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ROLE OF CALCIUM-REGULATING HORMONES, ADIPOCYTOKINES AND RENAL FUNCTION TEST IN THE PROGRESS OF TYPE 2 DIABETES MELLITUS IN A SAMPLE OF IRAQI PATIENTS

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ABSTRACT

A cross-sectional study was conducted on 80 type 2 diabetic patients aged 20-60 years in Baghdad and 20 non diabetic persons as controls. Laboratory assessment of glucose related parameters; Fasting blood sugar (FBS), Glycated hemoglobin (HbA1c), Insulin and Insulin resistance (IR), renal function test; Blood urea, serum creatinine, Calcium (Ca) and Phosphorus (P), Calcium regulating hormones; Parathyroid hormone (PTH), calcitonin and vitamin D, cytokines, Adiponectin and Tumor necrosis factor (TNF-α) and comparison these parameters between patients and controls. The results: a high significant (p<0.01) increase in FBG level in the patients (211.34 ± 11.20 mg/dl) as compared with control (85.89 ± 3.07 mg/dl). A high significant (p<0.01) increase in HbA1c in the patients (8.89 ± 0.24 %) than to control (4.813 ± 0.09 %), insulin and HOMA2-I.R levels showed a high significant (P< 0.01) increase of patients as compared to control (49.87 ± 15.78 vs. 12.16 ± 2.57 µIU/ml), (28.49 ± 10.77vs. 2.618 ± 0.56 µIU/ml) respectively. A high significant (P<0.01) increase in B. urea and S. creatinine in the T2DM patients (35.77±1.13 mg/dl and 0.84± 0.04 mg/dl, respectively) and control (30.04±0.69 mg/dl and 0.60±0.03 mg/dl, respectively). The calcium level (8.33±0.06 mg/dl vs. 8.59±0.09 mg/dl) shows a significant (P<0.05) decrease in patients. No significant differences in PTH and calcitonin levels between patients and control, vit. D level, there was a high significant (P<0.01) decrease in patients (16.27 ± 0.55 ng/ml) and control (21.42 ± 2.15 ng/ml). Adiponectin was lower significantly (P<0.05) in patients (11.23 ± 0.40 µg/ml) than in control (12.38 ± 0.61 µg/ml), while there was no significant deference between the patients and control in TNF-α. Conclusion: Development of T2DM characterized by hyperglycemia, hyperinsulinemia accompanied with elevated levels of HbA1c and IR, hyperglycemia is the major cause of progressive renal damge, and the decreased levels of vitamin D in the diabetic patients suggest that altered vitamin D and calcium homeostasis may play role in the development of T2DM.

Key words: Adiponectin, vitamin D, insulin, TNF-α, calcitonin.
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دور الهرمونات المنظمة للكالسيوم ، مستوى المحركات الخلوية الدهنية وفحوصات الوظائف الكلوية في تطور مرض السكر النوع الثاني في عينة من المرضى العراقيين.

