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IRON STATUS, RELATION TO OXIDATIVE STRESS IN DIABETIC PATIENTS

Mohamed M. El-Seweidy, Sousou I. Ali, Samy Saleh* And Dina M. Abo El-Maaty
Biochemistry Department, Faculty of Pharmacy,
University of Zagazig and Suez Canal* University, Egypt

ABSTRACT

Diabetes mellitus is a disease characterized by chronic hyperglycemia and many metabolic disturbances. The present work was undertaken to study certain iron related parameters (serum iron, total iron binding capacity, ferritin and transferrin) in diabetic individuals (type I and II). Also to correlate the degree of glycemic control by estimating glycated hemoglobin, lipogram pattern (total cholesterol, triacylglycerols, high and low density lipoprotein cholesterol) and lipid peroxidation (MDA). This may be of value in elucidation of many metabolic disturbances in diabetes mellitus. A total of 70 diabetic patients (25 females, 5 males, age 40 ± 9 Y of IDDM) and 32 females, 8 males, age 45 ± 7 Y of NIDDM) were participating in this study. They were selected from outpatient clinic, Zagazig University Hospital. 15 healthy volunteers (10 females and 5 males, age 40 ± 5 Y) were chosen as a control group. Full history and complete clinical examination for each patient and control were done. The present data showed that glycated hemoglobin, lipid peroxide and ferritin were significantly increased in both diabetic groups in comparison to controls. Transferrin demonstrated significant reduction, while serum iron and total iron binding capacity were non significantly changed. Total-, low density lipoprotein cholesterol and triacylglycerols showed significant increase, high density lipoprotein however demonstrated significant decrease in comparison to control group. These findings revealed that, poorly controlled diabetics usually suffer from hyperferritinemia and an increase in oxidation derived products, but we could not find a correlation between lipid peroxidation and iron homeostasis or glycated hemoglobin.

INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and metabolic disturbances associated with absolute or relative deficiences in insulin or insulin action [1,2]. Multiple cardiovascular risk factors and dyslipidemia usually coexist in diabetic subjects [3,4].

Certain studies indicated that hyperglycemia may lead to tissue hypoxia [5], a potential significant risk factor to atherosclerosis [3,6] . Additionally thrombotic strock risk and dyslipidema are higher in diabetic individuals in comparison to normals [7,8] .

Relation between oxidative stress and free radicals role in diabetic individuals is well supported now.Enzymes involved in oxygen utilization, formation and release of energy, usually have iron. Cellular sets of enzymatic and non enzymatic antioxidant systems have been arranged for removal of these radicals like O_2, OH and H_2O_2 . This may prevent harmful effects of these oxygen species that catalyzed by active iron. Under certain conditions, iron pool (low molecular mass) may be drastically increased, in consistent with a state of iron overloaded plasma . Accordingly free iron is detected, OH radicals, peroxidation of liposomal phospholipids are accelerated and transferin is completely saturated. All these are participating to tissues damage (liver, spleen, heart), cardiac abnormalities, skeletal , cran ial defects and lastly death. Oxidative stress and protein glycation are closely related process that may contribute to the development of diabetic complications [9,10]. Increased oxidative stress may arise from increased free radical production and reduced activity of antioxidant defences. There is evidence for both of these phenomena in diabetics [11,12].

Iron may play an important role in the production of reactive oxygen metabolites due to the generation of hydroxyl radical (Haber-Weiss- reaction) [13] . It can also react directly with oxygen species to form ferryl and perferryl compounds representing another toxic oxygen metabolites [14] . Ferritin is the major storage form of iron in the body [15] . Its level is variable within tissues, especially the pancreatic islets, liver and bone marrow [16] . Elevated ferritin level was observed in acute, chronic liver disease, uremia, hyperthyroidism, rheumatoid arthritis and patients receiving iron therapy. Transferrin is a glycoprotein derived from β , globulin acting as a carrier for iron in certain metabolic stages [17,18] .

Several other metals bind to transferrin, the highest affinity is for ferric ion and not the ferrous one [19] . Insulin defecient diabetics are usually suffering from hemochromatosis . Iron over loading disease is usually associated with iron deposition in β- cells , liver and spleen. The present work aimed mainly to configurate iron homeostasis in diabetics, in relation to glycemic control and lipogram pattern.

