IHJPAS. 36(1)2023

Ibn Al-Haitham Journal for Pure and Applied Sciences

Journal homepage: jih.uobaghdad.edu.iq

Evaluation of Osteocalcin and Some Biochemical Marker in Diabetes Mellitus Iraqi Women's patients with Osteoporosis

Abeer Abaas Jafar
Department of Chemstry , College of Education for Pure Sciences, Ibn Al – Haitham, University of Baghdad, Baghdad, Iraq.
abeermastar@gmail.com

Bushra Hameed Ali
Department of Chemstry , College of Education for Pure Sciences, Ibn Al – Haitham, University of Baghdad, Baghdad, Iraq.
drbushra750@yahoo.com

Article history: Received 18 Augest 2022, Accepted 27 Septeber 2022, Published in January 2023.

doi.org/10.30526/36.1.2984

Abstract

Diabetes mellitus is a set of metabolic diseases, the most prevalent of which is chronic hyperglycemia. The culprits include insulin synthesis, insulin action, or both. Osteoporosis is a progressive systemic skeletal disorder defined by decreased bone mass and micro architectural degeneration of bone tissue, resulting in increased bone fragility and fracture risk, according to the World Health Organization (WHO). The degree of Nervosa damage determines how much a diabetic patient's body has been compromised. The current study's goal is an estimation: Age, BMI, FBS, HbA1C, D3, ALP, Ca, P, and Osteocalcin in Iraqi T2DM Women's patients with and without Osteoporosis. Three vitamins are required for Osteocalcin biosynthesis: vitamin K for Gla formation, vitamin C for hydroxylation of Pro-9 to hydroxyproline, and vitamin D for Osteocalcin production stimulation. Vitamin D is known to function in calcium homeostasis and bone metabolism .

Osteocalcin is a hormone produced by osteoblasts and secreted into the extracellular matrix of bone and the bloodstream. It serves as a biological marker for bone formation. The work was classified into three groups. G1 (n= 40) is the control set that went to the Iraqi Ministry of Health's Endocrinology and Diabetes Center in Baghdad. G2 (n= 40) are patients with type 2 diabetes mellitus without Osteoporosis who visited the Endocrinology and Diabetes Center of the Iraqi Ministry of Health in Baghdad, and G3 (n= 40) are patients with type 2 diabetes Mellitus with Osteoporosis.

Keywords: Osteocalcin, Osteoporosis, T2DM.

1. Introduction

Diabetes mellitus is a group of metabolic illnesses, the most prevalent of which is chronic hyperglycemia. The culprits include insulin synthesis, insulin action, or both. Chronic hyperglycemia has been linked to ketoacidosis, nephropathy, high blood pressure, and foot diseases. A range of complementary and alternative treatments can be used to treat diabetes. [1,2] Diabetes mellitus, also known as DM, is one of humanity's oldest diseases. Diabetes mellitus (DM) is a metabolic disorder marked by a rise in blood glucose levels that necessitates close monitoring and efficient management. Insulin is a hormone produced by pancreatic beta cells that aid in the absorption of glucose into cells for energy and performs various other functions. However, a lack of insulin synthesis or sensitivity causes diabetes mellitus (DM). [3] Osteoporosis is a progressive systemic skeletal disorder defined by decreased bone mass and micro architectural degeneration of bone tissue, resulting in increased bone fragility and fracture risk. Fractures were less common in premenopausal women than in postmenopausal women. In addition, Osteoporosis is diagnosed in postmenopausal women when their hip or spine bone mineral density (BMD) are two and a half standard deviations or more below the young adult's mean (T-score 2.5). However, there is no consensus on diagnosing osteoporosis in premenopausal women. [4] Osteoporosis is a condition in which bone tissue's mass and mineral density are reduced, leaving bones more brittle and prone to shattering at minor force. Fragility fractures occur when a person slips or falls, usually standing or sitting. The hip, spine, and wrist are the most common fragility fractures. [5] Osteocalcin biosynthesis requires three vitamins: vitamin K for Gla creation, vitamin C for hydroxylation of Pro-9 to hydroxyproline, and vitamin D for osteocalcin production stimulation. Vitamin D is known to function in calcium homeostasis and bone metabolism. [6] Osteocalcin is a hormone produced by osteoblasts and secreted into the extracellular matrix of bone and the bloodstream. In osteocalcin, one or more glutamic acid residues (Glu) can be carboxylated (Gla). Osteocalcin is found in both carboxylated and uncarboxylated forms in serum. Negatively charged carboxyglutamic acid groups bind positively charged calcium on the surface of bone minerals. According to studies, undercarboxylated osteocalcin (ucOC) increases cell proliferation and insulin release in islet cells. The most frequent noncollagenous proteins in bone are noncollagenous proteins. [7] Because this little peptide, which comprises 49 amino acids in humans and 46 in mice, is generated mainly by osteoblasts during bone production, its serum concentration is low. It serves as a biological marker for bone formation [8].

