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Insulin resistance and specific biomarkers in blood and urine of type 2 diabetic patients with or without nephropathy in Basrah, Iraq

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Diabetic nephropathy (DN) is a master cause of all surplus death-rate among type 2 diabetes mellitus (T2DM) patients with microalbuminuria. This study aimed to find effective biomarkers for early predicting of DN. Present study included 63 patients with T2DM (31 patients with DN, 32 patients without DN) and 33 healthy controls. These three groups were matched for their glucose, urea, creatinine, insulin, L-Carnitine (LC), osteoprotegerin (OPG), sialic acid (SA), trace elements (Selenium, Zinc, Magnesium), albumin (Alb), and fibronectin (FN). Glucose, urea, and creatinine were determined by spectrophotometer. Insulin, LC, OPG, SA, Alb, and FN were assayed by enzyme-linked immunosorbent assay (ELISA). Insulin resistance (IR) was calculated by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) equation. Selenium was measured by hydride generation while Zinc and Magnesium were measured by flame atomic absorption spectrometer. Compared with controls, the results indicated that T2DM patients with or without DN had a significant increase in glucose, urea, creatinine, insulin, IR, OPG, SA, Alb, FN and a significant decrease in LC and trace elements levels. It was concluded that IR is strongly associated with obesity and had an important role in the pathogenesis and increased complication of diabetes which could be used as excellent indicators for early-stage DN in T2DM patients and thus decreasing mortality and morbidity.

Key words: Diabetic nephropathy, insulin resistance, obesity, oxidative stress, traces elements.

INTRODUCTION

Diabetic nephropathy (DN) is defined as the manifestation of incessant clinical albuminuria in a person with diabetes (for more than 5 years) and accompanying retinopathy in absence of urinary tract infection, other kidney diseases and heart failure. DN is a multi-stage state that takes many years to be clinically overt (Lim, 2014). Some changes are present in the kidney function like raised kidney blood flow, hypertrophy of the renal and glomerular hyperfiltration. By good glycemic control, most of these changes could be inverted at an early stage. But they persevere in several patients and can be crucial in the subsequent evolution of clinical nephropathy (Mise et

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al., 2017). Normal human urine consists of very little amounts of albumin (Alb) only because less than 30 mg of Alb is excreted by healthful adults per 24 h. The glomerular filter dysfunction leads to inclusive leak for plasma proteins. Prevalent modification of podocyte foot operations is seen in different shapes of glomerulonephritis (Campion et al., 2017).

The evolution of DN was characterized by elevates Alb secretion rate and glomerular hyperfiltration. Exact quantification of the quantity of Alb in the urine has a pivotal clinical notion, for example, the excretion quantities of Alb more than 300 mg/24 h is called macroalbuminuria while the excretion quantities of Alb between 30 and 300 mg/24 h is called microalbuminuria (MAU) (Debbarma et al., 2015). Once overt nephropathy happens, the glomerular filtration rate (GFR) declines gradually over a period of many years at a speed (around 2-20 mL/min/year) which vary highly from person to another. End stage renal disease (ESRD) enhances in about 50% of type1 diabetes mellitus (T1DM) people with overt nephropathy for 10 years whereas in more than 75% within 20 years (Drosos et al., 2018).

Many of people with T2DM may have MAU and overt nephropathy quickly following their diabetes diagnosis because diabetes is indeed existing, perhaps for several years, before the diagnosis. About 20 to 40% of T2DM patients with MAU advance to overt nephropathy, but only around 20% of them will progress to ESRD in 20 years after overt nephropathy onset (Elnajjar et al., 2016). The normal history of DN in T2DM patients may lead to death after 6 years of the persistent proteinuria onset. The aggregate series is identical in T2DM patients, but doubts may present due to imprecise date of the diabetes onset (Fiseha and Tamir, 2016). The decrease is more changeable in T2DM patients and the rate of progress to ESRD can be, as low as, 20% through 20 years. Ameliorations in the nephropathy management have expanded the course of time from continual proteinuria to kidney failure. Considering the raised happening of both DM and DN, the detection of early DN is of fundamental importance to supply suitable therapy that prevents or slows evolution towards ESRD (Sekulic and Sekulic, 2015).

Biomarkers have a crucial role in the first detection of DN. The detection of early DN comprises numerous new biomarkers. These biomarkers span the period of normoalbuminuria that predates MAU but also the development of renal involvement during MAU and macroalbuminuria (Kim et al., 2014).

L-Carnitine (LC) is a co-factor needed for the transportation of long chain fatty acids in the mitochondria for production of energy in peripheral tissues. LC may play a pivotal role in prohibiting generation of free radical, protecting tissues from impairment by fixing the oxidized lipids of membrane and preventing fatty acids beta-oxidation damage in mitochondria (Giudetti et al., 2016).