PATIENTS AND METHODS

Patient:

70 diabetic individuals (IDDM, 25 females and 5 males) (NIDDM, 32 females & 8 males) were diagnosed and selected from outpatient clinic, Hospital of the University of Zagazig . Criteria for their selection is poor diabetic control (glycated hemoglobin is more than

7%). Their age was ranged from 35-52 Y. Duration of the disease was 15 ± 2 Y for IDDM and 8 ± 2.5 Y for NIDDM respectively. 15 normal volunteers (10 females and 5 males) were used as controls. Full history and complete clinical examination for each patient and control were done.

Sampling:

Blood samples were collected in the morning after 10 hours of fasting. First portion (in EDTA - coated tubes) was directed to glycated hemoglobin determination, other portion was processed for serum preparation. Serum collected was divided into aliquots (0.3ml/each), stored at - 20°C, and processed later for the following determinations: lipid peroxidation, serum iron, total iron binding capacity, ferritin, transferrin, total cholesterol, triacylglycerols, high - and low density lipoprotein cholesterol.

Methods:

Serum iron, total iron binding capacity were done as described by Ceriotti and Ceriotti [20], ferritin was estimated according to Valberg [21] . The method of Hellsing [22] was applied for transferrin determination, Geiger and Binder method [23] for glycated hemoglobin and that of Jain et al., for lipid peroxidation [24].

Serum total cholesterol was estimated enzymatically according to The Study Group European Atherosclerosis Society [25] and HDL-cholesterol was carried out according to Burstein and Schoinick [26]. The method of Bergmenyer [27] was applied for LDL-cholesterol determination. Triacyglycerols were estimated enzymatically according to Wahlefeld procedure [28].

Statistical analysis:

Data were presented as mean ± S.D. The significance difference between groups was evaluated by Student's t-test.

RESULTS

General data of diabetic individuals and their classification were summarized in (Table 1). The present results demonstrated that, glycated hemoglobin, serum lipid peroxide (MDA) (Table 2) and ferritin (Table 3) were significantly increased in IDDM and NIDDM in comparison to control. Transferrin demonstrated significant decrease, while serum iron and total iron binding capacity showed no change (Table 3). No correlation was found between ferritin and glycated hemoglobin or MDA.

Lipid profile, serum cholesterol (total, LDL-cholesterol) and triacylglycerols showed significant increase, HDL-cholesterol, however demonstrated significant decrease in comparison to control group (Table 4).

Table 1. General data of the individuals studied and their classification. Values are expressed in terms of M ± SD.

Subjects	Control	IDDM	NIDDM
Number of individuals	15	30	40
Duration of diabetes "Years"	-	15 ± 2	8 ±2.5
Age "Years"	40 ± 5	40 ± 9	45 ± 7
Sex :Female Male	10 5	25 5	32 8

Table 2. Glycated hemoglobin and serum lipid peroxide (MDA) levels in IDDM, NIDDM and control individuals. Values are expressed in terms of M±SD.

Groups	Control	IDDM	NIDDM
Glycated hemoglobin %	6.58 ± 0.45	13.4 ± 2.5 *	13.19 ± 1.9*
Serum MDA (n mol/dl)	0.25 ± 0.04	0.45 ± 0.07*	0.6 ± 0.09*

*significantly different from control individuals at $p < 0.01$.

Table 3.Serum iron, Total iron binding capacity (TIBC), Ferritin and transferrin levels in IDDM , NIDDM and control individuals. Values are expressed in terms of M ±SD.

Groups	Control	IDDM	NIDDM
Serum iron (µg/dl)	95.6 ±37	97.6 ± 72	88.3 ±36
TIBC	333.8 ± 33.5	352.4 ± 61.5	338 ± 47
Serum ferritin (ng/ml)	56.6 ± 32.7	135.4 ± 69.7*	144.2 ± 83*
Serum transferrin (mg/dl)	309.4 ± 13.6	212.8± 53*	215.5 ± 56.7*

*significantly different from control individuals at $p < 0.01$

Table 4. Lipogram pattern : Serum Total, LDL , HDL cholesterol and Triacylglycerol levels in IDDM, NIDDM and control individuals. Values are expressed in terms of M ±SD.

Groups	Control	IDDM	NIDDM
T. Cholesterol (mg/dl)	163±4.8	322 ± 49.9*	352.9 ±46*
LDL Cholesterol (mg/dl)	100 ±17	250 ±36*	280 ±25*
HDL Cholesterol (mg/dl)	53.1 ±5.6	29.3 ±4.8*	26.1 ±5*
Triacylglycerol (mg/dl)	65.9 ± 7.8	207.9± 44*	205 ±52*

*significantly different from control individuals at $p< 0.01$.

