ISSN: 0067-2904

The Key Role of Bone Function Markers in Patients with Type (II) Diabetes Mellitus

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Received: 3/10/2021 Accepted: 6/2/2022 Published: 30/7/2022

Abstract

Complications associated with diabetes are a consequence of acute disturbance in glucose metabolism in a human body. The most significant complication of diabetes is bone disorders which contributes to high levels of bone disability. This study included 118 diabetic patients, 56 males, 62 females, and 60 healthy non-diabetic controls, 30 males, 30 females. The patients and controls were age matched. Circulating levels of bone function markers (osteoprotegerin, vitamin D, PTH, total calcium and inorganic phosphorus) were determined in all subject groups. The data obtained from this study showed that the serum levels of osteoprotegerin had significantly increased in both diabetic male & female in both age ranges which were 496.3±61.46 pg/mL & 335.7±29.33 pg/mL; 329.8±48.78 pg/mL & 219.9±18.72 pg/mL respectively, in comparison with its level in control matched age ranges 294.6±26.19 pg/mL & 226.8±28.07 pg/mL; 215.7±31.85 pg/mL & 171.9±14.19 pg/mL respectively. Serum calcium concentration had non-significantly increased both in the diabetic males and females in both age ranges which were 11.10±0.46 mg/dL & 11.76±0.74 mg/dL; 10.33±0.33 mg/dL & 10.28±0.48 mg/dL respectively, when compared with its level in control matched age ranges 10.46±0.34 mg/dL & 10.14±0.35 mg/dL;9.69±0.41 mg/dL & 10.08±0.45 mg/dL. Serum vitamin D, parathyroid hormone (PTH) and inorganic phosphorus concentrations had significantly decreased both in the diabetic male and female subjects in both age ranges which were 5.78±1.30 ng/mL and 2.47±0.12 ng/mL; 9.47±1.98 ng/mL, and 10.70±2.11 ng/mL; 74.78±7.42 pg/mL & 67.83±3.69 pg/mL;42.94±2.00 pg/mL & 15.51±1.98 pg/mL; 4.34±0.27 mg/dL & 4.76±0.35 mg/dL; 4.38±0.21 mg/dL; 5.12±0.44 mg/dL respectively when compared with their level in control matched age ranges 13.07±2.13 ng/mL & 15.53±3.40 ng/mL; 57.49±5.64 pg/mL & 62.61±3.71 pg/mL; 5.12±0.44 mg/dL & 5.35±0.37 mg/dL respectively. The current results suggest that circulating levels of osteoprotegerin play a crucial role in biological mechanism of type (II) diabetes, and are possible biomarkers of insulin resistance and progression of many serious health problems associated with diabetes.

Keywords: Type (II) Diabetes Mellitus, Markers of bone function, Osteoprotegerin, Microvascular Complications, Parathyroid hormone

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الدور الرئيسي لمؤشرات وظائف العظام في مرضى السكري من النوع الثاني