2. Patients and Methods

The present research was done at the Iraqi Ministry of Health's endocrinology and diabetes facility in Baghdad. The participants in this study were divided into three groups the first group (G1) was composed of 40 healthy individuals, each with an age range of 40 to 55 years. The second group comprises 40 people who have type 2 diabetes but do not have Osteoporosis. The third group consists of (40) patients with Osteoporosis and T2DM who visited the Iraqi Ministry of Health's endocrinology and diabetes center in Baghdad Blood was obtained in a volume of 5 mL from those with type 2 diabetes who do not have Osteoporosis. For Type 2 Diabetes Mellitus patients with Osteoporosis, then split into two portions. For additional measurements, 2 ml of the blood was inserted in a tube with an anticoagulant (EDTA). The leftover blood (3 ml) was put in a tube without an anticoagulant (EDTA) and allowed to coagulate for 30 minutes at room temperature. The serum was then retrieved by centrifuging it for 10 minutes at 3500 g to extract it for measuring

biomarkers. ALP, Ca, P, and FBS are measured by spectrophotometer, Mini Vidas measures D3, and Hblc measures HbA1C (RayBiotech.

Inc. is a manufacturer of the osteocalcin ELISA Kit.) in the USA. T-test, mean, and standard division were used to calculate the data. The patients and the control group were compared using the T-test and the patients and the control set. Both the patients and the control group are compared using $P<0$.

Table 1. Demonstrated BMI, FBS, HbA1C, D3, ALP, Ca, PO4 and Osteocalcin as newly biomarker in these samples of work

parameter	**G1 NO=40**	**G2 NO=40**	**G3 NO=40**	**G1 Vs G2**	**G1 Vs G3**	**G2 Vs G3**
Age	43.55±2.44	43.91±2.42	43.92±2.25	NS	NS	NS
BMI	25.74 ±3.35	30.26±2.89	28.50±3.39	HS	HS	S
FBS	82.65± 5.77	204.75 ±56.72	159.3± 28.86	HS	HS	HS
HbA1C	4.74±0.43	8.64±1.42	8.64±1.39	HS	HS	NS
Osteocalcin	9.40±0.89	27.60±5.85	47.04±5.50	HS	HS	HS
D3	33.47±3.80	26.55±5.71	8.48±0.48	HS	HS	HS
ALP	72.22±7.52	77.55±8.82	92.02±3.88	S	HS	HS
Ca	8.97±0.52	6.13±20.02	4.89±0.57	HS	HS	HS
PO_4	3.68±0.67	2.91±0.77	1.81±0.32	HS	HS	HS

Table 2. The association for the result study between Osteocalcin to (BMI, FBS, HbA1C, D3, ALP, Ca and PO_4)

parameter	**T2DM**		**T2DM with Osteoporosis**	
	r	**P value**	**r**	**P value**
BMI	0.307	HS	0.602	HS
FBS	-0.022	HS	-0.002	HS
HbA1C	-0.038	HS	0.306	HS
D3	0.140	HS	0.123	HS
ALP	0.517	HS	0.196	HS
Ca	-0.361	HS	0.242	HS
PO_4	0.102	HS	0.208	HS

