Estimation of Oxidative Stress and Some Trace Elements in Iraqi Men Patients with Type 2 Diabetes Mellitus

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Abstract

Type 2 diabetes mellitus (T2DM) is a chronic disorder that is associated with the imbalance of trace elements which are involved in many functions especially enzyme activities. Changes in the levels of serum elements probably can create some complications in type 2 diabetes mellitus. Previous experimental and clinical studies report that oxidative stress plays a major role in the pathogenesis and development of (T2DM). However, the exact mechanism of oxidative stress could contribute to and accelerate the development of (T2DM).

The aim of this study contained the following sections: firstly, to determine some biochemical parameters in subjects with type 2 diabetes mellitus (T2DM) like lipid peroxidation marker, malondialdehyde (MDA), total antioxidant capacity (TAC), and glycated haemoglobin (HbA1c). Secondly, to determine serum and urine levels of zinc as trace element and serum level of iron, then to compare those parameters with that for age matched healthy individuals.

The study performed on 45 Iraqi men, who attended Baghdad Teaching Hospital. They were grouped into to 2 groups based on fasting serum glucose (FSG), and (HbA1c) value first group was included 20 healthful persons with A1c <6.4% as control non diabetic group, second group was included 25 patients with A1c>6.4% as T2DM.

Outcomes of this study demonstrated a clear increase in T2DM in fasting serum glucose, (HbA1c), MDA values, urine concentration of zinc , and serum level of iron compare to control.T2DM shows a significant reduction in both TAC and serum level of zinc ,if compared with control group .

Keywords: Type 2 diabetes mellitus, Trace elements, Lipid peroxidation.

تقدير جهد التاكسد وبعض العناصر النزرة لمرضى عراقيين مصابين بداء السكري النوع الثاني

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الخلاصة

مرض السكر النوع الثاني هو اضطراب مزمن يترافق مع اختلال مستويات العناصر النزرة التي تشارك في العديد من الوظائف خاصة الفعاليات الانزيمية.ان التغيرات في مستوى مصل هذه العناصر ربما تولد بعض التعقيدات في مرض السكر النوع الثاني .الدراسات التجريبية والسريرية السابقة اظهرت بان جهد التاكسد يمكن ان يلعب دورا رئيسيا في مرضية وتطور مرض السكر النوع الثاني .ومع ذلك فان الالية الدقيقة لجهد التاكسد يمكن ان تساهم في تسريع وتطوير هذا المرض.

الهدف من هذه الدراسة تضمن ، أولا تقدير المعايير الكيموحيوية في المرضى الذين يعانون من مرض السكري النوع الثاني (T2DM)مثل ,مؤشر الاكسدة الفوقية , مالون ثنائي الألديهايد (MDA), السعة الكلية لمضادات الأكسدة(TAC) والهيموغلوبين المسكر(HbA1c) اضافة لفحص مستوى مصل سكر الصائم(FSG).ثانيا تقدير مستوى الزنك في مصل الدم وفي الادرار وكذلك تقدير مستوى الحديد في مصل الدم لدى هؤلاء المرضى , ومقارنة النتائج مع مجموعة اشخاص اصحاء مطابقة بالعمر لمجموعة المرضى.اجريت الدراسة على (٤٥) رجل عراقي ، من الذين أدخلوا الى مستشفى بغداد التعليمي لتلقي العلاج.تم تقسيمهم الى مجموعتين على اساس مستوى سكر الدم الصائم والهيموغلوبين المسكر (HbA1c).

المجموعة الاولى تالفت من (٢٠) فرد قياسA1c<6.4%كمجموعة ضابطة من الاصحاء,المجموعة الثانية تالفت من(٢٥) مريضا قياسAc1>6.4%باعتبارهم مرضى السكري النوع الثاني.

أظهرت نتائج هذه الدراسة ارتفاعا معنويا في مرض السكري النوع الثاني مقارنة مع المجموعة الضابطة في كل من مستوى مصل سكر الصائم(FSG) ,الهيموغلوبين المسكر(HbA1c),مؤشر الاكسدة الفوقية(MDA),مستوى الزنك في الادرار,ومستوى الحديد في مصل الدم. في حين اظهرت الدراسة انخفاضا معنويا لدى مجموعة المرضى مقارنة بالمجموعة الضابطة في السعة الكلية لمضادات الاكسدة(TAC) ومستوى الزنك في مصل الدم.

