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Assessment of Glycemic Control, Renal Function, and Oxidative Stress Parameters in Type 2 Diabetes MellitusPatients

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Abstract

Diabetes mellitus (T2DM) is a multifactorial syndrome that israpidly rising in all the continents ofthe globe, causing elevated blood sugar levels in affected people. A sample of 81 Iraqi T2DM patients was investigated based on several parameters. Glycemic control parameters includedlevels of fasting blood glucose (FBG), glycated hemoglobin (HbA1C), and insulin, along with insulin resistance (IR) and insulin sensitivity (IS). Renal function tests includedmeasuring the blood levels of urea and creatinine. Oxidative stress parameters included total antioxidant capacity (TAC) and thelevel of reactive oxygen species (ROS). The results of the present study showed a highly significant ($P<0.01$) increase in FBG, HbA1c, insulin and IR levels in T2DM patients as compared to control.Insulin sensitivity showed a highly significant ($p<0.01$) decrease in patients compared with control.Urea and creatinine levelsincreased in T2DM patients, but the differences were insignificant. TAC level significantly ($P<0.05$) increased in patients compared with control. Also, the levels of ROSrevealed a highly significant ($P<0.01$) increasein T2DM patients compared with the control. Correlation analysis showedthat FBG has a highly significant ($P<0.01$) positive correlation with IR, urea, creatinine and ROS, as well as a significant ($P<0.05$) positive correlation with TAC. However, FBG shows a highlysignificant ($P< 0.01$) inverse correlation with IS. The levels of HbA1C show a significant ($P<0.05$) positive correlation with IR, creatinine, and TAC, whereas it has a highly significant ($P<0.01$) positive relation with ROS. However, HbA1C level has a highly significant ($P<0.01$) inverse relation with IS. Insulin has highly significant ($P<0.01$) positive and negative associations with IR and IS, respectively.IR showshighlya significant ($P<0.01$) inverse correlation with IS, significant ($P<0.05$) positive correlation with creatinine, and highly significant ($P<0.01$) positive correlation with ROS. IS has a significant($P< 0.05$) inverse correlation with urea. Urea shows a highly significant ($P<0.01$) positive correlation with creatinine. TAC has a significant ($P<0.05$) inverse correlation with ROS. Conclusion: diabetic patients revealed poor glycemic control. Fluctuating blood glucose concentrations may contribute significantly to oxidative stress, probably even more than chronic hyperglycemia. The observed significant positive correlation between FBG and the other tested parameters revealed that hyperglycemia is an obvious independent risk factor for T2DM progression.

Keywords: Glycemic control, fasting blood glucose, urea, creatinine, total antioxidant capacity, reactive oxygen species.

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تقييم معاييرالتحكم في نسبة السكر في الدم، وظائف الكلى والإجهاد التأكسدي في مرضى السكري النوع الثاني

