ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Impact of Serum Fibroblast Growth Factor-23 & Undercaboxylated Osteocalcin Levels on Carotid Artery Calcification among Type2 Diabetics

Inas A Ali[1], Shatha H Ali[2*], Mohanad A Abdulrahman[3]
[1] *Department of Clinical Pharmacy, College of Pharmacy, University of Baghdad, Baghdad, Iraq*
[2] *Department of Clinical Laboratory Science, College of Pharmacy, University of Baghdad, Baghdad, Iraq*
[3] *Medicine department, Imam Hussain Medical City, Karbala health directory, Ministry of health, Karbala, Iraq*

Abstract

It was suggested that fibroblast growth factor-23(FGF-23) and undercarboxylated osteocalcin (ucOC) could be the link between bone loss and vascular calcification (VC). The target of this study is to investigate the potential effect of serum FGF-23 and ucOC on the incidence of carotid artery calcification in T2DM with normal kidney function in relation to mineral and glucose metabolism and also to assess the possible impact of obesity and hypertension.

This study included fifty-two men with calcification of carotid artery type 2 diabetes mellitus (T2DM) (diagnosed by a Doppler ultrasonography) designated as (Group P), aged 55.02±8.41 years and their body mass index (BMI) mean value was 28.85±4.27. Those patients were compared with apparently healthy control (Group C); included twenty- five healthy subjects (mean age & BMI of 46.84±7.60 years 30.10± 6.77, respectively). Fasting venous blood samples were collected for analysis of glycated hemoglobin (HbA_{1C}), glucose, insulin, homeostatic model assessment-insulin resistance (HOMA-IR),calcium, phosphate, parathyroid hormone (PTH), thyroid stimulating hormone (TSH), urea, creatinine, FGF-23, ucOC as well as C-reactive protein (CRP).

As the statistical analysis measured values of HbA_{1C}, fasting serum glucose (FSG) and HOMA-IR were significantly increased in diabetics when compared with control ($p<0.01$), mean values of fasting serum insulin were not significantly different ($p>0.05$). In addition, the serum levels of calcium, inorganic phosphate and calcium*phosphate product (Ca*Pi) were not shown significant difference between studied groups ($P>0.05$). Moreover, the mean value of serum ucOC was significantly decreased in diabetic patients as compared with control ($p<0.01$). Furthermore, mean serum FGF-23 level was, statistically, significantly elevated in diabetics, with highest values were evaluated among the hypertensive ones. Obese patients were presented with significantly elevated glomerular filtration rate (GFR) and C-reactive protein mean values.

In T2DM patients with carotid artery calcification and normal kidney function, elevated serum levels of FGF-23 has been observed, while ucOC levels were reduced. Both higher FGF-23 and lowered ucOC levels are relevant to modified bone metabolism in association with abnormal glucose metabolism which would participate in VC, and thereby cardiovascular complications in type 2 diabetics. Thus, more understanding of diabetes- related biochemical changes and its progressive complications for developing new treatment approaches is urgently needed.

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

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a metabolic disorder which is characterized by hyperglycemia with alteration of lipid metabolism, and it is mainly caused by the inability of islet B cells to compensate for the high levels of serum glucose, increased secretion of glucagon and decreased incretin response, increased production of endogenous glucose and the resistance of peripheral insulin (1).

Fibroblast growth factor-23 (FGF-23) is a hormone produced by the osteocyte in the bone (2). FGF-23 can prevent sodium-dependent phosphate re-absorption, as well as, 1-alpha-hydroxylase enzyme activity in the proximal tubule, causing hypophosphatemia and inappropriate production with a low level of vitamin D3 (3). Consequently, this phosphaturic effect will increase the phosphate excretion in relation to the reduced formation of mineralized bone (4).

On the other hand, osteocalcin (OC) is presenting as γ-carboxylated at the three glutamine terminals which interacts with the hydroxyapatite and it is called as undercarboxylated osteocalcin (ucOC) (an active form of OC) when less than three terminals are γ-carboxylated (5). In a feedback circle, the insulin signals back in osteoblasts to enhance the OC activity and, hence, the insulin secretion. Osteoblast expresses the receptor for insulin and when enhanced by insulin, it will promote osteoclast resorption, resulting in OC under-carboxylation (6)

Since, the impact of FGF-23 and ucOC in individuals with normal renal function is less known, the target of this study is to investigate the potential effect of serum FGF-23 and ucOC on the incidence of carotid artery calcification in T2DM with normal kidney function in relation to mineral and glucose metabolism and also to assess the possible impact of obesity and hypertension in these patients.

MATERIALS AND METHODS

This is a case-control study which was carried out at Imam Al-Hussain Medical City / Al-Hassan Specialized Center of Diabetes and Endocrine Disease in Karbala city from October/2017-March/2018. A total number of 100 diabetic male were chosen from the out-patient department clinic under the surveillance of an endocrinologist. Their age was ranging between 40-65years and they were non-smoker and non-alcoholic. Diabetes mellitus (DM) was diagnosed in agreement with the American Diabetes Association (ADA) criteria (7). Patients were selected after excluding the followings: acromegaly, Cushing's syndrome, hyperthyroidism and hypothyroidism, hyperparathyroidism and hypoparathyroidism, chronic liver disease (liver cirrhosis), chronic and acute renal failure, malignant hypercalcemia, multiple myeloma, Paget's disease, osteomalacia, fracture and those patients on medications (drugs that affect vitamin K status such as vitamin K, warfarin and ketoconazole), anticonvulsant, bisphosphonate, heparin, vitamin D3 and glucocorticoid.

