ORIGINAL ARTICLE

Osteocalcin serum levels in obese patients with type 2 diabetes mellitus: The virtual points observed in a case control study

Hala Akeel Shams, Hayder Mutter Al-Kuraishy, Ali Ismail Al-Gareeb

Abstract

Objectives: To elucidate the effect of metformin alone or in combination with sitagliptin on osteocalcin serum levels in obese patients with type 2 diabetes mellitus (T2DM).

Methods: This case-control study was conducted at the Department of Clinical Pharmacology and Therapeutic, College of Medicine, Mustansiriyah University, Baghdad, Iraq, from February to April, 2019. This study comprised 62 obese T2DM patients compared with 28 healthy controls, they were divided into three groups; Group I: Obese patients with T2DM on metformin (n=36), Group II: Obese patients with T2DM on metformin plus sitagliptin (n=26), and Group III: healthy controls subjects (n=28). Body mass index (BMI), blood pressure profile, lipid profile, Glycaemic indices, and serum levels of human osteocalcin were measured. The data analysis was done by using SPSS 20.

Results: Osteocalcin serum level was lower in patients with T2DM compared with the control (P=0.001), also it was relatively low in metformin (MT) group (21.04±3.16 ng/mL) compared to sitagliptin (ST) group (25.65±7.30 ng/mL), (P=0.04). In relation to the HbA1c, osteocalcin serum level was reduced in patients with T2DM with high HbA1c (HbA1c-H) compared to the patients with T2DM with low HbA1c (HbA1c-L), (P=0.03) and was not significantly different when compared with moderate HbA1c (HbA1c-M), (P>0.05). However, osteocalcin was negatively correlated with HOMA-IR(r=-0.78, P=0.0001).

Conclusion: Osteocalcin serum level was reduced in T2DM patients and negatively correlated with HOMA-IR and HbA1c and FBG. Combination of metformin with sitagliptin was more effective than metformin monotherapy in amelioration of osteocalcin serum level in patients with T2DM.

Keywords: Osteocalcin, Type 2 diabetes mellitus, Obesity, Metformin, Sitagliptin. (JPMA 71: S-4 [Suppl. 8]; 2021)

Introduction

Type 2 diabetes mellitus (T2DM) is a protracted metabolic disease characterized by advanced deterioration of lipid and carbohydrate metabolisms. T2DM is characterised by chronic hyperglycaemia as a result of increased hepatic gluconeogenesis, peripheral insulin insensitivity, and/or relative insulin insufficiency.[1] T2DM represent 90-95% of overall cases of DM, and linked with high mortality.[2]

Overweight and obesity can lead to substantial adipose tissue accumulation to the degree that may cause physical and psychosocial adverse effects. The genetic susceptibility of obesity are correlates with environmental influences resulting in a heterogeneous phenotype of obesity.[3] Nearly two thirds of the world´s adult population complain of overweight, and obesity which increases morbidity and mortality. In obesity, visceral adiposity leads to metabolic disorders including; insulin resistance (IR), hyperglycaemia, hypertension, dyslipidaemia, and development of T2DM.[4]

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Obesity is associated with chronic inflammatory disorders, due to irregular secretion of adipokines and stimulation of pro-inflammatory signaling pathways, leading to the activation of inflammatory reactions. These adipokines inhibit insulin receptors resulting in decrease of glucose transport with subsequent hyperglycaemia.[5] In T2DM, white adipose tissue is the major source of inflammatory adipocytokines including interleukins (ILs) such as, IL6, IL1, IL10, and other adipocytokines as leptin, adiponectin, and osteocalcin.[6] Bone has appeared recently as a newly important organ involved in the modulation of glucose metabolism through secreting cytokines and hormones like osteocalcin which plays an essential role in glucose metabolism.[7]

