Indications of Liver and Kidney Functions in Non-Insulin Dependent Diabetic Patients

Hazhar M. Balaky*[1], Ismail S. Kakey[2]

[1]Department of Chemistry,Faculty of Science, Soran University, Kurdistan Region, Iraq
[2]Department of Biology, Faculty of Science and Health, Koya University , Koya KOY45, Kurdistan Region , F.R. Iraq

Abstract

This study aimed at the investigation of abnormal liver and renal functions by biochemical manifestations of underlying metabolic abnormalities in relation to hyperglycemia in non-insulin-dependent diabetic patients. The study comprised 118 diabetic patients (56 males, 62 females) and 60 age-matched healthy non-diabetic controls (30 males, 30 females). All subjects were tested for serum levels of liver enzymatic indicators, which include aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP), as well as non enzymatic parameters, including total bilirubin and total proteins.Also, serum levels of renal function markers, including microalbumin, creatinine, urea, and uric acid were measured.

The findings of this study stated that serum ALT, AST, and ALP levels were significantly higher in diabetic males and females with both age ranges (40-59 & 60-80 years). Serum total bilirubin level showed a significant decrease in diabetic males and females of both age groups. However, total proteins level showed a significant increase in diabetic males and females of both age groups. The results also showed that the level of microalbumin in urine as well as those of creatinine, urea, and uric acid in the serum were significantly higher in diabetic males and females. The present study concludes that there is a bidirectional relationship of enzymatic and non enzymatic liver and renal functions markers with the hyperglycemic status in Type 2 diabetes mellitus.

Keywords: (Diabetes Mellitus (DM), liver functions markers, renal functions markers)

مؤشرات وظائف الكبد والكلى في مرضى داء السكري غير المعتمد على الأنسولين

هزار محمد[1]*، اسماعيل كاكي[2]

[1]قسم الكيمياء ، كلية العلوم ، جامعة سوران ، إقليم كوردستان ، العراق

[2] قسم الأحياء ، كلية العلوم والصحة ، جامعة كويا ، كويا ، إقليم كوردستان ، العراق

الخلاصة

استهدفت هذه الدراسة إلى تقييم مؤشرات وظائف الكبد والكلى من خلال المظاهر الكيميائية الحيوية المرافقة للتشوهات الأيضية الكامنة المتعلقة بفرط سكر الدم في مرضى السكري غير المعتمد على الأنسولين. اشتملت الدراسة على 118 مريضاً من المصابين بالسكري (56 ذكور و 62 إناث) و 60 شخصا سليما اعدت كمجموعة سيطرة (30 ذكور و 30 إناث) ، وتم مطابقة المرضى والضوابط مع العمر. قيست

*Email: hazharbalaky86@yahoo.com

مستويات المؤشرات الكبد الإنزيمية والتي شملت، الأسبارتيت ترانس امينيز (AST) والألانين ترانس امينيز (ALT)والقلويد الفوسفاتيز (ALP) ، وكذلك قيست المؤشرات غير الإنزيمية لوظائف الكبد والتي شملت ،البيليروبين الكلي والبروتينات الكلية ، كما وتضمنت الدراسة قياس مستويات مؤشرات وظائف الكلى والتي شملت،الميكروالبومين والكرياتينين واليوريا وحمض البوليك.

اظهرت نتائج هذه الدراسة ارتفاعا معنويا في مستويات ALT و AST و ALP والبروتين الكلي في مصل دم الذكور والإناث المصابين بداء السكري للفئتين العمريتين (40-59) و (60-80) عامًا، في حين أظهرت النتائج خفضا معنويا في مستوى البيليروبين الكلي في مصل دم الذكور والإناث المصابين بالسكري للفئتين العمريتين (40-59) و (60-80) سنة.

كذلك اظهرت النتائج ارتفاعا معنويا في مستويات مؤشرات وظائف الكلى لكل من الميكروالبومين البولي وكذلك مستويات الكرياتينين واليوريا وحمض البوليك في مصل الدم الذكور والإناث المصابين بمرض بالسكري للفئتين العمريتين (40-59) و (60-80) سنة.

وخلصت نتائج الدراسة الحالية إلى وجود علاقة ثنائية الاتجاه لمؤشرات وظائف الكبد والكلى الأنزيمية وغير الإنزيمية مع حالة فرط سكر الدم في داء السكري من النوع الثاني.