Also, osteoprotegerin (OPG) is a secreted glycoprotein which regulates the bone resorption. It is generated as a monomer (60 kDa) and congregated as a homodimer in the cell and then secreted, fundamentally, as a di-sulfide connected homodimer in the circulation. OPG was specified as a cytokine and member of the tumor necrosis factor (TNF) receptor superfamily (Xia et al., 2015).

Furthermore, sialic acid (SA), a general expression for a family of acetylated derivatives of neuraminic acids, is a vital element of glycolipids and glycoproteins. It works as a cofactor for several cell receptors and associates positively with many of the serum acute phase reactants (Zhu et al., 2017).

Moreover, fibronectin (FN), a high molecular weight (440 kDa) glycoprotein, is a substantial part of the glomerular extracellular matrix (ECM). It is generated in the platelets, liver and vascular endothelia. It shows in a soluble format in plasma and on the cell surface in fibrillar format (Uwaezuoke, 2017).

Although the scientific community has started resolving the secrets of the close linkage between insulin resistance (IR), some blood and urine biomarkers, some trace elements (Selenium [Se], Zinc [Zn] and Magnesium [Mg]) and their physiological impacts, a lot is still remaining to be discovered. In Basrah governorate (southern of Iraq), to date, no study has been investigated on IR and its effects on some blood and urine biomarkers in DN. Therefore, the present study is aimed to assess the effect of IR on some blood and urine biomarkers as excellent indicators for early-stage DN in patients with T2DM.

MATERIALS AND METHODS

Subjects

Sixty-three subjects (men and women) suffering from T2DM were enrolled in this study and they were divided into two groups: the first group consisted of 31 patients suffering from DN (15 men and 16 women) while the second group consisted of 32 patients free from DN (15 men and 17 women). These subjects were matched with 33 healthy controls (16 men and 17 women). This study is a cross-sectional clinical study and it was conducted based on the Helsinki ethical guidelines. Patients with the following inclusion criteria were enrolled in this study; age between 36 and 65 years old; T2DM patients (with or without DN) were diagnosed by clinicians in the diabetes and endocrine glands centre in Al-Mawany teaching hospital. The control group was health individuals, not suffering from T2DM nor having any family history of T2DM, not suffering from any acute or chronic cardiovascular diseases and not taking any drug believed to alter plasma glucose level. All the volunteers had a stable clinical course for at least 3 months. Patients who were pregnant (women), had angina or heart failure, renal failure, hypertension, alcoholics, T1DM, urinary tract infection, uncontrolled thyroid disorders, severe liver dysfunction, human immunodeficiency viruses (HIV) infections, pancreatic diseases, hormonal abnormalities, genetic syndromes and severe concurrent illness were excluded from the study. Demographical data were collected via a structural interview that was conducted during the visit. Standard self-administered questionnaire paper is used to

define the age, health habits (exercise, smoking or alcohol consumption), duration of DM and DN, medical history and current medications. Diagnosis of T2DM patients was based on the recommendation of American Diabetes Association (ADA, 2018). Duration of DM was considered as the time from which the patient was diagnosed with T2DM. DN was diagnosed on the basis of GFR level [<60 mL/min/1.73 m²] (Jerums et al., 2012) or persistent MAU (>19 µg/mL) in the morning urine samples of patients (Zanetti et al., 2020).

Samples collection and preparation

All samples (blood and urine) were collected in the morning between 09:00 and 10:00 am after 12 h fasting time and 30 min of rest in the supine position. 10 mL of venous fasting blood sample was collected from each subject by vein puncture then divided into two parts: the first part was whole blood obtained by adding 1 mL of blood into tubes (with anticoagulant) and shook gently to be utilized for the determination of Se level. The second part (9 mL) was moved to plain tube (without anticoagulant) which admitted clotting for 20 min at room temperature. After the blood had clotted, it was moved into a centrifuge at 402 × *g* for 20 min to get the serum. In addition, 20 mL of urine was collected from each subject as well and centrifuged at 402 × *g* for 20 min to remove all suspended particles and cell derbies aliquoted, the clear supernatant was collected carefully. Whole blood, serum and urine samples, for each participant, were utilized immediately in the estimation of variables in this study while the rest was stored in deep freezing (-80°C) until another use (Al-Fartosy and Mohammed, 2017).

Routine laboratory tests

Routine lab tests included determination of glucose, urea and creatinine levels were determined by UV-Vis Spectrophotometer (UV-EMC-LAB, Duisburg, Germany) by using the following kits (Randox, County Antrim, UK, Cat. No.: GL364; Linear, Barcelona, Spain, Cat. No.: 1156015; Randox, County Antrim, UK, Cat. No.: CR 511/S), respectively (Sirivole and Eturi, 2017).

