

Assessment of Total Procollagen Type 1 Intact N-terminal Propeptide, C-telopeptide of type 1 collagen, Bone Mineral Density and its Relationship to Body Mass Index in Men with Type 2 Diabetes

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Abstract:

Background: Type 2 diabetes negatively affects the biochemical parameters of bone turnover more than obesity and is associated with an increased risk of osteoporosis and fragility fractures. Obesity and type 2 diabetes (T2DM) are linked to increased fracturing risk; however, the effect of obesity on diabetes-related bone deficit is unknown.

Objective: The goal of this research is to compare the indications, bone density, and bone turnover in T2DM men and a control group, and to investigate the effect of body mass index on bone turnover levels.

Subjects, Material and Method: This case-control study was conducted on 120 men whose ages were from 40 - 69 years. They were grouped into two categories: T2DM (n=80) and healthy control (n=40). Serum samples from both groups were analyzed for blood glucose, Calcium and Albumin, by using (Cobas c111), PTH by (Cobas e 411), P1NP and CTX-1 levels in the serum using ELISA kits. Participants underwent (DEXA) measuring Bone Mass Density (BMD) at the lumbar spine.

Results: The control (obese and non-obese) participants had statistically significant higher levels of CTX-1 and P1NP than the patients (obese and non-obese) (P-value = 0.000). There was no significant differences in Spine BMD, T-score (p-value = 0.27 and 0.37 respectively).

Conclusion: Men with T2DM had a low bone turnover level and deteriorated bone quality compared to controls. The obese healthy controls can maintain healthy bone metabolism if T2DM is prevented.

Keywords: Type 2 diabetes, bone turnover markers (CTX-1, P1NP), bone mineral density, osteoporosis, osteopenia, DEXA.

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Introduction:

The World Health Organization (WHO) defines Diabetes as a "metabolic illness with numerous etiologies characterized by chronic hyperglycemia caused by abnormalities in insulin synthesis, insulin action, or both (1). T2DM is linked to a number of risk factors, including genetic influences, environmental influences, age, obesity, and lack of physical fitness (2). DM has been identified as a major risk factor for osteoporosis because it affects the skeletal system and bone metabolism through multiple pathways (3). Osteoporosis is a progressive systemic skeletal

condition that affects postmenopausal women. Low bone mass is a sign of osteoporosis and spatial micro-architectural bone tissue degeneration, with fractures being the most common result. (4). Obesity is considered an abnormal or excessive fat accumulation, which is known as a common osteoporosis risk factor and (T2DM) (5). Body weight and body mass index are thought to be strong predictors of osteoporotic fractures. There have been several studies on the relationship between body mass index and bone mineral density in women, but only a few on men so far (6). The preservation of the skeletal system is determined by calcium, which is one of the essential minerals involved in bone homeostasis and is linked to increased bone minerals (7-8). Reduced (BMI) is a good risk factor for future fracture (9). Similarly, despite having a high BMI, one major public health goal is to raise awareness among patients and health care providers about the risk of osteoporosis (OP) as well as fractures (10). The parathyroid hormone is a hormone that affects both bone formation and resorption (11). Bone tissue is a

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living, porous, anisotropic substance (12), which is continuously modified by a process called bone remodelling (13). Human Total Procollagen Type I Intact N-terminal Propeptide (P1NP) and Human C-telopeptide of type I collagen (CTX-I) are produced during bone remodelling and are considered a bone turnover marker as recommended by the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (14). The early identification of a reduction in BMD in a diabetic patient may be helpful in preventing bone loss and future fracture risk. BMD has been identified as a key determinant of future fracture risks (15). Bone turnover markers can assess bone quality, making them a useful supplement to BMD, which can only assess bone mass in fracture risk assessments (16). Few studies compare whether bone quality parameters differ between middle-aged and older men with T2DM and controls (obese and non-obese) and thus, understanding how to maintain normal bone metabolism.