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الخلاصة

تحدث المضاعفات المصاحبة لمرض السكري نتيجة لاضطراب حاد في المسار الأيضي في جسم الإنسان. إن أهم مضاعفات مرض السكري هي اضطرابات العظام ، والتي تساهم في ارتفاع مستويات العجز العظمي. اشتملت الدراسة على 118 مريضاً من المصابين بالسكري (56 ذكور و 62 إناث) و 60 شخصا سليما اعدت كمجموعة سيطره (30 ذكور و 30 إناث) ، وتم مطابقة المرضى ومجموعة السيطرة من حيث العمر. خلال هذه الدراسة ، تم تقييم تأثير ارتفاع السكر في الدم المصاحب لداء السكري من النوع الثاني على المستويات المنتشرة لمؤشرات وظيفة العظام (اوستيوبروتيجرين، هرمون جارات الغدة الدرقية، فيتامين د ،الكالسيوم والفوسفور غير العضوي). اظهرت البيانات التي تم الحصول عليها من خلال هذه الدراسة أن مستويات مصل osteoprotegerin قد ازادت بشكل معنوي في كل من الذكور والإناث المصابين بمرض السكري في كلا الفئتين العمريتين (496.3 ± 61.46 بيكوغرام / مل و 335.7 ± 29.33 بيكوغرام / مل ؛ 329.8) ± 48.78 بيكوغرام / مل & 219.9 ± 18.72 بيكوغرام / مل) على التوالي ، مقارنة بمستواه فى المجموعة الضابطة (294.6 ± 26.19 بيكوغرام / مل و 226.8 ± 28.07 بيكوغرام / مل ؛ 215.7 ± 31.85 بيكوغرام / مل) و 171.9 ± 14.19 بيكوغرام / مل) على التوالي). ازداد تركيز الكالسيوم في الدم بشكل غير معنوي في كل من الذكور والإناث المصابين بمرض السكري في كلا الفئتين العمريتين (11.10 ± 0.46 مجم / ديسيلتر و 11.76 ± 0.74 مجم / ديسيلتر ؛ 10.33 ± 0.33 مجم / ديسيلتر و 10.28 ± 0.48 مجم / ديسيلتر) على التوالي). عند مقارنته بمستواه في الفئات العمرية فى المجموعة الضابطة (10.46 ± 0.34 مجم / ديسيلتر و 10.14 ± 0.35 مجم / ديسيلتر ؛ 9.69 ± 0.41 مجم / ديسيلتر & 10.08 ± 0.45 مجم / ديسيلتر).انخفض مستوى مصل كل من فيتامين د , وهرمون الغدة الجاردرقية وتركيز الفوسفور غير العضوي انخفاضًا كبيرًا في كل من الذكور والإناث المصابين بمرض السكري في كلا الفئتين العمريتين (5.78 ± 1.30 نانوغرام / مل و 2.47 ± 0.12 نانوغرام / مل ؛ 9.47 ± 1.98 نانوغرام / مل) و (10.70 ± 2.11) نانوغرام / مل) ؛ (74.78 ± 7.42 بيكوغرام / مل & 67.83 ± 3.69 بيكوغرام / مل ؛ (42.94 ± 2.00 بيكوغرام / مل & 15.51 ± 1.98 بيكوغرام / مل) ؛ (4.34 ± 0.27 مجم / ديسيلتر & 4.76 ± 0.35 مجم / ديسيلتر) (4.38 ± 0.21 مجم / ديسيلتر ؛ (5.12 ± 0.44 مجم / ديسيلتر) على التوالي عند مقارنتها بمستواها في المجموعة الضابطة (13.07 ± 2.13 نانوغرام / مل & 15.53 ± 3.40 نانوغرام / مل ؛ 57.49 ± 5.64 بيكوغرام / مل & 62.61 ± 3.71 بيكوغرام / مل ؛ 5.12 ± 0.44 مجم / ديسيلتر و 5.35 ± 0.37 مجم / ديسيلتر) على التوالي .تشير النتائج الحالية إلى أن مستويات الاوستيوبروتيجرين في الدم تلعب دورًا مهمًا في الآلية البيولوجية لمرض السكري من النوع (II) وهي مؤشرات حيوية محتملة لمقاومة الانسولين وتطور العديد من المشاكل الصحية الخطيرة المرتبطة بمرض السكري.

Introduction

Type (II) Diabetes mellitus (T2DM) is one of the worldwide health problems that is featured via defects in the secretion of insulin and/or a reduction in the sensitivity to insulin, referred to as insulin resistance [1]. WHO considers diabetes as a main health problem globally. In recent years, the incidence of type II diabetes mellitus has increased significantly due to metabolic disorders caused by insulin secretion malfunction [2]. Studies which estimated bone mineral density (BMD) in diabetic patients and nondiabetic patients did not find differences in BMD between the 2 groups. However, those studies did find higher

osteoporosis prevalence in those with diabetes, elevated fragility of bone structure which increases the risk of fracture in T2DM [3]. Recent cohort studies established that resorption, replacement and skeletal integrity are affected irreversibly by diabetes, and that the diabetes is correlated with a higher risk of bone fracture [4]. Bone mineral density values alter among diabetes patients and can be elevated, declined or even remain normal. Although the mechanism of altered metabolism of bone in diabetic patients is known to be multifactorial but the pathogenesis is not entirely clear. Metabolism of bone in diabetic patient is influenced by various factors, including decreased osteoblast and numbers of osteoclasts due to abnormal release of insulin and/or action of insulin [5].

