

Impact of Osteocalcin Level on Vascular Calcification in Type 2 Diabetics in Relation to Fibroblast Growth Factor-23(FGF-23)

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Abstract

The present study aimed to assess the potential impact of serum concentration of undercarboxylated osteocalcin (the active form of osteocalcin) and fibroblast growth factor-23 on the incidence of cardiovascular diseases in type 2 diabetics with carotid artery calcification and the possible association with metabolic changes in relation to glucose and minerals homeostasis.

This study included 52 men with carotid artery calcification type 2 diabetes mellitus. These patients were categorized; as follows: group A includes 30 patients who had cardiovascular disease and group B includes 22 patients who had no cardiovascular disease. These groups were compared with 25 apparently healthy control (Group C).

It has been shown that fasting serum glucose, HbA_{1c}, homeostatic model assessment of insulin resistance and undercarboxylated osteocalcin values were significantly different in group A and B as compared with control. Also, undercarboxylated osteocalcin was negatively correlated with fasting serum glucose and HbA_{1c} in group A and B. Furthermore, mean serum fibroblast growth factor level was significantly different among the three studied groups, with highest levels in group A.

In type 2 diabetic patients with normal kidney function and carotid artery calcification, fibroblast growth factor-23 is associated with cardiovascular disease while undercarboxylated osteocalcin does not.

Keywords: Diabetes Mellitus Type 2, Vascular Calcification, Undercarboxylated Osteocalcin, Fibroblast Growth Factor-23

تأثير مستوى الاوستيوكالسين على تكلس الاوعية الدموية في مرضى السكري من النوع الثاني والمتعلق بعامل نمو الارومة الليفية - ٢٣ (اف جي اف - ٢٣) ايناس عبد المحسن علي*^١ و شذى حسين علي**

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الخلاصة

تهدف هذه الدراسة الى تقييم التأثير المحتمل لتركيز المصل من الاوستيوكالسين غير الكاربوكسيلي (الشكل الفعال للاوستيوكالسين) وعامل نمو الارومة الليفية-٢٣ على الاصابة بامراض القلب والاعوية الدموية في مرضى السكري من النوع ٢ والمصابين بتكلس الشريان السباتي وامكانية الارتباط مع التغيرات الابضية فيما يتعلق بتوازن الكالسيوم والمعادن.

شملت هذه الدراسة اثنين وخمسين رجلا مصابا بمرض السكري من النوع الثاني مع تكلس الشريان السباتي. تم تصنيف هؤلاء المرضى على النحو التالي: المجموعة أ تضم ثلاثين مريضا والذين يعانون من أمراض القلب والأوعية الدموية والمجموعة ب تشمل اثنين وعشرين مريضا والذين لا يعانون من أمراض القلب والأوعية الدموية. تمت مقارنة هذه المجموعات مع اشخاص على ما يبدو بصحة جيدة (المجموعة ج) شملت خمسة وعشرين شخصا من الاصحاء.

لقد لوحظ أن قيم جلوكوز المصل الصيامي، الهيموغلوبين السكري، قيم تقييم نموذج التوازن لمقاومة الانسولين والايوستيوكالسين غير الكاربوكسيلي كانت مختلفة بشكل معنوي في المجموعة أ و ب مقارنة مع مجموعة السيطرة (الاصحاء). ايضا الاوستيوكالسين غير الكاربوكسيلي كان مرتبطا سلبيا مع جلوكوز المصل الصيامي والهيموغلوبين السكري في المجموعة أ و ب. وعلاوة على ذلك، كان متوسط مستوى عامل نمو الأرومة الليفية - ٢٣ في المصل مختلفا بشكل ملحوظ بين المجموعات الثلاث المدروسة، مع أعلى المستويات في المجموعة أ.

في مرضى السكري من النوع الثاني والذين يعانون من تكلس الشريان السباتي ويتمتعون بوظيفة كلى طبيعية لوحظ ان عامل نمو الارومة الليفية- ٢٣ يرتبط مع أمراض القلب والأوعية الدموية في حين الاوستيوكالسين الغير كاربوكسيلي لا يرتبط.
الكلمات المفتاحية: مرض السكري النوع ٢، تكلس الأوعية الدموية، أوستيوكالسين غير الكاربوكسيلي، عامل نمو الارومة الليفية ٢٣.