Subjects, Material and Method:

From November 2021 to the end of February 2022, 80 (males) attending the Specialized Center for Endocrinology and Diabetes in Al-Rusafa / Baghdad were diagnosed with T2DM. Diabetes mellitus is diagnosed according to the American Diabetes Association (ADA) and the WHO criteria of using either plasma glucose (FPG or OGTT) or HbA1c estimation. The FPG of 126 mg/dL (7.0 mmol/L), 2-h OGTT plasma glucose of 200 mg/dL (11.1 mmol/L), HbA1c of 6.5 percent (48 mmol/mol), or random plasma glucose of 200 mg/dL (11.1 mmol/L) with symptoms of hyperglycemia is diagnostic of DM. Patients with T2DM, insulin treatment, and comorbidities (such as hyperparathyroidism, hypo or hypercalcemia, and kidney failure) were excluded from this study. The study groups were 80 T2DM male patients as cases and 40 healthy male controls, with an age range of 40-69 years. The blood samples were centrifuged at (3000 rpm for 10 minutes) to separate and collect the serum which is used to assay the levels of serum calcium, glucose, Albumin by COBAS C 111 analyzer, and parathyroid hormone (PTH) by Cobas e 411 immunoassay analyzer. The remaining serum was stored at (- 20 C°) to be used for assays of CTX-1, and P1NP by Automatic ELISA. HbA1c estimation was done by COBAS C 111 analyzer. The WHO definitions of osteoporosis and osteopenia are based on T-Scores from dual-energy X-ray absorptiometry (DEXA) scans of the spine, hip, and forearm in postmenopausal white women, depending on the T-Score result they are classified as: normal $T \geq -1$, Osteopenia $-2.5 < T < -1$, Osteoporosis

$T \leq -2.5$ (17). Statistical Analysis: The collected data were statistically analyzed using the Spearman correlation coefficient to assess the relationship between numerical data. A P-value of less than 0.05 is regarded as significant. SPSS version 25 software was used for all statistical analyses. Permission to conduct the research was obtained from the Iraqi Ministry of Health at the Specialized Center for Endocrinology and Diabetes, Rusafa, Baghdad.

Results:

The results show that nearly half of the controls are overweight (47.5%), while (32.5%) were obese, and (20%) had normal BMI. As for the cases, there were slight differences in percentages, as (22.4%) had normal BMI, (38.8%) were overweight and another (38.8%) were obese. The DEXA results revealed that (82.5%) of the controls had normal BMD, and 17.5% had osteopenia, while the patient's group BMD was found to be normal in (80.0%), (18.7%) had osteopenia and osteoporosis was found in (1.3%), table (1).

Table (1): The distribution of cases and controls by BMI and DEXA scan result

Variables	Categories	Patients		Controls	
		No.	%	No.	%
BMI (kg/m ²)	Normal	18	22.4	8	20.0
	Overweight	31	38.8	19	47.5
	Obese	31	38.8	13	32.5
Disorder	Normal	64	80.0	33	82.5
	Osteopenia	15	18.7	7	17.5
	Osteoporosis	1	1.3	0	0
	Total	80	100.0	40	100.0

*BMI=Body mass index

Table (2) shows that the mean \pm SE age was significantly higher than the mean \pm SE of the control group ($P = 0.000$), while the mean \pm SE of BMI was not significantly different ($P = 0.97$). The mean \pm SE disease duration among the cases was 6.7 ± 0.49 years. The mean \pm SE of FBS and HBA1C among the patients was statistically higher than the control group $P = 0.000$ for both). There were no statistically significant differences in mean \pm SE of BMD and T-score between the two groups ($P = 0.27$ and 0.37 respectively). Similarly, there were no statistically significant differences in mean \pm SE levels of PTH, Ca, and albumin between the two groups ($P = 0.18, 0.56, 0.61$ sequentially).

Table (2): Mean± SD values of selected study variable in the two groups.