Osteoprotegerin (OPG) is a new member of the tumor necrosis factor (TNF) receptor family. It is a secretion and acts like a cytokine. Generation of OPG takes place in a variety of tissues such as vascular smooth muscle cells, bone tissues and endothelial cells. In normal condition, serum level of OPG is lower in blood than in the tissues [6] but elevated circulating concentrations of osteoprotegerin have been found in T2DM with microvascular complications [7]. The balance between bone catabolism of and resynthesizing is controlled to a large extent by the releasing OPG soluble receptor. Recent research has clarified the crosstalk between ECs and osteoblasts during osteogenesis, hence linking angiogenesis with osteogenesis. It has been revealed that osteoprotegerin may be involved in the calcification of vascular which a risk factor of cardiovascular system [8].

Vitamin D plays a key role in bone metabolism and a variety of immunological and cellular processes. Increased importance of vitamin D assessment is due to the awareness of high osteoporosis incidence and its correlation with low vitamin D concentrations in adults [9]. In addition, among many other risk factors, a low concentration of vitamin D is considered as one of the most critical risk factors for osteoporosis and related fractures [10]. Adequate supplementation of vitamin D may play a key curative role in blocking the severity and progression of T2DM [11]. Parathyroid hormone is involved in minerals metabolism and reflects the action of the parathyroid gland. It has been reported that various fragments of parathyroid hormone in the body's circulation causes antagonistic influence on bone and the renal functions [5]. Parathyroid hormone is involved in regulating calcium circulation levels by enhancing resorption of bone, repressing urinary calcium loss and promoting the production of the active metabolite of vitamin D known as calcitriol [12].

It has been described as a risk factor for osteoporosis and cardiovascular disorder attributing to long term high dietary intake of phosphorus, bone fracture was linked with risk of T2DM [13]. Metabolic imbalance in phosphate (P) takes place from the early initiation of diabetes and may contribute in lowering of high energy phosphates and tissue ischemia. These changes occur in the cells and tissues in which the entry of glucose is not controlled by insulin and principally in poorly regulated diabetes patients with retinopathy, nephropathy, neuropathy, and macrovascular disease [14].

Calcium level has a relation with insulin resistance (IR) and they can affect each other based on metabolic mechanisms and pathophysiological insulin resistance, and insulin release based on homeostasis of calcium [15], alteration of Ca^{2+}levels may influence insulin release since initiation of insulin exocytosis from β-cells is based on calcium influx through voltage-operated calcium channels. On the contrary, insulin can influence calcium metabolism through blunting of calcium influx [16]. Circulating calcium and insulin resistance are extensively recorded to be linked with hypertension. However, their causal relation patterns influence high blood pressure and to what extent serum calcium is linked with high blood pressure through insulin resistance, is largely unknown [17]. Epidemiologic researches have proposed that increased serum calcium level may play a key role in diabetes progression [18]. Further studies have shown direct links of increased circulating calcium levels with markers of dysregulated sugar metabolism, such as elevated levels of fasting glucose, insulin

resistance (IR) and β-cells dysfunction, as well as T2DM [19]. In respect to the nutrition, inadequate calcium supply and a declined vitamin D condition are major risk factors of osteoporosis. Calcium is the basic mineral that exists in bones where it supplies skeletal strength and acts as a pool for preserving serum calcium concentrations in a physiological range. Calcium deficiency enhances bone loss through elevated resorption rate of bone in order to preserve the circulating calcium level [20].

Materials and Methods

Subjects and Study Design

This study was a case control study conducted during September 2018 -April 2019. The selected number of diabetic patients was 118. Out of which 56 were males and 62 females with age ranging between 40-80 years for males and females patients diagnosed as type (II) DM, and were attending Laila Qasim diabetic center in Erbil Governorate. The patient individuals were divided into four groups, depending on their ages and genders: males between 40-59 years and60-80 years, females between 40-59 years and 60-80 years. The controls group comprised of 60 individuals with their sexes and ages matching normal non diabetic subjects.

Blood Samples Collection

Venous blood specimens were collected from the study subjects, patients and healthy individuals, after 10-12 hours of fasting. The specimens were reserved in gold-top serum separator tubes (SST) and EDTA tubes before their centrifugation at 3500 rpm for 10 mins. The resulting serum and plasma samples were separated and preserved in Eppendorf tubes. After collection the serum and plasma samples were stored at -20° C to be ready for biochemical analyses.