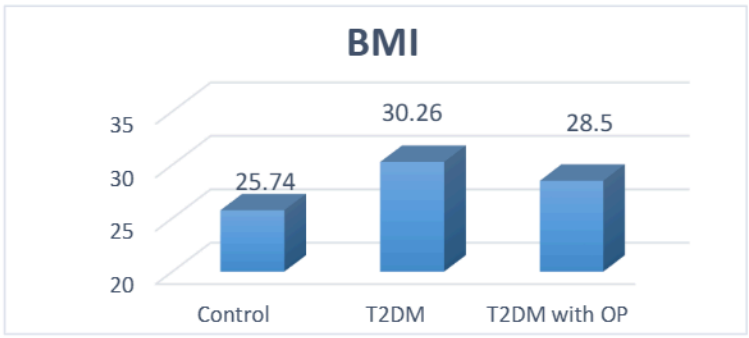

Figure 1. BMI levels for all studied groups

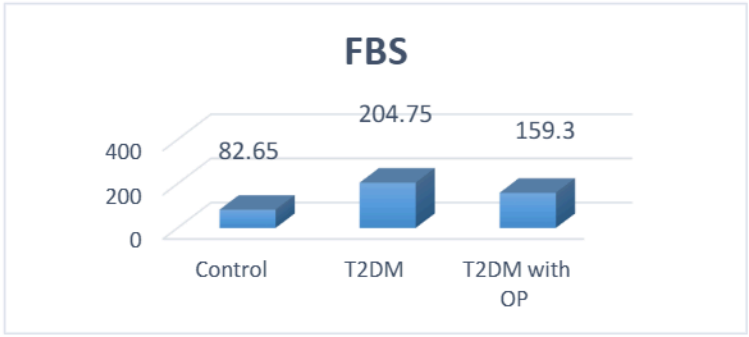

Figure 2. FBS levels for all studied groups

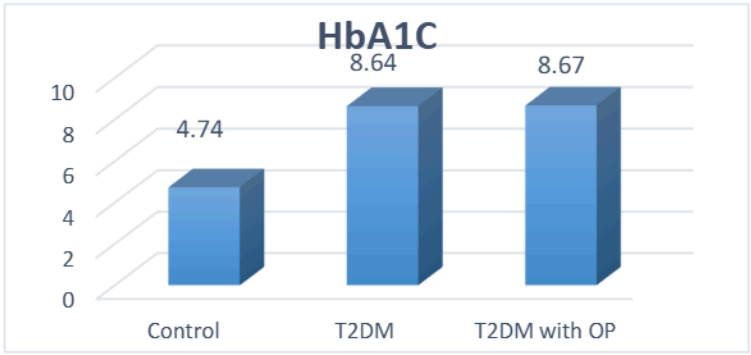

Figure 3. HbA1C levels for all studied groups

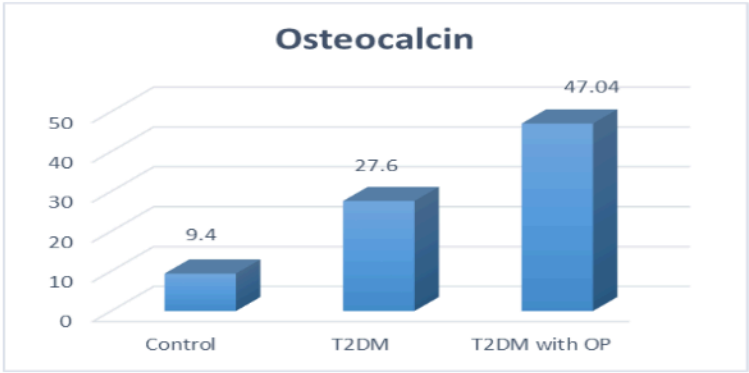

Figure 4. Osteocalcin levels for all studied groups

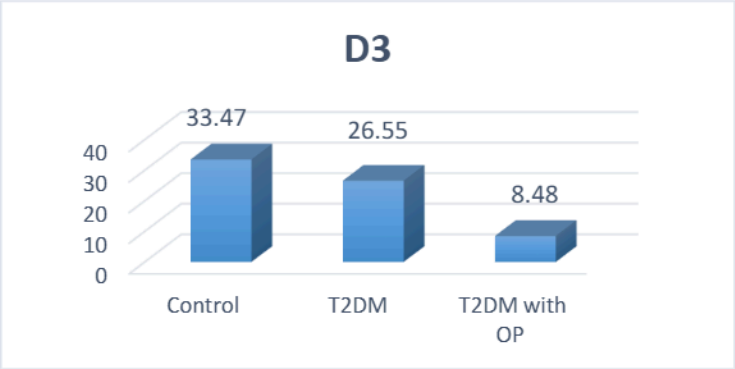

Figure 5. D3 levels for all studied groups

Figure 6. ALP levels for all studied groups

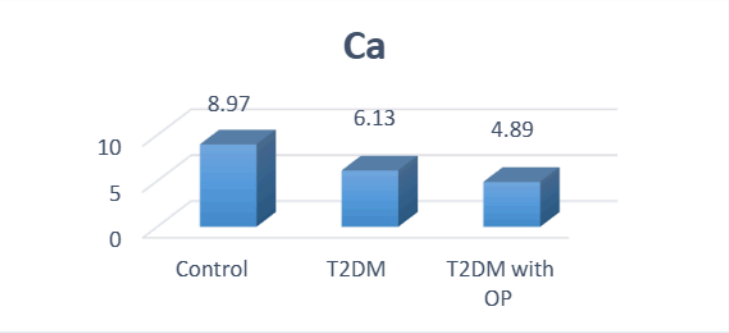

Figure 7. Ca levels for all studied groups

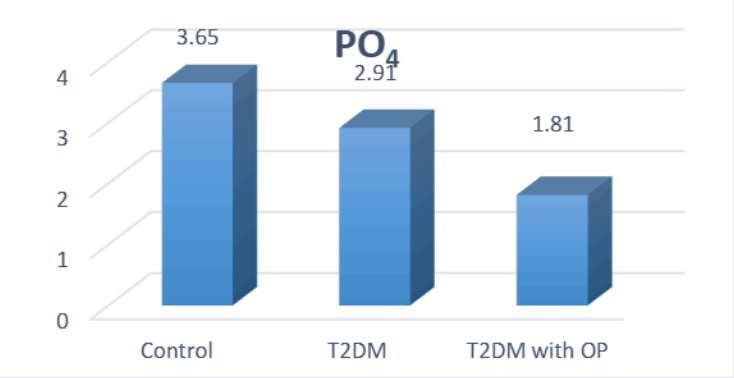

Figure 8. PO_4 levels for all studied groups

3. Results

One hundred twenty participants in the study were divided into three groups to describe the correlation between the groups: controls (G1), patients with type 2 diabetes mellitus (G2), and group G3 patients with osteoporosis and type 2 diabetes mellitus. The outcomes were presented as mean SD, student T. The extraction P-value and post-t-test were both utilized to show that the difference between the groups was significant when the P-value was ($P < 0.001$) in the test that compared the three study groups. **Table (1)** describes the correlation between the results of the comparison. BMI appeared in **Table (1)** and **Figure (1)**. We noticed a high signification decrease in G1 (25.74 ± 3.35) compared