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Evaluate the Plasma Iron Levels on Iraqi Type 2 Diabetic Nephropathy

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ABSTRACT

Diabetes is linked with a change of homeostasis for the plasma iron in human, that have the ability to produce reactive oxygen species (ROS) induced by this disease and its microangiopathy complication especially diabetic nephropathy (DN). In addition, iron indicators are associated with obesity and insulin sensitivity as contribute to the development and improvement of oxidative damage. The objective of the study was to measure and evaluate the level of iron in the plasma in diabetic patients with and without DN, and in normal individuals, The current study was conducted on sixty T2D patients diagnosed beforehand, these patients were categorized into two equal groups according to their albumin to Creatinine ratio (ACR), including patient with nephropathy (UAC=30-300 mg/g Creatinine), **(I)** and patients without nephropathy (UAC<30mg/g Creatinine), **(II).** Twenty four healthy persons were chosen as a control group**.** Each group included the same numbers of male and female. The age of patients ranged from (36-65) year . The results showed that the plasma level of iron showed a high significant increasing in patients without DN group (177.10±76.36 µg/dl) compared to patients with DN and healthy control groups (126.77±61.16 vs 116.79±26.16 µg/dl, respectively).

Introduction:

Diabetes is characterized by chronic hyperglycemia, which is caused by the defective insulin action or excretion or both, various pathogenic processes the β-cell, in the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced, primarily in type 1 diabetes (T1DM) – to abnormalities that produce resistance to insulin action in the liver and other peripheral tissue – primarily in type 2 diabetes (T2DM). The various forms of diabetes are classified in accordance with their etiology: T1DM and T2DM are the most common [1]. Diabetic kidney disease is characterized by a progressive increase of proteinuria that is a urinary albumin secretion of more than 300 mg in day collection, and abnormal kidney function due abnormality in serum Creatinine, glomerular filtration rate (GFR) and calculated Creatinine clearance, also represented in high blood glucose and a high risk of cardiovascular mortality and morbidity. Pathological lesions associated with T2DM and kidney disease are similar to those associated with kidney disease in T1DM. The conventional lesions, of diabetic kidney disease are mesangial extension, diffuse hyalinosis and thickened glomerular basement diaphragm of afferent and efferent arterioles [2]. These pathologies are used in a new classification system for diabetic kidney disease: Class I – GBM thickening only; Class II – mild to severe mesangial expansion; Class III – including nodular sclerosis; Class IV – all of the above changes and 50% globally sclerotic glomeruli [3]. Iron is an essential metal for hemoglobin synthesis of red blood cells, cellular proliferation, redox reactions and cellular proliferation, while the excess iron accumulation causes organ dysfunction through the generation of reactive oxygen species. The total amount of body iron is ~3–4 g, two-thirds of which is composed of erythrocytes iron and recycled iron by red blood cell destruction, and the remainder is stored in ferritin, whereas only 1–2 mg per day of iron is absorbed through the

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intestine, and circulated in the blood. In the circulation, iron is usually bound to transferrin, and most of the transferring-binding iron is used for bone marrow erythropoiesis [4].

A main reason is an unsuitable erythropoietin response to anemia, often accompanied by iron insufficiency and treatment with angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, iron reduction therapy, has been shown to increase hepatic insulin sensitivity, raise pancreatic insulin sensitivity, diminishHbA1c, development liver assignment tests, and progress low-grade inflammation, in patients with insulin resistance-correlated with hepatic iron overload syndrome. Anemia as a common complication of diabetic nephropathy, appearing prior than in nondiabetic renal disease and expansion the risks of cardiovascular and microvascular complications. [5].

Iron levels in the body when be high with elevated levels of oxidative strain that may increment the risk of T2DM [6], epidemiological research has indicated a positive correlation between high body iron stores, insulin resistant and the risk of T2DM, such as, gestational diabetes, metabolic syndrome and polycystic ovarian syndrome [7]. Elevated ferritin levels in T2DM are correlated with the improvement of diabetic microvascular complications, perhaps during the overlap with vascular endothelial growth factor or during endothelial dysfunction [8]. It has also been recommended that an elevated transferrin secretion in diabetic patients, with micro albuminuria may contribute to tubulointerstitial injuries as a result of transferrin reabsorption, by proximal tubular epithelial cells. This elevates intracellular iron concentrations leading to oxidative damage of the tubular cells [9].