Introduction

   Diabetes mellitus (DM) is the most common disorder of endocrine system in humans, characterized by hyperglycemia over a prolonged period of time. Hyperglycemia usually occurs due to insulin secretion impairment, defects in the metabolic action of insulin, or both. The long term hyperglycemia in diabetes is related to both micro and macrovascular disorders. Various organs, e.g., the liver, eyes, kidneys and nerves, are negatively affected by chronic hyperglycemia, which also causes an increased risk of cardiovascular disease (CVD) [1]. The liver has an essential role in glucose homeostasis through its ability for storing glycogen in the fed state and producing glucose by both processes of glycogenolysis and gluconeogenesis in the fasting state. An abnormal liver function test in itself may be a biochemical indicator for the diagnosis of metabolic disorders. For instance, lipid and glucose abnormalities, fatty liver disease, and increased activities of liver enzymes are markers of liver tissue damage. Increased levels of these markers was linked with type 2 diabetes, metabolic syndrome, and insulin resistance [2]. The scope of liver disease in type 2 diabetes includes abnormal liver enzymes and non-alcoholic fatty liver disease (NAFLD), liver cirrhosis, hepatocellular carcinoma (HCC), and acute liver failure [3]. Most patients with type 2 DM have some form of nonalcoholic fatty liver disease. However, it is becoming clear that the link between non-alcoholic fatty liver disease and type 2 DM is more complex than previously believed. Moreover, numerous pathophysiological and cardiometabolic risk factors (proinflammatory and profibrotic pathways) are shared between these two diseases. Additionally, strong epidemiological evidence suggests a bidirectional relationship between these two disease conditions and that non-alcoholic fatty liver disease may precedes and/or promotes the development of type 2 DM [4].

    Many studies indicated that DM, high blood pressure, lipid disorders, increased body fat (obesity), insulin impairment activity, and high level of glycosylated hemoglobin (HbA1c) are the main risk factors associated with diabetic nephropathy (DN) [5]. Moreover, the appearance of abnormally low levels of microalbumin in the urine is the first clinical renal complication in diabetes mellitus. Furthermore, the onset of microalbuminuria leads to macroalbuminuria, and the latter is followed by deterioration of the function of the kidney, with a progressive reduction in the glomerular filtration rate (GFR), which finally causes an end stage renal disease [6]. The kidney has a vital role in glucose homeostasis, which depends on the sufficient amount of insulin production from the pancreatic beta cells and adequate insulin action in the peripheral tissues. Both insulin hormone production and the sensitivity of tissues to insulin are diminished in the setting of chronic kidney disease [7]. The latest figures shown by the International Diabetes Federation (IDF) proved the enormity of the diabetes epidemic and indicated that the number of patients with kidney damage related to diabetes will continue to grow dramatically [8].

Materials and methods

Study population and design

    The current study was a case control study which was conducted during September 2018 -April 2019. The number of diabetic patients was 118, 56 of them were males and 62 were females, whose

age ranged was 40-80 years. Patients diagnosed as type (II) DM and attending Laila Qasim diabetic center in Erbil Governorate participated in the current study. The patients were divided into four groups depending on age and sex: female (40-59 years), female (60-80 years), male (40-59 years), and male (60-80 years). The group of controls comprised sixty sex and age-matched normal non diabetic subjects.

Venous blood specimens were collected from the study subjects (patients and healthy individuals) after 10-12 hours of fasting. The specimens were reserved in gold-topped serum separator tubes (SST) and EDTA tubes before centrifugation. The specimens were centrifuged at 3500 rpm for 10 min and the resulting serum and plasma samples were separated and preserved in Eppendorf tubes. Once the collection of all samples was completed, the serum and plasma samples were defrosted to be ready for biochemical analyses.

Biochemical assays

Serum glucose was determined by the enzymatic colorimetric method, using BIOLABO kit (France). Also, HbA1c (Hemoglobin A1c) in whole blood was determined by the fluorescence immunoassay (FIA) using Boditech Med kit. Serum ALT, AST, ALP, total protein TP, total bilirubin (TB) , creatinine, urea, and uric acid (UA) were measured by using the enzymatic colorimetric method using BIOLABO kit (France). Furthermore, the determination of microalbumin in urine was carried out by FIA.