to G2 (30.26 ± 2.89) and G3 (28.50 ± 3.39), respectively. But, it showed a significant ($P<0.005$) increase when comparing G2 (30.26 ± 2.89) to G3 (28.50 ± 3.39). The comparison of FBS was shown in **Table (1)** and **Figure (2)**, noticing a high significant ($P<0.001$) decrease as comparing G1 (82.65 ± 5.77) to G2 (204.75 ± 56.72) and G3 (159.3 ± 28.86), respectively. There was also a highly significant ($P<0.001$) increase when comparing G2 (204.75 ± 56.72) to G3 (159.3 ± 28.86). Results of HbA1C% in **Table (1)** and **Figure (3)** showed a highly significant ($P<0.001$) decrease when comparing G1 (4.74 ± 0.43) to G2 (8.64 ± 1.42) and G3 (8.64 ± 1.39), respectively. But, there was a nonsignificant ($P>0.005$) increase, when comparing G2 (8.64 ± 1.42) to G3 (8.64 ± 1.39). The result of Osteocalcin in table (1) and figure (4) showed a highly significant ($P<0.001$) decrease when comparing G1 (9.40 ± 0.86) to G2 (27.60 ± 5.85) and G3 (47.04 ± 5.50), respectively. Also, there was a high decrease ($P<0.001$) when comparing G2 (27.60 ± 5.85) and G3 (47.04 ± 5.50). Results of D3 in table (1) and figure (5) showed a highly significant ($P<0.001$) increase when comparing G1 (33.47 ± 3.80) to G2 (26.55 ± 5.71) and G3 (8.48 ± 0.48), respectively. Still, we showed a high significant ($P<0.001$) increase when comparing G2 (33.47 ± 3.80) to G3 (8.48 ± 0.48).

