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ORIGINAL ARTICLE

Macrophage Colony Stimulating Factor as Predictive Marker of Osteoporosis in T2DM Patients

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ABSTRACT

Background: Diabetes mellitus and osteoporosis are two common medical disorders that are becoming more common as the population ages. T2DM patients have a higher fracture hazard, having a high BMD, which is primarily due to the raise hazard of falling. Macrophage colony-stimulating factor (M-CSF) is one of the hematopoietic growth factor family, and It plays an important function in fracture repair by attracting stem cells to the fracture site and influencing the production of hard calluses by promoting osteoclast genesis.

Aims of study: The purpose of this research was to assess the blood level of macrophage colony-stimulating factor in Iraqi osteoporotic patients with and without type 2 diabetes. in addition, that M-CSF may be a predictive marker for osteoporosis in T2DM patients

Subjects & Methods: This study was conducted between October 2021 to March 2022 in Medical City of Baghdad Teaching Hospital. The current study included 92 individuals (females and males) aged 40-65 years' old, 67 of them are patients and 25 as a control. The lumber spine's bone mineral density was determined using dual energy x-ray absorptiometry (DEXA)scan to diagnose these patients. Patients divided into (20) person as T2DM patients, (27) person as osteoporosis patients, and (20) as osteoporosis patients with T2DM

Results: The current study showed an important increase in serum M-CSF of osteoporosis patients with and without T2DM groups when compared with control, also, there was no significance increase in M-CSF level in T2DM patients comparing with control. Also, there was an important negative relation between M-CSF and bone mineral density (BMD) In osteoporosis patients, there was a substantial positive connection between M-CSF, FBS, and HbA1C.

Conclusions: The current study demonstrated that serum macrophage colony-stimulating factor (M-CSF) levels was significantly elevated in osteoporosis patients with and without T2DM, Therefore, this parameter may be a diagnostic marker for osteoporotic patients. In addition, that diabetic patients may be prone to osteoporosis, and M-CSF may be a predictive biochemical marker for development of osteoporosis in type 2 diabetic patients.

Keywords: Macrophage colony-stimulating factor, Osteoporosis, bone mineral density, Type 2 diabetes mellitus.

INTRODUCTION

Bone is a complex natural material with a complicated hierarchical multiscale organization, crucial to carry out its functions. Bones assist and protect the body's various organs, manufacture red and white blood cells, store minerals, provide structure and support to the body, and allow mobility [1] . Diabetes mellitus type 2 (T2DM) is a widespread metabolic condition globally. [2]. It affects bone homeostasis leading up to 3-fold elevated hip fracture hazard comparing to those healthy individuals. Type 2 diabetes mellitus and osteoporosis are frequent metabolic disease mainly affect older population and both of them belong to the most important causes of mortality and morbidity. Although, patients with T2DM have normal or raised BMD but they have an increased danger of fractures [3].

The most frequent form of metabolic bone disease is osteoporosis (OP), It is described as "a skeletal dysfunction characterized by diminished bone strength that predisposes the person to an increased risk of fracture." Bone strength is also characterized as "mainly reflecting the integration of bone density and bone quality" [4] . It is a systemic skeletal illness defined by low bone mineral density (BMD), defective bone mineralization or microarchitecture, and/or low bone strength; it is a symptomatic condition that goes misdiagnosed until a fracture occurs [5]. Osteoporosis is a gradual metabolic or skeletal condition that increases the risk of fracture owing to degradation in bone mass and microarchitecture. Osteoporosis is defined as bone mineral density less than 2.5 SD below the reference range in young individuals of the same gender (t score - 2.5). [6]. The practical definition of osteoporosis is a bone mineral density (BMD), as suggested by the World Health organization (WHO), by measuring dual-energy X-ray absorptiometry (DEXA). It gives information on variation in bone mineral contents considered as the standard process to measure bone mineral density and is beneficial for the follow up of bone mass and studying of bone mass variation in the same patient [7].