Osteocalcin is a specific protein produced by osteoblast and secreted into the extracellular matrix of bone. Osteocalcin also modulates β-cells proliferation and insulin synthesis.[8] It has been reported that, osteocalcin enhances the differentiation of β-cells and insulin release; as it stimulates the synthesis of adiponectin in adipocyte which contribute to higher insulin sensitivity. These effects together demonstrate a positive feedback loop involving bone, β-cells and fat tissue, thus, osteocalcin improves peripheral insulin sensitivity and insulin release.[9]

Pharmacotherapies for diabetes as metformin and sitagliptin may affect osteocalcin synthesis and release. Metformin, which is the first line drug in the management of T2DM, acts through inhibition of hepatic gluconeogenesis and enhancement of insulin sensitivity.[10] Sitagliptin, a dipeptidyl peptidase-4 (DDP-4) inhibitor, enhances physiological levels of gastric inhibitory polypeptide (GIP-1) and glucagon like peptide (GLP), with anti-inflammatory, anti-oxidant and cardio-protective effects.[11] Since, both metformin and sitagliptin modulate adipose-tissue derived adipocytokines, therefore, objective of the present study was to elucidate the differential effect of metformin and/or sitagliptin on the osteocalcin serum in obese patients with T2DM.

Methods

This case-control study was conducted at the Department of Clinical Pharmacology in collaboration with