Variables	Patients (n=80)		Control (n=40)		P-value
	Mean ± SD	SE	Mean ± SD	SE	
Age (years)	52.0 ± 7.23	0.81	46.2 ± 6.63	1.09	0.000*
Duration of disease (years)	6.7 ± 4.44	0.49	-	-	-
FBS mmol/dl	11.3 ± 3.39	0.44	4.5 ± 0.42	0.06	0.000*
HbA1C%	8.7 ± 1.89	0.21	5.1 ± 0.22	0.03	0.000*
LSBMD g/cm ²	1.3 ± 0.26	0.03	1.2 ± 0.19	0.03	0.27
T-score	0.7 ± 2.11	0.23	0.3 ± 1.63	0.26	0.37
BMI (kg/m ²)	28.7 ± 3.77	0.42	28.7 ± 3.44	0.54	0.97
PTH pg/mL	38.9 ± 15.08	1.68	4.62 ± 12.99	2.05	0.18
Ca mg/dL	9.0 ± 0.51	0.05	9.1 ± 0.41	0.06	0.56
Albumin g/L	37.9 ± 3.06	0.34	37.6 ± 2.98	0.47	0.61

*p-value ≤ 0.05 significant

Table (3) shows the differences in mean and median levels of CTX-1 and P1NP between obese and non-obese participants for the cases and controls. Non-obese controls had significantly higher mean and median CTX

and P1NP levels than patients (P≤0.000 for both). Obese controls had significantly higher mean and median CTX and P1NP levels than patients (P≤0.000 for both).

Table (3): Mean±SD values of selected study variable in the two groups according to BMI

Parameter	Group	No.	Mean ± SD Median (1 st Q-3 rd Q)	SE	P-value
Non-obese	CTX-1 ng/ml	Control	24 16.5 ± 13.11 12.8 the (3.1-28)	2.523	< 0.000*† (< 0.000*†)
		Patient	49 2.2 ± 0.62 2.1 (1.8-2.5)	0.089	
	Total P1NP ng/ml	Control	24 918.7 ± 976.21 288 (96.0-2.40.0)	187.872	< 0.000*† (< 0.000*†)
		Patient	49 52.5 ± 11.86 51.0 (44.5-57.0)	1.695	
Obese	CTX-1 ng/ml	Control	16 16.8 ± 13.18 9.2 (6.3-27.9)	3.56	< 0.000*† (< 0.000*†)
		Patient	31 3.1 ± 1.611 2.5 (2.3-3.3)	0.289	
	Total P1NP ng/ml	Control	16 695.5 ± 690.56 122.6 (107.6-1379.5)	191.52	< 0.000*† (< 0.000*†)
		Patient	31 78.0 ± 98.14 58.08 (53.0-70.8)	17.627	

*p-value ≤ 0.05, † p-value of independent t-test, ‡ p-value of Kruskal-Wallis test

Discussion:

A variety of factors seem to contribute to the risk of bone circulation disorders. This study attempts to explore the relationship between BMI, bone mineral density and bone turnover markers in T2DM. The majority of both the cases and controls were overweight or obese, and the prevalence of osteoporosis was a close in the two groups, probably since both groups have a similar BMI distribution. The higher mean age among the cases than the controls may be explained by the fact that DM is more prevalent among the older than the younger groups. This study found no significant differences in mean ± SE of the lumbar spine bone mineral density (LSBMD) and T-score between the two groups, which agrees with previous studies (18) and may be due to the patients and controls having similar BMI (19). The LSBMD assessment by DEXA may be normal or high in obese and diabetic patients, which is an artefact that may impede scan interpretation because the large amount of soft tissue can augment the apparent density of bone and obscure bone edges, complicating

DEXA data analysis (20). A previous study (21) has found that an increased mechanical load on the bones of obese people, combined with hyperestrogenemia caused by increased aromatase activity in the expanded adipose tissue, are thought to account for the elevated BMD in this population. T2DM is associated with poor trabecular microarchitecture and bone strength in obese men. The higher fracture risk can be attributed to the effect of obesity on bone turnover markers (6). In the present study, the patient was selected based on the absence of diabetes complications with normal parathyroid and renal functions. Accordingly, there was no significant difference between the two groups in the level of PTH, Alb, and Ca used to evaluate the alteration of bone metabolism in the study groups without other complications. Obesity is a risk factor for the development of T2DM and insulin resistance (22) and its prevalence increases with increasing BMI (23). Therefore, the study of their isolated effects on the bone would be difficult. However, the combination of both may result in worse bone disease. In the current study,