Assay of blood and urine biomarkers

Serum (insulin, LC, OPG and SA) and urine (Alb and FN) biomarkers were assayed by human insulin ELISA kits. Sandwich ELISA technique was applied, and the level of each biomarker was measured by a standard curve. Insulin was assayed by the following kit (Calbiotech, California, USA, Cat. No.: IS130D) (Febbraio, 2017). While LC was assayed by the following kit (BT-Lab, Shanghai, China, Cat. No.: E3426Hu) (Bae et al., 2015). Whereas OPG was assayed by the following kit (BT-Lab, Shanghai, China, Cat. No.: E1558Hu) (Bernardi et al., 2016). Where SA was assayed by the following kit (BT-Lab, Shanghai, China, Cat. No.: E1620Hu) (El-Sayed et al., 2018). On the other hand, Alb was assayed by the following kit (Creative Diagnostics, New York, USA, Cat. No.: DEIA2299) (Campion et al., 2017). Finally, FN was assayed by the following kit (BT-Lab, Shanghai, China, Cat. No.: E2002Hu) (Indriani et al., 2020).

Ethical approval

All enrolled subjects signed informed consent to participate. The study was approved by the ethics committee of College of Science, University of Basrah (No.: 7/54/1879) and conducted in accordance with the Declaration of Helsinki.

Estimation of trace elements

Serum Zn and Mg were determined by using GBC 933 Plus flame atomic absorption spectrometry "AAS" (GBC, Braeside, Australia). While, whole blood Se was determined by Flame Atomic Absorption Spectrometer with Homemade Hydride Generation System (Shimadzu, Tokyo, Japan) (Al-Fartosy et al., 2019).

Calculations of some clinical parameters

Body mass index (BMI) was calculated by the standard BMI equation (Al-Fartosy et al., 2020a):

$$BMI\ (kg/m^2) = weight\ (kg)/height\ (m^2).$$

while insulin resistance (IR) was calculated by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) equation (Al-Fartosy et al., 2020b):

$$HOMA\text{-}IR = Fasting\ insulin\ (\mu IU/mL) \times Fasting\ glucose\ (mg/dL) / 405.$$

whereas glomerular filtration rate (GFR) was calculated by the Modification of Diet in Renal Disease Study (MDRD) equation (Chen et al., 2016):

$$GFR\ (mL/min/1.73\ m^2) = 186 \times Serum\ Cr^{-1.154} \times age^{-0.203} \times 1.212\ (if\ subject\ is\ black) \times 0.742\ (if\ subject\ is\ female).$$

where creatinine clearance (CrCl) was calculated by the Cockcroft-Gault equation (Chen et al., 2016):

$$CrCl\ (mL/min) = (140 - age) \times (weight, kg) \times (0.85\ if\ female) / (72 \times Serum\ Cr).$$

Statistical analysis

Results were analysed using the statistical package for the social sciences (SPSS) software (Version 21) and the values were expressed as mean ± standard deviation (SD). The values of $p > 0.05$ was considered statistically not significant, $p < 0.05$ was considered as significant differences and $p < 0.01$ for highly significant in comparison with the corresponding control value (Al-Fartosy et al., 2019).

RESULTS

The general characteristics of all subjects participated in the present study were given in Table 1.

Data obtained indicated that there was a non-significant change ($p>0.05$) in body mass index (BMI) level in T2DM patients with and without DN as compared to control group as shown in Table 2. In addition, compared with normal controls, the results indicated that T2DM patients with and without DN had high significantly ($p<0.01$) increased levels of serum biomarkers (glucose, insulin, HOMA-IR and SA) as mentioned in Table 2. Furthermore, patients with T2DM with DN had a high significant increase ($p<0.01$) and patients with T2DM without DN had a significant increase ($p<0.05$) in the levels of serum (urea, creatinine and OPG) and urine (Alb and FN)

Table 1. The demographic characteristics of the present study.

Characteristics		T2DM patients		Healthy control
		With DN	Without DN	
Total subjects number		31	32	33
Age (years) (mean ± SD)		57.2 ± 4.4	53.9 ± 4.7	51.7 ± 5.0
DM Duration (years) (mean ± SD)		13.5 ± 2.5	5.2 ± 1.6	-
DN Duration (years) (mean ± SD)		6.8 ± 0.6	-	-
Demographic Area	Urban	28	27	29
	Rural	3	5	4
Educational background	Learned	25	24	26
	Illiterate	6	8	7
Smoking habits	Positive	0	0	0
	Negative	31	32	33
Food habits	Vegetarian	5	6	8
	Non-vegetarian	26	26	25
Employment status	Employed	19	14	28
	Not Employed	12	18	5
Subjects' gender	Men	15	15	16
	Women	16	17	17

SD: Standard deviation, DM: Diabetes mellitus, DN: Diabetic nephropathy.

biomarkers, compared to healthy control as demonstrated in Table 2. On the other hand, our data reported that GFR, CrCl and LC levels were decreased significantly in T2DM patients ($p<0.01$ in patients with DN and $p<0.05$ in patients without DN) as illustrated in Table 2.

