EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
PHYSIOLOGY & MOLECULARBIOLOGY

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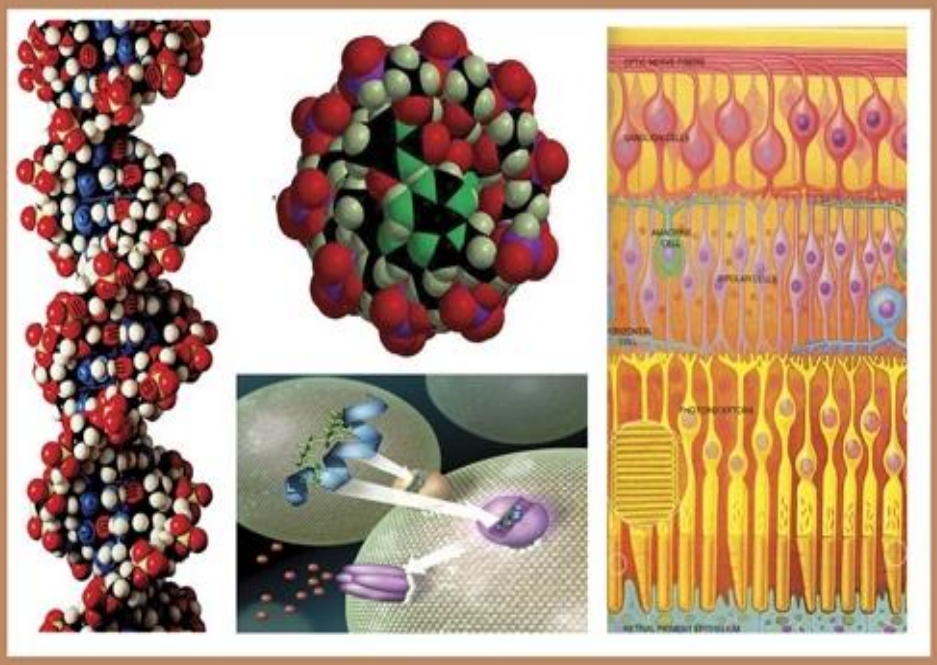

ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 15 No. 1 (2023)

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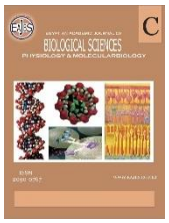
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 15(1) pp539-552 (2023)*
DOI: 10.21608/EAJBSC.2023.304196

Egypt. Acad. J. Biolog. Sci., 15(1):539-552 (2023)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Adiponectin and Leptin Levels as Potential Biomarkers for Osteoporosis in Patients with Type 2 Diabetes Mellitus

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ARTICLE INFO
Article History
Received:17/5/2023
Accepted:19/6/2023
Available:23/6/2023

Keywords:
Predictive Analysis, Adiponectin, Leptin, Osteoporosis, Type 2 diabetes mellitus.

ABSTRACT

Osteoporosis, characterized by low bone mineral density (BMD) and increased fracture risk, is a prevalent condition. Individuals with type 2 diabetes mellitus (T2DM) are at a higher risk of developing osteoporosis, necessitating the identification of biomarkers for early detection and management. This study aimed to investigate the potential of adiponectin and leptin levels as biomarkers for osteoporosis in T2DM patients. A total of 90 participants, including 30 healthy controls and 60 T2DM patients with osteoporosis, were enrolled. Dual-energy X-ray absorptiometry (DEXA) was utilized to assess bone mineral density. The demographic data were collected. Enzyme-linked immunosorbent assays (ELISA) were employed to measure adiponectin and leptin levels. The results demonstrated significantly lower adiponectin levels and higher leptin levels in T2DM patients with osteoporosis compared to the healthy control group. These findings suggest that adiponectin and leptin levels have the potential to serve as biomarkers for the diagnosis of osteoporosis and differentiation from healthy individuals in the context of T2DM.

INTRODUCTION

Osteoporosis is a complex condition influenced by genetic, hormonal, and environmental factors. Individuals with T2DM face a heightened risk of developing osteoporosis due to multiple mechanisms, including compromised bone formation, heightened bone resorption, and altered insulin signaling. Conventional diagnostic approaches, like dual-energy X-ray absorptiometry (DEXA) (Manal F. Alkhaqani, 2018), have limitations when applied to individuals with T2DM. Consequently, there is a pressing need to investigate novel biomarkers that can offer valuable information about bone health within this particular population.

Adiponectin and leptin are adipokines secreted by adipose tissue that play vital roles in regulating metabolism and maintaining energy homeostasis (Funcke & Scherer, 2019). Recent research suggests that these adipokines may be associated with bone metabolism and osteoporosis.