يمكن الاستنتاج ان الشد التاكسدي الذي تسببه الجذور الحرة مع انخفاض قيمة مضادات الاكسدة ,قد تلعب دورا هاما في مرض السكري النوع الثاني بسبب تزايد مستويات (MDA) ومستوى الحديد في مصل الدم وتناقص مستويات الزنك و(TAC)في مصل الدم

الكلمات المفتاحية: مرض السكري النوع الثاني، العناصر الضئيلة، الاكسدة الفوقية للدهون.

Introduction

Diabetes Mellitus is a long - term chronic disease marked by derangements in sugar, lipid, and protein metabolism caused by abnormalities in insulin release,

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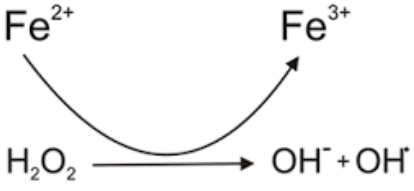
insulin action, or both. In 1979, the National Diabetes Data Group developed a categorizing and identifying plan for diabetes [(1)]. The level of HbA1c relies on the level of glucose in the blood stream and the time-period of hyperglycemia. The changes in the level of HbA1c in diabetic patients can be used to follow the efficiency of treatment for the diabetes.T2DM, is associated with increased metabolic processes and oxidative stress .The trace elements are important co-factors in these events. Impaired metabolism of these minerals play a role in the progression of T2DM and later development of complications. Trace element participate in production of reactive oxygen species (ROS), which contribute to oxidative stress. Oxidative stress contributes to the pathogenesis of many diseases including T2DM affect a cascade of reaction series resulting in cellular injury and illness [(2)]. Malondialdehyde is an indicator of fat peroxidation and rises in oxidative stress status. Free MDA, the chemically active form, helps as a marker of last injury [(3-6)].

Antioxidants are unique organic compounds that are active in the prevention of very rapid harmful chemical chain reactions with oxygen or nitric oxide, that is, oxidation reactions. Antioxidants interact with the free radicals before they are successfully interacting with other molecules, which can provide defense against oxidation reaction [(7)].

Trace elements form a part of daily eating are known to play major role in the maintenance of health [(8)]. Literature survey shows that some trace elements like zinc plays an important role in synthesis ,storage and secretion of insulin ,also increase the insulin ability to bind to its receptor[(9)], also serving as cofactor or components for enzyme systems included glucose metabolism [(10)].

On the other hand, serum iron effects on glucose metabolism even in non-existence of increased iron overload or even in a state of iron deficiency. Iron is a transition of metal and a possible catalyst in cellular reactions through Fenton reaction:



These free radicals can be quite harmful, particularly for cellular components such as lipids, proteins and nucleic acids. Free radicals, when oxygen present, reacts with membranes lipids double bonds generating lipid peroxides that can spread the damage throughout the membrane.

In erythrocytes, ROS can induce hemolysis. Free radicals can oxidize lateral amino acidic groups, impairing protein function by promoting cross-linkage formation such as disulfuric bond, causing mutation in protein structure or folding .Also DNA structure can be damage or modified by free radicals, through induction mutations in azotate bases (this show how ROS can be responsible of cellular aging and cancer induction), resulting in a state of oxidative stress and finally cell damage death [(11)].

Experimental part

Patient selection and blood sampling

In the present study, 45 Iraqi men, who attended Baghdad Teaching Hospital, classified into a couple of groups. First group includes 20 apparently healthy subjects {by clinical examination and with no history of diabetes (A1C <6.4%)}. Their age ranged (45± 10) years. Second group included 25 patients with type 2 diabetes (A1C>6.4%), their age ranged (52±12) years diagnosed by specialized physician. It was adopted HbA1c in the diagnosis of T2DM according to A report published in 2009 by an International Expert Committee on the role of HbA1c in the diagnosis of diabetes recommended that HbA1c can be used to diagnose diabetes and that the diagnosis can be made if the HbA1c level is ≥6.5%[(12).]

Blood was collected by vein puncture into plain tubes with no EDTA. Serum was separated from the cells by centrifuging at 3500 rpm, and then divided into small portions and kept frozen until analysis.