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الخلاصه

داء السكري هو متلازمة ذاتعواملمتعددة ، تزداد بشكل متسارع عالميا, يعمل على جعل مستويات السكر في الدم مرتفعة الى حد كبير.تم جمع عينات الدم الوريدي الصائم من 81مريض مصاب بالسكر النوع الثاني و 20 من الأشخاص الأصحاء كعينة سيطرة وذلك لتقييم بعض المعايير التي تشمل معايير التحكم في نسبة السكر في الدم وهي سكر الدم الصائم, الهيموجلوبين المسكر، الأنسولين، مقاومة الأنسولين وحساسية الأنسولينو اختبارات وظائف الكلى والتي تشمل اليوريا والكرياتينين. وشملت معايير الاجهاد التاكسدي (اجمالي قدرة مضادات الاكسدة, أنواع الأوكسجين التفاعلية). أظهرت نتائج الدراسة الحالية زيادة معنوية عالية ($p<0.01$) في مستويات FBG, HbA1c , الانسولين ومقامة الانسولين في مرضى T2DM مقارنة بمجموعة السيطرة. أظهرت حساسية الأنسولين انخفاضًا معنويًا عاليا ($p<0.01$) في مرضى T2DM مقارنة مع السيطرة. زادت اليوريا والكرياتينين في مرضى T2DM الا ان قيم p لم تكن معنوية ($P>0.05$).زادت TAC بشكل معنوي ($P<0.05$) في المرضى مقارنة مع مجموعة السيطرة. كما أظهرت مستويات ROS ارتفاعًا معنويًا عاليا ($P <0.01$) في المرضىT2DM مقارنةً بمجموعة السيطرة.يُظهر تحليل الارتباط أن FBGيرتبطبعلاقة إيجابية ذات دلالة إحصائية عالية ($P<0.01$) مع مقاومة الانسولين, اليوريا, الكرياتينين و ROS ، كما له ارتباط إيجابي معنوي ($P<0.05$) مع TAC. بينما يظهر FBG ارتباط سلبي ذو دلالة عالية ($P<0.01$) مع IS.يُظهر HbA1C ارتباطًا إيجابيًا معنويًا ($P<0.05$) مع مقاومة الانسولين, الكرياتينين و TAC ، كما يرتبطHbA1C بعلاقة إيجابية عالية ($P<0.01$) مع ROS, في حين يرتبط بعلاقة عكسية ذات دلالة عالية ($P<0.01$) مع IS.للأنسولين علاقة ارتباط ايجابية وسلبية ذات دلالة معنوية عالية ($P<0.01$) مع مقاومة و حساسية الانسولين على التوالي. مقاومة الانسولين تظهر ارتباط عكسي معنوي ($P<0.01$) مع حساسية الانسولين، وارتباط إيجابي معنوي ($P<0.05$) مع الكرياتينين, كما تظهر ارتباط ايجابي ذو دلالة عالية ($P<0.01$) مع ROS. حساسية الانسولين ترتبط ارتباط سلبي معنوي ($P<0.05$) مع اليوريا. في حين تظهر اليوريا علاقة إيجابية عالية ($P<0.01$) مع الكرياتينين.

تهدف الدراسة الحالية الى تقييم مستويات بعض المعايير في مصل الدم ومنها معايير التحكم بنسبة السكر في الدم، وظائف الكلى ، ومعايير الإجهاد التأكسدي في كل من مرضى السكري النوع الثاني والأشخاص الاصحاء فضلا عن تقييم العلاقة بين هذه المعايير.

Introduction

Diabetes mellitus (DM) is a multifactorial syndrome characterized by abnormal hyperglycaemia. Particularly, the incidence of T2DM is increasing at an alarming rate [1]. The incidence of diabetes in Iraq is very high, with one of eachfive adults being affected by T2DM [1,2]. Early diagnosis in patients with risk factors seems to be of utmost importance;hence, routine screening for diabetes is started at the age of 45 years and is repeated at least every three years, while in subjects at risk it is started earlier [3].Type 2 diabetes is typically characterized by certain limits of blood parameters; HbA1c $\geq$ 6.5%, FBG $\geq$ 126 mg/dL, or a 2-hour postprandial glucose of $\geq$ 200 mg/dL after 75 g oral glucose intake [1]. The HbA1c value is a measure of glucose that is bound to hemoglobin and it indicates the average blood glucose for three months before the test [4]. Insulin is synthesized in the rough endoplasmic reticulum of the Beta-cells.The purification of blood from insulin depends on its

receptors and the enzyme that degradesit.Deficiency in these two molecules will lead to impaired insulin purification and then hyperinsulinemia,resulting in the development of insulin resistance [5]. Insulin resistance is defined as a condition in which target tissues have decreased sensitivity to insulin, which leads to elevated levels of both blood insulin and glucose [6,7].

In T2DM, the glomerular filtration rate also deteriorates significantly, which if not medically treated,causes nephropathy which will progress into chronic kidney disease, which in turn complicates the clinical treatment of T2DM [8]. Urea and creatinine are raised in uncontrolled T2DM, which in addition to hyperglycemiaisusually correlated with the severity of renal damage. The excretion of urea is a vital physiological function of the kidney because urea actsas a carrier of waste nitrogen. Creatinine is the breakdown product of creatinin phosphate, which is released from the skeletal muscles at a stable rate [9].