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المستخلص

أجريت دراسة مستعرضة على عينة من 80 مريض من مرضى السكر النوع الثاني الذين تتراوح أعمارهم بين 20-60 سنة في بغداد و 20 غير المصابين بداء السكري كعناصر سيطرة. تم اجراء التقييم المختبري للمؤشرات المرتبطة بالغلوكوز (سكر الدم الصائم، الهيموكلوبين المسكر ، الأنسولين ومقاومة الانسولين) ، مؤشرات الوظيفة الكلوية (يوريا الدم، كرياتينين المصل ، الكالسيوم والفسفور) ، هرمونات تنظيم الكالسيوم (هرمون جار الغدة الدرقية ، الكالسيتونين) والفيتامين د. ،السيتوكنات (أديبونيكتين وعامل التخثر الورمي-الفا) ومقارنة هذه المؤشرات بين المرضى وعناصر السيطرة. النتائج: زيادة معنوية (0.01>p) في مستوى FBS في المرضى (211.34±11.20 ملغ / ديسيلتر) بالمقارنة مع السيطرة (85.89 ± 3.07 ملغ / ديسيلتر). زيادة معنوية عالية (0.01>p) في مستوى HbA1c في المرضى 8.89±0.24 ٪ مقارنة بالسيطرة 4.813±0.09 ٪ ، أظهر أنسولين ومقاومة الانسولين HOMA2-IR مستويات زيادة معنوية عالية (0.01>p) لمجموعة المرضى مقارنة بالسيطرة (49.87 ± 15.78 مقابل 12.16 ± 2.57 ؛ 28.49± 10.77   مقابل2.618 ± 0.56) على التوالي. تم العثور على زيادة معنوية عالية (0.01>p) في يوريا الدم والكرياتينين في دم مرضى السكري (35.7±1.13 ملغ / ديسيلتر و 0.84 ± 0.04 ملغ / ديسيلتر ، على التوالي) بالمقارنة مع السيطرة (30.04 ± 0.69 ملغ / ديسيترل و 0.60 ± 0.03 ملغ / ديسيلتر ، على التوالي). يبلغ مستوى الكالسيوم مقارنةً بالسيطرة (8.33 ± 0.06 ملغ / ديسيلتر في مقابل 8.59 ± 0.09 ملغ / ديسيلتر) مما يدل على انخفاض معنوي (0.05>P) في مستوى تلك المؤشرات لدى المرضى. اما فيما يخص فيتامين D ، فقد اظهر انخفاضا معنويا (0.01>p) في المرضى (16.2± 0.55 نانوغرام / مليلتر) مقرنة بالسيطرة (21.42 ± 2.15 نانوغرام / مليلتر). وثقت النتائج أن مستوى الأديبونيكتين في المصل كان أقل معنويا (0.05>P) في المرضى(11.23±0.40ميكروغرام/مليلتر) بالمقارنة مع السيطرة (12.38 ± 0.61 ميكروغرام/مليلتر).

الكلمات المفتاحية: ادييونيكتين ، فيتامين د ، الأنسولين ،α-TNF ، الكالسيتوني.

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INTRODUCTION

The increasing prevalence of Type 2 diabetes mellitus (T2DM) worldwide is reaching epidemic proportions and is becoming a major public health problem (1). Insulin secretion depends on disease status and duration and can vary from delayed but markedly elevated in response to a glucose challenge, to absolutely diminished (20). In Iraq, the prevalence of DM among adults is 10.4%, which means that around three million Iraqi individuals are suffering from DM (1). Insulin is a polypeptide containing two chains of amino acids linked by disulfide bridges. Insulin is synthesized in the rough endoplasmic reticulum of the β-cells. The actions of insulin on adipose tissue; skeletal, cardiac, and smooth muscle; and the liver. Insulin receptors are found on many different cells in the body, including cells in which insulin does not increase glucose uptake (8). HbA1c: Glycation is the non-enzymatic addition of a sugar residue to amino groups of proteins. Human adult hemoglobin (Hb) usually consists of HbA (97% of the total), HbA2 (2.5%), and HbF (0.5%).HbA1c is formed by the condensation of glucose with the N-terminal valine residue of each β-chain of HbA to form an unstable Schiff base. HbA1c is the major fraction, constituting approximately 80% of HbA1 (25). The glomerular filtration rate (GFR) also deteriorates significantly in this process. If not treated, and addressed medically, nephropathy progresses into chronic kidney disease (CKD). This association of T2DM and CKD complicates the treatment of T2DM clinically (34). Serum urea and creatinine are known to be raised with hyperglycemia in uncontrolled diabetics and usually correlate with severity of kidney damage. Creatinine is the breakdown product of creatinine phosphate is released from skeletal muscle at a steady rate. It is filtered by the glomerulus, and a small amount is also secreted into the glomerular filtrate by the proximal tubule (12). The reduction in serum calcium level in type2 diabetes mellitus is most probably due to hyperglycemia which increases calcium and phosphorus excretion in urine which is proportional to the degree of glucosuria, hypercalciuria by osmotic diuresis caused stimulation of bone resorption caused by secondary hyperparathyroidism (16). In response to urinary calcium loss, PTH secretion is mildly but significantly stimulated to maintain serum calcium concentrations (24). It is an element that plays an important role in many physiologic systems. Phosphate is a component of cell membranes and biological macromolecules including nucleotides, proteins, lipids and carbohydrates. Eighty-five percent of phosphorus resides in bone (mostly as hydroxyapatite), 14% is present in cells and less than 1% is represented in plasma. Acid-base balance, hormones and vitamin D modulate intestinal absorption and renal reabsorption of phosphorus. Parathyroid hormone (PTH) inhibits renal reabsorption of phosphorus, and growth hormone decreases renal excretion. Vitamin D increases renal reabsorption (20). Parathyroid hormone (PTH) in response to reduced calcium levels resulting in an increase in bone resorption and subsequently normalization of calcium levels. There are conflicting reports related to the role of PTH on glucose hemostasis. Limited studies suggest a role for this hormone in insulin sensitivity through its role in increases the production of 1, 25-dihydroxyvitamin D [1, 25(OH)2D] (33). Calcitonin is a 32 amino acid hormone secreted by the C-cell of the thyroid gland. The role of calcitonin in maintaining the serum calcium and stimulate renal vitamin D (1,25D) production. Calcitonin has an immediate effect on decreasing osteoclast activity and has been used for treatment of hyperkalemia (11). The main defects that determine the development of T2DM are insulin resistance, pancreatic β-cell dysfunction and systemic inflammation. Multiple studies strongly suggest a role of vitamin D in the wellbeing of β-cells, insulin production and secretion, tissue sensitivity to insulin and the susceptibility to T2DM. An inverse relationship betweenT2DM and vitamin D is suggested by cross-sectional and prospective studies pointing to a direct link between the risk of T2DM and vitamin D (15). Vitamin D deficiency is an important factor leading to reduced intestinal calcium absorption (20). Adiponectin is aprotein synthesized and secreted predominantly by adipocytes into peripheral blood, accounting for 0.01% of the total plasma protein in human