Determination of serum glucose levels

Glucose was determined after enzymatic oxidation in the presence of glucose oxidase [(13)]. The formed hydrogen peroxide composed reacts within the catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinone imine dye as indicator.

Determination of glycated hemoglobin (HbA1c)

Nycocard HbA1c is a boronate affinity assay traceable to the international federation of clinical chemistry and laboratory medicine (IFCC) reference method for measurement of HbA1c. The reagent contains agents called lyse erythrocytes and precipitates hemoglobin specifically, as well as a blue boronic acid conjugate that binds cis-diols of glycated hemoglobin [(14)].

Determination of malondialdehyde (MDA)

The concentration of MDA in serum was determined according to Buege and

Aust method of enzymology [15]. MDA formed from breakdown of poly unsaturated fatty acid (PUFA). MDA reacts with thiobarbituric acid (TBA) to give pink color that is read at λ max535nm. MDA was calculated according to the molar extinction factor of 1.56×105 [16].

Determination of total antioxidant capacity (TAC)

ABTS [2, 2-Azino-bis-(3-ethylbenzthiazoline sulphonate)] is incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation $ABTS^{\cdot +}$. This has a relatively stable blue-green color, which is measured at λ = 600nm. Antioxidants in the added sample cause repression of this color production to a degree which is proportional to their level [17].

Trace metals determination

Levels of the selected metals were estimated by flam atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan). The amount of radiant energy absorbed is proportional to its concentration. The serum diluted to 10 ml with hydrochloric acid in 10 ml centrifuge test tube. The diluted samples displayed directly to the flame. Zinc was measured at λ = 213.86 nm, whereas iron was measured at λ = 248.33nm.

Statistical analysis

Student's t-test was applied to compare the significance of the difference in the mean values of between studied groups, and ($p < 0.05$) was considered statistically significant, while $p<0.001$ was considered highly significant. (Microsoft office 2013–Excel).

Results and Discussions

Table (1) shows the serum levels of some biochemical measures in two studied groups. The fasting serum glucose levels show a high significant increase (P<0.001) for patients (190 ± 18.60) mg/dl compared to control (80±7.5) mg/dl.

Hyperglycemia plays a critical role in the growth and progression of diabetic nephropathy, but the mechanism is not clear. The possible sources of raised oxidative stress might involve increased production of free radicals or weakened antioxidant protection system, enhances free radicals formation in diabetes. Our results are in agreement with the recent study that reports an increase in oxidative stress as depicted by increase in MDA with the concomitant decrease in TAC as compared to controls. MDA concentration increases with the severity of T2DM. TAC summarizes the overall activity of antioxidants and antioxidant enzymes. The cooperation between different antioxidant pathways provides greater protection against attack by ROS, compared to any single compound. Thus the overall antioxidant capacity may give more relevant biological information compared to that obtained by the measurement of individual biomarkers [18].

Table (1): Levels of FSG, HbA1c, MDA and TCA in sera of patients with T2DM and healthy subjects.

Parameters	Control mean ±SD	patients mean ±SD	P-value
FSG(mg/dl)	80± 7.5	190± 18.60	P <0.001
HbA1c (% of total Hb)	5.40± 0.11	8.10± 1.30	P <0.001
MDA (µmol/L)	1.75± 0.20	5.60± 0.82	P <0.001
TAC (mmol/L)	0.80± 0.26	0.29± 0.12	P <0.001

P-value: Probability, SD: Standard Deviation.
FSG: Fasting Serum Glucose.
HbA1c: Glycated haemoglobin. +9
MDA: Malondialdehyde.
TAC: Total Anti-Oxidant.

Results of glycated hemoglobin display a significant increase (P<0.001) in sera of patients (8.10±1.30) opposed to control (5.40±0.11) values.

The classification of the groups in the current study was depending on the levels of HbA_1c to assess a diabetic from a non-diabetic individual. It has been reported that the prevalence and interference between intermediate hyperglycemia was defined by HbA1c (5.7-6.4) %. This range was proposed as an indicator of Type2 diabetes mellitus, since the advantage of using HbA1c is less day to day variability compared with glucose tolerance test and fasting glucose levels [19]. According to World Health Organization (WHO), the American Diabetes Association (ADA) has suggested 5.7 – 6.4% as the high risk range, therefore we can use HbA1c levels in diagnosis of T2DM [20].