Biochemical Assays

Serum level of osteoprotegerin was estimated by sandwich enzyme-linked immunosorbent assay (ELISA) technique using the human osteoprotegerin (OPG) ELISA kit manufactured by Sun Long Biotech, China. Circulating 25-OH vitamin D concentration was evaluated by solid-phase sequential enzyme immuno-assay (EIA) technique using a kit manufactured by Monobind, USA. The evaluation of serum parathyroid hormone (PTH) was done by using sandwich enzyme-linked immunosorbent assay (ELISA) technique using the human parathyroid hormone (PTH) ELISA kit manufactured by SunLong Biotech, China. The determination of serum inorganic phosphorous was done using the enzymatic colorimetric method using BIOLABO kit of France. And finally the assessing of serum calcium was performed by the colorimetric technique using BIOLABO kit from France.

Statistical Analysis

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

Results and Discussion

Serum Levels of Bone Function Markers

The serum level of bone function markers for both diabetic male and female patients and controls are presented in Tables 1 and 2.

Table 1-The levels of bone function markers in diabetic male patients

Parameters		Age (Years)	Controls	Patients	P-Value
Male	Osteoprotegerin (pg/mL)	40-59	294.6±26.19	496.3±61.46	0.0392
		60-80	226.8±28.07	335.7±29.33	0.0078
	25-OH Vitamin D (ng/mL)	40-59	19.06±1.85	5.78±1.30	<0.0001**
		60-80	19.79±2.36	2.47±0.12	<0.0001**
	PTH (pg/mL)	40-59	122.4±9.20	74.78±7.42	0.0004**
		60-80	93.31±4.61	67.83±3.69	0.0003**
	Inorganic Phosphorous (mg/dL)	40-59	6.39±0.48	4.34±0.27	0.0035
		60-80	6.62±0.54	4.76±0.35	0.0337
	Total calcium (mg/dL)	40-59	10.46±0.34	11.10±0.46	0.3575
		60-80	10.14±0.35	11.76±0.74	0.1228

Value expressed in Mean±SE

Table 2- The levels of bone function markers in diabetic female patients

Parameters		Age (Years)	Controls	Patients	P-Value
Female	Osteoprotegerin (pg/mL)	40-59	215.7±31.85	329.8±48.78	0.0409
		60-80	171.9±14.19	219.9±18.72	0.0369
	25-OH Vitamin D (ng/mL)	40-59	13.07±2.13	9.47±1.98	0.4969
		60-80	15.53±3.40	10.70±2.11	0.5087
	PTH (pg/mL)	40-59	57.49±5.64	42.94±2.00	0.0128
		60-80	62.61±3.71	15.51±1.98	<0.0001**
	Inorganic Phosphorous (mg/dL)	40-59	5.12±0.44	4.38±0.21	0.9413
		60-80	5.35±0.37	3.86±0.33	0.0331
	Total calcium (mg/dL)	40-59	9.69±0.41	10.33±0.33	0. 536
		60-80	10.08±0.45	10.28±0.48	0.7359

Value expressed in Mean±SE

Serum Levels of Osteoprotegerin

As indicated in Table 1, the serum osteoprotegerin level significantly increased (P=0.0392) & (P=0.0078) in diabetic males in both age ranges which were 496.3±61.46 pg/mL & 335.7±29.33 pg/mL respectively, in comparison with its level in control matched age ranges 294.6±26.19 pg/mL & 226.8±28.07 pg/mL respectively. Similarly, there was significant increase (p=0.0409) & (p=0.0369) in serum osteoprotegerin (OPG) level in diabetic females in both age ranges 329.8±48.78 pg/mL & 219.9±18.72 pg/mL respectively, in comparison with its level in control matched age ranges 215.7±31.85 pg/mL & 171.9±14.19 pg/mL respectively (Table 2).

The results of the present study shows that there was a remarkable elevation in serum OPG level in both diabetic male and female groups of both age ranges. This result is in consonance with that of Maser *et al.* and El Said *et al.*[7, 21] who reported increased serum OPG level in T2DM. Osteoprotegerin is an osteoclastogenesis blocking factor, an essential molecule for the morphogenesis and remodelling of bone. A number of researches have been done to evaluate its role with respect to the human skeletal system. Elevated OPG levels have been demonstrated in a variety of persistence diseases such as swelling and tenderness of one or more joints in T2DM adults [22]. The osteoprotegerin has been described as representing an indemnificatory response to bone and vascular damage. Patients with T2DM are at risk of declined bone mass and its associated complications later in life [23]. Patients with T2DM also appeared to show declined bone mass at the time of clinical diagnosis [24]. Cohort research demonstrated that diabetic patients may have a higher risk of fractures. Increased

levels of OPG have been documented in diabetic subjects and have been independently correlated with the microvascular complications of diabetes [25].