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Introduction

Diabetes mellitus (DM), notably, type 2 diabetes mellitus (T2DM) is a common chronic metabolic disease ⁽¹⁾. In the last two centuries; pathogenesis, epidemiology, prevention, and treatment of DM have been well established ⁽²⁾. Additionally, both types of diabetes (1&2) are associated with a higher risk of bone fractures ⁽³⁾. In T2DM, bone mineral density (BMD) is equivalent or increased according to a meta-analysis ⁽⁴⁾, but the risk of fracture is increased despite this BMD increment ⁽⁵⁾. The paradoxical increment in fracture rate in patients with T2DM with BMD increment might result from an increased falling rate ⁽⁶⁾. In addition, a higher risk of fracture in T2DM is thought to be attributed to the decreased bone formation and decreased bone quality ⁽⁷⁾.

Hyperglycemia can reduce the bone density through different pathways. Toxic effects caused by high serum glucose levels can directly decrease the function and number of osteoblasts ⁽⁸⁾. Generally, osteoclastogenesis is enhanced in DM as shown in human studies ⁽⁹⁾. Meanwhile, vascular calcification, as osteogenesis, involves multiple interactions of different cells that produce the matrix vesicles with subsequent mineralization ⁽¹⁰⁾.

The most important direct mechanism that combines the bone loss with the vascular calcification is an increased release of calcium and phosphate from the bone, in the form of calcium-phosphate complexes, by an enhancement of osteoclast-induced resorptive process. Subsequently, these mineral ions of calcium and phosphate and/or complex may be localized to the vascular sites and form a nidus for future mineralization or cause locally increased calcium and/or phosphate levels that induce precipitation of calcium-phosphate complexes in the artery ⁽¹¹⁾.

Osteocalcin (OC), a biomarker for the bone formation, has been well documented to be lower in T2DM patients than in non T2DM controls. Hence, suggesting that glucose levels would affect OC levels in T2DM patients, so apparently, the formation of bone is suppressed if compared with non T2DM controls ⁽¹²⁾. The OC can regulate energy metabolism through the enhancement of pancreatic β -cells to secrete insulin ⁽¹³⁾.

Furthermore, insulin signaling in the osteoblasts may regulate the hemostasis of whole-body glucose via control of OC activation ⁽¹⁴⁾. On the other hand, it has been found that decreased OC levels are associated with insulin resistance and T2DM reflecting the fact of OC ability to modulate the risk of cardiovascular disease ⁽¹⁵⁾.

While, fibroblast growth factor-23 (FGF-23) is a hormone predominately expressed in the osteocytes and has a role in the mineral homeostasis through induction of hyperphosphaturia, inhibition of calcitriol synthesis and inhibition of parathyroid hormone (PTH) secretion ⁽¹⁶⁾. It is well demonstrated that the higher levels of FGF-23 are associated with an increased risk of arterial stiffness and atherosclerosis even in patients with no renal impairment ⁽¹⁷⁾. FGF-23 is considered as an inhibitor factor of bone mineralization ⁽¹⁸⁾, and because, it can regulate the phosphate metabolism, it may be a good marker of the time-averaged hyperphosphatemia, and subsequently, the FGF-23 is found to be as a marker of the vascular calcification ⁽¹⁹⁾.

The goal of this study was to assess the potential impact of serum concentration of undercarboxylated osteocalcin (ucOC) (the active form of OC) and FGF-23 on the incidence of cardiovascular diseases in type 2 diabetics with carotid artery calcification and the possible association with metabolic changes in relation to glucose and minerals homeostasis.

Subjects and methods

A case-controlled study was carried out at Al-Hassan Specialized Center for Diabetes and Endocrine Diseases / Imam Hussain Medical City in Karbala (from October/2017 to March/2018). A total number of one hundred diabetic male patients were selected from the outpatient clinic under the supervision of an endocrinologist. Diabetes was diagnosed according to the American Diabetes Association criteria ⁽²⁰⁾. After excluding those with bone-related disorders :osteomalacia, fracture, tumors, Paget's disease, multiple myeloma, malignant hypercalcemia, hyperthyroidism and hypothyroidism, hyperparathyroidism and hypoparathyroidism ,or those on medication(drugs affect vitamin K status like warfarin, ketoconazole and vitamin K), bisphosphonate, glucocorticoid,1,25-(OH)₂-D₃, heparin and anticonvulsant. After a Doppler ultrasonography for all of the diabetic patients (one hundred) looking for carotid artery calcification. Only 52 out of these patients, who were recognized to have a carotid artery calcification, were included in our study.

The patients were arranged into two groups according to the presence of cardiovascular disease like ischemic heart disease [based on electrocardiograph (ECG) results] ⁽²¹⁾ and/or hypertension [depending on the history, blood pressure measurement and ECG changes] ^(22,23); as follows:

- **Group A:** Thirty diabetic patients with calcification of the carotid artery who had cardiovascular disease.
- **Group B:** Twenty- two diabetic patients with calcification of the carotid artery who had no cardiovascular disease.