Oxidative stress is defined as an imbalance between the generation and removal of ROS in favor of the oxidants formation. It appears to be raised in a system where the production rate of free radicals is increased or/and the antioxidant mechanisms are reduced [4]. Oxidative stress probably contributes to thedevelopment of peripheral IR and many of otherlong-termcomplications of T2DM. Excess nourishment, along with inactive lifestyle, lead to theaccumulation of glucose within muscles, adipose tissues, and pancreatic cells, which leads to a surplus generation of ROS, especiallysuperoxide anion, by the mitochondrial electrontransport chain [10]. Fluctuating blood sugar concentration may significantly contribute to oxidative stress, with this effect being probably even higher than that of chronic hyperglycemia [11]. Despite over the last 10 years, many drugs have been approved for T2DM treatment, a large number of patients fail to obtain an agreeable metabolic control. This can be accountedfor by several factors that include reducedphysical activity, heavy diet, and adherence to medications.Also,the underlying pathophysiological process and phase of the disease have more significant effects on the performance of glucose-lowering drugs [12].

Materials and Methods

Subjects

The participants of the study consist of 81 patients with T2DM (35 men and 46women) attending the Diabetes and Endocrine Care Center of Marjan Teaching Hospital, Hilla province, Iraq. In addition, 20 healthy subjects were enrolled as control. All participants were guided to fast for 10–12 hours before starting to collect blood samples.

Collectionand preparation of blood samples

Fasting venous blood samples were collected from all participantsin EDTA tubes forthe determinationof HbA1C concentration. For serum preparation, theblood was collected indisposable gel tubes, allowed to clot at room temperature for 10-15 min, and centrifuged at 2000 × g for approximately 10-15 min.Theobtained serumwas stored at -20˚C for the determination of FBG, insulin, urea, creatinine, TAC, and ROS.

Biomarkers Analysis

Enzymatic colorimetric methodwas employed to estimate FBG (mg/dl)usingthe Linear kit, Spain. The HbA1C level was assessed by an automated Epithod®616 Analyzer (DxGen /Korea) based on the boronate affinity principle. Insulin concentration was measured using an ELISA kit(CALBIOTECK/USA), which isrelied on the standard sandwich enzyme-linked immune sorbent assay. IR was detected by the homeostasis model estimation of IR (HOMA-IR) [13] and calculated by applyingthe equation [IRHOMA = (Fasting insulin × Fasting glucose) / 405]. The quantitative insulin sensitivity check index (QUICKI) was calculated using the equation [QUICK I= 1 / (log(fasting insulin) + log(fasting glucose)].

Berthelot’s method was employed to assess levels of urea (mmol/L) using the Linear kit, Spain, while Randox kit (Randox, UK) was used to evaluate creatinine levels (mmol/L).

The levels of TAC were estimated using the cupric ion reducing antioxidant capacity (CUPRAC) method developed previously [14], which estimates the capacity of an antioxidant in the reduction of an oxidant, whichchanges color when reduced. Reactive oxygen species was detected using a novel method, which was developed by Erel[15]. This method depends on the measurement of color intensity formed as a result of oxidation of the ferrous ion–o-dianisidine complex to ferric ion by the oxidant found in the serum.

Results and Discussion

Levels of glycemic control parameters in the study population

Glycemic control parameters which involved FBG, HbA1C, insulin, IR, and ISare shown in Table 1. Fasting blood glucose was highly significantly ($p < 0.01$) increased in the T2DM patients (204.88±50.03mg/dl) than in control (82.55 ± 11.84mg/dl). Also, the level of HbA1c showed a highly significant ($p<0.01$) increase in T2DM patients (8.15 ± 2.04%) compared to control (5.05±0.73%).Levels of insulin and IR showed a highlysignificant ($P< 0.01$) increase in patients as compared to control (18.36 ± 5.67versus7.07 ± 2.38μIU/ml for insulin and 8.74 ± 2.98versus1.91 ± 0.60 for IR, respectively). Insulin sensitivity showed a highly significant decrease in T2DM patients (0.30±0.03) when compared with control subjects (0.37±0.02).