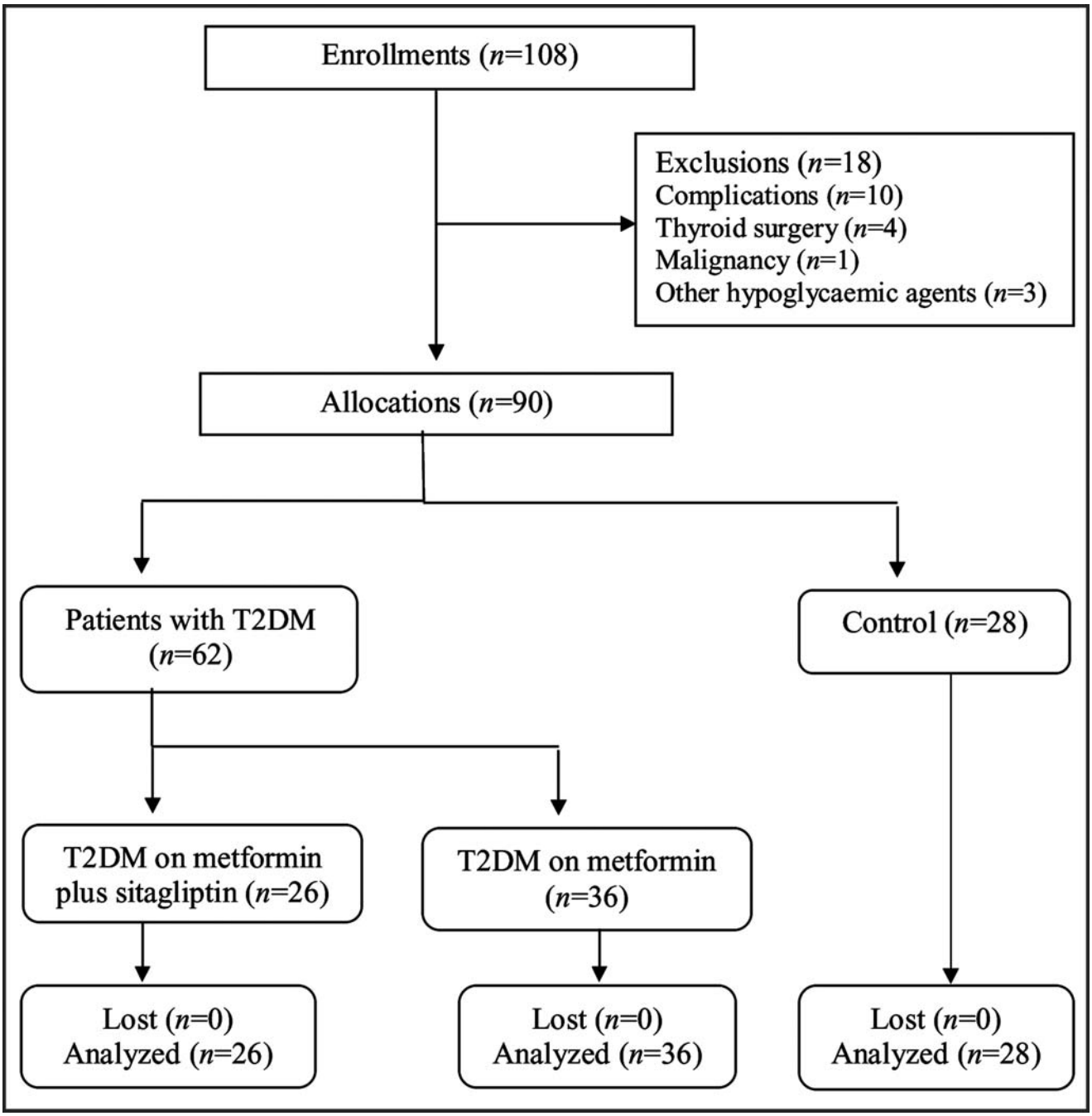


Figure-1: Consort flow-diagram of the present study.

Endocrinology center, Al-Yarmouk Teaching Hospital, College of Medicine, Al-Mustansiriyah University, Baghdad Iraq, from September 2019 till February 2020. It was approved by the Scientific Committee of Department of Pharmacology and certified by the committee of Medical College, according to the Declaration of Helsinki. The sample size was calculated according to the population size regarding 95% confidence interval and 5% marginal error. A total of 62 obese patients with T2DM with mean age of 48.62±8.87 years were compared with 28 healthy controls.

The recruited patients and controls were divided into three groups;

Group I: Obese patients with T2DM on metformin 1g / day, (n=36(, Group II: Obese patients with T2DM on metformin 1g / day plus sitagliptin 100 mg / day (n=26), and Group III: Healthy controls (n=28).

Any patient with chronic liver, renal, thyroid, pulmonary or heart, diseases or patients with T1DM, pregnancy or lactation, T2DM with complications, psychiatric and mental disorders were excluded from the study.

A detail medical history was obtained from all patients and routine investigations performed. Of the 108 patients initially recruited, 18 were excluded due to various complications and finally 90 were inducted in the study. Of these 62 had T2DM and 28 were healthy controls. Of the 62 subjects with T2DM, 26 were on metformin therapy and 36 were taking metformin plus sitagliptin, (Figure-1).

Anthropometric profiles: Weight and height of every patient was measured by means of the weight and height measuring scales. Body Mass Index (BMI); BMI= BW (kg) /Ht (m^2). Also, waist circumference (WC), hip circumference (HC) were measured in order to calculate waist to hip ratio by the following equation; WHR= waist (cm)/hip (cm). Blood pressure profile, including; systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured by automated digital sphygmomanometer, then, pulse pressure (PP), and mean arterial pressure (MAP) were calculated by the following equations; PP=SBP-DBP, MAP=SBP+2 (DBP)[3]. The normal value of PP is 30-40 mmHg; while that of MAP is 65-110 mmHg.[12]

Biochemical variables: Five milliliters of blood samples were withdrawn by venipuncture procedure after an overnight fasting. The blood samples were centrifuged 3000/ rpm and stored at (-20 C°) to be utilize later. The biochemical parameters that were used in the present study were lipid profile (triglycerides (TGs), total cholesterol (TC), and high density lipoprotein (HDL), by using instant cholesterol kit (Abbott, A.S.A) in ARCHITECT C 4000. However, low density lipoprotein (LDL), very low density lipoprotein (VLDL) were calculated by using the following equations; LDL=TC-HDL-(TG/5) and VLDL= TGs/5 respectively.[13]