Statistical Analysis

SPSS version 21 and GraphPad prism version 8 computer programs were used for statistical data analysis. Statistical test results and Bar graphs were expressed as Mean±SE. Unpaired T-test (Man-Whitney U) test was used for comparing the study parameter means between patient and control groups.

Results and Discussion

Serum levels of glucose and HbA1c%

The diabetic syndrome was confirmed by the determination of glucose and HbA1c levels in diabetic patients compared with normal healthy and age-matched controls. The diabetic males showed significantly higher ($P<0.0001$) glucose levels at both age ranges (40-59 & 60-80 years), which were 245.1±14.74 and 233.9±16.81 mg/dL, respectively, in comparison with their levels in the age-matched control groups (98.80±4.39 and 107.1±3.28 mg/dL, respectively). Similarly, significantly higher glucose levels in diabetic females at both age ranges were observed (285.9±17.01 and 282.1±14.70 mg/dL, respectively) as compared to the age-matched control groups (108.9±2.48 and 101.4±1.24 mg/dL, respectively). Also, there was a highly significant increase ($P<0.0001$) of HbA1c% levels in diabetic males at both age ranges (9.06±0.38 and 8.34±0.35 mg/dL, respectively) in comparison with their levels in the age-matched control (5.14±0.06 and 5.16±0.11 mg/dL, respectively). In addition, the mean serum HbA1c% levels were significantly higher ($P<0.0001$) in diabetic females at both age ranges (10.07±0.39 and 10.13±0.37 mg/dL, respectively) in comparison with their levels in the age-matched control (5.08±0.08 and 4.98±0.05 mg/dL, respectively) (Table-1).

Table 1-Levels of serum glucose and HbA1c% in diabetic patients

Parameters		Age (Years)	Controls	Patients	P-Value
Male	**Glucose (mg/dL)**	40-59	98.80±4.39	245.1±14.74	<0.0001
		60-80	107.1±3.28	233.9±16.81	<0.0001
	HbA1c (%)	40-59	5.14±0.06	9.06±0.38	<0.0001
		60-80	5.16±0.11	8.34±0.35	<0.0001
Female	**Glucose (mg/dL)**	40-59	108.9±2.48	285.9±17.01	<0.0001
		60-80	101.4±1.24	282.1±14.70	<0.0001
	HbA1c (%)	40-59	5.08±0.08	10.07±0.39	<0.0001
		60-80	4.98±0.05	10.13±0.37	<0.0001

Value expressed as mean ± SE, **Normal Values: (Glucose = 70-139 mg/dL), (HbA1c= 4.0-6.5%)**

The results of the current study showed that male and female diabetic patients have significantly higher blood glucose and HbA1c% levels as compared to those of the controls. The present results agree with those reported in a previous study [9], which indicated increased levels of serum glucose

and HbA1c% in patients with type 2 diabetes when compared to healthy control. Prospective and longitudinal studies indicated that higher glucose and HbA1c% levels were associated with a higher incidence of type 2 diabetes. The best hypothesis explaining the increased level of glucose in diabetic patients is that insulin resistance precedes the development of hyperglycemia in subjects who eventually develop type 2 diabetic. In addition, in diabetic patients, longstanding hyperglycemia contributes in the glycation of non-enzymatic proteins, which causes the production of reversible Amadori compounds and Schiff bases. This glycation mechanism subsequently yields irreversible advanced glycosylated end-products (AGEs) as a result of a series of molecular rearrangements of complex compounds. AGEs have the ability to precipitate in the blood stream and in various tissues. It is confirmed that the concentrations of HbA1c% in the blood represent the glucose levels to which the red blood cell has been exposed during its lifespan. Therefore, the HbA1c% test is valuable as it evaluates chronic glycaemia, instead of instantaneous blood glucose concentrations. HbA1c% has been used as a goal marker of average glycaemic control for many years, has a key role in the monitoring of patients with diabetes, and is dependent upon for clinical significant decisions, such as the initiation of treatment with insulin [10].