Results of ALP in **Table (1)** and **Figure (6)** showed a significant ($P<0.005$) decrease when comparing G1 (72.22 ± 7.52) to G2 (77.55 ± 8.82) and G3 (92.02 ± 3.88), respectively. But we showed a highly significant ($P<0.001$) increase when comparing G2 (77.55 ± 8.82) and G3 (92.02 ± 3.88). Results of Ca **Table (1)** and **Figure (7)** showed a highly significant ($P <0.001$) increase when comparing G1 (8.97 ± 0.52) to G2 (6.13 ± 2.02) and G3 (4.89 ± 0.57), respectively. Still, there was a highly significant ($P<0.001$) increase when comparing G2 (6.13 ± 2.02) to G3 (4.89 ± 0.57). Results of PO4 in **Table (1)** and **Figure (8)** showed a highly significant ($P<0.001$) increase when comparing G1 (3.68 ± 0.67) to G2 (2.91 ± 0.77) and G3 (1.81 ± 0.32), respectively but there was a highly significant ($P<0.001$) increase when comparing G2 (2.91 ± 0.77) to G3 (1.81 ± 0.32).

Correlation of Osteocalcin Vs BMI

The correlations between osteocalcin levels and BMI for the current investigation can be found separately in **Table (2)** for G2 and G3. These correlations were highly significant when $P \leq 0.001$ for G2 ($r=0.307$) and G3 ($r=0.062$).

Correlation of Osteocalcin Vs. FBS

Table (2) shows the correlations for the current study between Osteocalcin and FBS levels for G2 and G3 separately. These correlations are very significant when $P \leq 0.001$ for G2 ($r=-0.022$) and G3 ($r=-0.002$).

Correlation of Osteocalcin Vs. HbA1C

Table (2) shows the correlations between Osteocalcin and HbA1C levels for the current investigation, with results for G2 and G3 showing very significant negative and positive correlations when $P \leq 0.001$ for G2 (r= -0.038) and G3 (r=0.306), respectively.

Correlation of Osteocalcin Vs - D3

The correlations between osteocalcin and levels D3 for the current investigation are presented separately in table (2) for G2 and G3. These correlations were highly significant and positive when $P \leq$ 0.001 for G2 (r=0.140) and G3 (r=0.123).

Correlation of Osteocalcin Vs - ALP

The correlations between Osteocalcin and ALP levels for the current investigation can be found separately in **Table (2)** for G2 and G3. These correlations were highly significant and positive when $P \leq 0.001$ for G2 (r=0.517) and G3 (r=0.196).

Correlation of Osteocalcin Vs - Ca

Table (2) shows the relationships between Osteocalcin and Ca levels for the current study, with data for G2 and G3 showing extremely significant negative and positive correlations when $P \leq$ 0.001 for G2 (r=-0.361) and G3 (r=0.242), respective.

Correlation of Osteocalcin Vs -PO4

The correlations between Osteocalcin and PO4 levels for the current research can be shown in **Table (2)** for G2 and G3 individually. These correlations are highly significant positive, and negative when $P \leq 0.001$ for G2 (r=0.102) and G3 (r=-0.208).

4. Discussion

The study results show an increase in highly significant deference for osteocalcin levels in T2DM Iraqi patients groups with and without Osteoporosis compared to the control group. We suggested this finding was observed in this group due to these patients who were menopausal and diabetic. The third group was Osteoporosis. This group takes treatment to treat the OP. This is due to an increase in osteocalcin levels.