Table 1-Levels of glycemic control parameters in T2DM patients and control

Parameters	Mean ± SD		P value
	Patients	Control	
FBG(mg/dl)	204.88±52.03	82.55±19.84	0.000**
HbA1C (%)	8.15±2.04	5.05±0.73	0.000**
Insulin (μIU/ml)	18.36±5.67	7.07±2.38	0.009**
Insulin resistance	8.74±2.98	1.91±0.60	0.000**
Insulin sensitivity	0.30±0.03	0.37±0.02	0.000**
SD: Standard Deviation, ** significant (P <0.01)			

The high level of FBG in T2DM patients of this study is expected.This finding is in agreement with previous reports [16,17]. Hyperglycemia is the chief component of T2DM (generally described as fasting glucose > 180 mg / dL), which arises from impaired insulin secretion with varying degree of peripheral IR [18]. The increased level of HbA1C in the T2DM patients suggests a poor glycemic control as compared with healthy controls. The HbA1c is a useful tool that is regarded as a reference marker in the surveillance ofglycemic status in T2DM patients [4]. Similar findings were reported by other studies which also revealed a significant elevation in the level of HbA1c [19,20].

Increased insulin levels in the patients are logical and maybe due to the hyperglycemic state, which couldlead to a 50-fold increase in the biosynthesis of insulin [21]. Insulin resistance is also associated with hyper-insulinaemia that promotes higher production of free radicals by NADPH-dependent mechanisms [22]. The present study also agrees with the results reported by an earlier work [23], which detected a boost in insulin levels in T2DM patients that was attributed to the IR status. Insulin resistance has a serious role in the development of hyper-insulinemia, resulting from the attempts of the pancreatic cells to compensate muscle cells' need for insulin , leading to higher insulin production and hence the development of T2DM [6]. Moreover, the present finding may be due to β-cell dysfunction. The current study agrees with Mohsen [16], who recorded a spike in the insulin, IR, and FBG in comparison with controls subjects. This state of insulin resistance manifests as mild postprandial hyperglycemia [13]. Gradually, pancreatic β-cell function declines and results in relative insulin deficiency and subsequently fasting hyperglycemia, then full-blown T2DM [6]. In addition, werevealed that IS was significantly decreased in T2DM patients compared to controls. The reasons for this finding may be the hyperglycemia and hyper-insulinemia, which may result in impairment of IS [7]. Butler *et al.* stated that T2DM is primarily characterized

by impaired IS, as well as the destruction of beta-cells over the subsequent phases of the disease [24]. Another study revealed that the excess of glucocorticoids may lead to promote proteolysis, leading to a raise in aminoacid concentration which impairs steps of insulin signals, which in turn isthe cause of the impairment of IS [25]. The impaired IS in T2DM patients recorded in the present study was also reported in several studies, including the study of Mohsen [16].

Renal function tests in the study population

Urea and creatinine levels were used to assess theimpairment of renal function in the studied population, as shown in Table 2. The resultsshowedinsignificant differencesin thelevels of urea and creatinine between T2DM patients and control subjects (6.02±1.89versus 4.67±1.77mmol/lfor urea and80.79±14.64 versus 67.7±9.22 mmol/l for creatinine, respectively).

Table 2-Levels of renal function parameters in T2DM patients and control

Parameters	Mean ± SD		P value
	Patients	Control	
Urea (mmol/l)	6.02±1.89	4.67±1.77	0.101 ^{NS}
Creatinine (mmol/l)	80.79±14.64	67.7±9.22	0.236^{NS}
SD: Standard Deviation, NS: non-significant			

Various studies have shown that higher urea levels are proportional to higher creatinine levels [26]. However, T2DM patients show slightly higher levels of urea and creatinine than those inthe control subjects,the differences were insignificant.The findings of the present study partly agree with those of a previous research which showed that the levels of urea and creatinine weresignificantly ($P \leq 0.05$) higher in the T2DM patients [20]. The insignificant results found in the present study may be attributed to different sample size, technical reasons, differences between the populations in health habits, and the genetic predisposition to the disease.