Serum levels of liver function markers

The serum ALT level was observed to be significantly elevated ($P<0.05$) in diabetic males at both age ranges (40-59 & 60-80 years), with mean values of 33.19 ± 1.93 IU/L and 33.30 ± 3.03 IU/L, respectively, in comparison with their levels in age-matched control (27.87 ± 1.61 & 18.63 ± 2.27 IU/L, respectively). Similarly, a significant increase ($P<0.0001$) was observed in serum ALT levels in diabetic females at both age ranges (27.58 ± 2.73 & 28.05 ± 1.41 IU/L, respectively) in comparison with their levels in age-matched control (14.97 ± 0.77 & 14.66 ± 1.12 IU/L, respectively). The mean level of serum AST was significantly elevated ($P<0.05$) in diabetic males at both age ranges (31.11 ± 1.26 & 31.03 ± 1.30 IU/L, respectively) in comparison with their levels in age-matched control (26.69 ± 1.27 & 24.48 ± 1.71 IU/L, respectively). Furthermore, there was a significant increase ($P<0.05$) in mean serum levels of AST in diabetic females (29.02 ± 3.27 & 30.38 ± 5.12 IU/L, respectively) in comparison with their levels in the age-matched control (19.07 ± 1.58 & 18.35 ± 0.79 IU/L, respectively). There was also a highly significant elevation ($P<0.0001$) in the mean serum levels of ALP in diabetic males at both age ranges (344.8 ± 12.70 & 419.6 ± 33.90 IU/L, respectively) in comparison with their levels in age-matched control (159.8 ± 9.00 & 166.5 ± 8.49 IU/L, respectively). Additionally, the data also showed that diabetic females at both age ranges have significantly higher ($P<0.0001$) ALP levels (311.2 ± 9.82 & 360.1 ± 30.94 IU/L, respectively) in comparison with their levels in the age-matched control (157.1 ± 6.75 & 163.8 ± 6.32 IU/L, respectively). The results showed a significant decrease ($P<0.05$) of serum total bilirubin levels in diabetic males at both age ranges (0.79 ± 0.06 & 0.78 ± 0.06 mg/dL, respectively) in comparison with their levels in the age-matched control (0.98 ± 0.05 & 1.01 ± 0.07 mg/dL, respectively). On the other hand, a significant reduction ($P<0.05$) in mean serum levels of total bilirubin was also observed in diabetic females at both age range (0.77 ± 0.04 & 0.78 ± 0.06 mg/dL, respectively) in comparison with their levels in the age-matched control (0.96 ± 0.06 & (1.00 ± 0.04 mg/dL, respectively).

The concentration of total proteins showed a significant elevation ($P<0.05$) in mean serum levels in diabetic males at both age ranges (7.87 ± 0.23 & 7.88 ± 0.29 g/dL, respectively) in comparison with their levels in the age-matched control (6.93 ± 0.15 & 6.99 ± 0.12 g/dL, respectively). Additionally, there was a significant increase ($P<0.05$) in diabetic females at both age ranges (7.82 ± 0.38 & 7.84 ± 0.33 g/dL, respectively) in comparison with their levels in the age-matched control (6.76 ± 0.08 & 6.80 ± 0.13 g/dL, respectively) (Tabl-2).

Table 2-Levels of liver function parameters in diabetic patients.

Parameters		Age (Years)	Controls	Patients	P-Value
Male	**ALT (IU/L)**	40-59	27.87 ± 1.61	33.19 ± 1.93	<0.05
		60-80	18.63 ± 2.27	33.30 ± 3.03	<0.05
	AST (IU/L)	40-59	26.69 ± 1.27	31.11 ± 1.26	<0.05
		60-80	24.48 ± 1.71	31.03 ± 1.30	<0.05
	ALP (IU/L)	40-59	159.8 ± 9.00	344.8 ± 12.70	<0.0001

			60-80	166.5±8.49	419.6±33.90	<0.0001
	Total Bilirubin (mg/dL)		40-59	0.98±0.05	0.79±0.06	<0.05
			60-80	1.01±0.07	0.78±0.06	<0.05
	Total proteins (g/dL)		40-59	6.93±0.15	7.87±0.23	<0.05
			60-80	6.99±0.12	7.88±0.29	<0.05
Female	**ALT (IU/L)**		40-59	14.97±0.77	27.58±2.73	<0.0001
			60-80	14.66±1.12	28.05±1.41	<0.0001
	AST (IU/L)		40-59	19.07±1.58	29.02±3.27	<0.05
			60-80	18.35±0.79	30.38±5.12	<0.05
	ALP (IU/L)		40-59	157.1±6.75	311.2±20.62	<0.0001
			60-80	163.8±6.32	360.1±30.94	<0.0001
	Total Bilirubin (mg/dL)		40-59	0.96±0.06	0.77±0.04	<0.05
			60-80	1.00±0.04	0.78±0.06	<0.05
	Total proteins (g/dL)		40-59	6.76±0.08	7.82±0.38	<0.05
			60-80	6.80±0.13	7.84±0.33	<0.05