These groups were compared with apparently healthy control cases (**Group C**); included twenty-five healthy subjects.

After an overnight fasting, a blood sample was withdrawn to perform the required investigations. The study was authorized by The Local Research Ethics Committee and all subjects were provided with a written informed consent to participate in this study.

Serum glucose⁽²⁴⁾, urea⁽²⁵⁾, calcium⁽²⁶⁾, phosphate⁽²⁷⁾ and alkaline phosphatase (ALP)⁽²⁸⁾ were analyzed using specific kits purchased by Spinreact (Spain). While, glycosylated hemoglobin (HbA_{1c}) was measured by automated fluorescent immunoassay system (AFIAS) kit⁽²⁹⁾ purchased by Afias, Boditech Med Incorporated(Korea).Whereas, FGF-23⁽³⁰⁾ and ucOC⁽³¹⁾ were estimated using specific enzyme-linked immunosorbent assay(ELISA) Sandwich method kits, supplied by Cusabio(China) using ELISA plate reader Beckman Coulter(Austria). Insulin⁽³²⁾, PTH⁽³³⁾

and thyroid stimulating hormone (TSH)⁽³⁴⁾ were measured by specific electrochemiluminescence immunoassay (ECLIA) kits supplied by Cobas, Roche Diagnostics (GmbH, Switzerland) using Cobas e411 analyzer Roche, Hitachi(Switzerland). While serum Creatinine⁽³⁵⁾ was determined using a specific kit purchased by Shenzhen Mindray Bio-Medical Electronics Co., Ltd. (China). Glomerular filtration rate (GFR) was predicted to assess kidney function by the estimation of creatinine clearance (Ccr) based on serum creatinine (Scr) concentration in the adult male by using Cockcroft and Gault formula⁽³⁶⁾: $C_{Cr} = \frac{\{(140 - \text{age}(\text{yrs})) \times \text{weight}(\text{kg})\}}{(72 \times S_{Cr}(\text{mg/dl}))} \times 0.85$ (if female)

While the homeostatic model assessment of insulin resistance (HOMA-IR) is used to estimate insulin resistance from fasting glucose and fasting insulin, by applying the following formula⁽³⁷⁾:

$$\text{HOMA-IR} = \frac{\{\text{Fasting insulin}(\mu\text{IU}/\text{ml}) * \text{Fasting glucose}(\text{mg/dl})\}}{405}$$

Statistical analysis of data is presented as means \pm SD. Significance was set at $p < 0.05$. Cases and controls were compared employing either the t-test for independent samples or The Pearson coefficient, for normally distributed variables.

Results

The subjects' characteristics are illustrated in table-1.

Table 1. Subjects characteristics

Variable	Group	N	Mean \pm SD	p-value
Age (year)	A	30	58.40 \pm 7.81	0.001**
	B	22	50.41 \pm 6.98	
	C	25	46.84 \pm 7.60	
BMI (kg/m ²)	A	30	29.11 \pm 3.56	0.57
	B	22	28.51 \pm 5.15	
	C	25	30.10 \pm 6.77	
Systolic blood pressure (mmHg)	A	30	134.83 \pm 14.88	0.001**
	B	22	121.59 \pm 10.40	
	C	25	119.32 \pm 8.16	
Diastolic blood pressure (mmHg)	A	30	83.50 \pm 18.62	0.01**
	B	22	77.50 \pm 6.50	
	C	25	72.08 \pm 6.57	
Duration of diabetes (year)	A	30	7.83 \pm 7.35	0.001**
	B	22	6.50 \pm 6.23	
	C	25	0.00 \pm 0.00	

Continued table 1.

Variable	Group	N	Mean± SD	p-value
B.Urea(mg/dl)	A	30	32.20±6.59	0.02*
	B	22	29.41±7.15	
	C	25	27.64±3.78	
S.Creatinin(mg/dl)	A	30	0.86±0.19	0.27
	B	22	0.79±0.12	
	C	25	0.81±0.11	
GFR ^a (ml/min)	A	30	113.65±29.42	0.04*
	B	22	133.07±38.87	
	C	25	137.89±40.51	
PTH(pg/ml)	A	30	41.37±14.17	0.09
	B	22	41.76±17.48	
	C	25	49.37±11.53	
TSH(nmol/l)	A	30	1.85±1.14	0.88
	B	22	1.83±0.95	
	C	25	1.97±1.02	

a = GFR based on Cockcroft Gault equation. * = Significant difference ($p \leq 0.05$).

** : highly significant difference ($p \leq 0.01$). N: number of subjects, BMI: body mass index, GFR: glomerular filtration rate, PTH: parathyroid hormone, TSH: thyroid stimulating hormone.