(23). Adipocytokines, such as adiponectin, leptin and tumor necrosis factor-a (TNF-a). Adiponectin, the most abundant adipocytokine, was found to be decreased in conditions such as obesity, insulin resistance and type 2 diabetes (21). TNF- α is a pleiotropic cytokine that plays a central role in inflammation and apoptosis. It modifies the inflammatory reactions and immune reactions in response to injury and infection. TNF- α plays also a necessary and beneficial role as mediator of host resistance to infection and tumor formation (21).This study was conducted to estimate the prevalence and identify potential determinants of T2DM patients attending a Diabetes Center in Baghdad, Iraq, 2017.

MATERIALS AND METHODS

A cross-sectional study conducted on a systematic random sample of T2DM patients attending from AL-Kindi particular community for diabetes and heftiness treatment of Baghdad. The average age were (43.46±11.4 year) with range from (20-60) years old .The average disease duration were (66.47±59.3 month) and alternative disease duration from (1-240 months). In addition a control group which included 20 healthy subjects in terms of non-diabetic. Consent was taken before participation in this study from all subjects, and many parameters were measured from blood (serum) samples for patients and control groups. The diagnosis of T2DM was based on the American diabetes associated, 2016. The Diabetic Center in Eastern side of Baghdad during the period from December 2016 till May 2017. Laboratory investigations included FBS, HbA1c, insulin, IR, B.urea, serum creatinine, Ca, P, PTH, Calcitonin, Vit. D, Adiponectin and TNF-α.Venous blood sample (10 ml) has been collected from the studied subject and serum has been collected and kept at (-20°C) until used.

Measurement of parameters:
FBS, B.urea, S.creatinine, Ca, and P were spectrophotomertrically estimated using commercial kits. Cobas electrochemiluminescence immunoassay (ECLIA) e411 and e111 apparatus (Company Roche) were used to carry out HbA1c, insulin, PTH and calcitonin according to manufacture recommended procedure by using specific kit for each hormone. Vit. D, adiponectin and TNF-α were measured using enzyme-linked immunosorbent assay (ELISA) kits. The homeostasis model assessment (HOMA) was used to calculate insulin resistance (HOMA-IR) [FPI x FBS/405], where FPI is fasting plasma insulin concentration (μU/ml) and FBS is fasting blood sugar (mg/dl).

Statistical analysis:
The Statistical Package for Social Sciences (SPSS) version 18 (SPSS Inc., Chicago, IL, USA) used for data entry and analysis. Last significant difference-LSD was used to significant compare between mean. P ≤0.05 was considered significant.