Value expressed as mean ± SE, Normal Values: (ALT: Male = 7 - 55 IU/L, Female = 7 – 45 IU/L), (AST: Male = 8 - 48 IU/L, Female = 8- 43 IU/L), (ALP: Male (40-59) year= 90 – 395 IU/L, Male (60-80) year= 120-460 IU/L, Female (40-59) year=80-380 IU/L, Female (60-80) year=110-430 IU/L), Total Bilirubin = 0.2 - 1.3 mg/dL, Total Proteins = 6.4 - 8.3 g/dL.

Insulin resistance status in type II DM patients is responsible for the alterations of enzymatic activities, e.g., ALT, AST, and ALP, as well as levels of non-enzymatic liver parameters, e.g., total bilirubin and total protein. The data in the current study showed that diabetic patients (males & females) have significantly higher serum levels of liver enzymes as compared to those in the control. These results are in agreement with those obtained by others [11,12], who found that the activities of liver enzymes increase in diabetic patients compared to healthy subjects. AST and ALT are sensitive indicators of liver abnormality in various kinds of disease [13]. Increased levels of ALT could be due to impairment in insulin signaling rather than being purely because of liver cell damage. The pathophysiological mechanism responsible for these elevations in transaminases activities may indicate inflammation status that systemically impairs insulin signaling in the liver. It was reported that insulin resistance is caused by ALT regardless of age, body mass index, or gender [14]. A direct linkage was reported between transaminase enzymes elevation and hepatocyte injury. The rupture of the plasma membrane at high concentrations of the metabolites, loss of mitochondrial activities, and inactivation of the regulatory metabolic enzymes results in liver cell damages [15]. Elevated levels of ALT were independently associated with increased risks of type 2 diabetes. Furthermore, ALT is considered as a useful marker for diagnosing subjects with high risk of type 2 DM [16]. The high serum levels of AST in diabetic patients in the current study can be explained by the impact of both glycogen and insulin on liver tissues. The increases in the primary metabolic pathways, such as gluconeogenesis (synthesis of glucose from non-carbohydrate precursors) and glycogenolysis (degradation of stored glycogen for releasing of new glucose units), contribute in the elevation in the availability of substrate, e.g. alanine, and in its conversion to glucose might be regulated as a compensatory pathway for impaired hepatic insulin communication. Such impairment could permit the leakage of enzymes from hepatocytes, as a result of infiltration of accumulated fat as well as injury in the cells of liver.

However, AST is considered as a marker for normal functioning of liver, but it is less specific as compared to ALT and gamma-glutamyl transferase (GGT). Therefore, AST may be considered as a less specific marker of liver pathology that is related to the development of type 2 diabetes mellitus [17]. Moreover, the elevation in serum ALP level could be an indication of an increased deposition of fat in the liver, known as non-alcoholic fatty liver disease (NAFLD). Hepatic insulin resistance is mainly due to the accumulation of fatty substances in hepatic tissues, which is also involved in the development of systemic insulin resistance and hyperactivity of insulin. Thus, ALP could serve as a good marker for insulin resistance. Much evidence suggests that ALP is considered as a marker for both fatty liver and CVD. Cellular stress and normal cell turnover contribute in the leakage of ALP into the blood circulation. Elevation of the level of ALP is accompanied by an increased insulin

resistance and a greater risk for developing type 2 DM and poor blood glucose control. The relationship between serum ALP activity and some metabolic disorders related to type 2 diabetes, such as poor glycemic control and atherogenic dyslipidemia, could be explained by underlying, but not mutually exclusive, biological mechanisms, such as insulin resistance, fatty liver, and enhanced oxidative stress [18].