Date illustrated in table -2, showed that fasting serum glucose (FSG), HbA_{1c} and HOMA -IR values were significantly elevated in diabetic patients with cardiovascular disease (group A) and those without cardiovascular

disease (group B) as compared with the control (group C) ($p < 0.01$), but there were no significant differences between diabetic patients groups ($P > 0.05$). While fasting serum insulin mean values were not significantly different among the three studied groups ($p > 0.05$).

Table 2. Descriptive statistics of glycemic parameters and significant comparative studies between studied groups

Variable	Group	N	Mean± SD	P-value
FSG(mg/dl)	A	30	192.90±57.25	0.001**
	B	22	186.53±38.81	
	C	25	98.72±10.06	
HbA _{1c}	A	30	8.37±2.27	0.0001**
	B	22	8.59±2.09	
	C	25	5.44±1.23	
Insulin (μIU/ml)	A	30	14.08±10.68	0.32
	B	22	12.51±7.01	
	C	25	10.76±4.35	
HOMA-IR	A	30	7.11±7.08	0.004**
	B	22	5.65±3.41	
	C	25	2.63±1.14	

** : highly significant difference ($p \leq 0.01$). N: number of subjects, HbA_{1c}: glycosylated hemoglobin, FSG: fasting serum glucose, HOMA-IR: homeostasis model assessment of insulin resistance

As presented in the table-3, the mean serum calcium, phosphate, calcium×phosphate (Ca×Pi), as well as ALP values were not

significantly different among the studied groups (P>0.05).

Table3. Descriptive statistics of serum calcium, phosphorus, (calcium*phosphate) product and alkaline phosphatase among groups.

Variable	Group	N	Mean± SD	P-value
S.Ca(mg/dl)	A	30	9.27±0.64	0.63
	B	22	9.41±0.59	
	C	25	9.25±0.60	
S.Pi (mg/dl)	A	30	3.68±0.64	0.76
	B	22	3.80±0.63	
	C	25	3.70±0.45	
Ca×Pi (mg/dl) ²	A	30	34.34±7.30	0.66
	B	22	35.87±7.34	
	C	25	34.28±5.26	
ALP(U/l)	A	30	81.63±27.83	0.17
	B	22	101.91±65.10	
	C	25	83.84±20.91	

N: number of subjects, S.Ca: serum calcium, S.Pi: serum inorganic phosphate, Ca×Pi: calcium×phosphate, ALP: alkaline phosphatase.

As illustrated in figure-1, the mean of serum uOC level was significantly lowered in diabetic patients with cardiovascular disease (group A) and diabetic patients without cardiovascular disease (group B) when compared with control group (p<0.01), while there was no significant variation between diabetic patients groups (p>0.05).

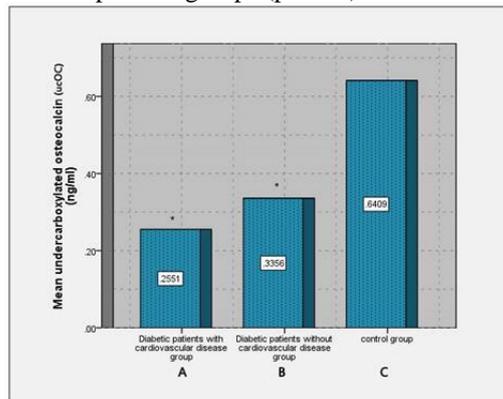


Figure1. Serum undercarboxylated osteocalcin levels in diabetics and control groups. *=significantly different from control

A correlation study was revealed that ucOC was negatively correlated with FSG and HbA_{1c} in the diabetic patient with cardiovascular disease group (p<0.01) [Figure-2&3] and diabetic patients without cardiovascular disease group (p<0.05) [Figure-4&5].

Serum human FGF-23 level was significantly elevated in diabetic patients with

cardiovascular disease (group A) as well as with diabetic patients without cardiovascular disease (group B) as compared to the control group (P<0.01). Besides a significantly higher mean value of FGF-23 in diabetic patients with cardiovascular disease (group A) over that of diabetic patients without cardiovascular disease (group B) (P<0.01)[Figure -6].