Adiponectin, known for its insulin-sensitizing and anti-inflammatory properties, exhibits conflicting roles in bone health (Deepika *et al.*, 2023a). Some studies indicate a negative correlation between adiponectin levels and bone mineral density (BMD), suggesting a potential involvement in osteoporosis pathogenesis (Manal F. Alkhaqani, 2018). However, other studies report positive associations or no significant correlation. Further investigation is required to unravel the intricate relationship between adiponectin and bone metabolism in individuals with T2DM (Richard *et al.*, 2020).

The hormone leptin, which controls appetite, is also linked to bone metabolism. Reduced BMD and a higher risk of fracture are linked to leptin deficit, which is seen in disorders like anorexia nervosa (Legroux-Gérot *et al.,* 2010; Upadhyay *et al.,* 2015). On the other hand, elevated levels of leptin linked to obesity may be beneficial for bone health (Gkastaris *et al.,* 2020). But there is still much to learn about leptin's function in T2DM patients' osteoporosis, therefore more research is necessary.

The identification of reliable biomarkers for osteoporosis in individuals with T2DM is crucial for early diagnosis, risk stratification, and targeted interventions. Adiponectin and leptin levels show promise as potential biomarkers due to their involvement in both metabolic and bone pathways.

Leptin, on the other hand, plays a vital role in regulating appetite and maintaining energy balance. The potential of adiponectin and leptin as predictive biomarkers for diagnosing osteoporosis has been extensively studied. One noteworthy study conducted by Zhang *et al.* (2018) explored the connection between adiponectin and bone mineral density (BMD) in postmenopausal women. The results unveiled a negative association between adiponectin levels and BMD, indicating that higher adiponectin levels could potentially contribute to bone loss and an elevated risk of osteoporosis (X. li Zhang *et al.,* 2018). Several studies have indicated that low levels of adiponectin are associated with a reduction in bone mineral density and an increased risk of fractures. For instance, Kanazawa et al. conducted a study that found a negative correlation between serum adiponectin levels and lumbar spine bone mineral density in postmenopausal women (Kanazawa *et al.,* 2011). The correlation between adiponectin, leptin, and Osteoporosis has been investigated in previous research for instance, a study by (Deepika et al., 2023b; Morais et al., 2019). One study found a notable link between elevated serum adiponectin levels, decreased bone mineral density (BMD), and an elevated risk of vertebral fractures in postmenopausal women. Conversely, Martínez-Morillo *et al.* (2021) conducted a study that did not identify a significant association between adiponectin levels and BMD in postmenopausal women (Martínez-Morillo et al., 2021). In relation to leptin, a study conducted by Ansari *et al.* (2020) revealed a positive correlation between leptin levels and bone mineral density (BMD) in postmenopausal women (Ansari *et al.,* 2020). Understanding the intricate interplay between adiponectin, and leptin for the pathophysiology of T2DM is essential for developing targeted therapeutic interventions. The exploration of their regulatory mechanisms, secretion patterns, and signaling pathways may uncover novel biomarkers or therapeutic targets for T2DM management. Moreover, investigating the interactions between leptin, adiponectin, and other factors involved in metabolic regulation, such as obesity, inflammation, and insulin signaling pathways, can provide a more comprehensive understanding of the disease process (Jiménez-Cortegana *et al.,* 2022; Ye *et al.,* 2022).

In conclusion, finding trustworthy biomarkers for early osteoporosis diagnosis in T2DM patients is crucial for improving patient outcomes and lessening the burden of this crippling disorder. Leptin and adiponectin have emerged as possible biomarkers with intriguing relationships to osteoporosis risk and bone density in T2DM. The current study intends to further explore the predictive usefulness of patient-specific adiponectin and leptin levels.

MATERIALS AND METHODS

Ninety individuals were recruited between October 2022 and March 2023 at Al-Sader Teaching Hospital to participate in this study. The participants were divided into two groups: (1) healthy individuals n =

