Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Osterix Gene Expression in Women with Osteoporosis and Its Association with Biomarkers of Bone Remodeling and Hormone Levels According to Menopausal Status

Abbas Fadhil Kazem[1*], Wefak Jbori Albazi[1], Mustafa Waleed Yahya[2]

[1]Department of Medical Analysis, College of Applied Medical Sciences, University of Kerbala, Karbala, Iraq

[2]College of medicine, Karbala, Karbala, Iraq.

[*]**Corresponding Author**

Abbas Fadhil Kazem: abbas.fadhil@s.uokerbala.edu.iq

Received: 02/04/2026
Accepted: 02/05/2026
Published: 30/06/2026

Keywords: Osteoporosis, *Osterix* (SP7), Estrogen, Vitamin D, PTH, Bone remodeling, Gene expression

DOI:10.62472/kjps.v17.i28.238-248

Abstract

Background: The aim of the current study is to reveal the gene expression of the *Osterix* gene and its relationship to bone regeneration and hormonal indicators in women with osteoporosis according to their physiological menopausal status, the current study was conducted after obtaining the necessary ethical approvals from the relevant authorities.

Methodology: Samples were collected from women with osteoporosis, aged 39 to 60 years, 40 of whom were premenopausal and 50 postmenopausal. Estrogen, vitamin D, and parathyroid hormone levels were measured, as were calcium, phosphorus, and alkaline phosphatase levels in the patients' serum using the appropriate kit for each test. *Osterix* gene expression was also measured using quantitative real-time polymerase chain reaction (RT-qPCR).

Results: The results showed that postmenopausal women with osteoporosis had statistically significant decreases in estrogen, vitamin D, and calcium levels, as well as statistically significant increases in parathyroid hormone and alkaline phosphatase levels, compared with premenopausal women with osteoporosis.

Conclusion: The results also showed a significant decrease in *Osterix* gene expression in postmenopausal women compared to premenopausal women, and correlation analysis revealed a positive relationship between estrogen levels and *Osterix* gene expression for all women.

تعبير جين الـ *Osterix* لدى النساء المصابات بهشاشة العظام وارتباطه بالمؤشرات الحيوية لإعادة تشكيل العظام ومستويات الهرمونات تبعاً لحالة انقطاع الطمث

عباس فاضل كاظم، وفاق جبوري البازي، مصطفى وليد يحيى

الخلاصة

المقدمة: تعد هشاشة العظام اضطراب في الجهاز الهيكلي حيث يؤدي الى تدهور بنية النسيج العظمي، مما يؤدي الى زيادة التعرض للكسور، لا سيما لدى النساء بعد سن اليأس. كما يعد جين *Osterix* منظم يعمل على تمايز الخلايا البانية للعظم. تهدف الدراسة الحالية إلى تقييم مستويات التعبير الجيني لـ *Osterix* ، و الكشف عن مدى تأثيره على المؤشرات البيوكيميائية و الهرمونية لإعادة تجديد العظام وفقاً لمرحلة انقطاع الطمث لدى النساء

المرضى وطرق العمل: تضمنت عينات الدراسة 90 عينة دم و شظايا عظمية لنساء تعرضن للكسور وشُخِّصت إصابتهن بهشاشة العظام ، قسمن إلى مجموعتين ، 40 امرأة في مرحلة ما قبل انقطاع الطمث و50 امرأة في مرحلة ما بعد انقطاع الطمث. خضعت عينات الدم لفحص مستويات الإستروجين، وفيتامين D ، وهرمون الجار درقية ، والكالسيوم ، والفسفور وانزيم الفوسفاتيز القلوي (ALP) ، وتم تقييم التعبير الجيني لـ *Osterix* في الأنسجة باستخدام تقنية التفاعل البوليميرازي المتسلسل (RT-qPCR)

النتائج :كشفت النتائج عن انخفاض ذو دلالة إحصائية في مستويات الإستروجين وفيتامينD والكالسيوم لدى النساء بعد سن اليأس، وفي المقابل كشفت النتائج عن زيادة في هرمون الغدة جار الدرقية وانزيم الفوسفاتيز القلوي . كما أظهرت النتائج انخفاض في التعبير الجيني لـ *Osterix* لدى النساء ما بعد انقطاع الطمث مقارنة بالنساء ما قبله. وأظهرت علاقة الارتباط علاقة ايجابية بين مستويات الإستروجين في الدم ومعدلات تعبير جين *Osterix* لدى جميع المريضات

الاستنتاج :أظهرت نتائج الدراسة الحالية أن انخفاض الإستروجين واختلال توازن الكالسيوم وفيتامين D يعدان اهم الأسباب لتثبيط التعبير الجيني لجين *Osterix* مما يؤدي إلى انخفاض في تمايز الخلايا البانية للعظم وقصور آليات البناء الخلوي على هذا الأساس من الممكن ان يكون جين *Osterix* مؤشر حيوي جزيئي واعد لتنبؤ المبكر عن هشاشة العظام قبل تفاقم الهشاشة في بنية النسيج العظمي المرتبطة بتقدم السن وانقطاع الطمث .

