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The Extensive Study of Magnesium Deficiency, 25-(OH) Vitamin D3, Inflammatory Markers, and Parathyroid Hormone in Relation to Bone Mineral Density in Iraqi Osteoporosis Patients: A Cross-Sectional Study

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ABSTRACT

Backgrounds and Aims: Magnesium is essential for bone development and mineralization and may influence osteoporosis progression. However, its relationship with low bone mineral density (BMD) and fracture risk is not well understood. This study aimed to identify the primary risk factors and the effect of magnesium deficiency on bone density in osteoporosis patients.

Methods: The study involved 162 adults categorized into normal, osteopenia, and osteoporosis groups, plus 50 healthy individuals. BMD of the lumbar spine (L1–L4) and femur neck, body mass index, and T-scores were assessed via dual-energy X-ray absorptiometry, while serum magnesium, 25-(OH) Vitamin D3, inflammatory markers, and other clinical tests were measured. The results showed significant variations in BMD, T-scores, magnesium, and vitamin 25(OH)D levels.

Results: Notably, osteoporosis patients exhibited a substantial decline in mean BMD along with an increase in mean T-scores. They also had significantly lower serum levels of magnesium, vitamin 25(OH)D, and calcium, compared to other groups, while parathyroid hormone levels slightly increased. Inflammatory markers were significantly elevated in osteoporosis patients. Magnesium and vitamin 25(OH)D showed an inverse relationship with T-scores and a direct positive correlation with BMD and bone mineral content. Additionally, a negative correlation between magnesium and inflammatory markers was observed. The findings highlighted a strong correlation between magnesium deficiency and osteoporosis, with a more significant odds ratio compared to factors like 25(OH)D, PTH, BMD, T-score, and calcium.

Conclusion: Magnesium deficiency has a more pronounced impact on bone health than vitamin D deficiency. Thus, magnesium deficiency emerges as a major risk factor for osteoporosis progression and a predictor of fracture incidence in patients with osteoporosis or osteopenia.

1 | Introduction

Osteoporosis is a serious problem that is increasingly influencing the global population's health standards; the incidence of osteoporotic fracture is gradually increasing, mainly in females [1]. Osteoporosis is a skeletal disorder characterized by compromised bone strength, predisposing a person to an increased risk of fracture [2]. According to a 2016 study by Angela, a dual-energy X-ray absorptiometry scan (DXA) primarily measures bone mineral density (BMD) to diagnose osteoporosis. It can also be identified through minimal trauma fractures, especially in the hip or vertebra [3]. Low vitamin D state and decreased exposure to sunlight contribute to malabsorption of calcium and vitamin D insufficiency, leading to osteoporosis; these factors are needed to maintain calcium homeostasis, skeletal integrity, and muscle strength [4]. Parathyroid hormone regulates $1,25(OH)_2D$ production, with serum calcium and phosphate also affecting it [5]. Low 25-(OH)D levels can lead to secondary hyperparathyroidism, causing low BMD and bone turnover [6, 7]. Nevertheless, studies show that not all patients with hypovitaminosis D, develop secondary hyperparathyroidism [8, 9].

In recent years, there has been increasing attention to understanding the relationship between trace elements and vitamin deficiencies associated with osteoporosis [10]. Regarding it, it is known that calcium, phosphorus, vitamin D, magnesium (Mg), and fluoride are crucial nutrient factors for osteoporosis prevention, with Mg being the least reported among these factors. In recent years, there has been increasing attention to understanding the relationship between trace elements and vitamin deficiencies associated with osteoporosis [10]. Regarding it, it is known that calcium, phosphorus, vitamin D, magnesium (Mg), and fluoride are crucial nutrient factors for osteoporosis prevention, with Mg being the least reported among these factors. The development of osteoporosis can be caused by deficient Mg, with several mechanisms involved. For example, when the mechanism of hydroxyapatite is altered, the bone mineralization process is affected. Also, the bone turnover rate can be enhanced by stimulating the osteoclasts' activities. Another mechanism is the alterations in the levels and functions of parathyroid hormone (PTH) and $1,25(OH)_2$-vitamin D, which might damage Ca homeostasis and ultimately, cause hypocalcemia. Also, osteopenia can be a result of changes in the levels of pro-inflammatory cytokines. One additional mechanism was reported to occur through triggering endothelial dysfunction [11, 12].

The literature included only a limited number of studies examining the relationship between low BMD and magnesium deficiency. We designed our study to identify the primary risk factors for fracture incidence in osteoporosis and explore the potential correlation between magnesium deficiency and reduced bone density, considering that osteoporosis has emerged as a significant public health concern affecting individuals across all age groups.

2 | Materials and Methods

2.1 | Study Design

A cross-sectional investigation was conducted on osteoporosis patients in Basrah city, south of Iraq, for the period from September 2022 to February 2023. Ethical approval was obtained from the ethical commitee of College of Pharmacy, University of Basrah, Iraq with reference No. CPBU208, December 31, 2023. The study complied with the ethical standards of the Helsinki Declaration of the World Medical Association in 2013. All participants were informed of the study objectives and provided written consent letters. A questionnaire form was utilized to collect basic data from the included subjects. The form covered several demographic (gender, age), lifestyle (smoking, alcohol), and health information (medications, genetic disorders, and other disorders such as those of blood, hypertension, diabetes mellitus, and kidney.

2.2 | Patients

Out of the 212 participants, the study included 162 patients (males and females) ranging in age from 21 to 80 years, who were divided into three groups based on the diagnostic guidelines for osteoporosis; the T-score for BMD was defined according to the World Health Organization definitions that use T-score evaluation for normal, osteopenia, and osteoporosis. The first group of patients was 42 with normal (40 females and 2 males); the second group included 62 osteopenia patients (57 females and 5 males); the third group included 58 patients with osteoporosis (56 females and 2 males), and the fourth group included 50 healthy subjects who were regarded as controls (47 females and 3 males), as shown in Figure 1. Each participant provided written informed consent. All subjects underwent a detailed medical examination, with BMD measurements at the lumbar spine's (L1–L4) site and femur neck. A detailed history and physical examination were performed on each subject using a pre-designed form. The collected information included physical examination, vital parameter assessment, anthropometry, and systemic examination. For patients who were confirmed to have osteoporosis, both clinical and epidemiological sorts of information were gathered in a private clinical laboratory and consulting clinic, followed by reviewing and verification by a specialized rheumatologist, as shown in Figure 1.