An inadequate compensatory self-defensive response to block dysfunction in vascular endothelial and the development of atherosclerosis contribute in the elevated circulating osteoprotegerin concentrations in subjects with diabetes [26]. It has been recorded that circulating osteoprotegerin levels significantly correlated with insulin resistance. However, the mechanisms behind their correlation are currently unknown. It is thought that inflammation may relate osteoprotegerin to insulin resistance. Unresponsiveness to insulin is a characteristic feature of T2DM and is considered as a persistent low-grade systemic inflammation [27]. It has been revealed that circulating osteoprotegerin is directly associated with markers of inflammation and has played a critical role in the mechanism of inflammation [28].

Osteoprotegerin/RANK/ RANKL system is believed to be correlated with the immune responses and controlling inflammation, and is directly involved in the controlling pro-inflammatory cytokine generation in macrophages [29]. Additionally, activation of NF-κB pathway and its downstream players attribute to OPG/RANK/RANKL system [30] which are closely linked to the mechanism of insulin resistance [31]. Thus, osteoprotegerin may play a key role in the mechanism of insulin resistance through NF-κB pathway. It has been documented that osteoprotegerin /RANK/RANKL system may have a possible role in the mechanism of diabetes. Inhibiting this pathway ameliorated insulin resistance in hepatic tissue and inhibited the progression of diabetes mellitus [32]. Various studies have also been achieved in T2DM establishing that OPG levels were remarkably higher in the patients compared to healthy controls [6, 33]. High levels of OPG have documented in T2DM patients with asymptomatic coronary artery disease. The process of calcification is due to over-expression of OPG by the vascular cells [34].

Singh *et al.* [35] reported that there is a high OPG levels in T2DM associated with foot vascular calcification. They suggest that high OPG may be a response to high 25 hydroxy vitamin D (25 OH D) levels and high circulating level of lipid generated calcification. O'Sullivan *et al.*[36] found that peripheral artery disease is correlated with high circulating osteoprotegerin, regardless of the coexistence of DM. This result, in addition to its association with severity of peripheral artery disease, proposes that circulating osteoprotegerin may be a novel marker for the presence of extremity of peripheral artery disease, potentially by reflecting the degree of fundamental calcification of vascular. Secchiero *et al.* [37] recorded that serum OPG is remarkably elevated in T2DM patients, stimulating expanded examination of the association between osteoprotegerin generation/levels and glycemic concentrations, and identifies the initially onset of diabetes mellitus and that it may also be involved in dysfunction of endothelial cells. Singh *et al.* [35] proposed that osteoprotegerin plays a major role in regulating the response of endothelial and vascular smooth muscle cells to the action of calcification promotors from the stage of endothelial cell dysfunction through to the progression of medial arterial calcification.

Niu *et al.* [38] in their study found that circulating osteoprotegerin concentrations were remarkably correlated with HOMA-IR in population living in China and that the serum OPG levels were remarkably higher in patients with impeded glucose controlling and diabetes than in those with normal sugar controlling. Serum levels of osteoprotegerin and RANKL were correlated with various factors, comprising PTH, 25 hydroxy vitamin D (25 OH D) and therefore may indicate an integrative parameter for different endocrine signaling disturbances found in T2DM. It is well confirmed that parathyroid hormone and vitamin D elevate RANKL gene expression, while vitamin D up regulates and parathyroid hormone down regulates osteoprotegerin expression [39].

Serum Level of 25-OH Vitamin D

The results represented in the Table 1 show, a highly significant decrease (P<0.0001) of vitamin D concentration in diabetic males int both age ranges 5.78±1.30 ng/mL and 2.47±0.12 ng/mL respectively, in comparison with its level in control matched age ranges 19.06±1.85 ng/mL and 19.79±2.36 ng/mL respectively. Meanwhile, the results indicated that vitamin D concentration non-significantly decreased (P=0.4969); (P=0.5087) in diabetic females in both age ranges which were 9.47±1.98 ng/mL and 10.70±2.11 ng/mL respectively, in comparison with their levels in control matched age ranges 13.07±2.13 ng/mL and 15.53±3.40 ng/mL respectively (Table 2).