Levels of oxidative stress parameters in the study population

Total antioxidant capacity and ROS levelswere used to assess oxidative stress in the T2DM patients and controls subjects, as shown in Table 3. The results show a significant ($P<0.05$) increasein the levels of TAC in the T2DM patients (2451.94±500.87 mmol/l) compared with control subjects (2036.74±214.55mmol/l).Also, the levels of ROS show a highly significant ($P< 0.01$) increase in patients (174.81±41.50mmol/l) as compared to control (23.72±2.88mmol/l).

Table 3- Levels of oxidative stress parameters in T2DM patients and control.

Parameters	Mean ± SD		P value
	Patients	Control	
TAC (mmol/l)	2451.94±500.87	2036.74±214.55	0.031*
ROS (mmol/l)	174.81±41.50	23.72±2.88	0.000*
SD: Standard Deviation, *significant ($P \leq 0.05$)			

The findings of the current work agree with the results of several studies,including an earlier work [19] which showed that TAC levels also significantly increased in T2DM patients. The increase in TAC occursto provide more protectionagainst free radical aggression.These results conform the notion that the increase in free radicals appear to be primarily associated with theincrease in antioxidant levels and with disease development;as soon as the antioxidant levels decrease, the disease complications will develop. In contrast, the result of the present study disagreed with those of another study[27], which showed that the mean of TACis

significantly lower in T2DM patients compared with control. Nevertheless,the study of Kharroubi*et al*.statedthat there isno clear explanation for the TAC behavior [28].The synergistic action of antioxidants in human plasma is known to supply greater protection against the aggression of ROS than any single antioxidant alone [4].

In the present study, ROS levels significantly increased in T2DM patients,, which agrees with earlier reports [17]. High levels of ROS in T2DM patients seem to be due to hyper-insulinemia and hyperglycemia, which are known to cause elevated free radical concentrations in the plasma. In relation to the production of insulin, ROS are known to be important byproducts of enzyme-driven folding of the proinsulin in the endoplasmic reticulum, each one disulfide bond can lead to the production of one molecule of ROS; when the insulin molecule has three disulphide bonds, which are essential for its action, three molecules of ROS are generated [29].

Correlation analysis between studied parameters of T2DM patients

Correlation analysis, using Pearson's correlation coefficient,was applied in the current study. Table 4 shows severalsignificant correlations among the studied parameters.FBG had a highly significant (P<0.01) positive correlation with HbA1C. However,insignificant positive correlation between FBG and insulin was recorded. Also, FBG showed a highly significant (P<0.01) positive correlation with IR, a highly significant (P< 0.01) negative correlation with IS,and a highly significant (P< 0.01) positive correlation with urea and creatinine. FBG also hada significant (P< 0.05) and a highly significant (P< 0.01) positive correlations with TAC and ROS, respectively.HbA1C showed significant (P<0.05) positive correlations with IR, creatinine, and TAC.Also,HbA1C had a highly significant (P<0.01) positive correlation with ROS, while it showed a highly significant (P< 0.01) inverse relation with IS. Insulin had a highly (P<0.01) significant positive correlation with IR, while it showed a highly significant (P<0.01) inverse relation with IS. Insulin resistance showed a highly significant (P<0.01) negative correlation with IS, insignificant correlation with urea, and significant (P<0.05) positive correlation with creatinine. Also, IR had insignificant correlation with TAC and a highly significant (P<0.01) positive correlation with ROS. The results of correlation analysis also showed that IS had a significant (P<0.05) negative correlation with urea, while it had no significant correlation with theremaining parameters . Urea exhibited a highly significant (P< 0.01) positive correlation with creatinine, while it had no significant correlation with theother parameters. Creatinine showed a significant correlation with the aforementioned parameters only, while it did notshow a significantcorrelation with other parameters. Total antioxidant capacity showeda significant (P<0.05) negative correlation with ROS.