2.3 | Exclusion Criteria

We excluded 138 subjects from the study for several reasons, including: subjects with estrogen replacement therapy within a year, abnormal thyroid function, and significant liver disease. The exclusion criteria also encompassed regular treatment with phosphate-binding antacids and any other medication that impacts the skeleton, such as steroids, anti-resorptive therapy, anticonvulsants, anticoagulants, coronary disease, cancer, kidney or liver failure, permanent arrhythmias, drug-resistant tuberculosis, acute rheumatic fever or rheumatic disease (acute phase), pulmonary arterial hypertension (greater than grade 2), decompensated heart disease, and advanced-stage mental illness. Children, individuals under 20 years, pregnant women, those with chronic illnesses or suffering from cancer, people taking supplements like vitamin D, calcium, or magnesium or all of them were excluded from this study, as shown in Figure 1.

2.4 | Bone Mineral Density

Assessment of BMD (g/cm^2) was achieved at the specified spine site with the use of dual-energy X-ray absorptiometry (DEXA,

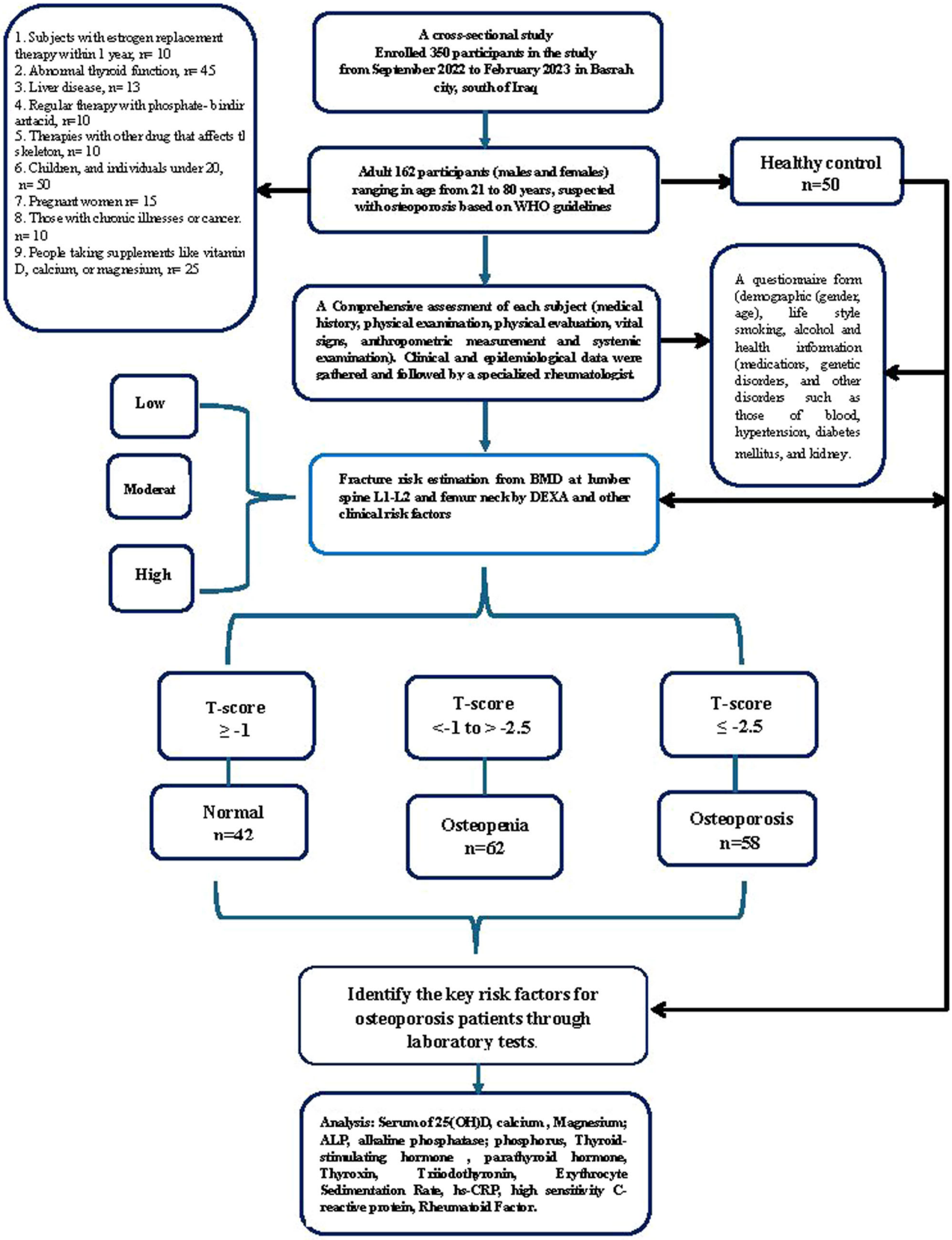

FIGURE 1 | Flowchart for diagnosing and identifying risk factors related to osteoporosis.

stratos, MEDIX90, DMS, France). Osteoporosis was diagnosed according to the WHO guidelines for the following: T- score categories; −2.5 SD or below the mean: osteoporosis; −1.0 and −2.5 SD: osteopenia; −1.0 SD or higher: normal.

2.5 | Body Mass Index

BMI is a measure for indicating nutritional status in adults. It is defined as a person's weight in kilograms divided by the square of the person's height in meters (kg/m^2). Patients were classified based on the WHO system for classification of obesity into underweight (BMI < 18.5 Kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2) and obesity (BMI ≥ 30.0 kg/m^2).

2.6 | Sample Collection

The participant provided 5 mL of whole blood via venipuncture during their morning visit to the private clinical laboratory and consulting clinic. Two milliliters of blood were placed in an EDTA tube (AFCO, Jorden) for complete blood count analysis, followed by 3 mL in simple gel tubes. The full blood samples were permitted to coagulate at room temperature (18°C–25°C) for 15–30 min. The serum was then isolated from the blood by centrifugation at 1000-2000 g for 10 min. Serum aliquots (1.5 mL) were preserved in microcentrifuge tubes and subsequently divided into three Eppendorf tubes (Eppendorf, Germany) with 0.5 mL in each, then stored in deep freeze at −80°C (Vest froze, Denmark) in the consulting clinic.

2.7 | Enzyme-Linked Immunosorbent Assay

2.7.1 | Determination of Quantitative 25-(OH) Vitamin D3

Vitamin D3 was evaluated in the sera of all participants by utilizing enzyme-linked immunosorbent assay (ELISA) technique by following the steps clarified in the kit leaflet (Euroimmun, UK). According to the measured levels of 25(OH) D, the participants were categorized into severe deficient (< 10 ng/mL), deficient (10–20), insufficient (20–30 ng/mL) and sufficient (> 30 ng/mL).