Moreover, levels of blood trace elements (Se, Zn and Mg) were high significantly ($p<0.01$) lower in T2DM patients with and without DN as compared to control group as seen in Table 3.

DISCUSSION

To the best of our knowledge, this is the first study climbed on the objective of assessing the effect of IR on some blood and urine biomarkers and some trace elements levels in T2DM patients with and without DN in Basrah governorate (southern of Iraq). In the current study, the data presented that all the patients and healthy control subjects were non-smokers. Also, most of the volunteers from both patients and healthy control were from urban, all of them acquired a well-education and they had a good work place, as shown in Table 1. The major variations between urban and rural regions are the differences in food habits, genetic, social, psychic, pollution, environments and others raising dramatically in urban areas (Al-Fartosy et al., 2020b).

Obesity (elevated BMI level) perhaps acts as a diabetogenic factor via elevating resistance to the action of insulin in those genetically predisposed to enhance T2DM. IR leads to higher plasma levels of insulin which bring about an increase in appetite. Consequently, people eat more and gain more weight. So, obesity could be one of the etiological factors in the development of T2DM, and mostly because of loss of early phase insulin secretion in response to glucose which happens relatively earlier in the development of T2DM (Park et al., 2018). This loss is critically crucial as the early blast of insulin secretion plays a substantial role in priming target tissues of insulin, especially the liver responsible for normal glucose homeostasis after food uptake and mealtime glucose deflection take place when this process was deteriorated (Boughton et al., 2017). Obesity is considered one of the modifiable cardiovascular risk factors that is far more predominant in those people with T2DM than in the general population. Moreover, obesity and physical inactivity are important independent risk factors for T2DM in middle aged men (Wang et al., 2016).

Insulin resistance (IR) can be known as a form of biological misinformation in the body in which the insulin hormone receptors on the cell membrane are not responding suitably to the insulin. Thus, the glucose in blood becomes unable to reach into cells which could lead to a hypoglycaemic reaction. This condition makes

Table 2. Levels of serum and urine biomarkers in men and women of healthy control and T2DM patients with and without DN.