RESULTS AND DISCUSSION

1.Glucose related parameters of the studied subjects
The Table 1 shows the glucose related parameters (FBS, HbA1c, insulin and HOMA2-IR) in the type 2 DM patients and control. A high significant (p<0.01) increase was found in the level of FBG in the patients (211.34 ± 11.20 mg/dl) as compared with control (85.89 ± 3.07 mg/dl). Also, a high significant (p<0.01) increase was found in the level of HbA1c in the group of patients (8.89 ± 0.24 %) compared to control (4.813 ± 0.09 %), insulin and HOMA2-I.R levels showed a high significant (P< 0.01) increase of patients as compared to control (49.87 ± 15.78 vs. 12.16 ± 2.57 μIU/ml), (28.49 ± 10.77vs. 2.618 ± 0.56 μIU/ml) respectively .

Table 1. Levels of glucose related parameters in diabetic T2DM patients and control

group	FBS (mg/dl)	HbA1c (%)	Insulin (μIU/ml)	IR HOMA2
		Glucose related parameters		
Patients	211.34[a] ± 11.20	8.89[a] ±0.24	49.87[a] ±15.78	28.49[a] ± 10.77
Control	85.89[b] ± 3.07	4.813[b] ± 0.09	12.16[b] ± 2.57	2.618[b] ± 0.56
LSD value	46.317 **	1.0005 **	16.162 **	8.429 **
P-value	0.0001	0.0001	0.0037	0.0052
		** (P<0.01).		

*Values are means ± standard error of means.
*Means in column carrying similar small letters indicate a non-significant difference (p> 0.05).
*Means in column carrying different small letters indicate a significant difference (p< 0.05).

The increased FBG level of T2DM patients in the current study is expected. These findings are in agreement with previous reports (12). It has been reported that age and the duration of diabetes are probably significant predictors for development of severing hyperglycemia in the patients with T2DM. It has been stated that with advancing β-cell failure, people with long duration of T2DM progressively resemble those with type 1 diabetes mellitus (39). The increase level of HbA1c in the patients of this study is parallel with FBS in these patients. This result is agreement with the (18) who found that FBS and HbA1c correlated positively. The ADA proposed HbA1C ≥ 6.5% for the diagnosis of diabetes and 5.7-6.4% for the highest risk to progress the diabetes (19). The HbA1c, also known as the glycosated hemoglobin level, measures glucose that is attached to hemoglobin and it provides an indication of what the average blood glucose has been for three months prior to the test (26).Since our current study recorded the highest age of patients in the largest category (50-59year), indicating a correlation between progression of patients age and increase of HbA1c level. Similar results were reported by other author (2). Regarding the levels of insulin, similar results were reported by (9) who demonstrated that insulin level was increased in T2DM patients compared with control subjects. The present finding is logical, since insulin testing is used to assist in diagnosing early type 2 diabetes. On the other hand, the current finding may be due to β-cell dysfunction. There is a study reported that β-cell function levels were significantly lower in T2DM patients compared with control group (28). In normal physiology, insulin secretion is induced by elevated plasma glucose levels. Concerning the IR, elevated levels were observed in T2DM patients which was associated with increased insulin levels. These results are agreement with (29) who reported an elevation insulin hormone levels, insulin resistance, and FBS than control group. This observation may be explained on the ground that in type 2 diabetes, insulin resistance is probably the first metabolic abnormality. Insulin resistance in turn causes elevated serum glucose, and hyperglycemia causes hyper-secretion of insulin by the pancreas. When hyperglycemia is chronic and prolonged the β-cells of the pancreas become impaired and they cease to function (37). Insulin resistance is defined as a series of clinical manifestation for diminished effectiveness of insulin in lowering blood sugar levels caused by decreased sensitivity to insulin of liver, muscle and adipose tissue in T2DM (25).

2. Renal function tests of the studied subjects

Table 2, a high significant (P<0.01) increase was found in levels of blood urea and serum creatinine in the T2DM patients (35.77±1.13 mg/dl and 0.84± 0.04 mg/dl, respectively) as compared with control (30.04±0.69 mg/dl and 0.60±0.03 mg/dl, respectively). The mean of serum calcium level of patients as compared to control are (8.33±0.06 mg/dl vs. 8.59±0.09 mg/dl) which shows a significant (P<0.05) decrease in patients group. In contrast, non-significant (P>0.05) differences were found in the mean of serum phosphorus level between the patients (4.11±0.06 mg/dl) and control (4.37±0.20 mg/dl).