Furthermore, the results of the present study demonstrate a significant increase in serum total protein levels. This approximates the results shown by others [19], who observed that total proteins level was increased in diabetics as compared to controls, while a significant reduction of serum TB level was recorded in diabetic male and female patients with both age ranges in comparison with those in control groups. These results are in accordance with those obtained by others [20, 21]. The observed increase in serum total protein in diabetic patients may be explained on the basis of the elevation of different acute phase proteins, globulins, and fibrinogen in DM, which is compounded by a decrease in the fractional synthetic rate of albumin due to insulin resistance or insulin deficiency, which contribute to the elevation in plasma proteins [11]. In contrast to the findings of the present study, an earlier work [22] showed that diabetic patients suffer from a significant reduction in serum levels of total protein due to a permanent elevation of blood glucose which leads to the production of an advanced glycosylation compounded with an elevated rate of glomerular filtration and enlargement of glomerular tissues. Moreover, many studies reported the association between the concentration of total bilirubin and the risk of complications of DM, such as diabetic nephropathy, diabetic retinopathy, and other macrovascular complications. The mechanisms which show the association between TB and the complications of diabetes are as follows; hyperglycemia can lead to mitochondrial superoxide overproduction in endothelial cells of both large and small vessels. Different pathways, which include increased formation of AGEs, increased expression of AGEs receptors and their activating ligands, activation of protein kinase C isoforms, and over activity of the hexosamine pathway, can be activated by increased superoxide production. These pathways are involved in the pathogenesis of complications of DM [23]. Chronic oxidative stress causes worse diabetic control through defective insulin secretion and gene expression. It was suggested that DM is correlated with an increasing degree of oxidative stress. Another study reported that microvascular and macrovascular complications of diabetes were deeply correlated with oxidative stress. Bilirubin is an endogenous antioxidant that decreases the levels of free oxygen radicals and, thus, ameliorates oxidative stress. Therefore, free oxygen radicals may increase by lowering bilirubin levels, thus causing worse diabetic regulation [24].

Serum levels of renal function markers

The results in Table-3 reflect a highly significant increase (P<0.0001) of microalbumin level in urine in diabetic males at both age ranges (40-59 & 60-80 years) with mean values of 50.15±9.10 and 75.70±22.19 mg/dL, respectively, in comparison with their levels in the age-matched control (5.73±2.81 and 5.94±0.25 mg/dL, respectively). Similarly, highly significant increases (P<0.0001 & P<0.05) of microalbumin levels in urine were observed in diabetic females at both age ranges (100.1±21.76 and 83.61±17.12 mg/dL, respectively) in comparison with their levels in the age-matched control (5.17±0.45 and 9.36±0.30 mg/dL, respectively).

The results in Table-3 indicate significant increases (P<0.05) in serum creatinine concentrations in diabetic males at both age ranges (1.25±0.06 & 1.31±0.77 mg/dL, respectively) in comparison with their levels in age-matched control (1.00±0.04 & 0.96±0.07 mg/dL, respectively). Similarly, there were significant increases (P<0.05) in serum creatinine concentrations in diabetic females at both age ranges (1.23±0.12 & 1.29±0.18 mg/dL, respectively) in comparison with their levels in age-matched control (0.82±0.04 & 0.79±0.06 mg/dL, respectively).

As shown in Table-3, the mean values of serum urea levels were observed to be significantly higher (P<0.05) in diabetic males at both age ranges (56.85±8.80 & 45.93±2.89 mg/dL, respectively) in comparison with their levels in the age-matched control (35.91±0.73 and 35.38±1.23 mg/dL, respectively). Similarly the serum urea levels were significantly increased (P<0.05) in diabetic females at both age ranges (40.74±3.56 and 48.84±5.15 mg/dL, respectively) in comparison with their levels in the age-matched control (31.36±1.79 and 32.80±2.50 mg/dL, respectively).

The mean values of uric acid concentrations in diabetic male and female patients and control subjects are presented in Table-3 and show a significant increase (P<0.0001) in diabetic males at both age ranges (8.38±0.49 and 9.74±0.72 mg/dL, respectively) in comparison with their levels in the age-

matched control (5.06±0.22 and 4.53±0.16 mg/dL, respectively). Similarly, a highly significant elevation (P<0.0001) in the mean level of uric acid was recorded in diabetic females at both age ranges (8.19±0.55 and 7.64±0.71 mg/dL, respectively) in comparison with their levels in the age-matched control (3.83±0.26 and 3.80±0.23 mg/dL, respectively).