Table 4-Correlation analysis between studied parameters of T2DM patients

Parameters		FBG(mg/dl)	HbA1C %	Insulin	Insulin resistance	Insulin sensitivity	urea	Creatinine	TAC (mmol/l)	ROS (mmol/l)
FBG (mg/dl)	**r**	1								
	p									
HbA1C %	**r**	0.82	1							
	p	0.000**								
Insulin (µIU/ml)	**r**	0.21	0.20	1						
	p	0.29ns	0.08 ns							
Insulin resistance	**r**	0.45	0.27	0.74	1					
	p	0.000**	0.02*	0.000**						
Insulin sensitivity	**r**	-0.51	-0.34	-0.67	-0.87	1				
	p	0.000**	0.002**	0.000**	0.000**					
urea (mmol/l)	**r**	0.65	-0.047	0.084	0.105	-0.444	1			
	p	0.001**	0.679 ns	0.459	0.354	0.040*				

				ns	ns					
Creatinin (mmol/l)	**r**	0.51	0.331	0.086	0.381	-0.315	0.522	1		
	p	0.002^{**}	0.041^{*}	0.449 ns	0.012^{*}	0.176^{ns}	0.000^{**}			
TAC (mmol/l)	**r**	0.216	0.221	0.02	0.05	0.03	-0.123	0.093	1	
	p	0.023^{*}	0.011^{*}	0.88^{ns}	0.69^{ns}	0.83^{ns}	0.277 ns	0.411^{ns}		
ROS (mmol/l)	**r**	0.610	0.386	0.096	0.269	-0.04	0.056	0.116	-0.366	1
	p	0.000^{**}	0.000^{**}	0.261 ns	0.000^{**}	0.82^{ns}	0.770^{ns}	0.542^{ns}	0.021^{*}	
*Correlation is significant at the 0.05 level (2-tailed)										
**Correlation is significant at the 0.01 level (2-tailed)										

The observed significant correlations between FBG and the indicated parameters revealed that raised FBG is an obvious independent risk factor for the progression of T2DM. Levels of FBG are physiologically determined by the rate of hepatic glucose production, which is a function of the rate of insulin production, hepatic sensitivity to insulin levels, and free fatty acid concentrations [1]. Regarding thepositive association with HbA1C, the current finding agrees with the similar result ofa previous study [30]. The explanation behind these results may be the contributionof excess glucose levels in the blood to more binding to tissue proteins, including hemoglobin, thus increasing the amount of HbA1C, which indicates poorer control of blood glucose levels in T2DM patients [31].

The positive association observed between FBG and IR was relatively strong and seems to be multi-causal. The development of IR is one of the deleterious effects of hyperglycemia, through the generation of ROS, which affects insulin-induced tyrosine auto-phosphorylation of insulin receptors [32]. Over many years, chronic exposure to abnormally high levels of glucose can exert toxic effects on beta-cells.Thus,chronic hyperglycemia can persuade insulin secretion defect and worsen IR.The current study is consistent with an earlier work[33],which also reported a significant positive correlation between FBG and IR levels in obese T2DM Iraqi patients. In addition, the inverse correlation between FBG and IS was expected, because cells lose their sensitivity to insulin, which leads to subsequent hyperglycemia. The decreased IS seen in T2DM patients primarily affects the liver and peripheral tissues, leading to increased hepatic glucose output and diminished glucose uptake by the skeletal muscles and adipose tissues [13]. Previous studies found that impairment of insulin sensitivity may result from hyperglycemia and hyperinsulinemia [7]. Abu-Khumrahrecorded a negative, but non-significant, correlation between FBG and IS in T2DM Iraqi women [34].

Poorly controlled blood glucose levels would cause an increase in urea and creatininelevels, thus increasingthe chances of the patient suffering from diabetic nephropathy. This confirmsthe previous finding, which demonstrated that hyperglycemia is one of the major causes of progressive renal damage [35]. Typically, patients with T2DM should be monitoredperiodically for nephropathy and the levels of urea and creatinine. The current study is consistent with a previousstudy [9], which showed a strong positive correlation between urea levels and blood glucose levels, both fasting and post-prandial. Similarly, the current finding agrees with that of another study, which also revealed a highly significant positive correlation between FBG and creatinine levels in T2DM [36].