The diagnosis of carotid artery calcification for all of selected diabetic patients was made by Doppler ultrasonography technique and only 52 male out of those patients (one hundred) were diagnosed to have a calcification of carotid artery and they were designated as group P in our study, in addition to 25 healthy subjects as group C (controls).Out of the 52 diabetics with carotid artery calcification: 19(36.53%) patients were obese (BMI≥30) &33 (63.46%) were non –obese (BMI<30). Furthermore, when these patients (52) were further categorized based on the presence of cardiovascular disease (CVD) like ischemic heart disease

(IHD) [depending on electrocardiograph (ECG)] (8) and/or the presence of hypertension, into two groups (30 patients with CVD and 22 without CVD , groups A&B, respectively) and also categorized according to the presence of only hypertension [based on blood pressure measurement and the patient history] as follows, 23 of them were hypertensive and 29 were non ,the subjects' characteristics are presented in table-1.

Venous blood sample was withdrawn from each subject after an overnight fasting to achieve the required investigations. This study was approved by (The Local Research Ethics Committee) and all patients were supplied with a written informed consent to be a participant in this study. Fasting serum glucose (FSG) (9), calcium (10), phosphorus (11) and urea (12) were tested using specific kits supplied by Spinreact, Spain. Whereas, glycated hemoglobin (HbA_{1C}) was determined by automated fluorescent immunoassay system kit (AFIAS) (13) (Afias, Boditech Med Incorporated,Korea). While, ucOC (14) and FGF-23 (15) were analyzed by specific enzyme linked immunosorbant assay sandwich method kits (ELISA) (Cusabio, China) utilizing ELISA plate reader (Beckman Coulter, Austria). Thyroid stimulating hormone (TSH) (16), Parathyroid hormone(PTH) (17) and Insulin (18) were tested by specific electrochemiluminescence immunoassay kits (ECLIA) (Cobas, Roche Diagnostics GmbH, Switzerland) utilizing Cobas e411analyzer (Roche, Hitachi,Switzerland). Whereas serum Creatinine was measured using specific kit (19) supplied by (Shenzhen Mindray, China). C-reactive protein (CRP)-turbilatex kit (20) (Accent-200) purchased by PZ Cormay SA,Poland.

Furthermore, kidney function was examined by the assessment of glomerular filtration rate (GFR) which was predicted by the estimation of creatinine clearance depending on serum creatinine concentration based on the Cockcroft and Gault formula (21):

Ccr=[{(140-age (yrs) x weight(kg))/(72xScr (mg/dl)}]x0.85(if female)

Ccr:Creatinne clearance ,Scr:Serum creatinine

The homeostatic model assessment-insulin resistance (HOMA-IR) is a method used to calculate insulin resistance by multiplying fasting glucose by fasting insulin. It was first determined by Matthews et al. in 1985 by using the following formula (22):

HOMA-IR={Fasting insulin (µIU/ml)x Fasting glucose (mg/dl)}/405 :

The results were illustrated as mean±standard deviation (SD). Student *t*-test was used to determine the degree of significance between two groups. P value equivalent to or less than 0.05 was considered with significance difference. Pearson's correlation coefficient (r) was used to examine the statistical correlation between measured parameters.

RESULTS

Data presented in the table -2, showed that $HbA_{1C,}$ FSG and HOMA-IR levels were significantly higher in diabetic patients (group P) when compared with the control group (group C) ($p<0.01$). Whereas, the mean values of fasting serum insulin were not significantly different between the two studied groups ($p>0.05$) . As reported in table-3, the mean serum values of calcium, phosphorus and (Calcium *Phosphate product) (Ca*Pi) were not significantly different between group P and C ($P>0.05$). As shown in the figure-1, the mean value of serum ucOC was significantly decreased in diabetic patients (group P) as compared with the control group (group C) ($p<0.01$).

Serum FGF-23 level was significantly higher in diabetic patients (group P) when compared with the control group ($P<0.01$) [Figure 2].

Table-1: Descriptive Statistics of Subjects' Characteristics with Comparative Significant Studies between Patient and Control Groups