Macrophages play a significance role in the activation and production of osteoclasts and are distinguished from monocytes by macrophage colony-stimulating factor (M-CSF), also known as colony-stimulating factor-1 (CSF-1) [8]. It is known to have a significance role in fracture recovery, for instance by recruiting stem cells to the fracture site and affecting the formation of hard calluses by enhancing osteoclastogenesis [9]. M-CSF was first characterized as a hematopoietic cell growth factor that induces macrophages from bone marrow progenitors to form colonies in semisolid medium and was produced by a variety of cells including macrophages, endothelial cells (ECs), fibroblasts, and osteoblasts. Later, it was shown that osteoblasts and bone marrow stromal cells are the predominant M-CSF-generating cells in the bone microenvironment, producing both soluble and membrane-bound M-CSF. M-CSF. [10].

Aims of Study: The objective of this research was to compare the blood levels of macrophage colony-stimulating factor (M-CSF) between Iraqi osteoporotic patients with and without type 2 diabetes mellitus, and to investigate if M-CSF is a predictor of osteoporosis in T2DM patients.

MATERIALS AND METHODS

Study design: This study was performed between October 2021 to March 2022 in Baghdad province. The current study included 92 individuals (females and males) aged 40-65 years' old, 67 of them are patients and 2**5** as a control. By using a dual energy x-ray absorptiometry, the bone mineral density (BMD) of the lumbar spine was measured in these individuals who visited a clinic at the Medical City of Baghdad Teaching Hospital (DEXA).

The individuals were classification according to FBS and T.score to four groups as :

1 (25) individuals as healthy control (C).
2 (20) individuals as T2DM patients (D).
3 (27) individuals as osteoporosis patients without T2DM (Poro).
4 (20) individuals as osteoporosis patients with T2DM (Dporo).

Data included various types of medical and demographic information including age, gender, weight, and length. Samples and data collection is subject to the ethics of scientific research.

Exclusion criteria were patients with thyroid diseases as well as DEXA scans of other regions aside from the lumbar spine and hip joint.

Sample collection: After detecting illness using a DEXA equipment, five milliliters of venous blood were extracted from each subject. After an overnight fast, blood samples from patients and healthy volunteers were taken. Then serum and whole blood were stored at (-20 C°) for using later in laboratory assessments, which encompassed fasting serum glucose (FSG), glycated hemoglobin (HbA1c), and macrophage colony-stimulating factor (M-CSF).

METHODS

DEXA stands for Dual Energy X-ray Absorptiometry: The World Health Organization (WHO) has defined diagnostic criteria for charic osteoporosis and DXA fracture risk assessment. Osteoporosis is defined as a BMD value at the spine or hip that is more than 2.5 standard deviations below the optimum mean for healthy young persons of the same race and gender (T- score -2.5) [11].

Determination of Body Mass Index (BMI) kg/m2: Body mass index was a value resulting from a mass (weight) in kilograms and height in meter of a person. BMI wascalculated using below formula [12].

$$\mathbf{BMI} = \mathbf{weight(kg)} / (\mathbf{height\ (m)})^2$$

Determination of Fasting Blood Glucose (mg/dl): Fasting blood glucose was determined by enzymatic colorimetric method using glucose kit based on the PAP (phenol+ aminophenazone) enzymatic measurement of glucose from (Randox Company, France). [13], as per the manufacture′s instruction.

Determination of Glycated Haemoglobin (HbA1c) %: The kit was obtained from Stanbio (USA) [14], to determine the glycohaemoglobin that formed progressively and irreversibly in the erythrocyte during its 120-day life cycle.

Determination of M-CSF (ng/ml) Levels in Blood Serum: The enzyme-linked immunosorbent assay (ELISA) kit was purchased from (Fine Test, China) and used for determination of the levels of M-CSF. Sandwich ELISA (Human, Germany) format was employed and performed as per the manufacture′s instructions.

Statistical Analysis: The results were done using means±SD; t-test was used to estimate the variances between different sets. P-values of ($p > 0.05$) & respectively, were considered statistically significance and non-significance. The correlation coefficient (r) was examined and used to describe the link between the numerous characteristics under consideration.

RESULTS

The levels of age, BMI, BMD, M-CSF, FBS, and HbA1C levels in control (C), patients with T2DM(D), patients with osteoporosis (Poro), osteoporotic patients T2DM (Dporo) groups were summarizes in Table 1. The results which expressed as (mean± SD), showed no significance ($p > 0.05$) different in ages levels in both (D) and (Poro) patient groups when comparing with control group (C), while there was a significance ($p \leq 0.05$) different in ages levels in (Poro) and Dporo groups when compared with (D) group, in addition there was a significance ($p \leq 0.05$) different between (Poro) and (Dporo) patients' groups.