The present findings show that there was a remarkable decline in the concentration of circulating vitamin D in both diabetic males and females. These results were closely comparable with Hussain's *et al.* study[40] which recorded significantly low circulating vitamin D concentration in patients with T2DM. The impacts of vitamin D on metabolic pathway of glucose mainly attribute to the dispersal of its receptors (VDR) on β cells of pancreas, fatty tissues and skeletal muscles. Human insulin receptor gene stimulator also influences insulin sensitivity due to the existence of 1α hydroxylase in β cells and the existence of vitamin D response element in the insulin receptor gene stimulator. Activating the transcription of human insulin receptor gene & activating peroxisome proliferator activator receptor attribute directly to calcitriol. Stimulation of insulin receptor expression and enhancement of insulin-mediated glucose uptake *in vitro* is due to vitamin D [41]. Certain allelic alterations in receptor gene of vitamin D and DBP might influence glucose tolerance and insulin release; thus, involving in the genetic risk for T2DM [42].

Serum Levels of Parathyroid Hormone (PTH)

Table 1 reveals the mean level of circulating PTH of diabetic patients and controls. The results showed a remarkable decline in the mean level of circulating PTH in diabetic males in both age ranges which were 74.78±7.42 pg/mL & 67.83±3.69 pg/mL; (p=0.0004) & (p=0.0003) respectively when compared with its level in control matched age ranges 122.4±9.20 pg/mL & 93.31±4.61 pg/mL respectively. Similarly, there was a remarkable decrease (p=0.0128) & (p<0.0001) in serum parathyroid hormone (PTH) level in diabetic females in both age ranges, which were 42.94±2.00 pg/mL & 15.51±1.98 pg/mL respectively, when compared with their level in control matched age ranges 57.49±5.64 pg/mL & 62.61±3.71 pg/mL respectively (Table 2).

The findings of present study showed that there was a remarkable decline in circulating level of parathyroid hormone (PTH) in both diabetic male and female groups of both age ranges. This result was consistent with those of Chaudhuri *et al.* and Rana *et al.*[43, 44] who recorded that serum levels of serum parathyroid hormone decreased in diabetic patients. It is possible that the decreased release of PTH taking place in patients with poorly controlled glycemic index is linked to low circulating level of magnesium. Inaba *et al.*[45] revealed that circulating PTH concentration in male hemodialysis patients with T2DM was remarkably lower than in patients without diabetes. It was proposed that impair in PTH release from the parathyroid gland attributes to certain conditions specific to the diabetic state, such as insulin-like growth-factor-1 and continuous high glucose state. Poor control of blood sugar level further declined the serum PTH concentration and good control of blood sugar level was correlated with higher serum PTH level. Correlation between circulating PTH concentrations and abnormal metabolic pathway of glucose has been supported through blocking of insulin signaling in fatty tissues by parathyroid hormone. Parathyroid hormone, through binding to a G-protein coupled receptor stimulates adenylate cyclase enzyme that raises the generation of cAMP. Increased level of cAMP through activation of protein kinases results in phosphorylation of insulin receptor substrate 1 on serine 307. Decrease of expression of insulin receptor substrate 1 and glucose transporter 4 (GLUT4), and decreases in insulin-

induced glucose transport, explains an association between high circulating concentrations of PTH, prevalence of diabetes and insulin resistance [46].

Serum Levels of Inorganic Phosphorus

As indicated in the Table 1, the mean circulating inorganic phosphorus concentration was significantly decreased (p=0.0035) & (p=0.0337) in diabetic males in both age ranges, which were 4.34±0.27 mg/dL & 4.76±0.35 mg/dL respectively, in comparison with its level in control matched age ranges 6.39±0.48 mg/dL & 6.62±0.54 mg/dL respectively. Meantime, the findings in Table 2 show that, the mean circulating inorganic phosphorus concentration had non-remarkably declined (p=0.9413) in diabetic females in age range of 40-59 years, (4.38±0.21 mg/dL) when compared with its level in control matched age range (5.12±0.44 mg/dL). Whereas significant decrease (p=0.0331) was observed of serum inorganic phosphorus level in diabetic females with age range between 60-80 years (3.86±0.33 mg/dL) in comparison with its level in control matched age range (5.35±0.37 mg/dL) (Table 2). The results of present study revealed that there was a remarkable decline in serum level of inorganic phosphorus in both diabetic males and females. These results are comparable with results of Ugwuja and Eze as well as with that of Raul *et al.* [47, 48]. They had determined the circulating phosphate concentration along with other measured parameters in 60 diabetic patients and compared that with 60 healthy individuals, age and sex matched controls. They observed that circulating phosphate concentrations are remarkably lower in patients with T2DM than in controls ($p<0.05$). In DM, elevated urinary loss attributed to osmotic diuresis may be the most principle cause of declined phosphate [49].