Glycaemic indices measured included; fasting blood glucose (FBG) level, measured by using an Instant kit (Abbott, Glucose, Germany), on the ARCHITECT C 4000 and HbA1c was estimated by utilizing an ELISA kit (Kono biotech, China). Serum insulin level was measured by using an ELISA kit (My Bio source, U.S.A.). Insulin resistance (IR) was calculated by Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) using the specific equation; HOMA-IR= FBI (μU/ml) × FBG (mg/dl)/405.[14] Osteocalcin serum level was measured by an ELISA kit (My Bio source, U.S.A.). Moreover, Atherogenic Index (AI) =log (TG/HDL), cardiovascular risk index (CVRI) = TG/HDL and cardiac risk ratio (CRR) =TC/HDL were calculated.[15]

Data was analyzed by using SPSS 20, and presented as mean ± standard deviation (SD). The variables were tested by using unpaired student t-test between the controls and the treated groups. One way analysis of variance (ANOVA) with post-hoc test was used to scrutinize the significance of differences among the groups, level of significance was considered when $P<0.05$.

Results

The demographic characteristics showed that 62 (68.88%)

Table-1: Characteristics of the present study.

Variables	n, mean ±SD, %
n	90
T2DM	62(68.88)
T2DM on metformin	36(58.06)
T2DM on metformin plus sitagliptin	26(41.93)
Control	28(31.11)
Age (years)	48.62±8.87
Gender (Male: Female ratio)	49(54.44):41(48.23)
Smoking	26(41.93)
Duration of T2DM (months)	4.3±1.94
Associated diseases	
Dyslipidaemia	17(27.41)
Hypertension	10(16.12)
IHD	3(4.83)
Medications	
Amlodipine	8(12.90)
ACEIs	3(4.83)
ARBs	4(6.45)
Aspirin	5(8.06)
Family history	
Positive	27(43.54)
Negative	29(46.77)

Data are expressed as N, mean ±SD, %, M: F: male: female, IHD: ischaemic heart disease, ACEIs: angiotensin converting enzyme inhibitors, ARBs: angiotensin receptor blockers.

of T2DM patients compared to 28(31.11%) of healthy controls were evaluated. The average age was 48.62±8.87 years with male-female ratio of 49:41. Also; 26(41.93%) of patients were active cigarette smokers, and had other associated diseases including; dyslipidaemia 17 (27.41%), hypertension 10(16.12%), and ischaemic heart disease 3(4.83%). The associated medications in addition to metformin and sitagliptin were angiotensin converting enzyme inhibitors 3(4.83%), angiotensin receptor blockers 4(6.45%), amlodipine 8(12.90%), and aspirin 5(8.06%). Besides, the family history for T2DM was positive in 27(43.54%) and negative in 29(46.77%), (Table-1).

Metabolic and anthropometric profiles in patients with T2DM: BMI was higher in patients with T2DM on metformin (MT) or sitagliptin plus metformin (ST) compared to the controls (P<0.01). Waist-hip ratio (WHR) was higher in patients with T2DM on MT or ST compared to the controls (P<0.05), likewise it was higher in patients with T2DM on MT (0.93±0.03) as compared with ST group (0.90±0.03), (P<0.05). Blood pressure profiles (SBP, DBP, PP, MAP) were higher in patients with T2DM on MT or ST compared to the controls (P<0.05), only SBP and DBP were higher patients with T2DM on MT as compared with ST group (P=0.03). Regarding lipid profile, it was higher in patients with T2DM on MT or ST compared to the controls (P=0.0001). TC, TG, non-HDL-c, LDL, VLDL, but not HDL-c were high in patients with T2DM on MT as compared with ST group, (P=0.03). AIP was higher in patients with T2DM on MT or ST compared to the controls (P<0.01), it was higher in patients with T2DM on MT (0.391±0.05) as compared with ST group (0.238±0.03), (P=0.02). As well, CRR and CVRI were higher in patients with T2DM on MT or ST compared to the controls (P=0.03) and (P=0.04) respectively, which were also low in patients with T2DM

Table-2: Metabolic profile in patients with T2DM on metformin or sitagliptin compared with control.