Patients and Methods:

The present study was conducted in the National Diabetes Station for Treatment and Academy at Al-Mustansiriya University between (February 2016-April 2016), our study included sixty T2D patients who were divided into two equal groups, group **I**, T2DM with normoalbuminuria ([albumin to Creatinine ratio] <30 mg/g Creatinine) and group **II,** T2DM with microalbuminuria (albumin to Creatinine ratio=30-300 mg/g Creatinine). The two diabetic groups were described according to urinary albumin secretion, their ages ranged within (36-65) years for both genders. The Urine specimens were collected from each individual into a sterile container and utilized for the measurement of U.Mic, U.Cr and ACR. Venous blood specimens were collected from diabetic patients and controls into two sorts of vacutainer tubes as follows: first venous blood sample was collected in plain tube and left to clot for (30min) at room temperature and then centrifuge to separate at (3000rpm) for (10min) utilized for the biochemical examines. Second tube with EDTA, mixed gently by the shaker and then centrifuged to separate the plasma for 15min at 1000xg (2-8 c°) within30 min of accumulation and then transferred to a new polypropylene tube, plasma samples were stored at -20°C until assayed.

Fasting plasma glucose (FPG) level was determined according to a Trinder reaction by utilizing the glucose oxidase method, Cat. No. REF87409). Blood hemoglobin was measured according to the colorimetric method of drabkin's (Cat. No. REF1134015).

The iron is dissociated from transferring-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions give with FerroZine a colored complex, the intensity of the color formed is proportional to the iron concentration in the sample.

$$\text{Transferrine} + Fe^{+3} \xrightarrow{\text{Ascorbic acid}} \text{Transferrine} + 2Fe^{+2}$$
$$Fe^{+2} \xrightarrow{\text{FerroZine}} \text{Coloured complex}$$

Statistical Analysis

All information are expressed as means SD, unless stated otherwise. In all cases, P< 0.05 was viewed significantly (two-tailed). Data were put away and processed by utilizing a commercially obtainable (SPSS for Windows, Version 20.0).

Results and Discussion

The statistical analysis of results showed that there are non-significant differences of ages between the two studied groups of diabetic patients (patients with DN=52.93±7.77 vs patients without DN=52.77±8.05 year) and healthy control groups (52.08±9.21 year), as shown in **Table 1,** as well as the patients with DN group showed a significant difference in BMI (29.02 ±3.75 kg/m^2**)** as compared to a healthy control group (23.69±2.29 kg/m^2), and the patients

without DN group also showed a significant difference in BMI (28.50 ±3.82, kg/m^2**)** as compared to a healthy control group (23.69±2.29 kg/m^2).

As apparent from the **Table 1** and the mean levels of FPG were significantly higher in patients with DN group (218.17±65.10 mg/dl) as compared to the healthy control group (91.21±7.64 mg/dl), as well as the patients without DN group showed a significantly higher in levels of FBG (184.30±42.85 mg/dl) as compared to a healthy control group (91.21±7.64 mg/dl), while the patients with DN and patients without DN groups showing a significant difference in the levels of FBG (218.17±65.10 vs 184.30±42.85 mg/dl, respectively). In the present study, the statistical analysis of results showed that there is a significant increase of plasma iron in T2DM without DN group (177.10±76.36 µg/dl) as compared to a healthy control group (116.79±26.16 µg/dl). On the other hand the patients with DN group also showed a significant increase of plasma iron (126.77±61.16 µg/dl) when compared to the patients without DN group (177.10±76.36 µg/dl), whereas the mean level of plasma iron of patients with DN group and healthy control groups did not show statistically significant differences (126.77± 61.16 vs 116.79±26.16 µg/dl, respectively), as shown in **Table 1 and Figure 1.**