Imperfection in phosphate handling takes place in the renal tubules due to depolarization of the electrochemical sodium gradient as a result of entry of excessive sodium-dependent glucose. Since inorganic phosphate uses the same driving force, but have less ability for binding to sodium than glucose, in poorly regulated patients the reabsorption of inorganic phosphate, particularly becomes impeded. The imbalance in paradoxical phosphate may cause failure of the hemoglobin to release oxygen to the tissues and may impede production of ATP. The possibility of late complications of diabetes mellitus attribute to elevated intracellular glucose to the lack of intracellular phosphate complementary that occurs in the insulin-insensitive cells (insulin resistance) and tissues [14]. Declined concentrations of serum phosphorus in patients with T2DM in comparison to healthy subjects may indicate a possible negative effect of hyperglycemia on serum phosphorus., In a study of diabetic patients [50], it was found that there is a remarkable correlation of low serum phosphate levels with high 2-hourserum sugar concentrations independent of anthropometric indicator like percent body fat, age and sex. The mechanism is believed to be due to an increased consumption of energy, in particular an increased consumption of sugar compound associated with low intake of protein. Two important factors affecting glucose homeostasis are insulin release and insulin resistance. One elucidating for the correlation of low level of phosphate with IGT is that the low level of phosphate may contribute in insulin resistance in subjects with defect in electrolytes homeostasis as well as in a healthy individual. Phosphate is required for ATP production and hence appears to be an essential constituent of ATP metabolism [50].

Serum Levels of Total calcium

Table 1 shows the mean serum total calcium concentration of patients and control groups. The results revealed non-remarkable elevation (p=0.3575) & (p=0.1228) in serum total calcium concentration in diabetic males in both age ranges which were 11.10±0.46 mg/dL & 11.76±0.74 mg/dL respectively, when compared with its level in control matched age ranges 10.46±0.34 mg/dL&10.14±0.35 mg/dL respectively. Whereas non-remarkable decrease (p=0.0536); (p=0.7359) in serum total calcium level was recorded in diabetic females in both age ranges which were 9.69±0.41 mg/dL & 10.08±0.45 mg/dL, in comparison with its level in control matched age ranges 10.33±0.33 mg/dL and 10.28±0.48 mg/dL respectively (Table 2).

Current results revealed non-remarkable differences in the concentration of total calcium in patients with T2DM in comparison to the healthy subjects. Metabolism of calcium includes a complex inhibitory loop that impacts its transport in bones, kidneys and intestinal tract. An increase in circulating levels of calcium activates the CaSR, which declines PTH release. This contributes in decreased resorption and tubular reabsorption of calcium within the bone and renal, as well as declined synthesis of vitamin D which then reduces calcium absorption. When calcium levels are restored, the inhibitory loop ends [51]. Disturbed calcium Metabolism may contribute in abnormality in cell functioning which could then participate to disturbed glucose metabolism. Even though serum calcium may not principally be of consideration of this pathophysiology, *in vitro* studies have recorded increased cytoplasmic calcium being involved in insulin resistance in skeletal muscle and fatty tissue [52].

Conclusion

Present results provide confirmation of altered mineral component of bone, phosphorus and calcium, metabolism of vitamin D and elevated rate of resorption and replacement processes in bones in patients with T2DM. Serum concentrations of osteoprotegerin was correlated with various factors comprising parathyroid hormone, 1,25-Dihydroxycholecalciferol and therefore may be described as an integrative indicator for several disturbances of endocrine signaling that were observed in T2DM. Hence, osteoprotegerin could be a novel marker of the severity of diabetes. Vitamin D3 deficiency is inversely associated with fasting blood glucose and is thus associated with type 2 DM.

Conflict of interest

The authors declare no conflict of interest during this study.

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