Biomarker	Gender	T2DM Patients										Healthy Control
		With DN					Without DN					
		Mean ± SD	SE	Range	95 % CI		Mean ± SD	SE	Range	95 % CI		Mean ± SD
					Lower	Upper				Lower	Upper	
BMI (kg/m²)	Men	31.2 ± 1.6	0.4	28.7 - 34.1	26.6	35.9	30.3 ± 2.5	0.6	26.3 - 34.6	23.1	37.4	31.5 ± 0.2
	Women	30.1 ± 0.5	0.1	29.8 - 32.1	28.7	31.5	30.8 ± 0.3	0.1	29.6 - 31	29.8	31.9	30.0 ± 1.7
Glucose (mg/dL)	Men	172.7 ± 3.3**	0.8	162.0 - 180.0	163.4	182.0	168.6 ± 3.0**	0.8	158.0 - 174.0	160.0	177.1	104.2 ± 3.8
	Women	162.3 ± 2.3**	0.5	159.0-171.0	155.8	168.8	158.7 ± 2.1**	0.5	151.0-163.0	152.8	164.7	99.6 ± 4.5
Insulin (µU/mL)	Men	29.0 ± 2.7**	0.7	24.6-33.4	21.5	36.6	28.1 ± 2.3 **	0.6	24.3-32.0	21.5	34.7	11.5 ± 1.8
	Women	28.7 ± 1.1**	0.2	26.8 - 30.6	25.6	31.9	27.9 ± 1.8 **	0.4	24.6 - 31.1	22.7	33.2	10.7 ± 1.1
HOMA-IR	Men	12.4 ± 1.3**	0.3	9.9 - 14.9	8.7	16.0	11.7 ± 1.1**	0.2	9.5 - 13.8	8.6	14.8	2.9 ± 0.5
	Women	11.5 ± 0.5**	0.1	10.5 - 12.9	10.0	13.0	10.9 ± 0.8**	0.2	9.2 - 12.5	8.7	13.2	2.6 ± 0.4
Urea (mg/dL)	Men	59.3 ± 3.9**	1.0	52.8 - 65.7	48.2	70.3	36.7 ± 2.5*	0.6	32.5 - 40.9	29.5	43.8	29.6 ± 4.0
	Women	53.9 ± 1.8**	0.4	51.0 - 56.8	48.9	58.9	30.1 ± 2.1*	0.5	24.9 - 37.0	24.2	35.9	25.0 ± 2.2
Creatinine (mg/dL)	Men	1.7 ± 0.1**	0.1	1.3 - 1.9	1.2	2.3	1.1 ± 0.1*	0.1	1.0 - 1.3	0.9	1.3	0.9 ± 0.1
	Women	1.4 ± 0.1**	0.1	1.3 - 1.5	1.2	1.5	0.9 ± 0.1*	0.1	0.8 - 1.0	0.8	1.1	0.8 ± 0.1
GFR (mL/min/1.73 m²)	Men	43.2 ± 6.9**	1.7	36.8 – 58.0	24.0	62.4	70.8 ± 7.3*	1.8	60.0 - 83.8	50.5	91.1	91.1 ± 6.8
	Women	40.4 ± 2.1**	0.5	37.0 - 44.1	34.3	46.4	64.2 ± 4.9*	1.1	56.6 - 72.9	50.6	77.9	80.1 ± 7.2
CrCl (mL/min)	Men	66.5 ± 8.8**	2.2	58.0 – 85.0	42.0	91.0	96.0 ± 10.9*	2.8	81.0 - 113.0	65.7	126.3	130.3 ± 12.1
	Women	62.8 ± 5.3**	1.3	54.0 – 71.0	48.1	77.6	99.7 ± 9.7*	2.3	85.0 – 116.0	72.8	126.7	126.0 ± 8.0
LC (nmol/mL)	Men	29.0 ± 2.5**	0.6	21.9 - 36.0	21.8	36.1	37.2 ± 4.4*	1.1	30.0 - 44.5	24.8	49.7	47.4 ± 5.3
	Women	28.2 ± 4.9**	1.2	20.1 - 36.3	14.4	42.0	35.3 ± 2.1*	0.5	27.7 - 39.6	29.4	41.	44.9 ± 4.1
OPG (ng/mL)	Men	3.2 ± 0.1**	0.1	3.0 - 3.4	2.9	3.5	2.7 ± 0.1*	0.1	2.4 - 2.9	2.2	3.1	1.9 ± 0.3
	Women	3.1 ± 0.5**	0.1	2.3 - 4.0	1.7	4.6	2.6 ± 0.2*	0.1	1.9 - 3.3	1.9	3.2	1.7 ± 0.1
SA (mg/dL)	Men	87.6 ± 4.4**	1.1	80.4 - 94.9	75.2	100.1	79.4 ± 4.7**	1.2	71.6 - 87.2	66.1	92.7	61.0 ± 2.9
	Women	85.7 ± 2.8**	0.7	80.8 - 90.4	77.7	93.7	77.3 ± 2.6**	0.6	69.2 - 84.5	70.0	84.5	60.3 ± 5.9
Alb (µg/mL)	Men	29.5 ± 4.6**	1.1	22.0 - 37.0	16.7	42.4	11.1 ± 3.1*	0.8	6.1 - 16.2	2.5	19.8	6.1 ± 2.1
	Women	28.5 ± 5.2**	1.3	19.9 - 37.0	13.9	43.1	10.6 ± 2.5*	0.6	6.4 - 14.9	3.4	17.8	5.8 ± 2.0
FN (ng/mL)	Men	16.4 ± 4.4**	1.1	9.2 - 23.5	4.2	28.6	12.2 ± 3.4*	0.8	6.6 - 17.8	2.6	21.8	8.9 ± 3.1
	Women	15.3 ± 4.1**	1.0	10.0 - 24.1	4.0	26.7	11.3 ± 3.4*	0.8	5.2 - 17.0	1.8	20.8	8.0 ± 0.8

Data are presented as mean ± standard deviation (SD); SE: Standard error, Range: is the difference between the highest and lowest values in the set, 95% CI: Confidence intervals (Lower and Upper); significance level is indicated by *, Where: $p > 0.05$: p-value not significant, * indicates $p < 0.05$ (p-value significant); ** indicates $p < 0.01$ (p-value high significant), in comparison with the corresponding control value. T2DM: Type2 Diabetes Mellitus, DN: Diabetic Nephropathy, BMI: body Mass Index, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, GFR: Glomerular Filtration Rate, CrCl: Creatinine Clearance, LC: L-Carnitine, OPG: Osteoprotegerin, SA: Sialic Acid, Alb: Albumin, FN: Fibronectin.

Table 3. Levels of blood trace elements in men and women of healthy control and T2DM patients with and without DN.