2.7.2 | High-Sensitivity C-Reactive Protein (hs-CRP) Test

The concentrations of hs-CRP in the sera of patients and healthy controls were determined by utilizing ELISA based on the guidelines specified by the hs-CRP ELISA kit (Demeditec, Germany), which included several ready-to-use components, namely antibody-coated wells, CRP conjugate reagent, TMB reagent, stop solution, hs-CRP sample diluent, and reference standard set. Hs-CRP values higher than 3.0 mg/L were regarded as high-risk.

2.8 | Erythrocyte Sedimentation Rate (ESR) Test

Unspecific screening of osteoporosis was achieved by applying the Westergren method to measure the erythrocyte sedimentation rate (ESR; in mm/h) [13].

2.9 | Latex Agglutination Slide

Additional laboratory tests for general health assessment were evaluated, including the RF latex agglutination slide test, which was performed using latex test kits (Salucea, Netherlands) according to the manufacturer's instructions. The normal reference value for RF is 0–20 IU/mL.

2.10 | Clinical Biochemical Tests

After fasting for 8–11 h, we collected serum from venous blood specimens (about 2 mL in plain tubes) for analysis. Biochemical inclusion criteria included serum levels of calcium (mg/dL), magnesium (mg/dL), phosphorus (mg/dL), alkaline phosphatase (U/L), parathyroid hormone (PTH) (pg/mL), thyroid-stimulating hormone (TSH) (μU/mL), triiodothyronine (T3) (nmol/L), and thyroxine T4 (nmol/L) that are within the normal ranges. We utilized an Abbott diagnostic machine (Architect, USA) as a fully automated system, following the guidelines provided by the manufacturer (Architect, USA).

2.11 | Statistical Analysis

Statistical Package for Social Sciences software version 24.0 (SPSS Inc, Chicago, IL, USA) was used to analyze the collected data, which initially displayed abnormal distribution. Normality testing for quantitative data was conducted using the Shapiro–Wilk and Kolmogorov–Smirnov tests. Continuous and categorical variables were reported as frequencies and percentages (n, %). Study group results (normal, osteopenia, osteoporosis, and healthy control) were presented as mean (standard deviation) and analyzed using one-way ANOVA, with significance assessed through two-tailed paired tests. Post Hoc test (Least Significant Difference LSD) is a statistical method for identifying significant differences between group means following an ANOVA. It calculates the smallest significant difference between two means, facilitating pairwise comparisons among groups, with significance set at $p < 0.05$. The Pearson correlation coefficient was used to examine correlations among variables. The odds ratio was used to determine the risk factors associated with osteoporosis, influenced by a 95% confidence interval (95% CI). p-values, with $p < 0.05$ was considered significant.

3 | Results

Table 1 describes the demographic and clinical characteristics of the total set of patients and the most important classifications approved in the study. A total of 162 patients with a mean age of 44.94 ± 13.50 years were studied. Among them, 153 (94.5%) were females and 9 (5.5%) were males. Overall, 58 (35.80%) of the total studied subjects showed osteoporosis. Osteoporosis frequency values between the two sex groups were significantly different ($p < 0.001$). Osteopenia was found among 62 (38.27%) of the subjects. According to the classification of BMI, the majority of patients have obesity, 88 (54.33%), with BMI of (≤ 30 kg/m^2). The fracture type value without BMD was 3.31%, and the fracture risk BMD values at spines L1–L4 were 0.71 ± 0.07, 0.90 ± 0.13, and 1.04 ± 0.05 g/cm^2, respectively, as shown in Table 1. Moreover, it was noted that most patients, that is, 60 (37%), were suffering from

TABLE 1 | Patient demographic and clinical characteristics.

Characteristic	Number	Percentage (%)	
Number of total patients	162		
Male	9	5.5	
Female	153	94.5	
Age, (years)	*44.94 (13.50)		
Range	(21–80)		
Body mass index BMI (kg/m^2) categories	*30.87 (5.86)		
Underweight	0		< 18.5 (kg/m^2)
Normal	27	16.67	18.5–24.9 (kg/m^2)
Overweight	47	29.01	25–29.9 (kg/m^2)
Obesity	88	54.33	≥ 30 (kg/m^2)
Fracture type	Without BMD%	With BMD%	
Osteoporatic	3.31	NC	
Bone mineral density g/cm^2			
Lumber spine (L1–L4) and femur neck	0.908 (0.15)	98.76	
Fracture risk BMD (g/cm^2) at spine/L1–L4			
Low	*1.04 (0.05)		
Moderate	0.90 (0.13)		
High	0.71 (0.07)		
Bone mineral density categorization			
Normal	42	25.93	T score ≥ −1.0
Osteopenia	62	38.27	−2.5 < T-score < −1.0
Osteoporosis	58	35.80	T-score ≤ −2.5 with facture
25(OH)D(ng/mL) categorization			
Severe deficiency	60	37	< 10
Deficiency	58	36	10–20
Insufficiency	24	12	20–30
Sufficiency	20	15	> 30
Exposure to sunlight			
< 20 min daily	122	75.30	
≥ 20 min daily	40	24.70	

Note: Categorical and continuous variables are expressed as the mean (SD) and number (%), respectively.
*$p < 0.05$.

severe vitamin D deficiency (< 10 ng/mL), while the deficiency, insufficiency, and sufficiency were 36%, 12%, and 15%, respectively. There were also significant differences between frequencies of vitamin D levels ($p < 0.001$), as shown in Table 1.

Table 2 shows the results of the comparison between the three main patient groups that were classified according to their DEXA scan T-score (normal, osteopenia, osteoporosis) and healthy control. Through the results of our study, it was confirmed that the proportion of females is higher than that of males for all groups of patients and healthy subjects. The overall mean age of subjects in the three patient groups (21–80 years) was 41.14 ± 11.98 years, as shown in Table 2. The results also confirmed that there is no significant difference in the BMI (kg/m^2) among the three patient groups, but a significant difference was found when comparing them with the healthy control group ($p < 0.001$), as demonstrated in Table 2. Moreover, from the data listed in Table 2, it is apparent that there were significant differences among patient groups in both BMD and T-score ($p < 0.001$). The mean value of BMD (−0.71 ± 0.07 g/cm^2) showed a significant decline in osteoporosis patients as compared to normal, osteopenia, and healthy control groups (0.90 ± 0.13, 1.04 ± 0.05, and 1.20 ± 0.33 g/cm^2, respectively, $p < 0.001$). This decline in BMD was accompanied by an increase in the mean T-score (−3.0 ± 0.52) for osteoporosis patients, with the results of the groups being significantly different from each other ($p < 0.001$).