Table 2. Levels of renal function tests in T2DM patients and control group

The group	Renal function tests			
	B.urea (mg/dl)	S. Creatinine (mg/dl)	Ca (mg/dl)	P (mg/dl)
Patients	35.77[a] ± 1.13	0.84[a] ±0.04	8.33[a] ± 0.06	4.11[a] ± 0.06
Control	30.04[b] ± 0.69	0.60[b] ±0.03	8.59[b] ± 0.09	4.37[a] ± 0.20
LSD value	4.723 **	0.1528 **	0.274 *	0.319 NS
P-value	0.0108	0.0024	0.054	0.099
	* (P<0.05), ** (P<0.01), NS: Non-Significant.			

*Values are means ± standard error of means.
*Means in column carrying similar small letters indicate a non-significant difference (p> 0.05).
*Means in column carrying different small letters indicate a significant difference (p< 0.05).

Impairment in renal function is assessed by estimating the levels of blood urea and serum creatinine. The elevated levels of blood urea and serum creatinine in the current study is in

agreement with previous study (22) who reported a strong correlation between the levels of FBS and blood urea. In this study, a significant increase which was found in blood urea level with increased blood sugar level may be due to poorly controlled blood sugar levels and thus increase the chances of the patient suffering from diabetic nephropathy. Also, the present findings corroborate with the findings of other study which reported that hyperglycemia is one of the major causes of progressive renal damage, while did not support the finding regarding serum creatinine, they mentioned that the association between hyperglycemia and the serum creatinine levels showed a weaker link (12). According to these results, effective control of blood sugar levels can stop progression to diabetic nephropathy and thus remarkably reduce the morbidity and mortality associated with this metabolic disease. The tendency of occurrence of renal function tests value at the higher reference limits in cases of T2DM reflects the initiation of nephropathy changes (35). Calcium (Ca^{2+}) and phosphorus (P) are essential to many vital physiological processes. The decreased levels of calcium in the patients of this study is in agreement with the results of (27), who found an association between calcium and DM. The explanation behind these results is that differences in the level of insulin lead to that insulin induced Ca^{+2} oscillations even in Ca^{+2}-free medium; insulin increases cytoplasmic Ca^{+2} by mobilizing intracellular Ca^{+2} stores. Most receptor tyrosine kinases (RTKs) increase Ca^{+2} by activation forms inositol triphosphate receptor (InsP3) to bind to and release Ca^{+2} from InsP3 receptors in the endoplasmic reticulum (13).The combination of cellular Ca^{2+} channels and pumps creates cytosolic Ca^{2+} oscillations that drive oscillatory insulin secretion in glucose-stimulated β-cells. In current study, there was no significant differences in the phosphorus level. This finding is in agreement with previous studies done by who reported that the calcium and phosphorus level didn't change in T2DM (27). While is in disagreement with (14) who reported That serum level of phosphorus in T2DM patients was significantly lower than that in the control. It is clear from the results of this study that most of the patients were in duration of disease (1-60 month) and those may be did not suffer from kidney failure and nephropathy complication that affect on phosphorus level. Phosphorus has important functions in the body and several mechanisms have evolved to regulate phosphate balance including vitamin D and parathyroid hormone. Hassan *et al*. (16) have shown that the reduction in serum calcium level in type2 diabetes mellitus is most probably due to hyperglycemia which increases calcium and phosphorus excretion in urine.

3. Calcium regulating hormones

The results of calcium regulating hormones are presented in Table (3).This table shows there is no significant differences in PTH and calcitonin levels between T2DM patients (49.12±3.70 pg/ml and 1.494 ± 0.25 pg/ml, respectively) and control group (46.70 ± 2.77 pg/ml and 1.404 ± 0.24 pg/ml, respectively)**.** Regarding the results of 1, 25(OH)$_2$D level, there was a high significant (P<0.01) decrease inT2DM patients (16.27 ± 0.55 ng/ml) as compared with the control (21.42 ± 2.15 ng/ml).