However, more osteocalcin is not always a sign of bone strength. Osteocalcin levels can increase as a result of widespread bone loss. In older people, high blood levels of osteocalcin predict lower bone density (particularly in the hip and spine) and fracture risk, including hip fractures [9]. A study by Urano, Tomohiko, et al. (2018) showed the significance of serum osteocalcin levels with T2DM in postmenopausal women. According to several studies, osteocalcin may improve glucose tolerance by encouraging insulin secretion. [10]. The survey conducted by Ferron, Mathieu, et al. (2008) noted that serum Osteocalcin with diabetic Mellitus was produced by the osteoblast and served as one of the markers of bone development, combining calcium and hydroxyapatite in the bone matrix, which was essential for bone mineralization and calcium homeostasis. In studies, recombinant Osteocalcin has been demonstrated to increase insulin secretion, speed up β-cell proliferation, and reduce insulin resistance and hyperglycemia, shielding them from obesity and T2DM [11]. Kanazawa, I. et al. (2011) measures the total Osteocalcin in diabetes mellitus and Osteoporosis significantly in control and patients. These results indicate that Osteocalcin may contribute to glycemic control and may be able to lower the risk of T2DM. Additionally, more research is required to evaluate whether raising osteocalcin levels could be used as a treatment for

T2DM patients. [12]. The study conducted by Lee, Ji Hyun, et al. (2020) agreed with the present study.

The current research findings showed a significant difference in BMI between the control and patient groups. This was due to the patients' group being diabetic Mellitus, and led to increased insulin resistance and decreases in insulin sensitivity to control glucose levels. At the same time, we noticed a significant decrease in a recent result study when compared between G2 and G3. This was because the third group was under the treatment with an antidiabetic agent decreased BMI to control hyper glucose and prevent DM progress, leading to OP. At the same time, other studies suggested lower BMI categories in both men and women of older ages, higher rates of current smoking, and lower rates of T2DM and hypertension. Along with the increase in BMI, the prevalence of T2DM also increased. [13]. The results of BMI by Roomi, Ali B., et al. (2022) found that BMI was the control group's high signification than Osteoporosis. In postmenopausal women in Iraq, the prevalence of Osteoporosis has increased to 22.8%.

Our study stresses the significance of creating risk prediction systems to avoid consistently underestimating the risk of Osteoporosis related fractures in diabetic adults. [14] The present study showed a highly significant increase in FBS and HbA1C when comparing the control and patients group due to diabetes. Still, there was a highly significant decrease compared to patients with diabetes with and without Osteoporosis. This was because patients were in a period of treatment via antidiabetic to decrease the effect of complication as Osteoporosis. Consequently, this result agreed with other studies, like Park, Sung Keun, et al. (2021). They found that the FBS was significant between T2DM and Osteoporosis. Fasting glucose, including the normal, impaired fasting glucose (IFG), and diabetes mellitus (DM) guideline fasting glucose categories, is frequently used to determine aberrant glucose metabolism (AGM) in Osteoporosis. To comprehend how AGM affects Osteoporosis, it may be helpful to examine the association between fasting glucose levels and the risk of the disease. The connection between fasting glucose and Osteoporosis needs to be better understood. AGM, which comprises IFG, IGT, and DM, is frequently diagnosed using fasting glucose two hours after a glucose load. The currently available evidence is insufficient to assess the effect of IFG on Osteoporosis. Our research shows that IFG builds bones and lowers the risk of osteoporosis, in contrast to other studies that linked AGM and bone quality. [15]

Previous studies by Fang, Lingna, et al. (2021) found no significant between Osteoporosis and T2DM. Several routes by which higher blood glucose or HbA1c levels are associated with an increased risk of low muscle mass. The main two risk factors are AGEs and insulin resistance. T2DM is characterized by insulin resistance and various inflammatory markers, including IL-6, tumor necrosis factor-alpha, and C-reactive protein (CRP), associated with it. Muscle protein metabolism includes both the synthesis and breakdown of muscle proteins.