the obese and non-obese men with T2DM had levels of bone turnover markers CTX-1, and P1NP lower than obese and non-obese controls, which agrees with other studies (21) and indicates that the T2DM patient experiences a higher shift in bone turnover than the obese control group, even though both groups are at risk of osteoporosis. Circulating biochemical markers of bone turnover, such as the bone formation marker P1NP and the bone resorption marker CTX-1, have been found to be lower in T2DM and may be predictive of fractures independent of BMD (24). The decreased bone turnover rate could explain the higher bone fragility in T2DM patients. With a lower bone turnover rate, the older bone is not replaced by new bone, resulting in a decrease in bone mechanical strength. (24).

Conclusion:

In men, although both T2DM and obesity are associated with osteoporosis. In this study, the effect of T2DM on bone turnover and quality was found to be greater than that of obese and non-obese healthy controls. It is possible, in an obese healthy population, to maintain healthy bone metabolism if the development of T2DM is prevented. Fractures may be predicted by BTM, independently of BMD.

Author's contributions:

Elaf Abed Oleiwi: Collecting samples, analysis of data, and writing the manuscript
Manal K. Rasheed: Study design and revision of the writing
Khalaf G. Hussein: Diagnosis and sampling

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تقييم إجمالي Procollagen من النوع 1 N-terminal Propeptide السليم ، C-telopeptide من النوع 1 من الكولاجين، كثافة العظام المعدنية وعلاقتها بمؤشر كتلة الجسم لدى الرجال المصابين بداء السكري من النوع 2

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الخلاصة

الخلفية: يؤثر مرض السكري من النوع 2 سلبيًا على المعلمات البيو كيميائية لدوران العظام أكثر من السمنة ويرتبط بزيادة مخاطر الإصابة بهشاشة العظام وكسور الهشاشة. السمنة ومرض السكري من النوع الثاني مرتبطان بزيادة مخاطر الكسور؛ ومع ذلك، فإن تأثير السمنة على عجز العظام المرتبط بالسكري غير معروف.

الهدف: كان الهدف من هذا البحث هو مقارنة المؤشرات، وكثافة العظام، ودوران العظام لدى الرجال المصابين بداء السكري النوع الثاني مع الرجال الاصحاء، وكذلك التحقيق في تأثير مؤشر كتلة الجسم على مستويات دوران العظام..

الحالات والمنهجية: تضمنت دراسة الحالات والشواهد هذه مائة وعشرين رجلاً تتراوح أعمارهم بين أربعين وتسعة وستين سنة وتم تقسيمهم إلى فئات: سكري النوع الثاني (عدد = ٨٠) والاصحاء (عدد = ٤٠). تم تحليل مصل الدم لقياس تراكيز سكر الدم والكالسيوم والألبومين والباراثايرويد هورمون باستخدام جهاز الكوباس، وفحوصات دوران العظام البيو كيميائية باستخدام تقنية الأليزا وخضع جميع المشاركون لفحص كثافة معادن العظم باستخدام جهاز الدكسا.

النتيجة: كان لدى المشاركين في مجموعة الاصحاء (البدناء وغير البدناء) مستويات دوران العظام البيو كيميائية عالية مقارنة بالمرضى (البدناء وغير البدناء)، بقيمة إحصائية قدرها (0.000)، وكذلك في المجموعتين لم يكن لديهم فرق كبير في كثافة معادن العظم وكان دلالتها الإحصائية قدرها (0.27), (0.37).

الخلاصة: الرجال الذين يعانون من داء السكري من النوع الثاني لديهم مستوى دوران منخفض للعظام وتدهور في جودة العظام مقارنة مع أولئك (البدناء وغير البدناء) في مجموعة الأصحاء. ويمكن للذين يعانون من السمنة أن يحافظوا على التمثيل الغذائي الصحي للعظام إذا تم منع الإصابة بداء السكري النوع الثاني.

الكلمات الرئيسية: داء السكري من النوع الثاني، علامات دوران العظام CTX-1، PINP، كثافة المعادن في العظام، هشاشة العظام