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Evalution of Biomarkers in Iraq Patients with Diabetes Mellitus Type 2

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ABSTRACT

Background: Diabetes Mellitus is a chronic condition that happens when the pancreas fails to produce sufficient insulin or when the body's insulin production is ineffective.

Objectives: This study's aim was to investigate into the effects of Diabetes serum oxidants and antioxidants .

Materials and Methods: Samples of blood were taken from (50) patients with diabetes , furthermore (50) healthy individuals served as the control group.. Those who participated were separated into two groups as follows:Group (control), consist of fifty healthy individuals aged (20-70years) and fifty people with diabetes were included in the patient group (20-70 years). The study included 45% females and 55% males. Regarding each group, 52% of participant in control group were males and 48% were females while in patients 58% were males and 42% were females. Marker for the lipid profile, The mean difference of fasting blood sugar, HbA1c and preventative antioxidants Total Protein, Albumin, Globulins, Albumin/Globulins ratio, serum urea, serum creatinine and Uric acid patients with diabetes were done.

Results: The findings indicate that there has been a significant increase in the level of FBS, HbA1c, TC, TG, LDL, VLDL, urea, creatinine and uric acid in patients groups when compared to the control group. A statistical significant lower mean of total protein and HDL in compare to control. result revealed a statistical significant higher mean of cupper in patient group in compare to control and a significant lower mean of iron in patient group in compare to control .

Conclusion: The results included in this study showed dyslipidemia, renal disfunction and icrease in copper and iron due to the long duration of diabetes mellitus and the complication of disease.

Keywords: Diabetes Mellitus , blood sugar, protein, lipid profile and renal function markers, antioxidant , trace elements .

INTRODUCTION

Diabetes Mellitus is a chronic condition that happens when the pancreas fails to produce sufficient insulin or when the body's insulin production is ineffective [(1)]. Diabetes currently has no known treatment, but its effects can be well-balanced with proper health management and regular medical checkups, according to prior medical data. Blood sugar levels that are raised are indicated by increased appetite, increased thirst, and frequent urination[(2)].

When diabetes is not controlled, a number of negative effects may result. Heart disease, stroke, and other serious long-term effects such as chronic renal dysfunction, foot ulcers, and vision impairment[(3)]. The most dangerous clinical signs were ketoacidosis or a non-ketotic hyperosmolar state, which can cause dehydration, unconsciousness and death in the absence of effective treatment [(4)].

Early detection of diabetes by the measurement of C-peptide might help distinguish between type I and type II diabetes[(5)]. According to the World Health Organization (WHO), diabetes mellitus is classified as following[(5)].

Diabetes mellitus type 1 is a form of the disease that is predominantly caused by the destruction of pancreatic beta cells and is prone to ketoacidosis. This category contains examples of autoimmune beta cells destruction with unknown cause. Diabetes mellitus type 2 (caused by an ongoing insulin secretion deficit in the face of insulin sensitivity). Gestational diabetes mellitus is a form of glucose intolerance that appears during pregnancy or is first identified there (pregnancy's second or third trimester), concealed diabetes . Other causes of diabetes include drug- or chemical-induced diabetes (such as following HIV/AIDS treatment or organ transplantation), exocrine pancreas diseases (such as cystic fibrosis), monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]) and exocrine diabetes syndromes[(6)].

Fasting blood glucose (FBG) (≥ 7.0 mmol/L) test needed to stay at least 8 hours without eating any calories. Glycated hemoglobin (HbA1C) (≥ 6.5% in adults), used when there are no variables influencing the accuracy of the HbA1C and when type 1 diabetes is not suspected. This high hepatic glucose production persists even after fasting plasma insulin levels are raised 2.5 to 3 times, demonstrating a substantial resistance to the insulin's inhibitory effects [(7)].

The adaptive response of the kidney to maintain glucose in diabetes turns maladaptive, making it difficult for the body to meet its energy needs, especially those of the brain and other neurological illnesses, which are absolutely dependent on glucose. To control hyperglycemia, the kidney stores the glucose rather than releasing it into the urine[(8)].

A significant intracellular antioxidant, glutathione (glutamyl-cysteinylglycine), was essential for minimizing the effects of oxidative stress[(9)]. Ceruloplasmin might function as a pro-oxidant by contributing free copper ions, which triggers the production of reactive oxygen species (ROS) and the

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oxidation of low-density lipoprotein (LDL). Additionally, as ceruloplasmin is a protein found in the acute phase, its amount represents both acute and chronic inflammation in an organism. Due to the high frequency of solid tumors and the considerable rise in blood ceruloplasmin levels in individuals with locally progressed or regionally expanding malignancies, ceruloplasmin was a possible marker for diabetes mellitus[10].

Numerous metabolic processes, including those involved in insulin and glucose metabolism, were made possible by trace elements. For insulin to be produced and packaged into vesicles effectively, zinc must be transported into the pancreatic beta cells. Additionally, zinc-alpha2-glycoprotein and zinc finger protein 407 [11].