Biomarker	Gender	T2DM Patients										Healthy Control
		With DN					Without DN					
		Mean ± SD	SE	Range	95 % CI		Mean ± SD	SE	Range	95 % CI		Mean ± SD
					Lower	Upper				Lower	Upper	
Se (ng/mL)	Men	63.5 ± 3.4 **	0.8	62.1 - 67.6	54.1	73.0	70.9 ± 1.8**	0.4	69.3 - 73.6	65.7	76.0	92.8 ± 1.0
	Women	50.8 ± 1.9 **	0.4	49.2 - 55.0	45.4	56.2	59.5 ± 1.7**	0.4	58.4 - 64.2	54.7	64.3	80.1 ± 3.2
Zn (µg/mL)	Men	0.8 ± 0.2 **	0.1	0.5 - 1.3	0.1	1.5	0.9 ± 0.1**	0.1	0.5 - 1.2	0.5	1.4	1.5 ± 0.3
	Women	0.7 ± 0.2 **	0.1	0.4 - 1.1	0.1	1.3	0.7 ± 0.1**	0.1	0.5 - 1.1	0.2	1.2	1.0 ± 0.3
Mg (µg/mL)	Men	16.7 ± 1.3 **	0.3	14.4 - 18.9	12.8	20.5	19.9 ± 1.1**	0.2	18.1 - 21.7	16.8	23.0	23.4 ± 3.7
	Women	12.9 ± 2.5 **	0.6	8.7 - 16.9	5.9	19.9	14.9 ± 1.4**	0.3	12.4 - 17.3	10.9	18.9	20.1 ± 2.68

Data are presented as mean ± standard deviation (SD); SE: Standard error, Range: is the difference between the highest and lowest values in the set, 95% CI: Confidence intervals (Lower and Upper); significance level is indicated by *, Where: $p > 0.05$: p-value not significant, * indicates $p < 0.05$ (p-value significant); ** indicates $p < 0.01$ (p-value high significant), in comparison with the corresponding control value. T2DM: Type2 Diabetes Mellitus, DN: Diabetic Nephropathy, Se: Selenium, Zn: Zinc, Mg: Magnesium.

the pancreas produces high doses of insulin to endeavour getting the glucose out of blood into cells. So, this leads to decrease in the ability of insulin to adjust and signal changes in the levels of glucose in the blood and perhaps grows IR (Al-Fartosy et al., 2020b). Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was used as a substitute measure of IR in our study. Although HOMA-IR was not the gold standard for assessment of insulin sensitivity, but it was a clinically useful index in many studies. Pancreatic β-cells function may be damaged during adolescence or later. Its deterioration ranges from hyperinsulinemia, secondary to IR, with normal glucose tolerance to β-cells failure with T1DM. When IR is present, or when insulin secretion is decreased in the later stages of the disease, free fatty acids (FFAs) are released in large amounts followed by an elevated production of glucose, triglycerides (TGs) and very low-density lipoprotein (VLDL) (Al-Fartosy and Mohammed, 2017). In addition, FFAs also decreased insulin sensitivity in muscles by discouraging insulin mediated glucose uptake. On the other hand, elevated blood glucose level, and to some extent circulating FFA, raise insulin secretion and lead to increase hyperinsulinemia. It is obvious that IR causes blood glucose and FFAs levels to rise, thus, worsening the IR and hyperglycemia with released FFAs further rises insulin secretion forming a wicked circle (Cicero et al., 2015). Furthermore, the pivotal role of the fat mass does not rule out the significance of heritability in the evolution of the metabolic syndrome. Some environmental factors, such as the obesity epidemic due to the lack of physical exercise and increased caloric intake, are obviously responsible for the present elevation in the incidence of the metabolic syndrome worldwide (Febbraio, 2017). So far, the predilection to get weight is highly individual and determined by genetic factors. It has been speculated that TG's accumulation in skeletal muscles plays a lineal role in the aetiology of IR. The results of several studies have demonstrated that the degree of IR is positively correlated with intramuscular TG's content. So, chronic hyperglycemia and dyslipidemia in T2DM can both produce hurtful effects on β-cell structure and function (Xin et al., 2019). Even though inter-relationships between lipotoxicity and glucotoxicity have not yet been illustrated, it is supposed that glucotoxicity could lead to β-cell apoptosis independently of dyslipidemia. While lipotoxicity may damage β-cells only in the presence of hyperglycemia. In the situation of normoglycemia, raised FFAs must oxidized easily in the mitochondrion and should not damage the β-cell of pancreas. Moreover, as DN progress, IR may be stimulating the decrease in renal function toward ESRD. High TG's levels are a risk factor for proteinuria development and TG-rich apolipoprotein B-containing lipoproteins clearly promote the progression of renal insufficiency (Mahfouz et al., 2016).

Our data revealed that urea and creatinine levels

were increased while GFR and CrCl levels were decreased in T2DM patients with and without DN when compared with healthy controls, this agrees with another study with the same results (Chen et al., 2016).