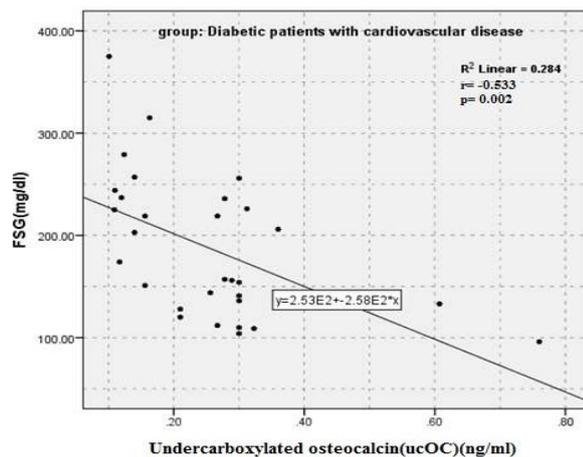


Figure 2. Correlation between undercarboxylated osteocalcin (ucOC) (ng/ml) and fasting serum glucose (FSG) (mg/dl) in diabetic patients with cardiovascular disease group.

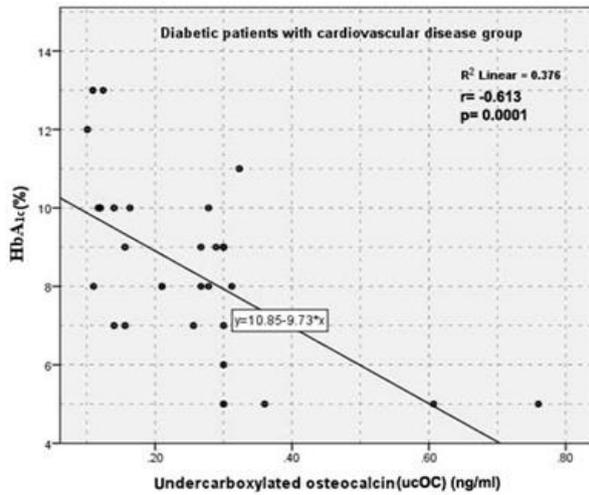


Figure 3. Correlation between undercarboxylated osteocalcin (ucOC) (ng/ml) and HbA_{1c} in diabetic patients with cardiovascular disease group

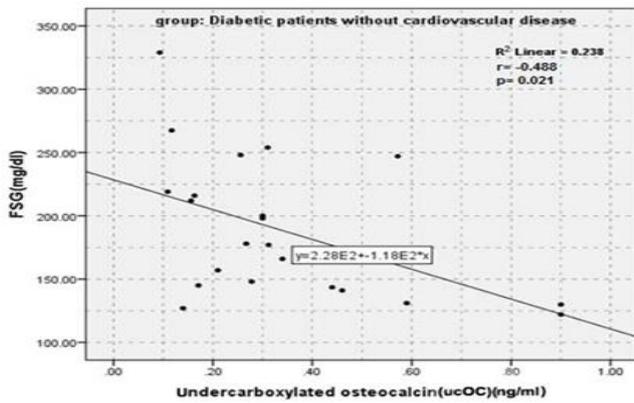


Figure 4. Correlation between undercarboxylated osteocalcin (ucOC) (ng/ml) and fasting serum glucose (FSG) (mg/dl) in diabetic patients without cardiovascular disease group.

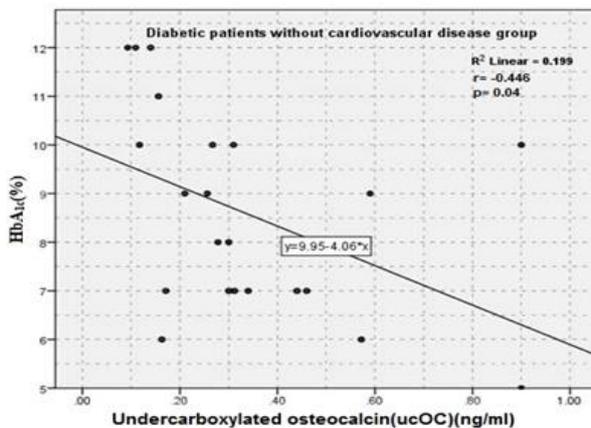


Figure 5. Correlation between undercarboxylated osteocalcin (ucOC) (ng/ml) and HbA_{1c} in diabetic patients without cardiovascular disease group.

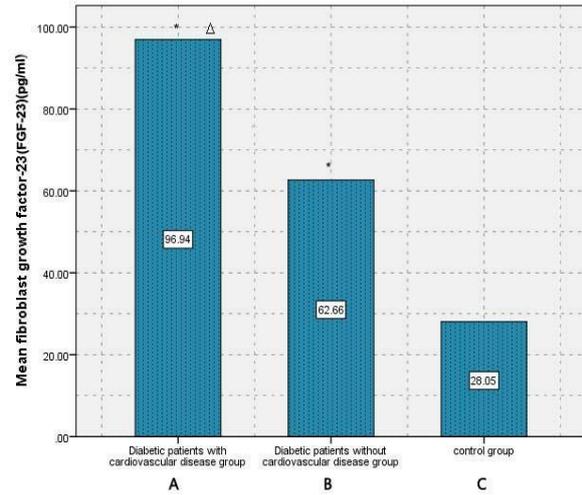


Figure 6. Serum fibroblast growth factor -23 in diabetics and control groups.