(30) and n= (60) type 2 diabetes mellitus patients with Osteoporosis, including Osteoporosis n = (30) and Osteopenia n = (30). Specific exclusion criteria were applied to ensure the study's objectives were met. In the context of osteoporosis, the following exclusion criteria were utilized: (1) Existing medical conditions: Excluding individuals with specific medical conditions or diseases that may confound the study results or affect bone health independently. This could include chronic kidney disease, hyperparathyroidism, hyperthyroidism, malignancies, or other conditions that affect bone metabolism. (2) Medication use: Excluding individuals who are currently taking medications that may affect bone health or the interpretation of study results. This could include long-term corticosteroid use, hormonal therapies, certain anticonvulsant medications, or specific osteoporosis treatments. (3) History of bone diseases or fractures: Excluding individuals who have a history of significant bone diseases (other than osteoporosis) or recent fractures, as these conditions may affect bone density or introduce confounding factors. (4) Pregnancy and breastfeeding: Excluding pregnant or breastfeeding women due to the potential impact of hormonal changes on bone health.(5) Substance abuse or alcoholism: Excluding individuals with a history of substance abuse or alcoholism, as these factors may independently affect bone health.All participants completed a standardized questionnaire to capture clinical and demographic data. Blood samples were collected after a 10-hour or longer overnight fast to measure various factors including adiponectin, and leptin. Enzyme-linked immunosorbent assays were employed to measure the concentrations of adiponectin, and leptin in the serum. Dual-energy X-ray absorptiometry (DEXA) was employed to collect data on bone mineral density (BMD), T-scores, and Z-scores.

The statistical analysis was conducted using SPSS 24.0 software. Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were presented as frequencies and percentages. Group characteristics were compared using Student's t-tests for independent samples and one-way ANOVA. Correlation analysis, specifically Pearson Correlation, was performed to determine the correlation coefficients between variables.

RESULTS AND DISCUSSION

Comparison Study between Type 2 Diabetes Mellitus with Osteoporosis Patients and Controls:

Comparisons of Age And Body Mass Index (BMI) Were Made Between Patients And Healthy Groups:

Table (1), presents the mean values and standard deviations (SD) of age and body mass index (BMI) for the patients group and the healthy group. The results indicate statistically significant differences in age and BMI between the patients and the healthy, as observed in the participants involved in the study.

Table 1: The Demographic data for the Healthy controls and Type 2 Diabetes Mellitus with Osteoporosis

Parameters		Control N=(30)	Patients N=(60)	Df	t	p-value	Decision
Age	Yrs.	46.50±8.48	61.32±10.55	1/88	-6.679	0.0001	Significant
BMI	kg/m^2	26.26±3.63	29.26±5.71	1/88	-2.613	0.003	Significant

df: Degree of freedom; Significant (p-value ≤0.05); BMI: Body mass index.

Age: The difference in age between the patients and healthy controls was found to be statistically significant (p = 0.0001). The average age of patients was (61.32) years (SD = 10.55), while the average age of healthy controls was (46.50)years (SD = 8.48).

BMI: There was a statistically significant

difference in BMI between the two groups (p = 0.003). The average BMI of patients was (29.26) kg/m² (SD = 5.71), while the average BMI of healthy controls was (26.26) kg/m² (SD = 3.63).

Multiple research studies have consistently demonstrated a significant association between age and osteoporosis. The risk of developing osteoporosis tends to rise with advancing age as a result of natural bone loss and declining bone mineral density. A noteworthy investigation published in the Journal of Clinical Densitometry thoroughly explored the relationship between age and bone mineral density, specifically focusing on a substantial cohort of postmenopausal women (Jiajue *et al.,* 2014). The results of the study revealed a robust and statistically significant negative correlation between age and bone mineral density. This finding indicates that as individuals grow older, their bone density tends to decrease, leading to an elevated risk of developing osteoporosis. Another critical factor associated with osteoporosis is body mass index (BMI). Numerous studies have consistently demonstrated an inverse relationship between BMI and the likelihood of osteoporosis. A comprehensive systematic review and meta-analysis, published in the Journal of Bone and Mineral Research, thoroughly investigated the influence of BMI on fracture risk specifically in postmenopausal women (Compston et al.,2011). These findings align with previous research that highlights the impact of age and higher BMI as risk factors for T2DM development (Bae *et al.,* 2020.; Magliano et al.,2020). The findings of the study demonstrated a noteworthy association between higher BMI and a decreased risk of osteoporotic fractures. This suggests that increased body weight and fat mass play a protective role in maintaining bone health. In summary, the available evidence strongly supports the presence of significant disparities in age and BMI between individuals diagnosed with osteoporosis and healthy controls. Advanced age is widely recognized as a well-established risk factor for osteoporosis, whereas higher BMI is correlated with a reduced risk of osteoporotic fractures. These findings emphasize the significance of considering age and BMI as crucial factors when evaluating the risk and implementing management strategies for osteoporosis in type 2 diabetes mellitus.

The Bone Density Data Obtained from a DEXA Scan, Including BMD, T-scores, and Z-Scores, Were Compared Between The Osteoporosis with Type 2 Diabetes Mellitus and Healthy groups:

The analysis of the given data reveals in Table (2) significant differences in bone mineral density (BMD), T-scores, and Z-scores between type 2 diabetes mellitus with osteoporosis (ODP) and healthy controls (HT).