Variable	Sample	Mean± S. D	P value
Age (year)**	patient	55.02 ±8.41	0.000
	control	46.84 ±7.60	
weight(Kg)	patient	81.51 ±13.02	0.426
	control	84.52 ±19.71	
Height(M)	patient	1.68 ±0.08	0.780
	control	1.68 ±0.07	
BMI (kg/m2)	patient	28.85 ±4.27	0.329
	control	30.10 ±6.77	
Systolic blood pressure** (mmHg)	patient	129.23 ±14.63	0.002
	control	119.32 ±8.16	
Diastolic blood pressure** (mmHg)	patient	80.96 ±14.95	0.006
	control	72.08 ±6.57	
Duration of Diabetes** (year)	patient	7.27 ±6.86	0.000
	control	0.00 ±0.00	
Duration of Cardiovascular Disease** (year)	patient	3.29 ±4.94	0.001
	control	0.00 ±0.00	
Serum Urea **(mg/dl)	patient	31.02 ±6.91	0.007
	control	27.64 ±3.78	
Serum Creatinine (mg/dl)	patient	0.83 ±0.16	0.627
	control	0.81 ±0.11	
GFR (ml/min)	patient	121.87 ±34.76	0.077
	control	137.89 ±40.51	
PTH * (pg/ml)	patient	41.54 ±15.49	0.028
	control	49.37 ±11.53	
TSH (nmol/l)	patient	1.84 ±1.06	0.611
	control	1.97 ±1.02	
CRP (mg/dl)	patient	3.81 ±2.33	0.76
	control	4.43±3.73	

Number of Patients =52; Number of Controls =25; (*): significantly different from control ($p<0.05$); (**): high significant difference from control ($p<0.01$).

Correlation study was reported that human ucOC was correlated negatively with FSG and HbA_{1C} in diabetic patient with CVD group (group A) ($p<0.01$), while FGF-23 was positively correlated with the duration of CVD($p=0.05$) [Table-4] .For diabetic patients without CVD group (group B), ucOC was similarly had negative correlation with FSG and HbA1c values ($p<0.05$), but FGF-23 was positively correlated with (Ca*P) values ($p<0.05$) [Table-5]. As shown in figures-3& -4, for obese diabetic patients compared with non- obese diabetic patients there were significant variation, for the mean values of GFR and CRP levels, although both GFR& CRP levels were not significantly altered in studied diabetics as compared to controls [table-1] .With significantly higher values in obese patients than non- obese patients ($p<0.01$) and ($p<0.05$) respectively. Meanwhile, the mean of FGF-23 level was with significant elevation in hypertensive diabetics, as compared with non- hypertensive within the diabetics group (group P) ($p<0.01$, figure-5).

Table 2: Descriptive Statistics of Glycemic Indices with Comparative Significant Studies between Patients and Control

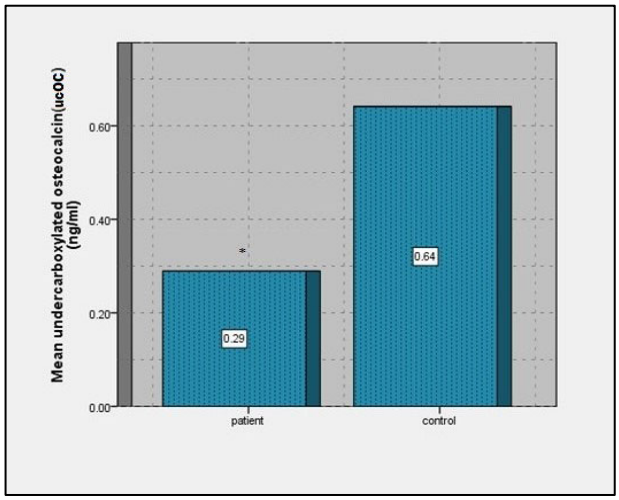
Variable	Group	Mean± S. D	P-value
HbA_{1C}** (%)	patient	8.46 ±2.17	0.0001
	control	5.44±1.23	
FSG**(mg/dl)	patient	190.20 ±49.94	0.0001
	control	98.72±10.06	
Insulin (µIU/ ml)	patient	13.42 ±9.26	0.177
	control	10.76±4.35	
HOMA-IR**	patient	6.49 ±5.82	0.002
	control	2.63±1.14	

**: high significant difference from control ($p<0.01$); Number of Patients =52; Number of Controls =25

Table -3: Descriptive Statistics of Serum Calcium, Phosphate and Calcium*Phosphate with Comparative Significant Studies between Patients and Control

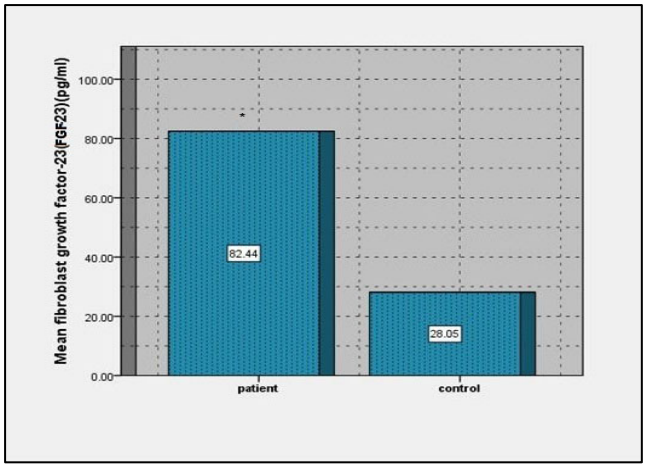
Variable	Group	Mean± S. D	P-value
Ca(mg/dl)	patient	9.33 ±0.62	0.591
	control	9.25±0.60	
Pi (mg/dl)	patient	3.73 ±0.63	0.830
	control	3.70±0.45	
Ca*Pi (mg/dl)	patient	34.99±7.28	0.667
	control	34.28±5.26	