*=significantly different from control.
Δ=significantly different from group b

Discussion

It has been observed that patients with T2DM are associated with high insulin resistance and may have apparently normal or elevated insulin levels, the higher levels of serum glucose in those patients would lead to even higher insulin levels with the normal function of their β-cells (20).

As agreed with other results, insulin levels appear within the normal range. Therefore, there was no significant difference between diabetic patient groups and control group. The association between insulin resistance and cardiovascular disease occur through its relation to hypertension, dyslipidemia, atherosclerosis, and hypercoagulability (38). Despite the non-significant higher value of HOMA-IR in diabetic patients with cardiovascular disease as compared with diabetic patients without cardiovascular disease group, this higher value may be responsible for the linkage between group (A) and the presence of cardiovascular disease. As mentioned previously, insulin resistance as assessed by HOMA-IR, was significantly higher in a diabetic patient with and without cardiovascular disease as compared with the control group. In patients with T2DM, the insulin resistance leads to increment in serum glucose levels; consequently, the prolonged hyperglycemia will reduce the bone formation by the osteoblasts(39). This will result in a decrement in the production of ucOC and OC that can cause a further increment in the insulin resistance via reduction of adiponectin formation in the adipocytes (40).

Based on these findings, both the high glucose and the high insulin levels can be included in the list of factors inducing vascular calcifications⁽⁴¹⁾. In this study, an interesting observation on the association between the insulin resistance and the vascular calcifications was provided as agreed with a previous study⁽⁴¹⁾ since HOMA-IR mean value was significantly higher in diabetic patients groups with vascular calcification when compared with control group.

In the present study, the mean serum calcium level was not significantly different among different studied groups ($p > 0.05$) as shown in the table-3. However, even though serum calcium was within the normal reference range, may play a role in developing vascular calcification. This result occurs in accordance with other studies that showed higher levels of the circulating calcium, even within the normal range, were found to be linked with the thickening of the carotid plaque which represents an initial marker of cardiovascular diseases⁽⁴²⁾.

Furthermore, as illustrated in the table-3, the mean serum phosphate level showed no significant difference among the studied groups ($p > 0.05$). However, it was suggested that phosphate, even within the normal reference range, acts as a contributing factor in the vascular calcification. This is consistent with Foley et al. who observed in multivariate models that the phosphate levels were significantly associated with the calcium level of the coronary artery and the higher levels of serum phosphate, even if it is within the normal range, may form a risk factor in the process of coronary artery atherosclerosis in the healthy young group⁽⁴³⁾.

Additionally, the current study showed that the values of $(Ca \times Pi)$ were not significantly different among the studied groups ($p > 0.05$) as shown in the table-3. As previously mentioned, the arterial calcifications are linked with an increased $(Ca \times Pi)$ product.

Serum phosphate may also act directly to increase the vascular calcification, especially when the $(Ca \times Pi)$ product levels are high as noticed in patients with chronic renal disease⁽⁴⁴⁾ or in subjects without chronic renal disease (implied to as a dystrophic calcification). An ectopic calcification is mainly called a dystrophic calcification if it is associated with a normal systemic mineral balance. Commonly, these areas show evidence of changes and/or necrosis in the tissues. Dystrophic mineralization is usually noticed in the soft tissues because of disease, injury, and aging⁽⁴⁵⁾. Additionally, the $(Ca \times Pi)$ products have a positive relationship with the risk of

cardiovascular disease in subjects free of chronic renal disease and cardiovascular disease in the community⁽⁴⁶⁾.

In addition, the data presented in table-3 showed that serum ALP was not significantly different when compared neither with control group nor within the patient's groups ($p > 0.05$). However, diabetic patients without cardiovascular disease group showed an elevation of mean serum ALP concentration level above the normal reference range while diabetic patients with cardiovascular disease group showed a high-normal level. This result is consistent with a Maxwell et al. study who had observed an elevated ALP level in diabetic patients⁽⁴⁷⁾. Since, mean FSG was significantly higher in the group with elevated ALP, indicating a relation between the severity of diabetes and diabetic bone disorder. Furthermore, an increment in the ALP levels is linked to the extensive vascular calcification, resulting in premature atherosclerosis and cardiovascular disease. ALP induces vascular calcification through its action on inorganic pyrophosphate (PPi) which is regarded as a powerful inhibitor for the passive calcium phosphate deposition. The biochemical actions of PPi are the prevention of the calcium and phosphate aggregation, and hydroxyapatite crystal growth. In vivo study, it was reported that the PPi is hydrolyzed into phosphate by the serum ALP. Consequently, an increment in the ALP activity can promote an imbalance between phosphate and PPi, inducing an ectopic calcification⁽⁴⁸⁾.