As well as the patients with DN and patients without DN groups also showed non-significant difference in hemoglobin (Hb) between the two studied groups (14.20±4.52 vs 13.09±1.30 g/dl, respectively) and healthy control groups (12.89±1.30 g/dl), as shown in **Table 1.**

In the current study, as shown in **Table 2:** The mean urine-microalbuminuria was significantly higher among patients with DN group (117.67±55.69 mg/l) as compared to the patients without DN and healthy control groups (12.67±6.92 vs 13.33±7.61 mg/l, respectively). The statistical analysis shows no significant differences in the concentration of urine-Creatinine between the two studied group of diabetic patients (patients with DN=9.01±6.84 vs patients without DN=9.58±4.76 mmol/l) and healthy control groups (11.03±4.54 mmol/l)**,** as shown in **Table 2.**

The results of the present study show that urinary ACR was significantly increased in diabetic patients with DN group (17.39±9.95 mg/mmol) compared to healthy control and diabetic patients without DN groups (1.25±0.44 vs 1.34±0.50 mg/mmol, respectively) as shown in **Table 2.**

Table 1: Mean and Standard Deviation for Serum Biochemistry in Controls, Cases of DM without DN and Cases of DM with DN.

Parameters	Factor	Mean±SD	Sig.
Age Years	T2DM with DN	52.93±7.77	**a**
	T2DMwithout DN	52.77±8.05	**a**
	Control	52.08±9.21	**a**
BMI Kg\m^2	T2DM with DN	29.02±3.75	**a**
	T2DMwithout DN	28.50±3.82	**b**
	Control	23.69±2.29	**a**
FPG mg/dl	T2DM with DN	218.17±65.10	**a**
	T2DM without DN	184.30±42.85	**b**
	Control	91.21±7.64	**c**
Fe µg/dl	T2DM with DN	126.77±61.16	**a**
	T2DM without DN	177.10±76.36	**b**
	Control	116.79±26.16	**a**
Hb g/dl	T2DM with DN	14.20±4.52	**a**
	T2DM without DN	13.09±1.30	**a**
	Control	12.89±1.30	**a**

Notes: Similar letters mean no significant difference between the groups at a level of less than **0.05**, and various letters mean the existence of a significant difference at a level of less than **0.05**

Table2: Mean and Standard Deviation for U.Albumin, U.Cr and ACR in Controls, Cases of DM without DN and Cases of DM with DN.

Parameters	Factor	Mean±SD	Sig.
U. Albumin mg/L	T2DM with DN	117.67±55.69	a
	T2DM without DN	12.67±6.92	b
	Controls	13.33±7.61	b
U. Creatinine mmol/L	T2DM with DN	9.01±6.84	a
	T2DM without DN	9.85±4.76	a
	Controls	11.03±4.54	a
ACR mg/mmol	T2DM with DN	17.39±9.95	a
	T2DM without DN	1.34±0.50	b
	Controls	1.25±0.44	b

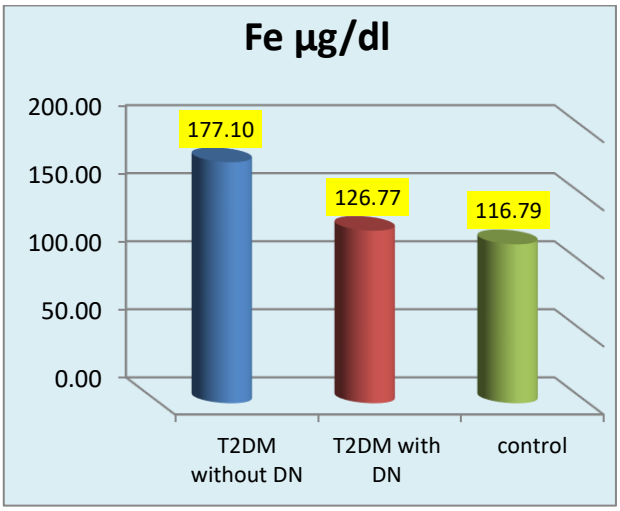

Figure 1. The Plasma Iron levels in T2DM Patients with DN, T2DM Patients without DN and Controls