Inflammatory signaling controls the four main proteolytic processes that break down muscle protein: Calpains are ATP-dependent ubiquitin-proteasome processes, such as macrophage autophagy and cell death.[16] Nevertheless, in the current study, we found highly significant decreases in vitamin D3.This view due to all patients with abnormality for glucose homeostasis because vitamin D was consider regulation for glucose metabolism by induce b-cell for pancreas to produce a lot of insulin to regulate glucose metabolism but when this pathway was cutoff this due to developed of complication of diabetes as osteoporosis spatially patients who not controlled

of his diet or systematically treated so as other study like the result of serum D3. Kuchuk, Natalia O., et al (2009) that found significate between healthy and osteoporosis .Serum 25(OH)D levels in osteoporotic postmenopausal women can be compared globally thanks to this study Based on season and latitude, you found significant differences in vitamin D levels between countries. Although it is thought that the skin's generation of vitamin D is the primary source of this vitamin, other factors besides latitude and sun exposure also affect the serum25(OH)D level. You should also incorporate vitamin D-rich foods in your diet, particularly oily fish like salmon, mackerel, herring, and sardines, which are thought to be the best sources. In the past, up to 60% of individuals used supplements like cod liver oil. Compared to the summer, 43 percent of women had 25(OH)D levels >75 nM, and just 34.3% of women did so in the winter. Up to 25% of postmenopausal women with osteoporosis need treatment for hypovitaminosis D in winter, compared to up to 35% in summer. Up to 10,000 IU per day, but no more than 50,000 IU per week, can be used to treat 25(OH)D deficiency. [17] Chen, Hailing, Jufen Li, and Qian Wang's (2018) results appeared was significant between control and osteoporosis patients. In the current study, we found a significant increase in ALP levels in type 2 DM patients without OP.

This agrees with another study suggested by Bones containing alkaline phosphatase. BAP is the name of the bone-specific isoform of the enzyme serum alkaline phosphatase. The glycoprotein that identifies how active osteoblasts are in bone metabolism is found on their surface . The BAP has long been considered a reliable indicator of bone metabolism. Osteoporosis, Paget disease, and fractures are metabolic bone diseases that happen when the rate of resorption in bone remodeling outpaces the formation rate. **[**18**]** Siddapur and Priyanka Ramappa's (2019) results showed a Non significant between T2DM and Osteoporosis. Likewise, we found a significant decrease in calcium levels in type 2 DM patients with and without OP. This agrees with other studies observing that serum calcium levels are typically maintained between 8.5 and 10.5 mg/dl, which is a constrained range. Serum calcium concentrations, however, are just 0.1 to 0.2 percent of extracellular calcium, or just 1% of the total body calcium [19] Shakoor, Sadaf, et al. (2014). As a result observed were high significant patients osteoporosis and control, serum calcium levels are a poor indicator of the total amount of calcium. In osteoporotic women, the study found a significant and distinct relationship between lean mass (muscle mass) and BMD. The relationship between muscle tissue and bone mineral thickness in osteoporotic women of all ages, which is invariably associated with less effective muscle tissue stimulation with bone, was neglected in women of all ages who did not have bone weakening as a result of reduced mass . In osteoporotic women, lean mass (muscle mass) was found to be highly and specifically correlated with BMD.[20] Raikou, Vaia D., Despina Kyriaki, and Sotiris Gavriil (2020) in the study measuring high signification between T2DM than control. Blood P levels were shown to be lower in T2DM patients due to metabolic abnormalities compared women with T2DM to hospitalized non-T2DM patients too we found highly significant decrease for p levels in type 2 DM patients with and without OP this noting produce because all those patients was suffering from vitamin D deficiency and this agree with other study who observed Lower blood P levels have been linked to higher insulin resistance in the healthy population. In a previous study of 881 nondiabetic subjects, low blood P levels were associated with high 2 hour serum glucose and decreased insulin sensitivity [21] .

5. Conclusion

We found a highly significant correlation between osteocalcin and BMI, FBS, HbA1C, D3, ALP, Ca, P, and in type 2DM patients group with and without OP. Based on the obtained data, we found an increased highly significant difference between osteoclcin and BMI, FBS, HbA1C, D3 ALP, Ca, and P for control and type 2 DM with and without OP. So, this new biomarker in this type of sample may be considered a vital role in the diagnosis of osteoporosis in diabetic Mellitus and utilized this biomarker to predict the developing osteoporosis in type 2 diabetic Mellitus menopausal for Iraqi women .

References

1. Al-Masoudi, Faris Ibrahim, Bushra H. Ali. Evaluation of Nesfatin–1 and Other Biochemical Markers in diabetic Neuropathy Iraqi patients before and after treatment with tegretol:*Annals of the Romanian Society for Cell Biology* ,**2021**, 9422-9429.