Table 3-Levels of renal function markers in diabetic patients

Parameters		Age (Years)	Controls	Patients	P-Value
Male	**Microalbumin (mg/dL)**	40-59	5.73±2.81	50.15±9.10	<0.0001
		60-80	5.94±0.25	75.70±22.19	<0.0001
	Creatinine (mg/dL)	40-59	1.00±0.04	1.25±0.06	<0.05
		60-80	0.96±0.07	1.31±0.07	<0.05
	Urea (mg/dL)	40-59	35.91±0.73	56.85±8.80	<0.05
		60-80	35.38±1.23	45.93±2.89	<0.05
	Uric acid (mg/dL)	40-59	5.06±0.22	8.38±0.49	<0.0001
		60-80	4.53±0.16	9.74±0.72	<0.0001
Female	**Microalbumin in Urine (mg/dL)**	40-59	5.17±0.45	100.1±21.76	<0.0001
		60-80	9.36±0.30	83.61±17.12	<0.05
	Creatinine (mg/dL)	40-59	0.82±0.04	1.23±0.12	<0.05
		60-80	0.79±0.06	1.29±0.18	<0.05
	Urea (mg/dL)	40-59	31.36±1.79	40.74±3.56	<0.05
		60-80	32.80±2.50	48.84±5.15	<0.05
	Uric acid (mg/dL)	40-59	3.83±0.26	8.19±0.55	<0.0001
		60-80	3.80±0.23	7.64±0.71	<0.0001

Values expressed as Mean±SE. Normal Values: (Microalbumin in urine: Male = <18 mg/dL, Female = <25 mg/dL), (**Creatinine**: Male = 0.7 - 1.3 mg/dL, Female = 0.6 - 1.1 mg/dL), (Urea: Adults = 13 – 43 mg/dL, >60 Years= 21 – 66 mg/dL), (Uric Acid: Male = 3.4 - 7.0 mg/dL, Female = 2.4 - 6.0 mg/dL).

Diabetic nephropathy is one of the main causes of morbidity and mortality in diabetes mellitus. Actually, the increase of death rate of diabetes mellitus occurs mainly in people with DM and proteinuria, not only from the end-stage renal disease (ESRD) but also from CVD, with the latter being particularly common in type 2 diabetic patients. Clinically, diabetic nephropathy is characterized by the development of kidney damage , which is reflected by increased albuminuria, renal function impairment (reduction in glomerular filtration rate GFR), high blood pressure, and increased morbidity and mortality rates related to complications of the cardiovascular system [25]. The results of the present study reported that all serum levels of renal function markers were significantly elevated in DM patients in comparison to healthy controls. These results are in accordance with those of other investigations [26,27] which found increases in the levels of renal function markers in DM patients in comparison to non diabetic subjects.

Variations in the prevalence of albuminuria were found by various cross-sectional and epidemiological studies. Variations in populations, microalbuminuria definition, urine collection methods, and the ways of measurement can explain the differentiation in the prevalence of microalbuminuria. Variations in the racial sensitivity to kidney disease (nephropathy) may also be used to clarify this differentiation. Furthermore, it was reported that male subjects, elder people, longer period of DM, hyperglycemia, and high blood pressure are risk factors of microalbuminuria. Many studies observed a strong association between blood glucose control and microalbuminuria [28]. The prevalence of microalbuminuria was high in type 2 diabetic patients. Therefore, the annual screening programmes for microalbumin in urine should be applied for diabetic patients.

Blood urea and creatinine are widely considered as markers for determining kidney functions. Good glycemic control is an absolute requirement to prevent the development of kidney failure. There is a strong correlation between blood sugar and blood urea levels and, hence, monitoring both parameters is very important. The reduction in filtering ability of the kidney in type 2 DM would cause the accumulation of waste products, which is a cause of the elevation in serum urea and creatinine

levels. Moreover, high blood glucose level causes damaging of millions of nephrons (very small filtering units in each kidney) and it is clear that nephrons are not working properly in DM patients, which leads to the elevation of serum creatinine level [29]. Furthermore, hyperglycemia leads to kidney damage and abnormal function. In this case, creatinine will not be cleared properly by the kidneys and leads to increased abnormality. The high level of glycated hemoglobin (HbA1c) can be lowered by intensive treatment plans, but high levels of serum creatinine and serum urea, which are set on increase because of permanent damage to the kidneys, would be difficult to reverse because the damage to the kidneys in diabetic patients is a permanent phenomenon. Increased blood urea and serum creatinine levels in diabetics clearly indicate prolonged hyperglycaemia which causes irretrievable damage to nephrons of the kidney. Raised serum creatinine and urea with reduced GFR have become fairly reliable indicators of kidney dysfunction. Effective control of blood sugar levels can stop the progression to diabetic nephropathy and thus remarkably reduce the morbidity and mortality associated with this metabolic disease [27].