The mean values for BMI in the same table show there were no significance difference ($p > 0.05$) between all control and patient's groups. Also, there is no significance difference in the BMD between diabetes group (D) and control group (C), while there was a significance ($p \leq 0.05$) different in BMD levels in both (Poro) and (Dporo) patient groups when comparing with control group (C), As well was a significance ($p \leq 0.05$) different between D with Poro and Dporo patients, in addition there was a significance ($p \leq 0.05$) different between (Poro) and (Dporo) patient's groups.

In the current study show no significance ($p > 0.05$) different in M-CSF levels in (D) patient groups when comparing with control group (C), while there was a significance ($p \leq 0.05$) different in M-CSF levels in (Poro) and Dporo groups when compared with (D) group, in addition there was a significance ($p \leq 0.05$) different between (Poro) and (Dporo) patient's groups.

The mean value of serum FBS and HbA1C levels in Table 1, showed no significance ($p > 0.05$) different in (Poro) patient groups when comparing with control group (C), and (Dporo) patient groups when comparing with (D) group, while there was a significance ($p \leq 0.05$) different in FBS and HbA1C levels in (D) and (Dporo) groups when compared with control group (C), Poro with D, in addition there was a significance ($p \leq 0.05$) different between (Poro) and (Dporo) patient's groups.

Table 1: Mean±SD of studied parameters levels in control and patient's groups.

Groups / Parameters	Control No.(25)	DM patients No.(20)	Poro patients No.(27)	Dporo patients No.(20)
Male/Female	9/16	7/13	7/20	8/12
Age (years)	47.2 ± 10.09	48.37 ± 6.81 aNS	52.52 ± 8.36 aNS bS	53.38 ± 6.69 aS bS cNS
BMI (kg/m2)	28.34 ± 3.3	30.82 ± 6.02 aNS	28.457 ± 3.54 aNS bNS	30.84 ± 5.79 aNS bNS cNS
BMD (g/cm2)	1.05 ± 0.05	1.04 ± 0.06 aNS	0.67 ± 0.08 aS bS	0.73 ± 0.06 aS bS cS
M-CSF (ng/ml)	272.4±124.9	313.38 ± 63.12 aNS	952.56±235.41 aS bS	777.12±217.54 aS bS cS
FBS (mg/dl)	85.5±8.31	170.81±73.57 aS	88.89±9.71 aNS bS	159.84±29.07 aS bNS cS
HbA1C %	5.45 ± 0.51	8.13 ± 1.21 aS	5.32 ± 0.58 aNS bS	8.13 ± 1.21 aS bNS cS

Significant (S): $p \leq 0.05$; Non-significance (NS): $p > 0.05$

a:t-test between control and patient's groups; b: t-test between D with Poro and Dporo patients groups;

and c: t-test between Poro and Dporo patient's groups Correlation of M-CSF with BMI, BMD, FBS and HbA1C

Correlation coefficients (r) and p- values between serum M-CSF with BMI, BMD, FBS and HbA1C ratio in all osteoporosis patients' groups with or without T2DM shown in Table 2.

There was a highly significance ($P \leq 0.001$) positive correlations between serum M-CSF with BMI ($r = 0.18$), FBS ($r = 0.27$) and HbA1C ($r = 0.38$), and highly significance negative correlation between M-CSF with BMD ($r = -0.01$) in control group (C).

Macrophage colony-stimulating factor showed a highly significance ($p<0.001$) positive correlation with BMI ($r =0.17$) and BMD ($r = 0.13$), also there are a highly significance negative correlation between M-CSF with FBS ($r = -0.17$) and HbA1C ($r = -0.02$) in T2DM group.

In osteoporosis group (Poro), there was a highly significance ($p<0.001$) positive correlation between M-CSF with BMI ($r = 0.38$), FBS ($r = 0.05$) and HbA1C ($r =0.40$), in addition to a highly negative ($r = -0.32$) correlation between M-CSF and BMD.

In the current study, there was a highly significance ($p<0.001$) positive correlation between M-CSF and BMD ($r = 0.04$), also there are a highly significance negative correlation between

M-CSF with BMI (r = - 0.17), FBS (r = - 0.16) and HbA1C (r = - 0.27) in osteoporosis patients with T2DM group (Dporo).