2. Salih, Nada Taha , Bushra H. Ali. Relation Study between Chromogranin a and Other Clinical Biomarker in Iraqi Children with Growth Hormone Deficiency Undergo Treated Via Recombinant Growth Hormone: *Annals of the Romanian Society for Cell Biology* **2021**, 11072-11077.

3. Tan, Sin Yee, et al. Type 1 and 2 diabetes mellitus: A review on current treatment approach and gene therapy as potential intervention. *Diabetes & metabolic syndrome: clinical research & reviews* **2019,***13,1*, 364-372.

4. Rozenberg, Serge, et al. How to manage osteoporosis before the age of 50. Maturitas. **2020**, *138,*14-25.

5.Ha, J. Y., ; Choi, E. Y. Health perception, health concern, and health promotion behavior of the elders. *Journal of Korean Gerontological Nursing* . **2013**,*15,3*, 277-85.

6. Hauschka, Peter V., et al. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. *Physiological reviews* .**1989**, *69,3*,990-1047.

7. Levinger, Itamar, et al. Multifaceted interaction of bone, muscle, lifestyle interventions and metabolic and cardiovascular disease: role of osteocalcin. *Osteoporosis International* . **2017**, *28,8,* 2265-2273.

8. Mizokami, Akiko, Tomoyo Kawakubo-Yasukochi, and Masato Hirata. Osteocalcin and its endocrine functions. *Biochemical pharmacology* . **2017**, *132,* 1-8.

9. Zoch, Meredith L.;Thomas L. Clemens, ; Ryan C. Riddle. New insights into the biology of osteocalcin. *Bone* . **2016**, *82* ,42-49.

10.Urano, Tomohiko, et al. Low serum osteocalcin concentration is associated with incident type 2 diabetes mellitus in Japanese women. *Journal of Bone and Mineral Metabolism* . **2018**, *36,4* 470-477.

11. Ferron, Mathieu, et al. Osteocalcin differentially regulates β cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. Proceedings of the National Academy of Sciences . **2008**, *105,13* 5266-5270.

12. Kanazawa, I., et al. Serum undercarboxylated osteocalcin was inversely associated with plasma glucose level and fat mass in type 2 diabetes mellitus. *Osteoporosis International* . **2011**,*22,1*, 187-194.

13. Lee, Ji Hyun, et al. Optimal body mass index for minimizing the risk for osteoporosis and type 2 diabetes. *The Korean journal of internal medicine*. **2020**, *35,6* ,1432.

14. Roomi, Ali B. et al. Evaluation of Bone Mineral Density, Serum Osteocalcin, and Osteopontin Levels in Postmenopausal Women with Type 2 Diabetes Mellitus, with/without Osteoporosis. *Journal of Osteoporosis* 2022. **2022**.

15. Park, Sung Keun, et al. Fasting glucose level and the risk of incident osteoporosis in the Koreans. *Bone* . **2021**, *142* , 115690.

16.Fang, Lingna, et al. A cross-sectional study: an assessment of low muscle mass and osteoporosis in type 2 diabetes mellitus patients with a high glycated hemoglobin level. Therapeutic Advances in Chronic Disease . **2021**, *12,* 20406223211026762.

17. Kuchuk, Natalia O., et al. Vitamin D status, parathyroid function, bone turnover, and BMD in postmenopausal women with osteoporosis: global perspective. *Journal of bone and mineral research* . **2009**, *24,4,* 693-701.

18. Chen, Hailing, Jufen Li, and Qian Wang. Associations between bone-alkaline phosphatase and bone mineral density in adults with and without diabetes. *Medicine* . **2018**, *97,17*.

19.Siddapur, Priyanka Ramappa. A study to compare serum calcium level between diabetic and non-diabetic post-menopausal women with osteoporosis. *Medicine* . **2019**, *97,17*

20. Shakoor, Sadaf, et al. Prevalence of osteoporosis in relation to serum calcium and phosphorus in aging women. *J Glob Innov Agric Soc Sci* . **2014**, *2,2* , 70-5

21. Raikou, Vaia D., Despina Kyriaki, ; Sotiris Gavriil. Importance of serum phosphate in elderly patients with diabetes mellitus. *World Journal of Diabetes* . **2020**, *11,10* 416.