Number of Patients =52, Number of Controls =25
Ca: calcium; Pi: inorganic phosphate; Ca*pi: calcium*inorganic phosphate



(*): Significant difference from control group

Figure 1: Serum Human Undercarboxylated Osteocalcin(ucOC) Levels among Studied Groups



(*): Significant difference from control group

Figure 2: Serum Human Fibroblast Growth Factor (FGF-23) Levels among Studied Groups

Table 4: Pearson's Correlations for Diabetic Patients with Cardiovascular Diseases (group A)

	Serum human undercarboxylated osteocalcin(ucOC) (ng/ml)	Serum human fibroblast growth factor-23(FGF-23)(pg/ml)
Variables that are significantly correlated	Fasting serum glucose(mg/dl) r= -0.533** p= 0.002	Duration of cardiovascular disease(year) r= 0.359* p= 0.050
	HbA_{1C}(%) r= -0.613** P= 0.0001	

*: Significant correlation is at the level ≤ 0.05, **: highly significant correlation is at the level ≤ 0.01.

Table 5: Pearson's Correlation for Diabetic Patients without Cardiovascular Disease (group B)

	Serum human undercarboxylated osteocalcin(ucOC) (ng/ml)	Serum human fibroblast growth factor-23(FGF-23)(pg/ml)
Variables that are significantly correlated	Fasting serum glucose(FSG) r=-0.488* p=0.021	(Ca*Pi) r=0.479* p=0.024
	HbA_{1C} r=-0.446* p=0.04	

*: Significant correlation is at the level < 0.05, **: Highly significant correlation is at the level < 0.01.

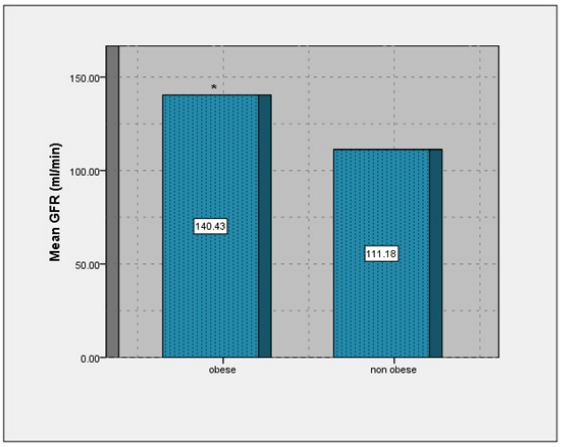


Figure 3 : Glomerular Filtration Rate (GFR) Mean Values for Obese and

*: significantly different from non obese group ($p<0.01$), - Number of obese patients=19 - Number of non obese patients=33

Non Obese Diabetic Patients

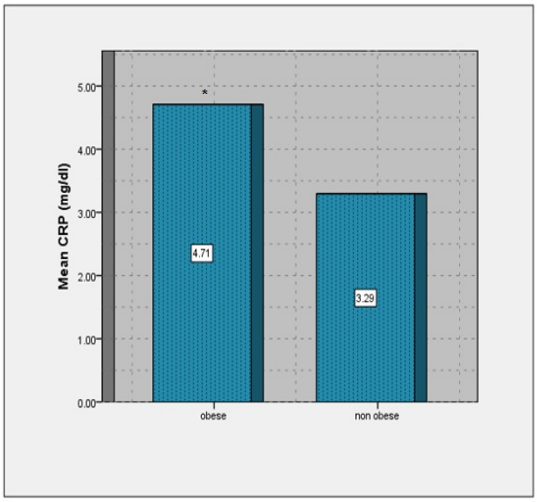


Figure 4: C-Reactive Protein (CRP) Mean Levels in Obese and Non Obese

*: significantly different from non obese group (p<0.05), - Number of obese patients=19 - Number of non obese patients=33

Diabetic Patients

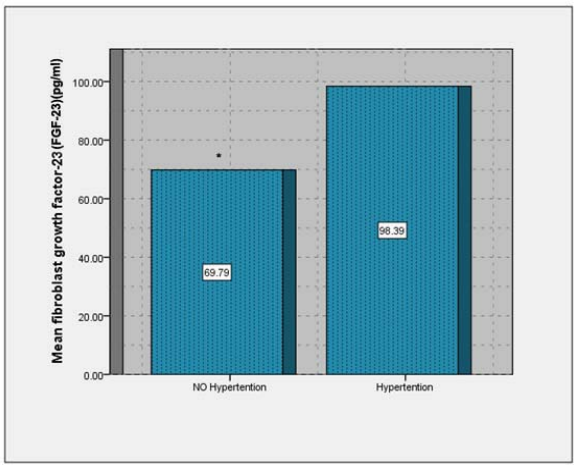


Figure 5: Fibroblast Growth Factor-23(FGF-23) Mean Levels in Hypertensive and Non Hypertensive Patients

*: significant difference from hypertensive group (p<0.01), -Number of non hypertensive patients=29 -Number of hypertensive patients=23

DISCUSSION

Variation of Glycemic Indices among Studied Groups:

The elevated mean value of HbA_{1C}, FSG and HOMA-IR in diabetic patients with carotid artery calcification (group P) as compared with the control (group C) (table -2), are indicative of disturbed glucose homeostasis for those patients of being diabetics, although fasting serum insulin levels were not different among these groups. However, fasting serum insulin levels in group P were within the normal reference range as consistent with a recent study (7). A previous study had appraised that despite the higher ambient insulin, insufficient insulinization would results from insulin resistance and it can unfavorably affect the function of cells that play an important role in atherogenesis resulting in endothelial dysfunction and oxidative stress(23). Therefore, the secretion of insulin in those patients is inadequate to compensate for the resistance of insulin (7) so that, the impact of insulin resistance in the development of vascular calcification (VC) can be predicted.