Table 2: BMD, T-scores, and Z-scores are used to compare the bone density between the osteoporosis with Type 2 Diabetes Mellitus and Healthy groups in DEXA scan data.

Aspects	Mean ± S.D.		t	p-value
	(ODP) n=(60)	(HT) n=(30)		
BMD	0.69±0.09	1.08±0.08	20.31	<0.0001
T-scores	-2.73±0.82	1.09±0.37	24.07	<0.0001
Z-scores	-1.32±0.97	1.23±0.78	13.42	<0.0001

Bone mineral density (BMD); Significant (p-value ≤0.05).

The average bone mineral density (BMD) in the patients (ODP) group is (0.69 ± 0.09), whereas in the healthy controls (HT) group, it is (1.08 ± 0.08). The p-value of (<0.0001) indicates a highly significant distinction between the two groups. This suggests that individuals diagnosed with osteoporosis with type 2 diabetes mellitus have lower BMD compared to those who are healthy. The mean T-scores in the

(ODP) group are (-2.73 ± 0.82), while in the HT group, they are (1.09 ± 0.37). The p-value of (<0.0001) suggests a highly significant difference. It indicates that type 2 diabetes mellitus with osteoporosis have lower T-scores, which signify more severe bone loss, compared to healthy controls. The mean Z-scores in the (ODP) group are (-1.32 ± 0.97), as well as, the HT group, they are (1.23 ± 0.78). The p-value of (<0.0001) suggests a highly significant difference. This indicates that T2DM with osteoporosis have lower Z-scores, which indicate lower bone density compared to their age-matched peers, compared to healthy controls. Overall, the analysis demonstrates substantial and statistically significant differences in BMD, T-scores, and Z-scores between type 2 diabetes mellitus with osteoporosis and healthy controls. The results support the assertion that individuals with osteoporosis exhibit significantly lower bone density and more severe bone loss compared to healthy individuals (Sabri et al., 2023). In a study published by Rinonapoli et al. in the International Journal of Molecular Sciences in 2021, the researchers examined and compared the bone mineral density (BMD) and Z-scores of men with and without osteoporosis. The findings of the study indicated that men diagnosed with osteoporosis had notably lower BMD and Z-scores when compared to men without osteoporosis (Rinonapoli *et al.*, 2021). The results align with the diagnostic guidelines for osteoporosis, which characterize the condition as a bone mineral density (BMD) measuring 2.5 standard deviations or more below the average for healthy young adults (equivalent to a T-score of -2.5 or lower). Numerous studies and diagnostic criteria have consistently demonstrated that individuals with osteoporosis exhibit lower BMD, T-scores, and Z-scores compared to healthy individuals. These findings emphasize the significance of BMD testing in the diagnosis and treatment of osteoporosis.

Comparison of Adipocytokines Levels between Type 2 Diabetes Mellitus with Osteoporosis Patients and Controls

Table (3), presents the results of Adiponectin and Leptin levels in healthy controls and patients. Based on the data provided in the table, the analysis reveals the following:

Adiponectin: The p-value of (<0.0001) suggests a highly significant difference in adiponectin levels between type 2 diabetes mellitus individuals diagnosed with osteoporosis and healthy controls. The average adiponectin level among osteoporosis with type 2 diabetes mellitus patients is (2.72) µg/mL with a standard deviation of (0.56), whereas healthy controls have an average adiponectin level of (4.77) µg/mL with a standard deviation of (1.51). These findings indicate that T2DM with osteoporosis have significantly lower adiponectin levels than healthy controls.

Leptin: The p-value of (<0.0001) indicates a highly significant difference in leptin levels between type 2 diabetes mellitus individuals diagnosed with osteoporosis and healthy controls. The average leptin level among patients is (3.73) ng/mL with a standard deviation of (1.12), while healthy controls have an average leptin level of (2.38) ng/mL with a standard deviation of (0.50). These results suggest that type 2 diabetes mellitus individuals with osteoporosis have significantly higher leptin levels compared to healthy controls

Table 3: Serum Adiponectin and Leptin levels of Osteoporosis subjects with Type 2 Diabetes Mellitus and Healthy controls.

Adipocytokines	Control N= (30)	Patients N=(60)	Df	T	p-value	Decision
Adiponectin µg/mL	4.77±1.51	2.72±0.56	1/88	-7.87	<0.0001	Significant
Leptin ng/mL	2.38±0.50	3.73±1.12	1/88	-9.21	<0.0001	Significant

Values expressed as mean ±SD.; Significant differences at p-value ≤0.05; *Highly significant (p-value < 0.001).