This study's aim is to evaluate oxidative stress by assessing the total protein, albumin (Alb), lipid profile and renal function markersin patients with diabetes, to measure ceruloplasmin (Cp) and uric acid (UA) in order to assess the serum antioxidant state in the mentioned disease, glutathione (GSH) copper; Iron; Zinc (Cu;Fe;Zn) and compared with the control group.

SUBJECTS AND METHODS

Study Design

This study was conducted on sixty diabetic patients group and forty apparently healthy control group. All studied groups (patients and controls) were from the same ethnic group admitted in Baghdad Teaching Hospital in Baghdad Teaching Hospital/ Diabetes department examination in Baghdad-Iraq in the period between September 2021- January 2022.

Blood Samples

Ten milliter of veinus blood were obtained from all patients and control groups; two ml was put into (EDTA) tubes and 8ml put into gel tubes containing gel help in separation of the serum. The EDTA tubes was stored in -20˚C in order to be used later in the genetic part of the study, while blood in the gel containing tubes was allowed to clot at room temperature for 30 minutes and then centrifuged at 2000×g for approximately 15 minutes then the sera were obtained and stored at -20˚C until analysis.

Biochemical Parameters

Determination of Total Protein

Assesment of serum total protein were performed according to biuret method The TP level was calculated as follows: TP con. (g/dl) = (A)Sample/(A)Standard ×6 (Standard conc.).

Determination of Albumin

In acidic buffer solution (pH 4.2), the greenish bromocresol binds albumin in the sample to form a colored reaction. Albumin was measured at λ=630 nm and the intensity of the resulted color is proportional to the albumin concentration in the specimen [12].

The level of albumin was calculated as follows:

Albumin conc. (g/dl) = (A)Sample/(A)Standard ×6 (Standard conc.)

Assessment of Globulin

Globulin in the serum samples was calculated from the following equation

Globulin conc. (g/dl) = TP conc. – Albumin conc.

Assessment of Glutathione (GSH) and Ceruloplasmin (CP)

Human Glutathione (GSH) and Ceruloplasmin (CP) were determined using Sinogene ELISA kit.

Determination of copper, Iron, Zinc (Cu;Fe;Zn)

Determination of copper, Iron, Zinc were done by analytical technique known as Flame Atomic Absorption Spectroscopy (FAAS) [13]

Ethical Approval

The study was approved by the Ethics Board of University of Baghdad and an informed written consent was taken from each participant in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The recorded data were examined using statistical software for social sciences, version 20.0. (SPSS Inc., Chicago, Illinois, USA). The Student t test was used to express and compare the mean and Standard Deviation (SD) of quantitative data. To compare qualitative data that was presented as frequency and percentage, Chi2 tests were conducted. As the level of confidence was maintained at 95%, a P value of 0.05 was considered significant.

RESULTS

The result revealed that patients had significant higher level of FBS, HbA1c, TC, TG, LDL, VLDL, urea, creatinine and uric acid level in compare to control group, p-value < 0.0001, < 0.0001, < 0.0001, < 0.0001, < 0.0001, 0.02, < 0.0001, 0.03, < 0.0001 and < 0.0001, respectively. A statistical significant dicrease of total protein and HDL in compare to control, p < 0.0001 and 0.04, as presented in Table 1.

Table 1: Mean difference of FBS, HbA1c, Total Protein, Alb., Glo., Albumin/Globulins ratio, TC, TG, HDL, LDL, VLDL, urea, creatinine and Uric acid between patients and control group

	Control	Patients	p-value
FBS (mg/dl)	91.36 ± 7.76	202.84 ± 5.21	< 0.0001*
HbA1c %	5.07 ± 0.41	9.62±1.51	< 0.0001*
Total Protein (g/l)	77.21±8.78	68.24 ± 12.20	< 0.0001*
Albumin (g/l)	45.23 ± 4.32	37.70 ± 7.77	< 0.0001*
Globulins (g/l)	31.88 ± 8.92	30.54 ± 5.89	0.47
Alb/Glo ratio	1.56 ± 0.30	1.37 ± 0.15	0.11
TC mg/dl	172.75 ± 23.91	199.14±31.36	< 0.0001*
TG mg/dl	109.49 ± 3.48	190.38 ± 8.16	< 0.0001*
HDL mg/dl	49.47 ± 10.84	45.37 ± 5.09	0.04*
LDL mg/dl	101.38 ± 22.69	115.69 ± 3.71	0.02*
vLDL mg/dl	21.89 ± 3.29	38.07 ± 5.63	< 0.0001*

*p-value ≤ 0.05

Regarding the mean of trace element and some antioxidant markers, our results revealed a statistically significant higher mean of cupper in patient group (0.68 ± 0.11) in compare to control (0.47 ± 0.05), p-value< 0.0001; and a significant lower mean of iron in patient group (3.5 ± 0.49) in compare to control (3.9 ± 0.01), p-value< 0.0001.

No statistical significant difference in mean of glutathione (0.23 ± 0.04), ceruloplasmin (0.27 ± 0.06) and zinc (0.75 ± 0.37) in compare to control (0.25 ± 0.06, 0.24 ± 0.06, 0.69 ± 0.53), p-value 0.13, 0.09 and 0.52, respectively, as presented in Table 2.