So, in this study, the elevated level of ALP could be considered as one of the contributing factors in the vascular calcification. However, these results lack the significant difference between the different studied groups. Statistically, the lack of significance can be attributed to the sample size of this study in comparison with the other previous studies.

Many studies had appraised that as the active form of OC, ucOC has an effect on the glucose and lipid metabolism⁽⁴⁰⁾. Serum OC was observed to be reduced in patients with hyperglycemia, DM, obesity, insulin resistance, and metabolic syndrome⁽⁴⁹⁾.

As consistent with these studies, the mean of serum ucOC level was significantly reduced in diabetic patients groups (A and B) when compared with control group ($p < 0.01$) as illustrated in figure-1

The reduction of serum ucOC level in patients with T2DM can be explained by the following causes:

1. Presence of insulin receptors on the osteoblasts, hence, reduction of insulin secretion and insulin resistance in DM can affect the function of the osteoblasts⁽⁵⁰⁾. Also, insulin stimulates the decarboxylation of OC indirectly through osteoclast activation⁽¹³⁾. So, in T2DM, decreased insulin sensitivity can decrease the decarboxylation of OC and ucOC levels.
2. The serum level of vitamin D is significantly lower in patients with T2DM; nevertheless, it can organize the processes of the transcription and translation of the OC gene⁽⁵¹⁾.
3. Hyperglycemia prevents the osteoblast function and may have a direct toxic effect on the osteoblast⁽⁸⁾.
4. It was reported that the reduction of OC was associated with the suppressed level of the PTH in DM and a low level of vitamin D could explain this relation⁽⁵²⁾ because vitamin D stimulates OC production by the osteoblast⁽⁵³⁾.

Decreased plasma OC levels were demonstrated to be linked with higher incidence of the pathological cardiovascular events, such as arterial and valvular calcification, more carotid intima-media thickness, and carotid atherosclerosis⁽⁵⁴⁾. The ectopic cardiovascular calcification is considered as one of the pathological vascular alterations that lead to the development of the cardiovascular disease⁽⁵⁵⁾. On the contrary to a study by Guo et al.⁽⁵⁶⁾, the current study did not show significant variations in the mean ucOC level between diabetic patient groups ($p > 0.05$). However, the mean level of ucOC in diabetic patients with cardiovascular disease was lower than the mean of diabetic patients without cardiovascular disease. Despite this difference, statistically, it was of no significance as presented in figure-1. Guo et al. supposed a possible association between the serum levels of ucOC with the severity of a vascular complication in T2DM, showing the relationship between ucOC and the cardiovascular diseases, as well as, providing a clinical evidence that serum ucOC level is reduced in individuals with vascular complications of T2DM, and there was an inverse relationship between ucOC and T2DM in Chinese men involving ucOC as a future therapeutic target to treat the vascular complications in T2DM⁽⁵⁶⁾. The possible mechanisms that can explain the relationship between the OC and the risk of cardiovascular disease are still unclear. It has been shown that OC can enhance the proliferation of pancreatic β -cell, secretion of insulin, and release of

adiponectin, thus increasing the insulin sensitivity. Therefore, the lower levels of OC may be combined with more resistance of insulin or T2DM and lower levels of adiponectin, thereby, promoting more cardiovascular damage. However, it has been demonstrated that the lower levels of OC may directly influence the cardiovascular risk⁽⁵⁴⁾.

So, a partial explanation of these conflicting data can be done by the gender and ethnic differences that present among the individuals. Moreover, the status of the vitamin D and vitamin K, which may influence the circulating levels of OC and ucOC, are actually not taken into account in the available and many of the cited clinical studies. The vitamin K is considered as a cofactor needed for the carboxylation process of OC. Hence, the dietary factors can have an essential role in OC synthesis. However, the current study did not investigate the effect of diet on the serum OC or ucOC.

Moreover, different risk factors for the cardiovascular disease like hypertension and dyslipidemia had existed in this and other studies, so the potential effects of these associated diseases with the corresponding therapeutic agents may influence the results of the current study.

As observed in this study from the figures (2-5), serum ucOC level was significantly and negatively correlated with FSG and HbA_{1c} in diabetic patient with and without cardiovascular disease, that was consistent with previous studies^(57,58) revealing that the ucOC acts as a hormone that promotes responsiveness to the insulin and glucose tolerance which was consistent with the results of Fulzele et al. study⁽¹⁴⁾.