The results also show that serum levels of 25(OH)D, calcium, and magnesium (10.73 ± 5.31 ng/mL, 7.78 ± 0.72 mg/dL, and 3.32 ± 0.06 mg/dL, respectively) were significantly lower among those suffering from osteoporosis when compared with normal, osteopenic, and healthy control groups ($p < 0.001$; Table 3). The

TABLE 2 | Comparative analysis of patients' distribution based on DEXA scan T-score.

	Bone mineral density categorization (no. of cases)											
Parameter	**Group 1 Control**	**Group 2 Normal**	**Group3 Osteopenia**	**Group 4 Osteoporosis**	[a]***p* value**	[b]**2 vs. 3**	**3 vs. 4**	**4 vs. 2**	**4 vs. 3**	**1 vs. 4**	**2 vs. 4**	**3 vs. 1**
No. of patients	50	42	62	58		—	—	—	—	—	—	—
Sex, F/M	47/3	40/2	57/5	56/2	0.001	—	—	—	—	—	—	—
Female (%)	94	95.24	91.94	96.56	—	—	—	—	—	—	—	—
Male (%)	6	4.76	8.06	3.44	—	—	—	—	—	—	—	—
Age (year)	40.72 (12.09)	41.14 (11.98)	45.38 (14.58)	49.36 (12.10)	0.001	0.091	0.132	0.007	0.134	0.004	0.007	0.06
Range	21–67	21–64	21–67	33–80	—	—	—	—	—	—	—	—
Height (cm)	159.90 (5.57)	160.07 (5.52)	160.20 (6.73)	158.2 (6.20)	0.6	0.911	0.145	0.206	0.145	0.244	0.206	0.805
Weight (Kg)	80.04 (15.90)	80.52 (16.09)	77.08 (16.11)	75.46 (14.18)	0.001	0.269	0.641	0.175	0.641	0.214	0.175	0.334
BMI (kg/m^2)	31.38 (6.14)	31.51 (6.25)	30.20 (5.63)	30.93 (5.83)	0.001	0.261	0.574	0.676	0.574	0.002	0.676	0.001
BMD (g/cm^2)	1.20 (0.33)	1.04 (0.05)	0.90 7(0.13)	−0.718(0.07)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
BMC (g)	57.19 (6.85)	57.32 (6.93)	51.55 (9.72)	40.05 (6.72)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
T-score	0.48 (0.04)	0.24 (0.07)	−1.4 (0.15)	−3.0 (0.52)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Z-score	0.19 (0.10)	0.20 (0.10)	0.803 (0.16)	−2.166 (0.84)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Abbreviations: BMC, bone mineral contents; BMD, bone mineral density; BMI, body mass index; F, female; M, male.
[a]*p* value was assessed using one-way analysis of variance (ANOVA). Categorical and continuous variables are expressed in terms of mean (standard deviation) and numbers (%).
[b]Calculates mean differences between two groups using post hoc test (least significant difference LSD), with significance at the $p < 0.05$ level.

TABLE 3 | Comparison of values of inflammatory markers, minerals, and biochemical parameters among the studied groups categorized according to their DEXA scan T-score.

	Bone mineral density categorization											
Parameter	**Group 1 Control**	**Group 2 Normal**	**Group3 Osteopenia**	**Group 4 Osteoporosis**	**[a] P value**	**[b] 2 vs. 3**	**3 vs. 4**	**4 vs. 2**	**4 vs. 3**	**1 vs. 4**	**2 vs. 4**	**3 vs. 1**
25(OH)D (ng/mL)	36.49 (9.57)	22.17 (16.96)	13.23 (9.54)	10.73 (5.31)	0.001	0.001	0.314	0.001	0.314	0.001	0.001	0.001
Ca (mg/dL)	10.15 (0.66)	9.30 (1.01)	9.03 (1.05)	7.78 (0.72)	0.001	0.156	0.001	0.001	0.001	0.001	0.001	0.001
Mg (mg/dL)	5.21 (0.49)	5.20 (0.48)	4.22 (0.42)	0.32 (0.06)	0.001	0.777	0.001	0.001	0.001	0.001	0.001	0.03
ALP (U/L)	78.60 (17.31)	82.42 (12.20)	90.39 (13.12)	101.09 (27.06)	0.007	0.139	0.445	0.597	0.445	0.429	0.597	0.071
P (mg/dL)	3.23 (0.87)	3.82 (0.22)	3.52 (0.75)	3.65 (0.65)	0.2	0.061	0.03	0.061	0.03	0.04	0.061	0.954
TSH (μU/mL)	2.48 (0.36)	2.56 (0.38)	2.64 (0.28)	2.58 (0.30)	0.8	0.780	0.740	0.940	0.740	0.988	0.940	0.711
PTH (pg/mL)	45.13 (10.41)	57.63 (18.55)	56.28 (14.96)	82.53 (20.22)	0.007	0.046	0.005	0.001	0.005	0.001	0.001	0.002
T3(nmol/L)	1.89 (0.31)	2.48 (0.57)	1.97 (0.46)	1.94 (0.95)	0.1	0.191	0.933	0.242	0.933	0.927	0.242	0.839
T4 (nmol/L)	104 (21.20)	108.3 (27.65)	105.70 (16.91)	95.35 (18.53)	0.001	0.01	0.02	0.01	0.02	0.081	0.01	0.675
Glucose (mg/dL)	101.07 (36.60)	109.25 (15.80)	111.25 (40.70)	123.80 (27.30)	0.1	0.154	0.154	0.166	0.839	0.433	0.166	0.244
ESR (mL/h)	11.52 (3.19)	22.5 (1.98)	27.83 (2.77)	29.16 (4.50)	0.002	0.140	0.741	0.01	0.839	0.001	0.01	0.001
hsCRP (mg/mL)	0.63 (0.03)	6.21 (1.56)	7.16 (1.78)	13.56 (0.37)	0.04	0.165	0.03	0.02	0.03	0.001	0.02	0.005
RF IU/mL	4.89 (0.54)	8.63 (0.30)	9.53 (1.04)	12.85 (1.61)	0.001	0.007	0.05	0.628	0.05	0.01	0.628	0.001

Abbreviations: 25(OH)D, 25-hydroxy vitaminD ALP, alkaline phosphatase; Ca, calcium; ESR, erythrocyte sedimentation rate; hs-CRP, high sensitivity C-reactive protein; Mg, magnesium; P, phosphorus; PTH, parathyroid hormone; RF, rheumatoid factor; T3, triiodothyronin; T4, thyroxin; TSH, thyroid-stimulating hormone.

[a]the p value was assessed between groups using one-way analysis of variance (ANOVA). Categorical and continuous variables are expressed in terms of mean (standard deviation) and numbers (%).