Results of MDA showed highly significant increase (P<0.001) in patients (5.60±0.82) µmol/L compared to control (1.75±0.20) µmol/L.

The present results are compatible with reported data that strongly confirmed the evidence that diabetic patients were susceptible to oxidative stress and higher blood glucose level had an association with free radical-mediated lipid peroxidation. There appears to be mismatch between oxidant and antioxidant defence systems in type 2 diabetic individuals [(21)].

Results of the present study consist with others who found that oxidative stress is increased in diabetes and the over generation of reactive oxygen species (ROS) is a direct consequence of hyperglycaemia. Diabetes patients have more severe oxidative stress than normal persons [(22)].

Total antioxidant capacity values showed highly significant decrease (P<0.001) for patients (0.29±0.12) mmol/L as compared to the controls, (0.80±0.26) mmol/L.

Our results are in good agreement with the recent study that reports an increase in oxidative stress as depicted by increase in MDA with the concomitant decline in TAC as compared to controls [(23)].

Total antioxidant capacity summarizes the total activity of antioxidants and antioxidant enzymes. The collaborating between diverse antioxidant pathways gives greater defense against assault by reactive oxygen species, compared to any solo compounds. Therefore, the general antioxidant capacity may provide further relevant biological data compared to that achieved by measuring of individual biomarkers [(24)].

Results of the present study were in agreement with a study indicated that the oxidative stress was boosted and there was an imbalance between oxidant – antioxidant defense according to the seriousness of the diabetic nephropathy. In patients at the acute phase of the disease decreased total antioxidant capacity may produce to abnormal lipid peroxidation, resulting in a high rate of glomerular injury. On the other side, continued lipid oxidation may produce to diminished antioxidant activity [(25)].

As shown in Table (2) serum of zinc level is significantly decrease (P <0.05) for patients (0.58±0.11)mg/dl as compared with control (0.90±0.52) mg/dl, whereas urine level of zinc increases significantly (P <0.05) for patients (0.71±0.31) mg/dl compared with control (0.43±0.12) mg/dl.

Serum zinc levels of diabetics were obviously lower than those of non-diabetics; also, urine zinc levels were higher in diabetics than non-diabetics studied. The cause of decreased level of serum zinc in diabetes may be due to increased urinary excretion due to reduction in renal function associated with disease, gastrointestinal malabsorption genetic factors or signs of function during which zinc acts as a defence mechanism [(26)].

Zinc and insulin levels in the pancreas modify in the same orientation in a diversity of conditions in humans [(27)], it is helpful in creation, store, and secretion of insulin [(28)]. The current results demonstrate that the level of zinc is reduced in the serum of diabetic patients (Table-2). The loss of these metals might be assigned to weakened absorption or to the excess excretion of these minerals in urine in these individuals, which may indicate a deficiency of these metals in serum of diabetic subjects and that is compatible with the literature [(29)].

Results of serum Fe in Table (2) showed a significantly increase (P<0.05) in patients (0.83±0.01) mg/dl compared to control (0.69±0.05) mg/dl, which including the radical forms due to oxidation of ferrous form.

Recent studies shows an increase in iron could expect the danger of evolving type 2 diabetes, while the reduction in iron level is protective [(30)]. Tissue iron excess will rise the generation of free radicals which in turn magnifies the steps implicated in inflammatory damage [(31)]. Iron is closely related to oxidative stress. Highly toxic free radicals, such as hydroxide and the super oxide anion, which leads to lipid peroxidation, which generated by iron through the Fenton reaction [(32)].

Table (2): Levels of serum Zn and Fe concentration, urine Zn concentration in patients with T2DM and healthy subjects.

Parameters	Control (mean ±SD)	patients (mean ±SD)	P-value
Zn(mg/dl) (serum)	0.90 ±0.52	0.58 ±0.11	P <0.05
Zn(mg/dl) (urine)	0.43 ±0.12	0.71 ±0.31	P <0.05
Fe (mg/dl)	0.69 ±0.05	0.83 ±0.01	P <0.05

Conclusion

Oxidative stress, which caused by free radicals production with impairment of antioxidant, play an importance role in T2DM, because of the increasing levels of MDA and serum level of iron and declining levels of TAC and serum level of zinc.

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