Variables	Control (n=28)	T2DM on MT (n=36)	T2DM on MT+ST (n=26)	P
BMI(kg/m^2)	24.34±3.72	31.22±5.40#	32.43±5.59¶	0.01
WHR(cm) SBP(mmHg)	0.88±0.05	0.93±0.03 #	0.90±0.03*	0.001
DBP(mmHg)	111.64±12.85	145.61±15.80#	128.42±13.91*¶	0.0001
PP(mmHg)	75.93±9.75	97.42±11.73#	81.72±10.81*	0.0001
MAP(mmHg)	35.71±6.94	48.19±7.56	46.70±8.52¶	0.0001
TC mg/dL)	87.83±8.63	113.48±12.96#	97.29±11.86*¶	0.0001
TG(mg/dL)	143.67±23.27	176.43±54.88#	150.62±44.14*	0.0001
HDL-c(mg/dL)	132.25±16.54	184.00±13.85#	139.47±16.73*	0.0001
non-HDL-c	39.29±3.43	32.62±4.81#	35.17±4.22¶	0.001
LDL(mg/dL)	104.38±9.07	143.81±13.81#	115.45±11.73*¶	0.0001
VLDL(mg/dL)	77.90±8.52	107.00±13.85#	87.60±11.71*¶	0.0001
AIP	26.45±12.61	36.85±9.44#	27.89±9.73*	0.0001
CRR	0.16±0.01	0.391±0.05#	0.238±0.03*¶	0.0001
CVRI	3.65±1.89	5.40±1.88#	4.28±1.07*	0.03
	3.36±1.08	5.64±2.31#	3.96±1.67*	0.04

Data are presented as mean ±SD, ANOVA test and Tukey HSD Post-hoc Test, BMI: body mass index; WHR: waist hip ratio, BFM: body fat mass, BF% body fat percentage, BMR: basal metabolic rate, SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; MAP: mean arterial pressure; TC: total cholesterol; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein; AI: atherogenic index, CRR: Cardiac Risk Ratio, CVRI: cardiovascular risk index; T2DM: type 2 diabetes mellitus; MT: metformin; MT+ST: metformin plus sitagliptin.
* P <0.05 compared to metformin group.
¶# P<0.05 compared to the control.

Table-3: Glycaemic indices in patients with T2DM on metformin or sitagliptin compared with control.

Variables	Control (n=28)	T2DM on MT (n=36)	T2DM on MT+ST (n=26)	P	N.V
FBG(mg/dL)	88.96±15.98	153.92±39.50#	163.77±52.42¶	0.001	70-100
HbA1c	5.56±0.23	7.59±0.71#	7.56±0.77¶	0.001	5.7-6.4
FSI(μU/mL)	2.97±1.05	14.59±3.97#	10.55±3.41¶*	0.001	<25
HOMA-IR	0.65±0.01	5.54±1.06#	4.26±1.04¶*	0.001	1.0-1.9
IS%	263.65±11.93	47.4±9.42#	64.2±8.53¶*	0.001	200-300
HOMA-β %	41.18±5.87	57.76±7.58#	37.68±11.83*	0.001	35-45