[b]Calculates mean differences between two groups using post hoc test (Least Significant Difference LSD), with significance at the $p < 0.05$ level.

TABLE 4 | The correlation between bone markers, inflammatory markers, and various parameters in osteoporosis patients with severe 25(OH)D deficiency.

	PC	BMI	BMD	BMC	CRP	25(OH)D	Ca	Mg	RF	ESR	PTH	ALP	T3	T4	Age
ESR	*r*	0.042	−0.294*	0.170	0.893	0.376**	−0.799**	−0.307*	0.179	1	0.826**	0.169	0.056	−0.285*	0.363**
	p	0.825	0.023	0.370	0.001	0.003	0.006	0.017	0.345		0.003	0.371	0.767	0.025	0.027
hsCRP	*r*	0.022	0.155	0.018	1	0.021	0.261	−0.660*	0.660**	0.893**	0.336	0.309	0.658**	0.702*	0.321*
	p	0.908	0.412	0.926		0.912	0.163	0.038	0.0001	0.001	0.069	0.097	0.001	0.002	0.014
RF	*r*	0.011	0.177	0.031	0.162	0.839**	−0.211	−0.783**	1	0.179	0.304*	0.303*	0.094	0.119	0.543*
	p	0.952	0.348	0.869	0.391	0.002	0.264	0.007		0.345	0.018	0.018	0.622	0.530	0.012

Abbreviations: 25(OH)D, 25-hydroxy vitamin D; ALP, alkaline phosphatase; BMC, bone mineral contents; BMD, bone mineral density; BMI, body mass index; Ca, calcium; ESR, erythrocyte sedimentation rate; hs-CRP, high sensitivity C-reactive protein; Mg, Magnesium; P, phosphorus; PTH, parathyroid hormone; RF, rheumatoid factor; T3, triiodothyronin; T4:thyroxin; TSH, thyroid-stimulating hormone.
*Pearson Correlation is significant at the $p < 0.05$ level (two-tailed).; **Pearson Correlation is significant at the $p < 0.01$ level (two-tailed).

data from Table 3 reveal that serum levels of magnesium (0.32 ± 0.06 mg/dL) were significantly lower in those suffering from osteoporosis when compared with normal, osteopenia, and healthy control groups ($p < 0.001$). The analysis of biochemical data revealed that one of the most interesting results is the slight increase in the mean level of PTH (82.53 ± 20.22 pg/mL) in osteoporosis patients ($p < 0.001$), whereas no significant differences were observed among the other groups, as shown in Table 3. In addition, differences in the parameters of ALP, P, TSH, T3, T4, and glucose among patients and healthy controls were insignificant, as listed in Table 3. Regarding the inflammatory parameters, as shown in Table 2, each of ESR, hs-CRP, and RF exhibited significant alterations, with a slight increase in osteoporosis patients when compared with other groups ($p < 0.05$).

The uniqueness of this study is reflected by the fact that there are many interrelationships between the factors adopted in the study. It appears from Pearson correlation coefficients that numerous correlations were conducted among the study variables, which revealed direct positive correlations between ESR and each of hs-CRP, 25(OH)D, PTH, and age ($r = 0.893$, $p = 0.001$; $r = 0.376$,$p = 0.003$; $r = 0.826$, $p = 0.003$ and $r = 0.363$, $p = 0.027$, respectively). While an inverse correlation was found between ESR and each of BMD, Ca^+ , Mg, and T4 ($r = -0.294$, $p = 0.023$; $r = -0.799$, $p = 0.006$; $r = -0.307$, $p = 0.017$ and $r = -0.285$, 0.025, respectively), as listed in Table 3. Moreover, our results established strong direct correlations between hs-CRP and each of RF, T3, T4, and age ($r = 0.660$, $p = 0.0001$; $r = 0.658$, $p = 0.001$; $r = 0.702$, 0.002 and $r = 0.321$, $p = 0.014$, respectively), as demonstrated in Table 4.

Furthermore, Table 5 confirms direct positive correlations between vitamin 25(OH) D and each of BMD and BMC ($r = 0.750$ and $r = 0.734$, $p = 0.001$, respectively), while it has an inverse relationship with T-score, $p = 0.031$, as demonstrated in Table 5. In contrast, the results confirmed strong inverse correlations between T-score and the bone markers BMD, BMC, and BMI ($p = 0.001$). In addition, Table 6 shows several positive and inverse relationships among the values of the different bone markers and minerals: direct positive correlations were observed between BMD and each of Ca^+ and Mg ($p < 0.05$), as well as inverse correlations between T-score and Ca^+ and Mg ($p < 0.001$). The results also confirmed inverse relationships between ALP and each of BMD, BMC, T-score, BMI, 25(OH)D, and Ca^+ ($p < 0.05$). Moreover, there were inverse relationships between P and each of BMD, BMC, T-score, and Ca^+ ($p < 0.05$). The most important result is the direct correlation between the levels of magnesium with those of vitamin 25(OH)D and Ca^+. No significant correlation was found between BMI and Ca, Mg, ALP, and P ($p > 0.05$) as shown in Table 6. Finally, the most significant correlation we want to concentrate on is that between PTH and BMD ($p < 0.001$). An inverse correlation was recorded between PTH and each of BMD, BMC, Ca^+, and 25(OH)D,($p < 0.05$). Likewise, an inverse relationship was found between TSH and each of BMD and Ca^+,($p < 0.05$), as shown in Table 6.

Table 7 lists the most important and predictive risk factors for fracture in osteoporosis patients. The results reveal that Mg represents the strongest factor associated with osteoporosis, with an elevated odds ratio (OR: 95% CI: 53.47–475.34,

TABLE 5 | Correlations for serum level of 25(OH)D and bone turnover markers (BMD, BMC, BMI and T-score).

Parameter	PC	BMD	BMC	T-score	BMI
25(OH)D	*r*	0.750*	0.734*	−0.555	0.006
	p	0.001	0.001	0.031	0.977
T-score	*r*	−0.984**	−0.838**	1	−0.299
	p	0.001	0.001		0.021

Abbreviations: 25(OH) D, 25-hydroxy vitamin D; BMC, bone mineral contents; BMD, bone mineral density; BMI, body mass index.
*Pearson Correlation is significant at probability of 0.05 (two-tailed).; **Pearson Correlation is significant at probability of 0.01 (two-tailed).

TABLE 6 | Correlation between the values of bone markers of spine L1–L4, some minerals, phosphorus, and alkaline phosphatase enzyme, PTH, TSH, and T4.