Table 2. Correlation coefficient (r) and P-value between M-CSF level and others studied parameters

Groups Parameters	Correlation coefficients (r)				P-value			
FBS	0.27	-0.17	0.05	-0.16	0.000	0.000	0.000	0.000
HBA1C	0.38	-0.02	0.40	-0.27	0.000	0.000	0.000	0.000

Highly Significant: $p \leq 0.001$

DISCUSSION

Osteoporosis is a metabolic skeletal disease characterized by reduced bone fine structure and quantity, generally as a consequence of an increase in osteoclastogenesis and/or an increase in osteoclastic bone resorption, culminating in uncontrolled bone loss in postmenopausal women. The balance between bone resorption by osteoclasts and bone formation by osteoblasts is critical for bone homeostasis [15].

Serum M-CSF levels in healthy adults vary from around 12 ng/mL in their early twenties to about 20 ng/mL in their nineties, which matches to the concentration range where we detected a three- to four-fold increase in resorption in vitro. C-telopeptide, a serum bone resorption marker, rises approximately linearly in women aged 21 to 71, although bone mineral density declines dramatically with age. As a consequence, increasing circulating M-CSF may play a role in "normal" age-related bone loss. Furthermore, as previously stated, M-CSF levels are raised in a variety of inflammatory and neoplastic illnesses associated with bone loss, both systemically and locally [16].

The risk of developing osteoporosis increased with age, with the post-old group having a 6.2 times greater likelihood of having osteoporosis than the pre-old group. This is consistent with a maturing concept that said that the pinnacle bone mass is attained during the 30s in the bone remodeling process. After the age of 30, bone mass thickness decreases due to an increase in osteoclast activity, which dwarfs the osteoblast. As a result, the bone remodeling process failed to achieve the optimal level of bone strength. This event increases the likelihood of bone thickness disintegration (osteopenia) and the risk of bone corruption (osteoporosis) [17].

Obesity and intensive physical exercise lowered the prevalence of osteoporosis in general. Obesity reduced the risk of osteoporosis by 70%, but rigorous physical exercise reduced the risk by 50%. Osteogenesis is stimulated by muscles that apply greater mechanical pressure, such as osteoblast activity stimulation on the skeletal. BMI is important in the prevention of osteoporosis. Mechanical pressure, gravity, and stretching may assist preserve mineral homeostasis and accelerate bone formation by decreasing apoptosis and increasing osteoblast and osteocyte proliferation. [18].

T2DM was connected favorably with osteoporosis occurrences in a Taiwanese population, with the association being stronger among the elderly, and T2D was strongly linked to the risk of osteoporosis., regardless of gender non-elderly participants, in particular [19]. Osteoporosis can affect men and women, especially those who are older. Diabetes has a number of different impacts on bones. Prior research on the link between T2DM and the danger of bone mineral density (BMD) loss has been contradictory, with much of evidence pointing to a higher baseline BMD in T2DM patients [20, 21]. Aging and lifestyle changes have a deleterious impact on both T2DM and osteoporosis. Most importantly, multiple studies have shown that the risk of fracture increases in individuals with T2DM, being high with long peroid of T2DM, poor glycemic control, and the presence of diabetes comorbidities [22].

The main findings on the influence of T2DM on BMD are described in table 1. Because the development of T2DM is delayed and may range from 5 to 10 years before the actual diagnosis of T2DM, the phrase "time since diagnosis" is more accurate than "duration of illness." The biggest research on BMD in T2DM, which included 792 older persons with T2DM who had DXA-based BMD and fracture data, found that the presence of treated T2DM increases the risk of fracture despite increased BMD at the femoral neck and lumbar spine. Only treated T2DM patients had an increased fracture risk, whereas individuals with impaired glucose tolerance had a decreased fracture risk [23]. This is consistent with the findings of Strotmeyer et al. [24]. These results were also confirmed in a study of younger individuals. In Korea research of 185 T2DM women, lumbar spine BMD was somewhat higher than in a healthy and age-matched control group, and BMD values were negatively associated with age, years after menopause, and, to a lesser degree, illness duration. [25].

According to this research, T2DM may impact various bone areas differently. Another research found that in Japanese people with T2DM, mean T-scores were 0.8 lower in 64 men and 1.1 lower in 81 women compared to 95 nondiabetic controls in cortical bone locations like the distal radius. [26]. There was no link between BMI and OP in the current study. Few other studies, like ours, have been unable to find any link between BMI and osteoporosis [27, 28]. Higher BMI, on the other hand, appears to protect against OP, as increased mechanical loading of the bones leads to increased bone mass. [29].