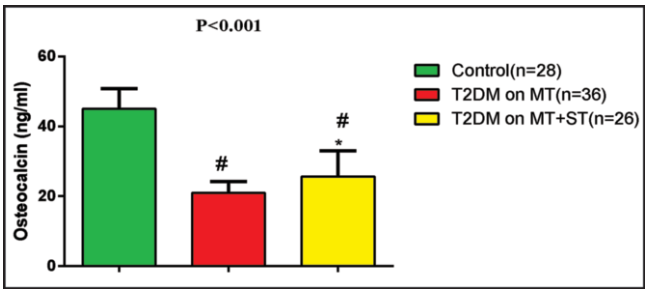
Data are presented as mean ±SD, ANOVA test and Tukey HSD Post-hoc Test, FBG: fasting blood glucose; FSI: fasting serum insulin; IS: insulin sensitivity; HOMA-IR: homeostatic model assessment-insulin resistance; HOMA-β: homeostatic model assessment- pancreatic β-cell functions; T2DM: type 2 diabetes mellitus; MT: metformin; MT+ST: metformin plus sitagliptin, N.V: normal value.
* P <0.05 compared to metformin group.
¶# P<0.05 compared to the control.

on ST as compared with MT group (P=0.04), (Table-2).

Glycemic indices in patients with T2DM: FBG and HbA1c were higher in patients with T2DM compared with controls (P=0.001), however they did not significantly differ in patients with T2DM on MT or ST therapy (P>0.05). Fasting serum insulin (FSI) was higher in patients with T2DM on MT or ST compared with the healthy controls (P=0.001), nonetheless FSI but did not significantly differ from the ST group (P=0.02). HOMA-IR was higher in patients with T2DM on MT or ST compared with the healthy controls (P=0.001), it was lower in ST group compared with MT group (P=0.02). In addition, both HOMA-β and insulin sensitivity were better in the ST group compared with MT, was not significantly differed compared to the controls group (P=0.06), (Table-3).

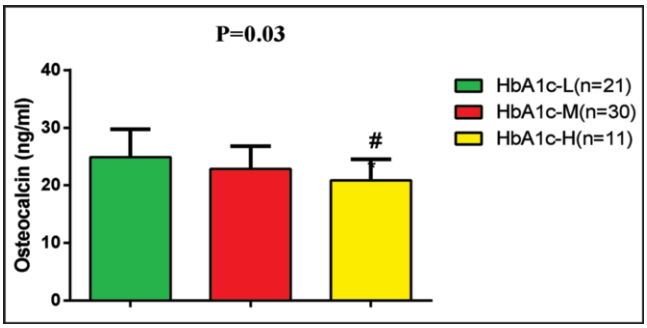
Osteocalcin serum level in patients with T2DM: Osteocalcin serum level was lower in T2DM patients compared with the controls (P=0.001), however it was relatively low in MT group (21.04±3.16) compared to ST group (25.65±7.30), (P=0.04), (Figure-2).

Osteocalcin serum level in relation to HbA1c in patients with T2DM: Osteocalcin serum level was low in T2DM patients with high HbA1c (HbA1c-H) compared to



*P<0.05 compared with metformin group
#P<0.01 compared with the controls
MT: Metformin; MT+ST: Metformin plus sitagliptin

Figure-2: Osteocalcin serum levels in patients with T2DM.



#P<0.05 compared to HbA1c-L and HbA1c-M.

Figure-3: Osteocalcin serum levels in relation to HbA1c in patients with T2DM.

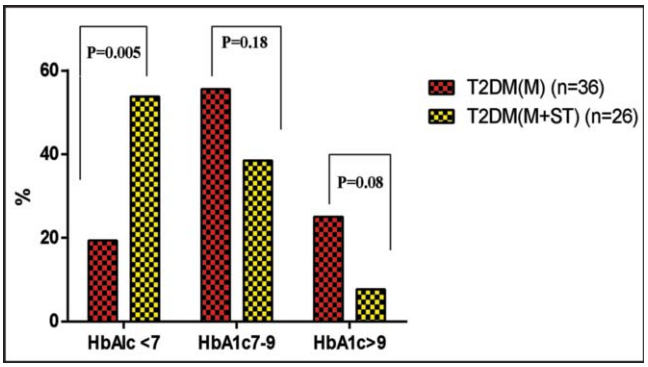


Figure-4: HbA1c levels in relation to diabetic pharmacotherapy.