L-carnitine (LC) is an essential co-factor for FA metabolism and other metabolic pathways, with body stores maintained primarily in skeletal muscle. The reduction in some catalysts and co-factors used by humans to biosynthesize LC from L-lysine and L-methionine via a series of reactions in the liver, kidney and brain such as S-adenosylmethionine, α-ketoglutarate, oxygen, ascorbic acid, iron, glycine, vitamin B6 and γ-butyrobetaine hydroxylase could lead to decrease in LC level in T2DM patients with and without DN (Giudetti et al., 2016). Moreover, the combination of impaired glucose tolerance (IGT) with simple obesity may stimulate the progress of hepatic ketogenosis in coupling with a raised SCAC (short-chain acylcarnitine) and an elevation in carnitine acylation with a decrease in LC levels (Bae et al., 2015).

Osteoprotegerin (OPG), a soluble glycoprotein composed of 380 amino acid residues, may be found in osteoblasts of the bone, vascular endothelial cells and smooth muscle cells. Obesity could increase the OPG level in T2DM patients with and without DN as OPG expression has been assured in adipose tissues as well. Abdominal adipose tissue is the largest fat tissue store in the body and correlates with metabolic syndrome, cardiovascular disease risk and other systemic inflammatory markers and may influence atherosclerosis. Adipose tissue releases many adipokines but also there is elevating evidence that there is a hormonal cross-link between adipose tissue and bone which leading to raise OPG level (Bernardi et al., 2016). In addition, insulin resistance (IR) may have a pivotal role in increasing OPG level in T2DM patients with and without DN because it could be the potential mechanism for elevating serum lipid levels especially levels of TG and this led to increase OPG levels. Moreover, increased creatinine and cystatin C levels may elevate the OPG level in T2DM patients with and without DN because cystatin C is a cysteine proteinase inhibitor that declines osteoclastogenesis by interfering at a late stage of pre-osteoclast differentiation which led to decreased protein clearance and decline in GFR as a probable cause of age-linked OPG elevation (Xia et al., 2015).

Sialic acid (SA) is a fundamental component of glycoproteins and glycolipids; it has a major role as a co-factor for receptors of many cells. Increased levels of SA in blood serum of T2DM with and without DN could be attributed to the fact that SA is a crucial acute phase reactant and maintains the negative charge of kidney glomerular basement membrane that is one of the master organizers of membrane permeability. Therefore, vascular endothelium holds higher levels of SA and hence comprehensive pathological states when there is tissue deterioration, tissue reproduction and inflammation connected with T2DM accounts for its sloughing to the circulation leading to raise vascular permeability and overall elevated SA level (El-Sayed et al., 2018). From the other hand, obesity, hyperglycemia and IR could develop inflammation and this may play a factor connecting DM to the development of diabetic complications. Increased glucose levels could enhance inflammation by raising oxidative stress. Another probability is that inflammatory response is a result of vascular complications after DM. Hence, diabetic vascular complications can lead to a severe tissue deterioration which may trigger excretion of large levels of local cytokines from cellular infiltrates like endothelial cells and macrophages. Moreover, this excretion of cytokines could stimulate an acute phase response with release of acute phase glycoproteins with SA from the liver tissue into the general circulation again which raised the levels of SA in blood (Zhu et al., 2017).

Selenium (Se), a trace element, is a major component in glutathione peroxidase (GPx) which is one of the main antioxidant enzymes in the human body and responsible for preventing the production of free radicals, decreasing their activity or destroying them (Al-Fartosy et al., 2020a). Se levels were found to be significantly lower in T2DM patients with and without DN when compared with healthy control group. It is known to work as an antioxidant and peroxynitrite scavenger when integrated into selenoproteins. This lack in Se levels may lead to oxidative stress, decline insulin secretion and elevated IR in some empirical models; thereby, perhaps taking a causal function in the forward and pathogenesis of T2DM. Moreover, elevated oxidative stress and glycosylation play a main pathogenic role in diabetic endothelial cell dysfunction in T2DM patients with and without DN (Al-Fartosy et al., 2019).

Zinc (Zn) is one of the fundamental trace elements which are involved in the synthesis, secretion, conformational integrity and storage of insulin. Our study reported that Zn levels in T2DM patients with and without DN were lower than the control group. The probable explanation of the current findings may come as the following reasons: in the mammalian pancreas, Zn is fundamental for the correct processing, secretion, storage and action of insulin in beta cells. Insulin is stored in secretory sacs or granules, where two Zn^{2+} ions coordinate six insulin monomers to generate the hexameric form on which maturated insulin crystals are based (Al-Fartosy et al., 2017a). It is also known that many other chronic disorders like DM could decrease Zn levels, for example, DM increases the excretion of minerals such as Zn in urine or may decrease gastrointestinal absorption of Zn. Also, hyperglycemia in DM is often linked with hyperzincuria, which is of kidney origin, and raise urinary loss of Zn^{2+} and declines of its level in entire body Zn^{2+} (Al-Fartosy et al., 2017b). Kidney tubular flaw in handling Zn and glucose-induced, osmotic diuresis are other probabilities. Furthermore, Zn may

enhance glycaemia and a restored Zn status in patients with T2DM (with or without DN) may oppose the harmful effects of oxidative stress which help to prevent complication beneficial antioxidant effects in people with T2DM. Zn has been illustrated to have particularly importance in the light of the mischievous outcomes of oxidative stress in subjects with DM. Zn has antioxidant properties, thus, it can balance macromolecules against radical stimulated oxidation (Al-Fartosy et al., 2020a).