In the current study, there was an important decrease in BMD in osteoporosis; The present study revealed BMD measured of osteoporosis is a better test than any other factor measured in the diagnosis of the disease, with incidence of low (BMD), Z-score and T-score in osteoporosis group more than controls, and this result is supported by other workers [30]

The results of the statistical analysis in table (1) for HbA1C showed there is no an important different between healthy groups and the osteoporosis without diabetes group, while there is an important difference when compared healthy groups and osteoporosis with diabetes group.

That means the HbA1c test provides valuable information that can be used for the management of diabetic. HbA1C is a valuable biomarker for long-term blood sugar control as well as a good indication of lipid profile. As a result, utilizing HbA1c to monitor blood sugar control may have additional advantages [31]. Increasing blood sugar prevents bone formation due to its effect on osteoblast by releasing large quantities of sclerostin which has a role in inhibiting osteoblasts [32].

The results of the statistical analysis in table (1) for FBS showed there is no an important difference between the healthy groups and the osteoporosis without diabetes group, and this is identical with the work in the study [33], while there is an important difference between the healthy individuals and osteoporosis with diabetes group that is also ensured in the following studies [34, 35].

The findings of the Jason et al. research reveal that M-CSF regulates many stages of human osteoclastogenesis, including precursor proliferation, differentiation, and fusion. M-CSF modulates osteoclastresorbing activity late in osteoclastogenesis, although it is not required for survival. Modulation of M-CSF signaling might be a therapeutic focus for bone resorption diseases. As a result, under physiological settings, M-CSF seems to have a major role in osteoclastic bone resorption [36]. Furthermore, as previously stated, M-CSF levels are raised in a variety of inflammatory and neoplastic illnesses associated with bone loss, both systemically and locally [37].

M-SCF has been shown to impact numerous aspects of osteoclastogenesis in human cells in vitro, including precursor proliferation, differentiation, and fusion, as well as mature osteoclast resorbing activity and cytoplasmic pervasion. Our

results, however, reveal that M-CSF is not required for osteoclast survival. It is conceivable to target M-CSF or it signaling pathways in the development of novel antiresorptive drugs.

Despite the knowledge that M-CSF is essential for osteoclastogenesis, no systematic investigation of how it influences human osteoclast growth and activity in vitro has been conducted. Many phases of human osteoclastogenesis have been reported to be influenced by M-CSF, including precursor proliferation, differentiation, and fusion. M-CSF modulates osteoclast-resorbing activity late in osteoclastogenesis, although it is not required for survival. Modulation of M-CSF signaling might be a therapeutic focus for bone resorption diseases. M-CSF production was increased by advanced glycation end product (AGE), which should result in an increase in monocyte synthesis and activation. Diabetes, for example, is associated with atherosclerosis and microangiopathy [38]. This study demonstrates that M-CSF is a major regulator of the inflammatory response and may regulate the production of proinflammatory cytokines by macrophages. M-CSF is thought to be the substance responsible for increased inflammation and bone resorption in type 2 diabetes inflammatory tissue development [39]. M-CSF, also known as CSF-1, was identified in serum, urine, and other physiological fluids as a chemical that stimulates macrophage colony formation by hematopoietic ancestor cells in the bone marrow. M-CSF is a growth factor that stimulates the proliferation and differentiation of mononuclear phagocytes. M-CSF is a development operator that assists in the differentiation of osteoclast precursors, which is necessary for osteoclastogenesis. [40].

Conclusions: When compared to prior studies, this is the first to investigate the relationship between M-CSF and type 2 diabetes mellitus in osteoporosis patients. The present investigation found that serum macrophage colony-stimulating factor (M-CSF) levels in osteoporosis patients with and without T2DM were considerably higher., Therefore, this parameter may be a diagnostic marker for osteoporotic patients. In addition, the significance negative correlation between M-CSF with bone mineral density (BMD), and the significance positive correlation between M-CSF with FBG and HbA1C indicate that diabetic patients may be prone to osteoporosis, and M-CSF may be a predictive biochemical marker for development of osteoporosis in type 2 diabetic patients.

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