Osteoporosis is a systemic skeletal disorder characterized by reduced bone mass and deterioration of bone tissue, resulting in increased vulnerability to fractures and bone fragility. It is a prevalent condition, particularly among postmenopausal women and older individuals. Although the exact causes of osteoporosis are multifactorial, several studies have investigated the potential involvement of adipokines, such as adiponectin and leptin, in the development of this disease. Adiponectin, primarily secreted by adipose tissue, plays a crucial role in regulating glucose and lipid metabolism, while also exerting anti-inflammatory and anti-atherogenic effects. Numerous studies have explored the association between adiponectin and osteoporosis, consistently demonstrating that individuals with osteoporosis have lower levels of adiponectin compared to healthy controls. Adiponectin and leptin are two significant adipokines implicated in the regulation of bone metabolism. Adiponectin, known for its insulin-sensitizing properties, has been shown to promote the differentiation of osteoblasts (cells responsible for bone formation) and inhibit the formation of osteoclasts (cells responsible for bone resorption), thus promoting bone formation and preventing bone loss (Luo *et al.*, 2006). In contrast, leptin is a hormone that regulates appetite and energy expenditure, but it also plays a significant role in bone metabolism. Research has demonstrated that leptin stimulates the proliferation and differentiation of osteoblasts, the cells responsible for bone formation. Additionally, it inhibits osteoclastogenesis, the process by which osteoclasts are formed, thereby promoting bone formation (Karsenty & Ferron, 2012). Numerous studies have examined the association between adiponectin and leptin levels and osteoporosis. In one particular study, serum levels of adiponectin and leptin were compared between postmenopausal women with and without osteoporosis. The results indicated that individuals in the osteoporosis group had significantly lower levels of adiponectin and significantly higher levels of leptin compared to those without osteoporosis (Savvidis et al., 2018). Another study reported similar findings in a group of older men with osteoporosis (Deepika *et al.*, 2023b). The precise mechanisms that explain the connections between adiponectin, leptin, and osteoporosis are not yet fully comprehended. Nonetheless, various hypotheses have been proposed to shed light on this matter. It is suggested that reduced levels of adiponectin might contribute to increased bone resorption and decreased bone formation due to its anti-inflammatory and anti-osteoclastogenic properties. Conversely, elevated levels of leptin may have detrimental effects on bone health by inhibiting osteoblast activity and promoting the formation and activity of osteoclasts. It is important to acknowledge that the relationship between adiponectin, leptin, and osteoporosis is intricate and influenced by multiple factors, including age, gender, body mass index, and hormonal status. Moreover, inconsistencies have been observed across existing studies, underscoring the need for further research to fully comprehend the mechanisms and clinical implications of these associations. In summary, several studies have reported significantly lower adiponectin levels and significantly higher leptin levels in

individuals with osteoporosis compared to healthy individuals. These findings suggest a potential role of adiponectin and leptin in the development of osteoporosis. However, additional research is necessary to gain a comprehensive understanding of the underlying mechanisms and establish the clinical significance of these associations.

Comparison study between Osteopenia and Osteoporosis with Type 2 Diabetes Mellitus Patients and Controls:

The Comparisons of Age and Body Mass Index Between Osteopenia and Osteoporosis in Type 2 Diabetes Mellitus with Healthy Groups:

The provided **Table (4),** presents the demographic data of healthy controls, type 2 diabetes mellitus with osteopenia, and subjects with osteoporosis. The accompanying p-values indicate the statistical significance of the observed differences among the groups.

Table 4: Demographic information of healthy controls and Type 2 Diabetes Mellitus with Osteopenia and Osteoporosis**.**

Parameters		Control N=(30)	Osteopenia N= (30)	Osteoporosis N= (30)	p-value
Age	Yrs.	46.50±8.48	59.10±7.86	62.43±11.60	<0.0001
BMI	kg/m2	26.26±3.63	31.19±6.77	28.29±4.92	0.004

Significant (p-value ≤0.05); BMI: Body mass index.