Parameters	PC	BMD	BMC	T-score	BMI	25(OH)D	Ca
Ca^+	*r*	0.385**	0.301	−0.387**	0.073	0.370	1
	p	0.002	0.01	0.002	0.700	0.04	
Mg^+	*r*	0.256	0.234**	−0.660**	0.168	0.765	0.846
	p	0.04	0.002	0.004	0.376	0.003	0.001
ALP	*r*	−0.773**	−0.277*	−0.668	0.234	−0.289*	−0.734**
	p	0.009	0.03	0.03	0.212	0.02	0.001
P	*r*	−0.687	-0.753	−0.671	0.238	0.274	−0.303
	p	0.02	0.01	0.03	0.205	0.143	0.01
PTH	*r*	−0.353**	−0.679*	0.222	0.190	−0.653	−0.734
	p	0.007	0.03	0.238	0.315	0.03	0.01
TSH	*r*	−0.336	0.190	0.129	0.127	0.080	−0.901
	p	0.01	0.315	0.496	0.502	0.676	0.001
T4	*r*	0.128	0.302	0.140	0.059	0.126	0.055
	p	0.499	0.01	0.460	0.756	0.506	0.773

Abbreviations: 25(OH)D, 25-hydroxy vitamin D; ALP, alkaline phosphatase; BMC, bone mineral contents; BMD, bone mineral density; BMI, body mass index; Ca, calcium; ESR, erythrocyte sedimentation rate; hs-CRP, high sensitivity C-reactive protein; Mg, magnesium; P, phosphorus; PTH, parathyroid hormone; RF, rheumatoid factor; T3, triiodothyronin; T4, thyroxin; TSH, thyroid-stimulating hormone.
*Pearson Correlation is significant at probability of 0.05 (two-tailed).; **Pearson Correlation is significant at probability of 0.01 (two-tailed).

TABLE 7 | The odds ratios and confidence intervals for fracture risk factors in osteoporosis patients.

		95% Confidence interval		
Factors	**Odds ratio***	**Lower**	**Upper**	***p* value**
Mg (mg/dL)	151.20	53.47	475.34	0.0001
25(OH)D (ng/mL)	2.429	0.91	6.41	0.001
PTH (pg/mL)	2.094	0.97	4.47	0.002
Ca^+ (mg/dL)	11.08	4.64	26.44	0.258
BMD (g/cm2)	130.01	34.77	385.92	0.247
T-score	82.60	76.20	236.18	0.347

Note: * odds ratio (OR) analysis for osteoporosis risk factors was carried out using logistic regression, $p < 0.05$.
Abbreviations: 25(OH)D, 25-hydroxy vitamin D; BMD, bone mineral density; Ca, calcium; Mg, magnesium; PTH, parathyroid hormone.

$p = 0.0001$), which is higher than the odds ratios of other factors shown in Table 7.

4 | Discussion

Recent studies have explored the potential relationship between magnesium and osteoporosis, but there is limited research on the link between serum magnesium levels and osteoporosis. Our study is among the few addressing this issue and is the first to demonstrate the combined predictive value of serum magnesium and BMD for fracture risk. We investigate whether integrating BMD with serum magnesium can improve fracture prediction accuracy and help classify patients by fracture risk, especially those with osteoporosis. The significance of magnesium in reducing fracture risk is particularly evident in patients

with low lumbar spine and femur neck BMD. To improve the specificity and sensitivity of DEXA-measured BMD for fracture risk, there is growing interest in incorporating clinical risk factors.

The results of the present study show, based on the demographic and clinical characteristics, that the percentage of females in the total study sample was 94.5%, which is higher than that of males (5.5%). Also, of the total number of patients, only 35.80% had osteoporosis, 96.5% were females, and 3.44% were males. In addition, analysis of data based on sex groups demonstrated a significant difference in the ratio of osteoporosis incidence. The study reveals that Iraqi women are over ten-times more likely to have osteoporosis than men, a finding consistent with previous studies [14, 15]. The most prominent finding is that the osteoporosis patient group has low BMD accompanied by an increase in T-score with a severe deficiency in the concentrations of vitamin D, calcium, as well as magnesium. The primary cause of this issue is inadequate sun exposure, which is the primary source of vitamin D for most humans, and its status is influenced by diet, with vitamin D synthesis varying throughout the year [16]. The research found that deficient vitamin D is not limited to osteoporosis but also observed in osteopenia, with osteopenia being more prevalent than osteoporosis, but no age-related increase in prevalence. This result is inconsistent with previous studies [17, 18]. Ström et al. also demonstrated that osteopenia was more prevalent as compared to osteoporosis regardless of age, with no age-related change [18]. This is what our current study confirmed: that osteoporosis can affect all age groups. A significant finding of our research is that osteoporosis affects various ages ranging from 21 to 80 years. This can be attributed to two types: primary osteoporosis, resulting from the normal aging process, and secondary osteoporosis, which arises from specific, potentially reversible clinical conditions. Properly addressing an underlying cause may reduce fracture risk and prevent unnecessary antiresorptive drug treatment [3].

Prior studies indicate that individuals in sunny countries like Iraq are more prone to hypovitaminosis D and osteoporosis. This can be attributed to various factors, including different lifestyles, frequency of physical activity, and limited direct sunlight exposure, and the higher likelihood that Iraqi women prefer Arab/Muslim dresses [19–22]. Notably, 75.30% of patients in our study reported sun exposure of less than 20 min per day. This high percentage suggests a significant prevalence of osteoporosis, particularly among women, potentially linked to the preference for traditional Arab/Muslim clothing that covers the skin and limited direct sunlight exposure. Furthermore, our findings show that osteoporosis is not age-dependent, as evidenced by the inclusion of individuals aged 33–80 in our study who also suffer from the condition. Sunlight exposure is crucial for improving BMD in osteoporosis patients by facilitating vitamin D production, which is vital for calcium absorption and bone health. Regular sun exposure can enhance BMD and lower fracture risk, with studies indicating that cumulative UV radiation may protect against hip fractures. While the direct effects of sunlight on bone metabolism, beyond vitamin D synthesis, are not fully understood, evidence suggests that increased sun exposure correlates with reduced fracture risk in older adults [23, 24].

The results of the association of serum 25(OH)D with BMD show controversy; part of the investigations could not show correlations, whereas other reports demonstrated that these two factors are positively correlated [25–27]. A similar finding was published by Arya et al., who found a significant correlation between serum 25(OH)D levels and BMD values at the proximal femur, suggesting subclinical 25(OH)D deficiency negatively impacts bone mass and therefore is linked with low BMD in these subjects [20]. This is supported by Villareal et al.'s study, which suggests that females with decreased serum 25(OH)D levels may be the only symptom of osteoporosis, possibly due to a correlation between 25(OH)D and BMD, which may vary depending on densitometry sites and variations in trabecular and cortical bone tissue composition [28].