Calcium, Phosphate and Calcium*Phosphate Product:

Our data in table -3 reported that there were no significant variation of serum calcium levels among studied groups, (group P) and (group C). This results is in consistance with Schaerström et al. study who documenting that there is no significant difference in the serum levels of calcium in DM patients as compared with the healthy subjects (24). In a study by Rubin et al. had shown that serum calcium levels in community dwelling, multiethnic normocalcemic subjects are positively correlated with carotid plaque thickness, a potential early estimator of atherosclerosis and CVD(25). The higher concentration of ambient calcium might change the vascular microenvironment to be more similar to the bone and prefer a plaque formation. The relationship between the serum calcium and plaque reflects that the plaque is calcified (25). For this reason, serum calcium even within the normal range has a role in carotid artery calcification.

Although our data (table -3) showed no significant difference in serum phosphate levels between diabetic patients (group P) and control (group C). However, serum phosphate level was within the higher normal range. It was concluded that the upper normal level of serum phosphate (3.5-4.5 mg/dl) (26) was considered as an independent triggering factor in the occurrence of VC, as well as, the CVD (27). Since VC is commonly observed in DM, aging and in several genetic defects with the existence of normal plasma concentration of phosphate and calcium, these observations suggest that VC can occur at normal plasma concentrations of these minerals (28).

Furthermore, the (Ca*Pi) product mean values were not significantly varies in diabetic patients (group P) from that of control (group C) (table -3). It is necessary to notice that none of the participants in this study had levels of (Ca*Pi) product more than 55 mg^2/dL^2, refers to a threshold level, as reported previously that any reading above it will be associated with the increased adverse events in patients with chronic renal disease (29).

However, *in vivo*, a study by Kuro et al. concluded that in the availability of normal serum levels of creatinine, cholesterol, albumin, and triglyceride and with a mild increment in the serum levels of calcium would result in a two-fold increment in the serum phosphate levels leading to an increment in the (Ca*Pi) product, subsequently, occurrence of VC and osteoporosis which were obviously of no relation to the abnormal lipid metabolism, malnutrition, or chronic renal failure (30). In accordance with these studies which excluded the kidney disease, the participation role of (Ca*Pi) product can be a predictor in the development of VC.

Undercarboxylated Osteocalcin:

There are several evidence suggesting that serum levels of ucOC to be negatively correlated with diabetes, obesity, insulin resistance and metabolic syndrome markers (31)(32). In agreement with previous studies (5), serum ucOC mean value was significantly decreased in diabetic patients group (group P) as compared with the control group (group C), (figure-1). As agreed with this result, a study in T2DM men, but not women, OC and ucOC levels were negatively correlated with abdominal aortic calcification score (33). Also, Zhang et al. showed that there is a negative association between ucOC level and the carotid plaque and intima-media thickness in chronic renal disease patients with no dialysis (34). It has been documented that the metabolism of glucose is regulated by ucOC directly by increasing the proliferation of pancreatic β-cells and enhancing the synthesis and secretion of insulin (35). Furthermore, the OC promotes energy expenditure and insulin sensitivity by other mechanisms (36), it acts on the adipocytes and promotes the secretion of adiponectin. Adiponectin can up-regulates the insulin sensitivity (37). In DM, bone integrity will be affected, since hyperglycemia weakens mature osteoblastic cells. Consequently, the decreased osteoblast function and mass inhibits OC synthesis and secretion(38).

Therefore, decreased levels of circulating OC which is associated with the availability of the insulin resistance, metabolic syndrome, and T2DM, obviously linked to the development of

atherosclerosis reflecting the fact of OC ability to modulate the risk of CVD (39).

Meanwhile, in the present study, serum ucOC level was showed a significant negative correlation with FSG and HbA_{1C} in group (A and B) as illustrated in table-4&5 respectively. Iki et al. confirmed our results by describing such correlation in community-dwelling older age Japanese males(40).

Fibroblast Growth Factor-23:

The results of the current study revealed that the mean of HOMA-IR and FGF-23 levels were both significantly elevated in the diabetic group (group P) as compared with the control group (group C). It has been observed that insulin has the ability to increase renal phosphate reabsorption by promoting renal sodium-phosphate cotransporter. Therefore, since insulin resistance induces disrupted renal phosphate homeostasis, an increment in the FGF-23 level has been detected (41). Furthermore, some workers had suggested that the phosphate is considered as an independent predictor of the CVD and it has a role in the development of the arterial calcification. Additionally, the insulin resistance is found in many subjects with the obesity and T2DM and is associated with the incidence of the VC (42). So, the mechanism by which FGF-23 can cause VC can be predicted.