According to some scientists, systemic iron overload contribute to abnormal glucose metabolism leading to T2DM by insulin deficiency as a result of oxidative stress on pancreatic beta cells, leading to cell death and decrease insulin secretion or insulin resistance caused directly by iron overload and hepatic dysfunction, iron is a potential catalyst in cellular reactions that produces oxygen reactive species such as hydroxyl radical (OH^-) and superoxide anion (O_2^-) that can initiate and propagate the cascade leading to oxidative stress and finally cell death, a significant impact of tissue iron excess on systemic effects of diabetes mellitus is suggested by recent research studies in which iron appears to influence the development of multiorgan dysfunctions in T2DM [10,11]. The Present study reveals that there is no difference in level values of plasma iron in patients without DN between male and female. Elevated levels of glucose accelerate, the generation of advanced glycation end-products (AGEs), AGEs are generated from the oxidation of proteins, lipids and nucleic acids and through the nonenzymatic glycation [12]. Increase transferrin saturation due to hemolysis or impairment of erythrocytes Hyperglycemia in T2DM it changes, in the osmolarity of the blood, which may be one of the factors for excess blood iron [13].

Elevated transferrin saturation also signals non effective erythropoiesis, iron accumulate in human tissues which may be damaging the insulin activity. As a result that may have lead to mild iron rise in type 2 diabetes without nephropathy [14]. Iron is a reactive metal ion, which reason the damage of cellular macromolecules by generating highly reactive oxygen radicals. Iron reduction of ferric state (Fe^{3+}) to the ferrous state (Fe^{2+}) state plays a central role in lipid peroxidation process. As the concentration of iron increases, eventually it combines in the liver. Ferritin, an iron storage protein may work as a source of iron for improvement of superoxide-based on lipid peroxidation. [15]

Conclusions

In concoction. Elevated plasma iron level in type 2 diabetic patients without DN is associated with a high risk of nephropathy and excess tissue iron increases the production of free radicals. Iron plays a pathogenic role in diabetes and its complexity, such as atherosclerosis and microvascular damage.

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تقييم مستويات الحديد في بلازما الدم لدى العراقيين المصابين بالسكر الكلوي من النوع الثاني

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الخلاصة

يرتبط مرض السكري مع توازن الحديد في الإنسان. حيث أن له القابلية على توليد الجذور الحرة وأنواع الأوكسجين التفاعلية (ROS) وترتبط مؤشرات الحديد مع السمنة والحساسية للانسولين والتي يمكن أن تسهم في تطور وتقدم الإعتلال الكلوي السكري . الهدف من هذه الدراسة تقدير ومقارنة مستوى بلازما الحديد في الأشخاص الطبيعيين وفي مرضى السكري مع أو بدون اعتلال الكللى السكري. أجريت الدراسة الحالية على ستون مريضاً مصاباً بمرض السكري النوع الثاني حيث كانت تتراوح أعمارهم بين (36–65)سنة لكلا الجنسين تم تشخيصهم مسبقاً. تم تقسيم المرضى الى مجموعتين متساويتين وفقا الى نسبة الألبومين–كرياتينين ضمت المجموعة الأولى المرضى الذين يملكون إعتلال الكلى وسميت مجموعة مرضى إعتلال الكلى السكري في حين إحتوت المجوعة الثانية المرضى الذين لديهم نسبة الألبومين طبيعية وسميت مجموعة المرضى بدون إعتلال الكلى السكري, ولغرض المقارنة تم إختيار أربع وعشرون شخصاً سليماً كمجوعة أصحاء. في المقابل مستوى بلازما الحديد أظهر زيادة معنوية عالية لدى مرضى السكري الذين لايمتلكون إعتلال الكلى السكري (177.10 ±76.36 مايكرجرام/ديسيلتر) بالمقارنة مع مجوعتي المرضى بدون إعتلال الكلى السكري والأصحاء(126.77 ± 61.16 و 116.79 ± 26.16, مايكرجرام/ديسيلترعلى التوالي).