Furthermore, the recent study proved that higher serum PTH was associated with lower serum 25(OH)D, BMD, Ca, and Mg concentrations in osteoporosis patients compared to other groups. The present study exhibited that serum PTH levels and BMD, BMC, Ca, and 25 (OH)D values are inversely correlated, in particular at the spine (L1–L4). Dual energy X-ray absorptiometry is a versatile tool for measuring BMD at various sites. While lumbar spine measurements are reproducible, they can be affected by artefacts. Current evidence indicates that hip bone mineral density is the most reliable predictor of hip fracture risk, while spinal density is useful for monitoring treatment [3]. These results are consistent with several previous studies [6, 29–33]. Our data also showed an association between declined levels of serum calcium and lower BMD. Consistent to our outcomes, Hosseinpanah et al. exhibited that PTH levels and BMD values are negatively correlated at the femoral neck. However, such correlation in other sites was absent [34]. Sadat Ali et al. reported a significant impact for vitamin D levels on BMD values in Saudi subjects, noting that 25(OH)D and BMD values are positively correlated, whereas 25(OH)D and parathyroid hormone are negatively correlated, with both correlations being significant [35]. It was reported that the main contribution of deficient vitamin D3 status to bone loss is achieved through the condition of secondary hyperparathyroidism [32]. Higher serum iPTH levels result in elevated bone turnover, a condition that has a primary association with cortical but also trabecular bone loss [26, 36].

Previous reports indicated that only a portion of patients with hypovitaminosis D develop secondary hyperparathyroidism. Compared to patients with hypovitaminosis D and secondary hyperparathyroidism, patients with hypovitaminosis D and a blunted PTH response showed decreased serum calcium levels, reduced bone turnover, and reduced bone density protection [9]. However, our results revealed that 25(OH)D levels higher than 20 ng/mL can predict iPTH levels, whereas higher PTH levels showed an association with declined BMD at the hip and lumbar spine. These connections point to secondary hyperparathyroidism as a possible cause of bone loss in people with low vitamin D levels, and it may help explain why BMD is lower. This result is consistent with those reported by a previous study [6].

The most significant findings that caught our attention, Patients with osteoporosis and osteopenia exhibit magnesium deficiency, which may be more significant than vitamin D, as they

have lower serum magnesium concentrations compared to normal and control groups. A DEXA scan showed that osteoporosis patients with higher T-scores had lower serum magnesium levels, with no age or sex dependence. Mg deficiency positively correlated with BMD, BMC, Ca, and 25(OH)D, while an inverse correlation was found with T-score, ESR, RF, and hs-CRP. The study reveals that only six factors significantly contribute to disease exacerbation in osteoporosis patients, with magnesium deficiency being the most significant and predictive risk factor for fracture in these patients. Magnesium deficiency is strongly linked to osteoporosis, with a high odds ratio. Systematic studies are needed to understand its effects on bone quality, as low magnesium levels are correlated with higher crystal size and decreased bone mass, traditionally contributing to osteoporosis. The value of BMD in fracture prediction can be reinforced by using Mg levels [37].

The study discovered a link between serum magnesium levels below 2.5 mg/dL and a higher risk of fracture in spines L1–L4 and femur neck, with a BMD T-score of ≤ -2.5. The rate of fracture prediction was better when Mg level and BMD value were used together. The study discovered significant differences in PTH values between osteoporosis patients and other groups, which contradicts previous research [37], that suggested magnesium could inhibit PTH secretion from parathyroid glands, particularly when the extracellular calcium concentration is moderately lower. However, decreased magnesium levels can also increase fracture risk through their influence on PTH [38].

Mangnesium (Mg) has emerged as one of the most intriguing research subjects in recent years, for a variety of reasons. A proportion of one-third of Mg preserved in the skeleton is present in the cortical bone, where it has the function of an exchangeable Mg reservoir that can be utilized for the maintenance of physiologically normal cation levels in the extracellular space [12]. Magnesium is an essential nutrient for bone development and mineralization, playing a role in osteoporosis progression. This multifactorial disease is marked by significant bone microstructure deterioration and loss. Magnesium deficiency indirectly impacts bone structure by influencing the key regulators of calcium homeostasis: parathyroid hormone and vitamin D [39].

Magnesium, the fourth most abundant mineral in the body, acts as a cofactor for numerous metabolic reactions, assisting enzymes in functions such as protein synthesis, muscle and nerve function, blood sugar regulation, blood pressure control, and energy production [40]. A critical role of Mg is in building and maintaining healthy bones; if dietary intake is insufficient, Mg is drawn from bones to meet the body's needs [11].

Research suggests that increasing magnesium intake can enhance BMD [41]. A deficiency in Mg may hinder bone formation and increase fracture risk due to its significance in bone mineralization [42]. Moreover, Mg significantly contributes to the activation of ATP, the primary energy source for cellular activities. Deficient Mg might result in disturbed release of parathyroid hormone, in addition to its potential to affect the production of vitamin D and 1,25(OH)2-vitamin D. The latter three materials are of major importance for regulating calcium and bone homeostasis [11]. Furthermore, deficient Mg deficiency can influence bone in a direct manner via the reduction of bone stiffness, the increase of osteoclasts, as well as the reduction of osteoblasts, whereas indirect influence can be exerted via its interference with PTH and vitamin D, leading to the stimulation of inflammation/oxidative stress and the subsequent bone loss [38, 43].

Numerous studies have demonstrated the significant role of Mg as a crucial cofactor in the proper synthesis and activation of vitamin D. In contrast, a feedback mechanism can be exerted by vitamin D on Mg to be more easily absorbed in the intestine [42, 44]. Several factors influence Mg absorption in the body, including dietary composition, gastrointestinal health, presence of other minerals, age and gender, magnesium status, pharmacological factors, and food preparation. Certain dietary compounds, such as phytate and oxalate, can inhibit Mg absorption by binding to it. A deficiency in vitamin D may also impair magnesium absorption, while elevated calcium levels can interfere as well. Conversely, some soluble fibers like fructo-oligosaccharides can significantly enhance magnesium absorption. Therefore, it is essential to consider these factors to maintain adequate magnesium levels [42]. Magnesium serum homeostasis is mainly secured through the degree of efficiency by which it is absorbed in the intestine and excreted in the kidneys [45].