Table 2: The comparison of mean difference of trace element and anti-oxidant markers according to study group

Parameter	control	Patients	P-value
GSH(µmol/L)	0.25 ± 0.06	0.23 ± 0.04	0.13
Cp (µmol/L)	0.24 ± 0.06	0.27 ± 0.06	0.09
Cu (µmol/L)	0.47 ± 0.05	0.68 ± 0.11	< 0.0001*
Fe (µmol/L)	3.9 ± 0.01	3.5 ± 0.49	< 0.0001*
Zn	0.69 ± 0.13	0.75 ± 0.17	0.05

*p-value ≤ 0.05

DISCUSSION

Estakhri M et al [14] study observe higher mean level of FBS and HbA1c in DM patients that agreed with current finding.

Reduction albumin and total protein level was noticed with no change in globulin and A/G ratio. Those finding were compatible with Hasan et al. [15] study. However, the current study also observed an increase in the mean of globulin in DM patients. Chronic hyperglycaemia increased glycosylated hemoglobin production, decrease glucose uptake by liver and muscle, hyperfiltration (perhaps resulting in an increase of 5%–10% in GFR) and glomerular hypertrophy in diabetic individuals and low production of protein. Alteration of protein half-life in DM was another cause for the decrease of total protein level [16].

Albumin is one of the most prominent plasma proteins that synthesis in the liver. Loss of the normal hepatic feedback inhibition of gluconeogenesis increase proteins and lipids destruction as well as the conversion of some amino acids (glucogenic) to glucose in the liver. Hemodiultion, loss of extravascular space due to increased vascular permeability in the inflammatory region in DM had been reported to cause decrease in albumin level [17].

Diabetic patients had higher mean level of all lipid biomarkers in comparison to control which was found in previous studies [18],[19],[20]. The dyslipidemia pattern often manifested with increased triglycerides, LDL, and decreased levels of HDL cholesterol (HDL-C). In patients with diabetes, insulin resistance is contributor of lipid abnormalities by increase adipose tissue releases of free fatty acids that were then taken up by the liver as a consequence of peripheral resistance to insulin; increase triglyceride production which require very low density lipoprotein cholesterol (VLDL) and in ApoB 19. This occurred through the action of the cholesterol ester transfer protein, triglyceride-rich VLDL enrich LDL and HDL, making them more cholesterol-rich. After being digested by hepatic or lipoprotein lipase, these triglyceride-rich LDL molecules were transformed into tiny dense LDL [21].

Both urea and creatinine were higher in DM patients in comparison with control group. In a case control study by Hassan *et al.* [22], both male and females diabetic patients had higher urea and creatinine in compare to control.

No difference in mean of glutathione between both studies group which against the finding of **Lutchmansingh *et al.* [23] and Sekhar *et al.*** [24] studies, both found a reduction in glutathione in DM patients. The difference between current finding and comparative studies could be related to small sample size, as the difference between the groups was presented but not approved statically.

Previous studies demonstrate a significant decrease in level in metabolic and type 2 DM patients in compare to control [25-27]. Despite the difference was not proved statistically, the current study also observed decrease in ceruloplasmin level in type 2 DM. Other studies as Sarkar A et al. [28] and Ramachandrayya SA et al. observed increase in cupper mean level in DM patients that matched the current finding[29].

Ceruloplasmin plays a part in the metabolism of copper (as transporter in the plasma), iron and act as neutralizer of the excess reactive oxygen species created in diabetes mellitus by interfering with ceruloplasmin and release copper and potential rise in copper-mediated ROS formation, which may have contributed to the reduction in serum ceruloplasmin and increased cupper that was found in our investigation [(30)].

A reduction in serum iron was observed in DM patients, which was consistent with Sowjanya et al [(31)], a case control study that observe a reduction in iron, total iron binding capacity mean and increased serum ferritin.

Numerous essential minerals as Cu, Zn, Mn, Cr, Mg, and Fe are closely related to DM pathogenesis, as well an alteration in their metabolism can be occur due to poor insulin release, insulin resistance, and glucose intolerance. Many of iron metabolic pathways were influenced by glucose metabolism indicate a reciprocal connection [(32)].

Both current study and **Atari-Hajipirloo *et al.*** observed increase in zinc level in DM patients[(33)]. Another study observed a reduction in zinc level in diabetic patients with diabetic polyneuropathy and without in comparison to control group. As well, lower zinc level associated with poor DM control and more complication [(33)]. There were several concepts that explain zinc deficit in diabetes individuals. The involvement of zinc in the metabolism of lipids and glucose, by increasing glucose metabolism and storage while reducing glucose absorption and synthesis. Facilitating the formation of insulin and its effective packing into vesicles, zinc also played a crucial role in the healthy operation of the pancreatic islet cells. Zinc was also discovered to improve insulin sensitivity by enhancing insulin's capacity to attach to its receptors [(34)].

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