An earlier study observed that diabetic patients have significantly elevated levels of serum creatinine and urea with longer periods of diabetes mellitus [30]. The authors concluded that the longer duration of diabetes mellitus is another risk factor for renal failure progressions. Moreover, elevations in the levels of renal function markers like creatinine, urea, and uric acid may be an implication of a wide range of diseases, such as kidney insufficiency which leads to elevated levels of creatinine and uric acid because of decreased blood flow to the kidneys. Renal artery stenosis also causes reduction of blood flow to the kidney. It could also cause a rise in blood pressure, retention of fluid, and cardiovascular problems [31]. Biologically, as a systemic indicator of oxidative status, serum uric acid is closely correlated to insulin resistance (a pathologic mechanism of type 2 diabetes mellitus) by blocking the synthesis of nitric oxide or by increasing C-reactive protein expression. Such practice depends on activating platelet adhesiveness which induces endothelial dysfunction that blocks glucose uptake through stimulating insulin- signaling. In contrast, some studies found that lowering the levels of serum uric acid might not be an effective strategy for restoring endothelial function and might not decrease the risks of progression of type 2 diabetes mellitus. Additionally, it was reported that serum uric acid is not responsible for the development of type 2 diabetes mellitus. Limited expectations were stated that drugs which are lowering uric acid will be effective in the prevention of type 2 diabetes mellitus [32]. The principle mechanism of type 2 diabetes mellitus include insulin resistance, hyperactivity of insulin, and various metabolic disorders, which also lead to the elevation of serum uric acid levels. Insulin resistance, which is characterized by a functional deficit in insulin secretion despite high plasma levels, is inversely associated with 4 h urinary uric acid clearance. Insulin resistance could cause elevation of serum uric acid level by both decreasing renal uric acid secretion by renal proximal tubular and uric acid reabsorption enhancement in humans due to an active transport mechanism strongly correlated to the tubular reabsorption of sodium and precipitation substrates for uric acid production [33]. Furthermore, a previous study [34] stated that there is a link between elevated levels of uric acid with T2DM. Moreover, obesity, which is a very common characteristic in T2DM, has an impact on the reabsorption of salts by kidney. This causes an increased pressure on glomerular capillary as well as an increased rate of glomerular filtration. Insulin resistance causes low urine pH due to impairment in the production of ammonia. Such acidic urine contributes in the formation of uric acid calculi. Elevated serum levels of urea (Hyperuricemia) is a major risk factor for type 2 diabetes mellitus, because it causes disturbances of the proinflammatory endocrine mechanisms in the vascular smooth muscle cells and adipose tissue, which leads to cell surface morphological alterations and insulin resistance. The elevated levels of uric acid and creatinine contribute to the presence of some of the symptoms of kidney dysfunction in diabetic patients, which causes lower back pain as a result of infection of the urinary tract and formation of uric acid calculi that accumulate in the renal tubules [35].

Moreover, the findings of the current research are in agreement with various studies which showed that raised renal function markers in Type 2 DM patients may refer to the presence of renal dysfunction. Thus, strict glycemic management helps to prevent renal impairment and diabetic nephropathy, which is associated with structural and functional kidney abnormalities in patients with diabetes.

Conclusions

Elevated serum concentrations of liver function enzymatic and non-enzymatic markers in type 2 diabetes support the hypothesis that the liver is important in the pathogenesis of type 2 diabetes and that hepatic enzymes may be useful additional markers of subjects at high risk for the development of diabetes.

High serum levels of renal profile markers were associated with an increased risk of type 2 diabetes mellitus and dysglycemia. As Diabetes mellitus is the major cause of renal morbidity and mortality, a good control over the sugar level can halt the progression of renal damage.

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