As observed in this study (figure-6), serum FGF-23 level was significantly elevated in diabetic patients with cardiovascular disease (group A) as well as in diabetic patients without cardiovascular disease (group B) as compared to the control ($P < 0.01$). The increased levels in T2DM could be attributed to:

Firstly: there is a strong relation between the FGF-23 and the BMD in T2DM patients. The serum level of FGF-23 may give an idea about the number of osteocytes and a higher BMD can be found in T2DM as agreed with Reyes-Garcia et al. study⁽⁵⁹⁾.

Secondly: the circulating FGF-23 levels are elevated as the kidney function decreases as revealed by Larsson et al. ⁽⁶⁰⁾. FGF-23 is a powerful biomarker of early chronic renal disease and may reflect the incident kidney disease in diabetics ⁽⁶¹⁾.

Thirdly: the insulin resistance had an independent association with FGF-23 levels. An increment in HOMA-IR levels is associated with an increment in FGF-23 levels among subjects with normal kidney function ⁽⁶²⁾. Because a patient with increased insulin resistance may reabsorb more phosphate, thus, a higher level of FGF-23 is reached in order to eliminate the phosphate ⁽⁶³⁾.

Moreover, the FGF-23 has the ability to conserve the calcium despite suppressed hormonal synthesis of vitamin D that induced by FGF-23 itself. So FGF-23 have a role in the development of vascular calcifications ⁽⁶⁴⁾. The increment in the circulating FGF-23 levels, which was also noticed in the diabetic animal models, may escalate the existing endothelial dysfunction and induce a vascular calcification, subsequently, resulting in atherosclerosis in DM patients ⁽⁶⁵⁾.

As reported in the figure (6), there was a significant elevation in FGF-23 level in diabetic patients with cardiovascular disease group when compared with diabetic patients without cardiovascular disease group ($P < 0.01$). This difference was related to the role of FGF-23 in the development of cardiovascular disease. Previous studies demonstrated a strong dose-response link between the increased FGF-23 levels and the future cardiovascular disease and death in both patients with chronic renal disease ⁽⁶⁶⁾ and with no chronic renal disease ⁽¹⁹⁾.

In addition, the higher FGF-23 level was associated with increased arterial stiffness as demonstrated in patients with T2DM ⁽⁵⁹⁾ and even in the general population ⁽⁶⁷⁾. Furthermore, Voigt et al. study reported that the FGF-23 was detected in the calcified carotid atherosclerotic lesion from individuals with normal renal function ⁽⁶⁸⁾.

On the other hand, in vivo study showed that FGF-23 effect on the blood vessels is indirect because FGF-23 can suppress the production of vitamin D hormone which is an important factor in the regulation of the endothelial function ⁽⁶⁹⁾ and proliferation of the cardiomyocyte ⁽⁷⁰⁾. The reduction in 1,25(OH)₂-D₃ can elevate the angiotensin II production through an increment in the renin expression, which leads to cardiac hypertrophy and hypertension ⁽⁷¹⁾. Moreover, FGF-23 may enhance endothelial dysfunction through direct interference with the nitric oxide-induced vasodilation ⁽⁷²⁾.

An important limitation of this study is the lack of generalizability of the results to both sexes and they are only limited to elderly men. Further studies about the same issue can be conducted on both sexes. Also, another limitation is the sample size of this study was relatively small. Therefore, we premise that the results of this study could be a base for future studies in larger groups of subjects.

In conclusion, the present study suggested that the higher serum FGF-23 level was associated with carotid artery calcification and cardiovascular disease (ischemic heart disease and/or hypertension) in T2DM patients with normal kidney function. While lowered serum ucOC level was associated with carotid artery calcification but does not associate with cardiovascular disease. Even so, by connecting the dots, both elevated FGF-23 and reduced ucOC levels are related to altered bone metabolism in relation to abnormal glucose homeostasis which would contribute to vascular calcification, and consequent cardiovascular complications in T2DM patients. Since ucOC was inversely correlated with FSG and HbA_{1c}, confirming the role of ucOC in glucose metabolism and thereby cardiovascular risk. For this reason, further studies are needed with a big sample size to investigate the role of ucOC in the development of cardiovascular disease in diabetics. Finally, serum ucOC and FGF-23 may supply a reliable marker for carotid artery calcification in T2DM patients with normal kidney function. For this reason, more investigations are required to understand the total functions of ucOC as a hormone in energy homeostasis that could offer new hopeful future studies for ucOC to be a constitutional basis in healing strategies for T2DM related metabolic and cardiovascular disorder.

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