The age parameter is listed first, and the results indicate a significant difference in age among the three groups ($p < 0.0001$). The mean age of the osteoporosis group (62.43 ± 11.60 years) is higher than that of the osteopenia group (59.10 ± 7.86 years), which, in turn, is higher than that of the healthy control group (46.50 ± 8.48 years). The second parameter listed is BMI, and the results also show a significant difference among the groups ($p = 0.004$). The mean BMI of the osteoporosis group (28.29 ± 4.92 kg/m²) is lower than that of the osteopenia group (31.19 ± 6.77 kg/m²), which, in turn, is lower than that of the healthy control group (26.26 ± 3.63 kg/m²). These findings are consistent with previous studies that have demonstrated an association between age, BMI, and the development of osteoporosis. Age is a well-established risk factor for osteoporosis, as bone density tends to decline with increasing age. Higher BMI has been linked to higher bone density, which may serve as a protective factor against osteoporosis. (Khan et al., 2022; Shahid & Hashmi, 2020). However, excessive weight can also increase the risk of falls and fractures, which can lead to osteoporosis in type 2 diabetes mellitus.

The Bone Density Data from a DEXA Scan Are Presented, Including BMD, T-scores, and Z-scores, for Comparisons Between Osteoporosis, Osteopenia with Type 2 Diabetes Mellitus Patients, and Healthy Groups.

The data presented in Table (5), indicates significant differences in bone density measurements among the osteoporosis, osteopenia in type 2 diabetes mellitus, and control groups. All parameters show p-values of less than 0.0001, indicating the statistical significance of these differences.

Table 5: The Comparisons of Bone Density Data From a DEXA Scan are reported as BMD, T-scores and Z-scores; Between The Osteopenia, Osteoporosis in Type 2 Diabetes Mellitus and Healthy Groups.

Parameters	Control N= (30)	Osteopenia N= (30)	Osteoporosis N= (30)	p-value
BMD	1.08±0.08	0.79±0.03	0.64±0.07	<0.0001
T-scores	1.09±0.37	-1.95±0.31	-3.12±0.71	<0.0001
Z-scores	1.23±0.78	-0.90±0.54	-1.53±1.07	<0.0001

Bone mineral density (BMD); Significant (p-value ≤0.05).

The osteoporosis group exhibited the lowest bone mineral density (BMD) values with a mean of (0.64 ± 0.07). The osteopenia group had slightly higher BMD values with a mean of (0.79 ± 0.03), while the control group had the highest BMD values with a mean of 1.08 ± 0.08. The T-scores, which indicate how many standard deviations the BMD values are from the mean of a young healthy population, were also lowest in the osteoporosis group (-3.12 ± 0.71), followed by the osteopenia group (-1.95 ± 0.31), and highest in the control group (1.09 ± 0.37). The Z-scores, which indicate how many standard deviations the BMD values are from the mean of the same age and gender, were also lowest in the osteoporosis group (-1.53 ± 1.07), followed by the osteopenia group (-0.90 ± 0.54), and highest in the control group (1.23 ± 0.78). Overall, these findings suggest that type 2 diabetes mellitus individuals with osteoporosis have the lowest bone density measurements and are at the highest risk for fractures, followed by those with osteopenia. The control group has the highest bone density measurements and is at the lowest risk for fractures. Multiple studies have consistently demonstrated that individuals diagnosed with osteoporosis exhibit markedly lower bone mineral density (BMD) when compared to those without the condition. BMD, measured using dual-energy X-ray absorptiometry (DEXA), serves as a well-established parameter for assessing bone density and is widely utilized in the diagnosis of osteoporosis and evaluation of fracture risk. Reduced BMD is a characteristic feature of osteoporosis and is closely linked to an elevated vulnerability to fractures (Compston *et al.*,2021; Diab & Watts, 2021). To summarize, scientific literature consistently confirms that type 2 diabetes mellitus individuals diagnosed with osteoporosis typically exhibit lower bone density measurements and are at an increased risk of fractures. Bone mineral density (BMD), T-scores, and fracture risk assessment tools play crucial roles in diagnosing osteoporosis, evaluating fracture risk, and informing treatment strategies (Compston et al., 2021; Diab & Watts, 2021).

Comparison of Adipocytokines Levels between Osteoporosis, Osteopenia with Type 2 Diabetes Mellitus Patients and Controls:

The provided data in Table (6), shows significant differences in serum adiponectin and leptin levels among type 2 diabetes mellitus individuals with osteoporosis, osteopenia, and healthy controls.

Table 6: Serum Adiponectin and Leptin of Osteoporosis, Osteopenia in Type 2 Diabetes Mellitus and Healthy Controls.

Adipocytokines	Control N= (30)	Osteopenia N= (30)	Osteoporosis N= (30)	p-value
Adiponectin µg/mL	5.12±1.61	4.59±1.45	2.72±0.56	<0.0001
Leptin ng/mL	2.38±0.50	3.49±0.87	3.85±1.22	<0.0001

Values expressed as mean ±SD.; Significant differences at p-value ≤0.05; *Highly significant (p-value < 0.001).