T2DM patients with low HbA1c (HbA1c-L), (P=0.03), which was not significantly different when compared with moderate HbA1c (HbA1c-M), (P>0.05), (Figure-3). Regarding HbA1c in relation to MT or MT plus ST pharmacotherapy in patients with T2DM, HbA1c <7 was showed in 19.44% in MT group compared to 53.84% in MT plus ST group (P=0.005). HbA1c level 7-9 was illustrated in 55.56% in MT group compared to 38.46% in MT plus ST group (P=0.18). HbA1c >9 was observed in 25.00% in MT group compared to 7.69% in MT plus ST group (P=0.08), (Figure-4).

Discussion

T2DM is a progressive metabolic disorder associated with macro and micro-vascular complications as a consequence of pancreatic β-cells dysfunction and IR. HbA1c which reflects the glycaemic status within the last 3months, was higher in patients with T2DM as compared to controls. In the T2DM subjects, those on the combination therapy had a lower HbA1c (L- HbA1c) as compared to patients on metformin monotherapy (P=0.005). However, moderate HbA1c (M- HbA1c) and high HbA1c (H- HbA1c) were not significantly different within these two groups (P>0.05). This is comparable with the results of a recent study.[16]

Osteocalcin is a non-collagenous hormone protein produced by osteoblasts. There are two forms of osteocalcin; carboxylated which binds Ca, and uncarboxylated form, which is considered a circulating hormone. This enhances adiponectin and insulin secretions as well as improves β-cell function and insulin responsiveness.[17] In the present study, osteocalcin serum level was lower in T2DM patients compared to healthy controls. It has been reported that osteocalcin is negatively correlated with development of T2DM, FBG, IR and the elevated inflammatory biomarkers such as CRP and IL-6 that adversely affect bone metabolism and insulin release.[18]

The present study revealed that there was a significantly beneficial effect of metformin plus sitagliptin on the level of serum osteocalcin compared to metformin monotherapy. Park et al., identified favourable influence of metformin on the improvement of osteoblast proliferation and glycaemic control, since metformin enhances glycaemic status through induction of adenosine monophosphate protein kinase (AMPK), which is also involved in bone marrow proliferation and osteoblast activation.[19] Zhang et al., demonstrated that metformin preserves bone micro-architecture and bone marrow density through activation of AMPK.[20] However, the current study revealed higher osteocalcin levels in the combination group compared to metformin monotherapy group. This finding is well-matched with earlier studies that showed a potential role of GLP-1 in the osteogenesis and synthesis/release of osteocalcin, since; DPP-4 inhibitors impede osteoclast activation and prevent bone loss.[21] Moreover, Zhu et al., proved that sitagliptin preserves bone formation through improvement of osteocalcin.[22]

Therefore, combination of metformin with sitagliptin was more effective than metformin monotherapy in amelioration of osteocalcin serum level in patients with T2DM. Besides, the present results demonstrated a negative correlation between HOMA-IR and osteocalcin. Wu et al., reported an inverse correlation of HbA1c, and HOMA-IR with osteocalcin, since poor glycaemic status adversely affects osteocalcin levels in T2DM patients.[23] The negative correlation between osteocalcin, HbA1C, HOMA-IR and FBG, is due to the low levels of osteoblast derived protein in T2DM.[24,25]

The present study had several limitations including; a small sample size and not estimating pro-inflammatory cytokines like tumour necrosis factor alpha (TNF-α), which influences both insulin sensitivity and osteocalcin. However, the present study provided an evidence of the association between T2DM patients and osteocalcin. A large prospective clinical trial is warranted in this regard.

Conclusion

Osteocalcin serum level was reduced in T2DM patients and negatively correlated with HOMA-IR, HbA1c and FBG. Combination of metformin with sitagliptin was more effective than metformin monotherapy in improving osteocalcin serum level in T2DM patients.

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