Various proteins and signaling molecules regulate bone homeostasis, with the RANK/RANKL/OPG axis widely recognized as crucial to the molecular mechanisms of osteoporosis [46]. Magnesium contributes to osteoporosis by modulating parathyroid hormone and vitamin D levels, thereby affecting the RANK/RANKL/OPG axis. Factors that enhance OC function and disrupt this axis result in bone loss and damage to bone tissue microstructure. During OC differentiation and activation, OBs regulate this process by expressing RANKL and OPG [47]. RANKL binds to RANK, activating downstream signaling pathways for OC differentiation, whereas OPG inhibits this response by blocking the RANKL-RANK interaction. RANK expression in vascular endothelial cells within the bone microenvironment is upregulated by vascular endothelial growth factor (VEGF), boosting the angiogenic response to RANKL. OBs can express VEGF receptors 1 and 2 (VEGFR1 and VEGFR2) and release VEGF when stimulated by vitamin D3 [48]. In human osteoblasts (OBs), parathyroid hormone modulates the expression of receptor activator of nuclear factor-κ B ligand (RANKL) and osteoprotegerin (OPG), which affects osteoclast (OC) formation. Additionally, Mg may influence vitamin D3-mediated bone remodeling, which coordinates OB and OC activation. Imbalanced OC activation can lead to increased bone resorption [39]. According to recent studies [49, 50], it has been confirmed that hormonal balance significantly affects Mg status and BMD in osteoporosis patients, with key hormones like estrogen, cortisol, and growth hormone (GH) playing crucial roles. Estrogen regulates the ratio of RANKL, which promotes bone resorption, to OPG, a protective factor against bone loss, thereby reducing resorption and enhancing BMD. In contrast, increased cortisol levels, often associated with aging, can lower BMD and worsen osteoporosis by promoting bone resorption. GH is vital for maintaining bone density; its deficiency negatively impacts bone structure, but GH treatment can improve BMD in osteoporosis patients. Mg

status is also important as it aids hormone function and bone remodeling. Insufficient Mg levels can worsen the effects of hormonal imbalances on BMD. Therefore, osteoporosis patients should monitor their hormonal levels and Mg status as part of their management plan, consulting healthcare professionals for personalized advice [49, 50].

It should be noted from the above literature review, however, that limited studies are available on the relationship between T-score, BMD, and serum Mg deficiency levels with multiple clinical factors—a fact that has motivated the present study. To assess fracture risk related to Mg status, clinicians should investigate the relationship between magnesium levels and bone health. Lower serum magnesium levels significantly increase fracture risk, highlighting its critical role in bone health. While BMD is the standard measure for osteoporosis and fracture risk, assessing magnesium status can offer additional insights into an individual's risk [51]. Clinicians can combine tools like the Fracture Risk Assessment Tool (FRAX) with magnesium evaluation for more precise fracture probability estimates. Nevertheless, magnesium status should be considered alongside overall nutritional and health factors, as it is only one of several elements impacting fracture risk and bone metabolism [52]. However, the majority of research questioning how nutrition and hormonal factors affect bone health has focused on calcium and vitamin D deficiency, as well as altered PTH levels [53]. In addition, all the previous studies on serum Mg concentrations have focused on postmenopausal women to understand how BMD and serum Mg are associated [54, 55]. Research indicates that lower magnesium concentrations are linked to a higher fracture risk in osteoporosis patients, suggesting that magnesium is important for bone health. However, the direct connection between magnesium intake and reduced fracture risk requires further investigation [54, 55]. Clinical studies imply that adequate magnesium levels may enhance BMD and overall bone health, potentially offering protection against fractures, and Mg supplementation can somewhat alleviate osteoporosis symptoms [39]. Additionally, recent research by Dominguez et al. found that lower serum Mg concentrations significantly increased the risk of fractures (RR = 1.579; 95% CI: 1.216–2.051; $p = 0.001$; I2 = 46.9%) [51]. The daily magnesium intake recommendations are about 420 mg for adult men and 320 mg for adult women, but many older adults struggle to meet these levels, with average intakes significantly lower. For menopausal women, magnesium supplements combined with calcium yield better results in improving BMD. An optimal calcium-to-magnesium ratio of 2.2–3.2 is crucial for osteoporosis protection, underscoring the importance of balanced nutrient intake [56]. Foods rich in magnesium, such as leafy vegetables, legumes, nuts, and seeds, also promote bone health. Overall, clinical evidence suggests that magnesium supplementation may help manage osteoporosis symptoms [57].

5 | Limitation and Future Directions

Although the study had strengths, it was limited by its focus on the lumbar spine's BMD and T-score over the femoral neck site due to a small sample size. Furthermore, the impact of Mg supplementation was not thoroughly explored, and critical signaling molecules involved in the regulation of bone homeostasis were not assessed. More research is needed to replicate these results in different populations and evaluate the role of serum magnesium in fracture prevention, as fractures are on the rise and pose a significant health burden due to associated disabilities.

6 | Conclusion

This study emphasizes the multifactorial nature of osteoporosis, with a particular focus on the crucial role of magnesium deficiency in maintaining bone health. It identifies that osteoporosis patients often have low BMD and deficiencies in magnesium, vitamin D, and calcium. Elevated parathyroid hormone (PTH) levels, associated with low calcium and vitamin D, exacerbate declines in BMD, particularly in the spine. The results indicate that magnesium deficiency has a more significant effect on bone health than vitamin D deficiency. Both deficiencies can lead to reduced bone density, but they do so through different mechanisms. Vitamin D directly affects calcium homeostasis, while magnesium influences both bone mineralization and the metabolism of vitamin D. The study establishes a correlation between low magnesium levels and reduced BMD and other vital bone health indicators, serving as a strong predictive risk factor for fractures. This highlights the need for further investigation into dietary strategies to improve magnesium intake. Recognizing magnesium deficiency as a significant fracture risk factor underscores the importance of integrating nutritional and therapeutic approaches in osteoporosis management to enhance patient outcomes and reduce fracture risk. To build on these findings, further studies should be conducted, including animal experiments and clinical trials, to investigate the coordination and interaction between osteoclasts and osteoblasts in maintaining bone homeostasis. Additionally, research should focus on elucidating the molecular mechanisms underlying osteoporosis, particularly the roles of the RANK/RANKL/OPG pathway.

Author Contributions

Eman T Ali: conceptualization, writing – original draft, writing – review and editing. **Ali N Mohammed:** conceptualization, writing – original draft, writing – review and editing. **Amer S Khudairi:** conceptualization, writing – original draft, writing – review and editing. **Ghassan M Sulaiman:** writing – review and editing. **Hamdoon A Mohammed:** writing – review and editing. **Ali M Abomughayedh:** writing – review and editing. **Mosleh M Abomughaid:** writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw data can be obtained on request from the corresponding author.

Transparency Statement

The lead author Ghassan M. Sulaiman affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any

discrepancies from the study as planned (and, if relevant, registered) have been explained.

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