Table 3. Levels of calcium regulating hormones in T2DM patients and control group

The group	Calcium regulating hormones		
	PTH (pg/ml)	Calcitonin (pg/ml)	Vit. D (ng/ml)
Patients	49.12^a ± 3.70	1.49^a ±0.25	16.27^b ± 0.55
Control	46.70^a ± 2.77	1.40^a ±0.24	21.42^a ± 2.15
LSD value	15.50 NS	1.062 NS	3.053 **
P-value	0.757	0.866	0.0012
** (P<0.01), NS: Non-Significant.			

*Values are means ± standard error of means.
*Means in rows carrying similar small letters indicate a non-significant difference (p>0.05).
*Means in rows carrying different small letters indicate a significant difference (p< 0.05).

The parathyroid glands secrete PTH in response to reduced calcium levels resulting in an increase in bone resorption and subsequently normalization of calcium levels (33)**.** The present finding is in agreement with the results of previous study (3) which did not

reported an increase in PTH secretion in patients with diabetic type2. While it is in disagreement with (33) who suggested that high level of PTH is associated with abnormal glucose metabolism and is related with the prevalence of diabetes. Poorly controlled diabetics tend to present lower calcium ion levels, and deficient PTH secretion (2), which disagrees with our result in which there is no significant deference in parathyroid hormone in diabetic patients when compared with control subjects. This conflicting in the results may be due to the fact that the level of calcium varied between T2DM patients and control, but the difference not high significantly. It is well documented that PTH is associated with calcium and the raised PTH inhibits insulin synthesis and secretion in β-cells and IR in target cells by regulating intracellular calcium (15). Physiologically, the high PTH and $1,25(OH)_2D$ levels contribute to the maintenance of normocalcemia by stimulating renal calcium reabsorption and by promoting calcium release from the skeleton with a positive flux to the serum calcium raise (20). It has been reported that calcitonin secretion is stimulated by increases in the serum calcium concentration, as well correlation were observed with PTH (11). Although in the present study no significant difference was found in the calcitonin level between T2DM patients and control, while as mentioned previously a significant decrease was found in the level of calcium in T2DM patients as compared with control. Insulin and calcitonin are also known to influence phosphate excretion. The mechanism of calcitonin effects on insulin secretion remains unclear. It can be hypothesized that calcitonin-induced hypocalcaemia reduces intracellular Ca^{2+} concentration in β-cell cytosol, decelerates the release of secretory granules localized in microfilament network near the cell membrane , thus delaying insulin secretion during glucose tolerance test (GTT) (30). A correlation between blood glucose level and antibodies to calcitonin was found. That one-time calcitonin injection led to the decreasing of functional state of β-cells under the obesity and in elderly age. Overall, elevated levels can also be found in conjunction with hyperparathyroidism, hypergastrinemia, and renal failure and

chronic inflammatory disease (13). The decreased levels of vitamin D in the T2DM patients of this current study is in agreement with the results of (17) who found that 54% of the study participants with T2DM had inadequate sera levels of vitamin D. Also 1, $25(OH)_2D$ levels were significantly lower in the T2DM patients than in the control group (6). Rafiq and Jeppesen (32) stated that some systematic review and meta-analysis of observational studies support an inverse association between hypovitaminosis D and type 2 diabetes. The higher frequency of hypovitaminosis D could be attributed to several factors. Mostly people do not take vitamin D fortified food and vitamin D supplements, so they depend solely on UV-B rays coming from sun for their vitamin D production. Deficiency of vitamin D is already found to be involved in the methylation of many genes (24) and also β-cells have the receptors for vitamin D. The treatment of vitamin D to isolated islets and β- cells can increase insulin secretion (38). The action of vitamin D is mediated by its receptors, it conforms the role of vitamin D in the functions of the β-cells (20) and vitamin D deficiency can cause insulin resistance and type 2 diabetes (24). Vitamin D deficiency and the onset of type 2 diabetes could be related to genetics. It is evident that genetic factors can play a very important role in the impairment of glucose metabolism (32). A positive role for vitamin D in the modification of the function of β-cells of the pancreas has been reported. A response of insulin to glucose load appears to be exclusively influenced by vitamin D. This role is mediated through several pathways, including direct stimulation of insulin secretion by vitamin D through the presence of vitamin D receptors (VDRs) in β-cells of the pancreas, and their expression of 1-α-hydroxylase enzyme. Also, 1, 25-(OH) 2D is able to activate transcription of the gene of human insulin and thus play an essential role in insulin secretion (10).