Various mechanisms have been suggested to explain the association between FGF-23 and the VC. It has been observed that elevated levels of FGF-23 are associated with elevated serum phosphate levels (43), and the higher levels of serum phosphate to be associated with the VC and systemic cardiovascular events in the community-living sample with or without the presence of severe renal disease. Therefore, a chronic hyperphosphatemia is considered as a risk factor for the VC and disease of the cardiovascular system (44). However, different studies have demonstrated that the high serum level of FGF-23 might be an essential biomarker of the VC, even in the subject with normal serum phosphorus concentration (26).

In this study, in diabetic patients without CVD group (group B), a significant positive correlation has been observed between FGF-23 and (Ca*Pi) product as presented in table-5. Indeed previous study had reported such correlation (45) which supported the concept that mineral parameters and (Ca*Pi) products contribute to the evolvement of the carotid artery calcification in accordance with elevated serum FGF-23 levels. However, this correlation was absent in the diabetic patient with CVD group (group A) and it may be due to the small sample size of this study .Furthermore, a significant positive correlation was noticed in diabetic patients with CVD group (group A) between FGF-23 and duration of CVD (table-4); such association demonstrates that FGF-23 contributes in the incidence, development and progression of CVD.

Effect of Obesity:

In this study, serum mean values of CRP were presented with no significant difference among diabetic patients (group P) and control (group C) as illustrated in table-1. The impact of obesity on CRP levels in T2DM patients group was presented with significantly higher mean levels of CRP in obese diabetic patients compared to non-obese diabetics (figure-4).

A weak relationship between CRP concentration and the vascular complications was noticed in this study and remained unclear. However, it is possible that non elevated CRP levels in DM group with VC (group P) may be related to the drugs that used in treating these CVD. Some of these drugs such as aspirin, angiotensin-converting enzyme inhibitors, beta-blockers, and angiotensin receptor blocker had been shown to decrease the CRP level (46)(47). Additionally, statins can have a similar effect (48) and were used frequently in those patients with CVD.

As described in the figure-3, the mean of GFR levels was significantly higher in obese patients than non-obese patients and obese diabetic patients were presented with glomerular hyperfiltration which is related to the impact of obesity on GFR. In animal studies, it was found that there is a relationship between obesity and the glomerular hyperfiltration. Moreover, human studies also demonstrated abnormal renal haemodynamics in the obese individuals, revealing an elevated GFR, elevated renal blood flow or both. Usually, the obesity is combined with stimulation of the renin-angiotensin-aldosterone system enhanced by multiple factors, consisting of secretion of renin and the precursors of renin by the adipocyte cells. This may lead to an increment in the angiotensin II resulting in an increased proximal sodium reabsorption. Meanwhile, in a diabetic kidney, the increment in the proximal sodium reabsorption can stimulate the tubuloglomerular feedback, hence, elicit a glomerular hyperfiltration (49).

Effect of hypertension:

In this study, figure-5 display the effect of hypertension on FGF-23 levels, as the mean of FGF-23 levels was significantly higher in the hypertensive diabetic patient as compared with the corresponding non hypertensive diabetic patient group indicating the role of FGF-23 in the evolvement of CVD and these results were compatible with a previous study by Seiler et al. who observed that dyslipidemia, obesity, hypertension, and smoking, were associated with the higher levels of FGF-23 and all of these factors are highly linked to the morbidity and mortality of the CVD and this can explain the relation between FGF-23 level and the adverse outcomes (50).

It is supposed that the increment of FGF-23 levels will lead to a reduction in vitamin D3 levels (51) and thereby, an elevation in the production of angiotensin II through an elevation in the expression of renin which consequently lead to hypertension and cardiac hypertrophy (52). FGF-23 may also retard nitric oxide-induced vasodilation in the endothelial cells(53).

CONCLUSION

This study supposed that T2DM patients with carotid artery calcification and normal renal function had an elevated FGF-23 and lowered ucOC levels. Such, elevated FGF-23 levels were related to carotid artery calcification and CVD (hypertension) whereas the reduced ucOC levels were related to the calcification of carotid artery but was not associated with CVD (hypertension). However, both hormones are relevant to modified bone metabolism in association with abnormal glucose metabolism which would participate in VC, and thereby cardiovascular complications in type 2 diabetics. Thus, more understanding of diabetes- related biochemical changes and its progressive complications for developing new treatment approaches is urgently needed.

REFERENCES

1. Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: from pathophysiology to prevention and management. Lancet. 2011;378(9786):169–81.
2. Quarles LD. Evidence for a bone-kidney axis regulating phosphate homeostasis. J Clin Invest. 2003;112(5):642–6.
3. Shimada T, Kakitani M, Yamazaki Y, Hasegawa H, Takeuchi Y, Fujita T, et al. Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. J Clin Invest. 2004;113(4):561–8.
4. Feng JQ, Ward LM, Liu S, Lu Y, Xie Y, Yuan B, et al. Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. Nat Genet. 2006;38(11):1310.
5. Motyl KJ, McCabe LR, Schwartz A V. Bone and glucose metabolism: a two-way street. Arch Biochem Biophys. 2010;503(1):2–10.
6. Fulzele K, Riddle RC, DiGirolamo DJ, Cao X, Wan C, Chen D, et al. Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. Cell. 2010;142(2):309–19.
7. Association AD. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2018. Diabetes Care. 2018;41(Supplement 1):S13–27.
8. Herring N, Paterson DJ. ECG diagnosis of acute ischaemia and infarction: past, present and future. J Assoc Physicians. 2006;99(4):219–30.

9. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst. 1972;97(1151):142–5.
10. Kajalkar RV, Gaikwad AD. Colorimetry Based Calcium Measurement. Int J Eng Res Dev. 2013;7(8):8–11.
11. Rubino L, Catapano V, Guerra G. Determination of inorganic phosphorus in serum: Evaluation of three methods applied to the Technicon RA-1000 analyzer. J Anal Methods Chem. 1989;11(4):164–7.
12. Taylor AJ, Vadgama P. Analytical reviews in clinical biochemistry: the estimation of urea. Ann Clin Biochem. 1992;29(3):245–64.
13. Nathan DM, Singer DE, Hurxthal K, Goodson JD. The clinical information value of the glycosylated hemoglobin assay. N Engl J Med. 1984;310(6):341–6.
14. Vergnaud P, Garnero P, Meunier PJ, Breart G, Kamihagi K, Delmas PD. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. J Clin Endocrinol Metab. 1997;82(3):719–24.
15. Heijboer AC, Levitus M, Vervloet MG, Lips P, Wee PM ter, Dijstelbloem HM, et al. Determination of fibroblast growth factor 23. Ann Clin Biochem. 2009;46(4):338–40.
16. Sanchez-Carbayo M, Mauri M, ALfayate R, Miralles C, Soria F. Analythical evaluation of TSH & thyroid hormones by electrochemiluminescent immunoassay. Clin Biochem. 1994;32:395–403.
17. Ohe MN, Santos RO, Kunii IS, Abrahão M, Cervantes O, Carvalho AB, et al. Usefulness of intraoperative PTH measurement in primary and secondary hyperparathyroidism: experience with 109 patients. Arq Bras Endocrinol Metabol. 2006;50(5):869–75.
18. Sapin R, Le Galudec V, Gasser F, Pinget M, Grucker D. Elecsys insulin assay: free insulin determination and the absence of cross-reactivity with insulin lispro. Clin Chem. 2001;47(3):602–5.
19. Fossati P, Prencipe L, Berti G. Enzymic creatinine assay: a new colorimetric method based on hydrogen peroxide measurement. Clin Chem. 1983;29(8):1494–6.
20. Sung HJ, Kim J-H, Park R, Lee KR, Kwon OH. Evaluation of Denka-Seiken turbidimetric high-sensitivity C-reactive protein assay. Clin Chem Lab Med. 2002;40(8):840–5.
21. Cockcroft DW, Gault H. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31–41.
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9.
23. Tanaka M, Fukui M, Tomiyasu K, Akabame S, Nakano K, Hasegawa G, et al. U-shaped relationship between insulin level and coronary artery calcification (CAC). J Atheroscler Thromb. 2010;17(10):1033–40.
24. Schaerström R, Hamfelt A, Söderhjelm L. Parathyroid hormone and calcitonin in diabetes mellitus. Ups J Med Sci. 1986;91(1):99–104.
25. Rubin MR, Rundek T, McMahon DJ, Lee H-S, Sacco RL, Silverberg SJ. Carotid artery plaque thickness is associated with increased serum calcium levels: the Northern Manhattan study. Atherosclerosis. 2007;194(2):426–32.
26. El-Abbadi MM, Pai AS, Leaf EM, Yang H-Y, Bartley BA, Quan KK, et al. Phosphate feeding induces arterial medial calcification in uremic mice: role of serum phosphorus, fibroblast growth factor-23, and osteopontin. Kidney Int. 2009;75(12):1297–307.
27. Foley RN, Collins AJ, Herzog CA, Ishani A, Kalra PA. Serum phosphorus levels associate with coronary atherosclerosis in young adults. J Am Soc Nephrol. 2009;20(2):397–404.
28. Lomashvili KA, Garg P, Narisawa S, Millan JL, O'neill WC. Upregulation of alkaline phosphatase and pyrophosphate hydrolysis: potential mechanism for uremic vascular calcification. Kidney Int. 2008;73(9):1024–30.
29. Block GA, Hulbert-Shearon TE, Levin NW, Port FK. Association of serum phosphorus and calcium x phosphate product with mortality risk in chronic hemodialysis patients: a national study. Am J Kidney Dis. 1998;31(4):607–17.
30. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature. 1997;390(6655):45.
31. Kindblom JM, Ohlsson C, Ljunggren Ö, Karlsson MK, Tivesten Å, Smith U, et al. Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish men. J Bone Miner Res. 2009;24(5):785–91.
32. Pittas AG, Harris SS, Eliades M, Stark P, Dawson-Hughes B. Association between serum osteocalcin and markers of metabolic phenotype. J Clin Endocrinol Metab. 2009;94(3):827–32.
33. Ogawa-Furuya N, Yamaguchi T, Yamamoto M, Kanazawa I, Sugimoto T. Serum osteocalcin levels are inversely associated with abdominal aortic calcification in men with type 2 diabetes mellitus. Osteoporos Int. 2013;24(8):2223–30.
