the control group.

CONCLUSIONS

Iron elevation in diabetic patients, the major cause of increased oxidative stress, liver damage, lipid peroxidation and especially high level of LDL-C suggest it's an important role and influence in development of disease, especially in lipid metabolism and represent a main risk factor of further complications of diabetes.

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Summary/Sažetak

Nedavne studije ukazale su važnu ulogu povišenih vrijednosti željeza u patogenezi Tip 2 *Diabetes mellitus* (T2D) i insulinske rezistencije. Cilj ove studije bio je odrediti koncentraciju slobodnog željeza u serumu kod T2D pacijenata i naći asociranost iste sa lipidnim profilom. U studiji je bio uključen 51 participant (27 zdravih kontrola i 24 netretirana dijabetičara), starosne dobi 45-65 oba spola. Kao što je očekivano, nađene su povišene vrijednosti koncentracija željeza u serumu kod dijabetičara u poređenju sa zdravim osobama dok je statistički značajna razlika pokazana između nivoa željeza u kontrolnoj grupi i grupe sa dobrom kontrolom glikermije ($p<0.05$). Nadalje, postojala je značajna pozitivna korelacija između koncentracije slobodnog željeza i LDLcholesterol nivoa i negativna signifikantna korelacija između koncentracije željeza s HDL holesterolom kod dijabetičara. Rezultati sugeriraju da povišene koncentracije serumskog željeza mogu imati važnu ulogu i uticaj na razvoj oboljenja, posebno na metabolizam lipida i lipidnog profila u riziku od budućih komplikacija dijabetesa.