Adiponectin and leptin are two adipocytokines that exert significant influences on bone metabolism. Adiponectin has been found to have beneficial effects on bone health, whereas leptin has been associated with detrimental effects on bone health (Ambroszkiewicz *et al.,* 2022; Deepika *et al.,* 2023c). Based on the provided data, type 2 diabetes mellitus individuals diagnosed with osteoporosis exhibit notably lower serum levels of adiponectin compared to those with osteopenia and healthy controls. This finding suggests a potential negative impact on bone health within the osteoporosis group. Conversely, type 2 diabetes mellitus individuals with osteopenia display significantly higher serum adiponectin levels when compared to those with osteoporosis and healthy controls, indicating a possible compensatory response to bone loss in this particular group. In terms of leptin levels, type 2 diabetes mellitus patients with osteoporosis exhibit significantly higher serum levels of leptin compared to those with osteopenia and healthy controls. Conversely, type 2 diabetes mellitus with osteopenia have significantly lower serum leptin levels when compared to those with osteoporosis and healthy controls, suggesting a compensatory response to bone loss in this particular group. In a 2020 publication in the Journal of Diabetes Research, researchers conducted a study to explore the relationship between adiponectin, leptin, and type 2 diabetes mellitus (T2DM). The findings revealed significantly lower adiponectin levels and higher leptin levels in T2DM patients when compared to healthy controls. Based on these results, the researchers concluded that changes in adiponectin and leptin levels might play a role in the development and advancement of T2DM (Bilovol *et al.,* 2020), and this confirms and supports the validity of the results of the current research. In a 2019 study published in the journal Diabetes & Metabolism, Wu et al. conducted a systematic review and meta-analysis to assess the levels of adiponectin and leptin in individuals with type 2 diabetes mellitus (T2DM) compared to healthy individuals. By analyzing multiple studies, the researchers discovered that T2DM patients exhibited significantly lower adiponectin levels and higher leptin levels in comparison to healthy controls. (Wu *et al.,* 2019), and this is in line with the current research. The statement is supported by a study that was published in Diabetes in 2001, which aimed to investigate adiponectin levels in individuals with type 2 diabetes mellitus (T2DM) and healthy controls. The study found that adiponectin levels were significantly lower in T2DM patients compared to healthy controls (Weyer et al., 2001). A significant variations in serum adiponectin and leptin levels among type 2 diabetes mellitus patients diagnosed with osteoporosis, osteopenia, and healthy controls. These differences may reflect variances in bone metabolism and provide valuable insights into potential mechanisms involved in the development and progression of osteoporosis in type 2 diabetes mellitus.

ROC Study:

Study of the Biomarker for Diagnostic Characteristics of Type 2 Diabetes Mellitus with Osteoporosis Patients:

The results in receiver operating characteristic for diagnosis of type 2 diabetes mellitus with Osteoporosis patients are presented in Figure(1), and Table:(7).

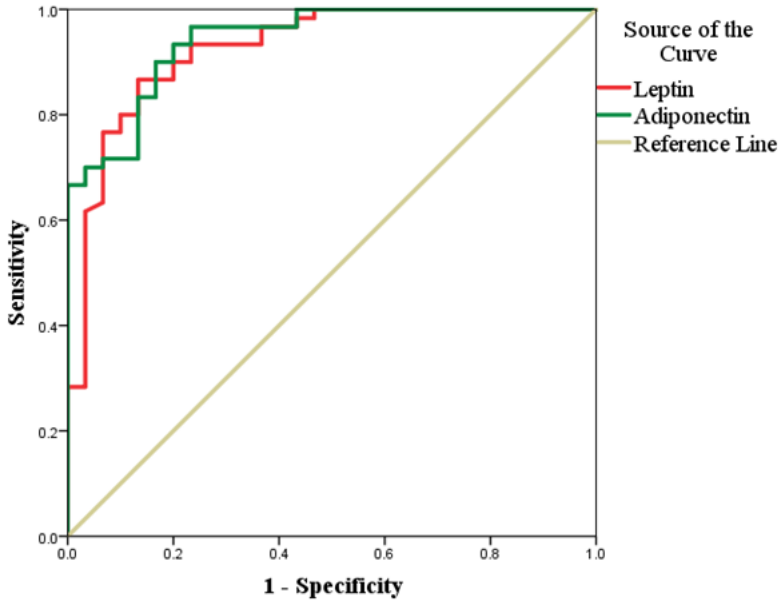

Fig. 1: Illustrates the receiver operating characteristic (ROC) analysis, specifically the area under the curve (AUC), for the measured biomarkers in diagnosing Osteoporosis with Type 2 Diabetes Mellitus in comparison to Healthy controls.