Magnesium (Mg) is a trace element which is responsible for maintaining some body functions. Mg is demanded for several enzymes' activities and for neuromuscular transmission. In our current study, the serum level of Mg showed a high significant decreasing in T2DM patients with and without DN when compared with healthy subjects. Mg is essential for many enzymes that play a key role in glucose metabolism (Al-Fartosy et al., 2020b). The hypomagnesaemia in T2DM patients with and without DN in our study may be due to poor dietary intake, elevated urinary loss because of hyperglycemia, impaired absorption of Mg, imperfect Mg reabsorption from kidney tubules, osmotic diuresis and loss of plasma protein bound to Mg. Mg diminution is said to decrease the insulin sensitivity, thereby, raising the risk of secondary complications. Hyperglycemia leads to decline cellular Mg levels. Hypomagnesaemia leads to decreased function of Mg dependent enzymes, collagen and ADP-induced platelet convenience and oxidative stress. Moreover, insulin lack and resistance lead to decreased tubular reabsorption of Mg and resulted in hypomagnesemia which prefer the onset and development of diabetic microangiopathy via a decrease in activity of Na^+/K^+ ATPase pump. So, hypomagnesemia alone foretells the progression to ESRD in patients with advanced DN (Al-Fartosy et al., 2017a).

Fibronectin (FN), a protein with high molecular weight, is an extracellular matrix (ECM) protein and is congregated by cells into elastic and insoluble fibrils. The significant increase in FN level in T2DM patients with and without DN in our study might be due to hyperglycemia because it increases the mRNA levels of FN in the kidney cortex and leads to the overproduction of FN in kidney tissues. Hence, the glycemic disruption is a crucial factor that raises FN synthesis in the kidney, eventually leading to diabetic glomerular injury. As FN is produced by kidney mesangial cells, it was also found that FN in these cells is elevated proportionately in the disease states characterized by mesangial expansion including diabetic nodules (Lee and Choi, 2015). Furthermore, the significant elevation in FN level in T2DM patients with and without DN in our study could be due to connective tissue growth factor (CTGF) because it plays a key role in glomerular alteration in diabetic sclerosis because this mediator stimulates transient actin cytoskeleton disassembly in mesangial cells, high production of FN and mesangial cell hypertrophy (Indriani et al., 2020).

Additionally, protein kinase C (PKC) may play a pivotal role in raising FN levels in T2DM patients with and without DN through a complex mechanism involving its isoforms (PKC-α, PKC-β, and PKC-ε). These isoforms have been engaged as mediators of kidney fibrosis and mesangial expansion via upregulating of vascular endothelial growth factor (VEGF) expression in mesangial cells, as well as transforming growth factor-β (TGF-β) and FN in the glomeruli. Also, NADPH oxidase-driven renal oxidative stress stimulates mesangial expansion and albuminuria by elevating the expression of renal FN (Uwaezuoke, 2017).

Conclusion

The results of this study illustrate that obesity is a case accompanied by elevated levels of insulin and glucose which are the vigorous indicators for evaluation of the IR syndrome in diabetic patients especially with kidney disease. In addition, kidney disease is linked with the raising of IR and BMI levels in T2DM patients which had been observed via decreasing the levels of GFR, CrCl and increased serum urea and creatinine levels. Furthermore, LC had a renoprotection features through anti-inflammatory and anti-sclerotic effects as well as its ability to improve insulin sensitivity in insulin resistant diabetic patients. Therefore, decreased levels of serum LC may represent a powerful indicator for evaluation of the oxidative stress syndrome in diabetics than non-diabetics. Additionally, the increase in OPG levels is an independent risk factor for MAU and may be involved in vascular calcification independently of progression of DN in patients with T2DM. Further, elevated levels of serum SA are strongly associated with the presence of nephropathy and it could be representing a predictor of kidney dysfunction in DN. Besides, the decrease in some essential antioxidant trace elements levels, such as Se, Zn and Mg, is a powerful indicator for evaluation of the oxidative stress syndrome in diabetic patients with and without DN than non-diabetics. Moreover, urinary biomarkers like Alb and FN and the combination of these two biomarkers demonstrated an excellent diagnostic value for early-stage of DN in patients with T2DM. Finally, these investigated clinical biomarkers must be used in the future for early detection of DN in men and women of T2DM.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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