DISCUSSION

In diabetes mellitus, glycation of serum and tissue proteins is accelerated non enzymatically [29]. Hemoglobin, ferritin, albumin and low density lipoprotein are examples [30] . Glycation of ferritin not only modifies the properties of circulating protein but may also influences its removal from the plasma. Glycated ferritin is cleared more slowely (half life 50 hours) than non glycated one (half life 5 hours) [31] This may explain the high level of ferritin observed.

Transferrin demonstrated significant decrease of both diabetic categories. This may be due to its high urinary excretion rate. Previous reports supported such hypothesis [31,32] .

Up to 60% of patients suffering from idiopathic hemochromatosis (excessive iron stores in the body) may develop diabetes mellitus. Accordingly iron deposition in certain organs especially liver and pancreatic parenchyma may be a causal factor [33] .

The reason for the abundance of (apo) ferritin in the β- cells is not clear till now. Such amount is more than sufficient, essential to complex with iron present in the islets. Accordingly, metals other than iron may present with ferritin and complexing with them [34]. Prominent application here is the presence of zinc in β--cells, such zinc may displace iron from ferritin [35,36]. Another potential reason here, ferritin has antioxidant properties, again these cells are very sensitive to oxygen radical attack [37] .

Human and experimental studies demonstrated that lipid peroxidation products showed significant increase in diabetes [38,39] . This was observed in the present work. Gerli et al [40]. hypothesized that oxygen free radicals may explain the long-term complications of diabetes mellitus. Baynes [10] postulated that, incomplete scavenging of reactive radicals leads to oxidation of cellular lipids, proteins, nucleic acids and glycoconjugates. The resulting damage at the cellular level was observed as oxidation, fragmentation and cross linking.

Hyperferritinemia in diabetics may be attributed to a reactive and compensatory defense mechanism by the body to elevate antioxidant scavenger capability in those patients in comparison to normal control [37 - 39].

It was suggested that hypertriglyceridaemia in diabetics was associated with either a decrease in lipoprotein lipase (LPL) activity (insulin dependent) involved in triacylglycerols removal [41,42] or due to over production of LDL -cholesterol by the liver [43]. LPL is also relevant to HDL -cholesterol production. It was reported that enhanced lipolysis of triglyceride-rich lipoprotein may lead to an increase in HDL-cholesterol. Therefore a precursor product relationship exists between the two [44].

As a conclusion, this study demonstrated that, poorly controlled diabetic patients usually suffer from hyperferritinemia with normal iron concentration, additionally an increase in oxidation derived products.

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دراسة علاقة حديد الدم ومركباته مع التوتر المؤكسد فى مرضى البول السكرى

محمد السويدى – سوسو إبراهيم – سامى صالح * – دينا أبو المعاطى
قسم الكيمياء الحيوية – كلية الصيدلة – جامعة الزقازيق وجامعة قناة السويس* – مصر

تستهدف الدراسة الحالية العلاقة بين نسبة حديد الدم ومركباته والدهون فوق المؤكسدة ونسبة الهيموجلوبين المسكر فى مرضى البول السكرى سواء المعتمدين أو غير المعتمدين على الإنسولين .

وقد شملت هذه الدراسة عينات عشوائية من مرضى البول السكرى من الجنسين (٣٠ مريضا معتمد على الأنسولين منهم ٢٥ أناث ، ٥ ذكور) (٤٠ مريضا غير معتمد على الأنسولين منهم ٣٢ أناث و ٨ ذكور) من المترددين على العيادة الخارجية لمستشفي الزقازيق الجامعى ، تتراوح أعمارهم ما بين ٣٥ – ٥٢ سنه كما تم إختيار مجموعة ضابطة من الأصحاء مولفة من ١٥ متطوعاً (١٠ أناث ، ٥ ذكور) وقد تم قياس المعدلات التالية فى بلازما الدم : الحديد ، الفريتين ، الترانسفيرين ، الهيموجلوبين المسكر ، الدهون فوق المؤكسدة ، الكوليسترول (الكلى ، منخفض الكثافة ، عالى الكثافة) والجليسريدات الثلاثية . وقد سجل كلا من الهيموجلوبين المسكر والدهون فوق المؤكسدة والفريتين والكلوليسترول (الكلى ومنخفض الكثافة) وكذا الجليسريدات الثلاثية ارتفاعاً معنوياً بينما انخفضت مستويات كل من الترانسفيرين والكلوليسترول عالى الكثافة.

ويستخلص من هذا البحث أن مرضى البول السكرى الغير الخاضعين للرعاية الطبية عادة ما يعانون من زيادة نسبة الفيرتين ونواتج أكسدة الدهون.