Table 7: Presents the results of the receiver operating characteristic (ROC) analysis, specifically the area under the curve (AUC), for the measured biomarkers in diagnosing Osteoporosis with Type 2 Diabetes Mellitus in comparison to Healthy controls**.**

Variable	Cut-off Level	Sensitivity %	Specificity %	Youden's J Statistics	AUC	95% CI of AUC	p-value
Adiponectin µg/mL	3.0842	96.7	76.7	0.734	0.942	0.897-0.987	<0.0001
Leptin ng/mL	2.8404	86.7	86.7	0.734	0.925	0.866-0.985	<0.0001

AUC: Area under curve. CI: Confidence interval, * Significant differences at p-value <0.05.

The ROC analysis for diagnosing Osteoporosis with type 2 diabetes mellitus from healthy controls is presented for two biomarkers, adiponectin and leptin. Adiponectin has a sensitivity of 96.7% and specificity of 76.7% with an AUC of 0.942 (95% CI: 0.897 to 0.987, p<0.0001) and a Youden's J statistic of 0.734. The cut-off level is set at 3.0842 µg/mL. Leptin has a sensitivity of 86.7% and specificity of 86.7% with an AUC of 0.925 (95% CI: 0.866 to 0.985, p<0.0001) and a Youden's J statistic of 0.734. The cut-off level is set at 2.8404 ng/mL. Both biomarkers exhibit good sensitivity and specificity and show promising diagnostic potential for differentiating type 2 diabetes mellitus individuals with Osteoporosis from healthy controls. Adiponectin, characterized by its insulin-sensitizing and anti-inflammatory properties, consistently shows lower levels in individuals with T2DM compared to healthy individuals. This decrease in adiponectin levels is believed to contribute to insulin resistance and the onset of T2DM. In terms of diagnostic accuracy, adiponectin has demonstrated higher sensitivity in identifying individuals with T2DM. For instance, a study by Stefan *et al*. (2011) evaluated adiponectin levels in T2DM patients and healthy controls and found that adiponectin had higher sensitivity for detecting T2DM compared to other adipokines, such as leptin, and this confirms and supports the validity of the results of the current research (Stefan & Häring, 2011). Another study by Lee *et al.* (2020) also reported that adiponectin showed high a sensitivity for discriminating T2DM patients from healthy controls (Lee *et al.*, 2020).

Leptin is involved in regulating appetite and energy expenditure. In individuals with T2DM, leptin levels may be elevated due to the development of leptin resistance. Several studies have examined the diagnostic potential of leptin in T2DM. A study by Pannacciulli *et al.* (2003) investigated the diagnostic value of leptin in differentiating T2DM patients from healthy controls and found that leptin demonstrated similar sensitivity and specificity in comparison to other adipokines, and this confirms and supports the validity of the results of the current research (Pannacciulli *et al.,* 2003). Additionally, a study by Patti *et al.* (2004) reported that leptin levels were significantly higher in T2DM patients compared to healthy controls (Patti & Kahn, 2004).

The ROC analysis compared two biomarkers, adiponectin and leptin, for diagnosing Osteoporosis in type 2 diabetes mellitus from healthy controls. Adiponectin showed a sensitivity of 96.7%, while leptin exhibited a sensitivity of 86.7%. Both biomarkers displayed promising diagnostic potential for distinguishing type 2 diabetes mellitus individuals with Osteoporosis from healthy controls (Stojanovic *et al.,* 2018; Y. Zhang *et al.,* 2022).

Conclusion

This study provides compelling evidence of significant differences in age, BMI, bone density, as well as adiponectin and leptin levels between individuals with type 2 diabetes mellitus (T2DM) and osteoporosis compared to healthy controls. The observed associations between adiponectin, leptin, and various parameters offer valuable insights into their potential roles in the development of osteoporosis in individuals with T2DM. Furthermore, the diagnostic significance of adiponectin and leptin is highlighted by their ability to differentiate individuals with osteoporosis and T2DM from healthy individuals.

Acknowledgements: The authors express their gratitude to all the participants who agreed to take part in this study.

Author Contributions: Manal F. AL-Khakani contributed to the design and conceptualization of the study, as well as the execution and planning of the statistical analysis. Muna Sadeq Hameed provided the data for the study. The authors take full responsibility for all aspects of the research, including addressing any concerns regarding the accuracy or integrity of any part of the study and conducting appropriate investigations.

Conflict of Interest: The authors state that they have no conflicts of interest related to the publication of this manuscript.

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