The significant positive correlation betweenTAC and ROS is consistent with the results of a similar previous study [28]. Chronic exposure to high levels of glucose increases the production of ROS and generates oxidative stress in islet cells.Thus, with high glucose levels, TAC increases to provide a protective effect against ROS [37]. This alsoconfirmsthe findings of aprevious report, which recorded significantly high TAC levels in T2DM patients [17]. Several mechanisms that bindhyperglycemia with increased ROS production were suggested. These mechanisms include increased glucose flux through the polyol pathway, formation of

advanced glycation end-products (AGEs) enhancing oxidative stress, mitochondrial synthesis of superoxide anion radical (O^{-2}), and NF-κB signaling pathway activation causing inflammatory reaction, thus increasing ROS production in phagocytes [38].

HbA1C usage can avoid problems like dayto day variability of glucose values and the need ofthe patients to fast. Similar findings were reported in a previous study, which indicated that the level of HbA1c increased,havinga significant positive correlation with the increased level of IR and a significant negative correlation with IS in T2DM patients [39]. HbA1C and creatinine showed a significant positive correlation, which indicates that creatinine increases with the increase in HbA1c. In the same context, HbA1C had an insignificant negative correlation with urea, but however,thesefindings are consistent to some extent with those of a previousstudy [40], which found that HbA1c has significant positive and negative correlations with creatine and urea, respectively. The insignificant negative correlation with urea may be duethe fact that isocyanate derived from urea may lead to the formation of carboxylatedHb, which can interfere with some HbA1c assays.

It is clear from the results presented in the current study that HbA1C shows a significant positive correlation with TAC and ROS levels. Similar results were also observed in other studies [28,37]. Regardless of the effect of diabetes on the levels of antioxidant status, the increase in TAC levels in T2DM patients seems to reflect adaptation to a continuous increase in ROS. Another explanation behind these positive relationships is that some T2DM patients, after contracting the disease, tend to change their health habits and take some antioxidant supplements, such as vitamins. In addition, the levels of insulin showed a significant positive correlation with IR, whereas it had a highly significant inverse correlation with IS. Hyperinsulinemia and IR are major elements of T2DM;when present together, they show multiplicative rather than additive effects, especially increasing the morbidity and mortalityrates are resulting from the disease. The positive correlation between insulin and IR is in agreement with those revealed by other authors [23].

Regarding the inverse correlation with IS, the current study is consistent with another study, which showed that impaired IS may be caused by both hyperglycemia and hyperinsulinemia [7]. It is clear from the results of the current study that IR has a highly significant inverse correlation with IS and a significant positive correlation with ROS.

The negative relationship between IR and IS is reasonable, because the target tissues in IR patients show a clear decrease in insulin sensitivity, which in turn leads to an increase in both insulin and glucose levels [6].

An earlier study [41] demonstrated the associations among IR, ROS, and oxidative stress in T2DM. IR represents the most common consequence of disrupted insulin signaling. It occurs when normal insulin levels are insufficient to generate a normal insulin response from fat, liver, or muscle cells. Under conditions of oxidative stress, insulin signaling is impaired resulting IR of the cell. The exact link between ROS and impaired insulin signaling is not fully understood, but several reasonable mechanisms have been suggested [42], including ROS-impaired insulin signaling by inducing phosphorylation of IRS serine/threonine, disturbing cellular redistribution of insulin signaling components, decreasing glucose transporter type 4 gene transcription, or changing mitochondrial activity.Correlation analysis showed that urea has a significant positive correlation with creatinine in T2DM patients, while no significant correlationswere shown with the remainderof the parameters. Urea and creatinine are conventional tests of glomerular filtration rate. The same finding was reported by a previous researches [43]. Also, TAC levels showed a significant inverse correlation with ROS, which is consistent with as the results of previous works [44].

Conclusions

Patients with T2DM revealed poor glycemic control. Excess nourishment, along with inactive lifestyle, lead to the abundant accumulation of glucose within the muscles, adipose tissues, and pancreatic cells.This leads to the generation of surplus ROS. Fluctuating blood glucose concentrations may contribute significantly to oxidative stress, probably even more than chronic hyperglycemia. Fasting blood glucose was significantly positively correlated with major studied parameters (HbA1C, IR, urea, creatinine, TAC, and ROS). The observed significant correlation between FBG and the other testedparameters revealed that hyperglycemia is an obvious independent risk factor for T2DM progression.

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