34. Zhang M, Ni Z, Zhou W, Qian J. Undercarboxylated osteocalcin as a biomarker of subclinical atherosclerosis in non-dialysis patients with chronic kidney disease. J Biomed Sci. 2015;22(1):75.
35. Zanatta LCB, Boguszewski CL, Borba VZC, Kulak CAM. Osteocalcin, energy and glucose metabolism. Arq Bras Endocrinol Metabol. 2014;58(5):444–51.
36. Ferron M, McKee MD, Levine RL, Ducy P, Karsenty G. Intermittent injections of osteocalcin improve glucose metabolism and prevent type 2 diabetes in mice. Bone. 2012;50(2):568–75.
37. Lihn AS, Pedersen SB, Richelsen B. Adiponectin: action, regulation and association to insulin sensitivity. Obes Rev. 2005;6(1):13–21.
38. Dawod OY, Elhussein AB, Ahmed MAE-R, Elbadawi NEE, Musa OAA. Correlation of Osteocalcin Level with Blood Glucose Concentration and Insulin Level in Type II Diabetic Sudanese Patients. Heal Sci. 2017;6(10):87–92.
39. Magni P, Macchi C, Sirtori CR, Romanelli MMC. Osteocalcin as a potential risk biomarker for cardiovascular and metabolic diseases. Clin Chem Lab Med. 2016;54(10):1579–87.
40. Iki M, Tamaki J, Fujita Y, Kouda K, Yura A, Kadowaki E, et al. Serum undercarboxylated osteocalcin levels are inversely associated with glycemic status and insulin resistance in an elderly Japanese male population: Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) Study. Osteoporos Int. 2012;23(2):761–70.
41. Garland JS, Holden RM, Ross R, Adams MA, Nolan RL, Hopman WM, et al. Insulin resistance is associated with Fibroblast Growth Factor-23 in stage 3–5 chronic kidney disease patients. J Diabetes Complications. 2014;28(1):61–5.
42. Winther K, Nybo M, Vind B, Pedersen SM, Højlund K, Rasmussen LM. Acute hyperinsulinemia is followed by increased serum concentrations of fibroblast growth factor 23 in type 2 diabetes patients. Scand J Clin Lab Invest. 2012;72(2):108–13.
43. Parker BD, Schurgers LJ, Brandenburg VM, Christenson RH, Vermeer C, Ketteler M, et al. The associations of fibroblast growth factor 23 and uncarboxylated matrix Gla protein with mortality in coronary artery disease: the Heart and Soul Study. Ann Intern Med. 2010;152(10):640–8.
44. Dhingra R, Sullivan LM, Fox CS, Wang TJ, D'Agostino RB, Gaziano JM, et al. Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. Arch Intern Med. 2007;167(9):879–85.
45. Wesseling-Perry K, Pereira RC, Wang H, Elashoff RM, Sahney S, Gales B, et al. Relationship between plasma fibroblast growth factor-23 concentration and bone mineralization in children with renal failure on peritoneal dialysis. J Clin Endocrinol Metab. 2009;94(2):511–7.
46. Prasad K. C-reactive protein (CRP)-lowering agents. Cardiovasc Drug Rev. 2006;24(1):33–50.
47. Palmas W, Ma S, Psaty B, Goff DC, Darwin C, Barr RG. Antihypertensive medications and C-reactive protein in the multi-ethnic study of atherosclerosis. Am J Hypertens. 2007;20(3):233–41.
48. Sathyapalan T, Atkin SL, Kilpatrick ES. Disparate effects of atorvastatin compared with simvastatin on C-reactive protein concentrations in patients with type 2 diabetes. Diabetes Care. 2010;33(9):1948–50.
49. Chagnac A, Herman M, Zingerman B, Erman A, Rozen-Zvi B, Hirsh J, et al. Obesity-induced glomerular hyperfiltration: its involvement in the pathogenesis of tubular sodium reabsorption. Nephrol Dial Transplant. 2008;23(12):3946–52.
50. Seiler S, Reichart B, Roth D, Seibert E, Fliser D, Heine GH. FGF-23 and future cardiovascular events in patients with chronic kidney disease before initiation of dialysis treatment. Nephrol Dial Transplant. 2010;25(12):3983–9.
51. Liu S, Tang W, Zhou J, Stubbs JR, Luo Q, Pi M, et al. Fibroblast growth factor 23 is a counter-regulatory phosphaturic hormone for vitamin D. J Am Soc Nephrol. 2006;17(5):1305–15.
52. Li YC, Kong J, Wei M, Chen Z-F, Liu SQ, Cao L-P. 1, 25-Dihydroxyvitamin D 3 is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest. 2002;110(2):229–38.
53. Silswal N, Touchberry CD, Daniel DR, McCarthy DL, Zhang S, Andresen J, et al. FGF23 directly impairs endothelium-dependent vasorelaxation by increasing superoxide levels and reducing nitric oxide bioavailability. Am J Physiol Metab. 2014;307(5):E426–36.