4. Cytokines related with T2DM

The results in Table 4 shows the results documented that serum adiponectin level was lower significantly (P<0.05) inT2DM patients (11.23 ± 0.40 µg/ml) in comparison with control (12.38 ± 0.61 µg/ml); while there was

no significant deference between the T2DM patients and control group regarding to serum TNF-α level (111.06 ± 5.19 ng/ml vs. 107.43 ± 6.36 ng/ml).

Table 4. Levels of adiponectin and TNF-α in T2DM patients and control group

Group	Cytokines related with T2DM	
	Adiponectin (μg/ml)	TNF-α (ng/ml)
Patients	$11.23^b \pm 0.40$	$111.06^a \pm 5.19$
Control	$12.38^a \pm 0.61$	$107.43^a \pm 6.36$
LSD value	1.018 *	22.238 NS
P-value	0.0498	0.746
* (P<0.05), NS: Non-Significant.		

*Values are means ± standard error of means.
*Means in column carrying similar small letters indicate a non-significant difference (p> 0.05).
*Means in column carrying different small letters indicate a significant difference (p< 0.05).

The decreased level of adiponectin in T2DM patients in this study is in line with the results of other previous studies (7, 8). There was a fact that adiponectin is a major modulator of insulin action for its role in enhancing insulin sensitivity, it is therefore of critical importance to note that factors decreasing adiponectin levels such as obesity, and specially the abdominal obesity, could correlate with IR (7). It was suggested that patients with insulin receptor dysfunction, including diabetics, may have unexpectedly high levels of adiponectin (6).These previous study disagreement with present study. Therefore, a reduced level of serum adiponectin seems to be not just a mere biomarker of DM but may also play a causal role in the development of IR, and metabolic syndrome (8). The latter finding clearly delineates a potential role for adiponectin in modulating the interaction between insulin and its receptor (7). Inflammatory adipocytokines like TNF-α was important in the etiology of the diseases associated with obesity, in particular, IR and T2DM. Increased levels of TNF-α in T2DM compared to healthy controls (21). Non-significant differences of TNF-α in diabetic patients as compared to controls consistent with (5) who observed an increased but not significant for TNF-α level in T2DM patients as compared to controls. The current result disagree with (6) who reported that TNF-α concentrations increased significantly in diabetic patients compared with the non-diabetic control. The variations in the present's results and these results could be attributed to the differences in duration of the disease, sample size, and the differences in age and sex of the studied groups (31). Role of the proinflammatory cytokine with the presence of obesity, IR and T2DM and the assay and assessment of sera levels of TNF-α could be beneficial in early detection of T2DM and prevention of its unfavorable consequences especially the cardiovascular complications and atherosclerosis. It was still unclear whether altered inflammatory cytokines were a cause or compensatory mechanism to IR and T2DM (31). TNF-α reduces the expression of GLUT4 and serine phosphorylation of IRS-1 which are the important enzymes for the synthesis of insulin. To prevent the inflammatory disorders by blocking inflammatory responses is one of the most effective treatment strategies for the prevention and control of development of insulin resistance and pathogenesis of T2DM (4). Clear decrease was found in the levels of vitamin D3 in all studied T2DM patients compared with control group. The serum adiponectin level was lower significantly in T2DM patients in comparison with control. All T2DM patients should have regular screening, and the health facilities need to provide the necessary equipment to conduct this investigation. Thus, large, well designed, controlled randomized interventional studies of vitamin D and calcium in potential prevention and management of T2DM are urgently required to clarify the relationship between vitamin D and glucose homeostasis in T2DM. From the result of this study could be concluded: Development of T2DM characterized by hyperglycemia, hyperinsulinemia accompanied with elevated levels of HbA1c and IR, hyperglycemia is the major cause of progressive renal and the decreased levels of vitamin D in the diabetic patients suggest that altered vitamin D and calcium homeostasis may play role in the development of T2DM.

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