1. Introduction

Osteoporosis is a disorder that affects the skeletal system, leading to a decrease in bone mass and a deterioration in bone structure, which in turn increases bone fragility and susceptibility to fractures. The disease is more prevalent among postmenopausal women due to hormonal changes that affect bone metabolism and the bone remodeling process (Baseem *et al*., 2024; Zhivodernikov *et al*., 2023). Bone remodeling is a continuous physiological process that depends on the balance between osteoblastic bone formation and osteoclastic bone resorption. This disorder also causes deterioration in bone structure and leads to the development of osteoporosis. This condition occurs through interactions between hormones and genetic factors (Schini *et al*., 2023). The *Osterix* gene is an important transcription factor in osteoblast formation and also acts as a signaling pathway post-RUNX2 gene, contributing to the regulation of several genes that play a crucial role in bone regeneration. This leads to impaired osteoblast activity and abnormal bone remodeling (Liu *et al*., 2020). Bone metabolism is also affected by biomarkers and hormonal regulators such as estrogen, parathyroid hormone and vitamin D, which are important factors for maintaining skeletal balance (Abdul-Amir *et al*., 2024). Therefore, the current study was conducted to evaluate the gene expression of *Osterix* in women with osteoporosis, and to study its association with bone regeneration markers and hormones across the stages of menopause.

2. Materials and Methods

The study included peripheral blood samples and bone fragments from women who had experienced fractures and undergone surgery. The women were divided into two groups based on their osteoporosis: premenopausal women (n=40) and postmenopausal women (n=50). They were diagnosed with osteoporosis using a special device designed for that purpose.

2.1. Patient Samples Collected

Blood samples (5 mL) were collected from women with osteoporosis before and after menopause, taking sterilization protocols into account. Samples were distributed into gel tubes for plasma separation and serum separator tubes for biochemical analysis. Following collection, samples were gently inverted, centrifuged in 3000 × g for 10 m4 °C, and the stored at −80 °C until further analysis (Zhivodernikov *et al*., 2023). Bone tissue samples were obtained intraoperatively from osteoporotic patients undergoing orthopedic procedures for fracture repair. Trabecular bone fragments were collected under sterile conditions by the operating surgeon and immediately rinsed with sterile saline to remove blood and debris. The samples were divided into two groups. The first group was preserved in TRIzol reagent for RNA extraction and gene expression assessment, and the second group was preserved in 10% formalin for histological examination (Faraldi *et al*., 2022).

2.2. Measurement of Bone Metabolism Markers in Serum

The tests included measuring several biochemical and hormonal indicators closely related to bone metabolism. Estrogen levels in the blood were measured, as this hormone plays a key role in regulating bone metabolism. Vitamin D and parathyroid hormone (PTH) levels (pg/mL) were also assessed, as these hormones regulate calcium, phosphorus and bone regeneration. Additionally, blood calcium concentrations were measured to assess mineral stability (Pederick and Bruning, 2021). Alkaline phosphatase (ALP) activity was also analyzed as a marker of bone formation. All measurements were performed using commercially available assay kits according to the manufacturer’s instructions under standardized laboratory conditions to ensure accuracy and reproducibility of the results (Schini *et al*., 2023).

2.3. *Osterix* (*SP7*) Gene Expression Analysis

2.3.1. Preparation of Primers

The primers were prepared according to the manufacturer's instructions. The primers were dissolved in deionized distilled water (ddH_2O) to a concentration of 100 picomoles/microliter and used as a stock solution, which was stored at −20°C. From this, a concentration of 10 picomoles/microliter was prepared as primary working primers, as shown in Table1, different annealing temperatures were applied for each gene based on primer melting temperatures to ensure optimal amplification efficiency and specificity, *Osterix* (*SP7*) was amplified at 63°C, while GAPDH was amplified at 68°C (Zhao *et al.,* 2021), as presented in Table1.

Table1: Primer Characteristics, Amplicon Size, and Annealing Temperatures Used for RT-qPCR Analysis

Gene	Primer	Sequence (5′→3′)	Primer Length (bp)	Amplicon Size (bp)	Annealing Temperature (°C)
Osterix (SP7)	Forward	TCCCTGGATATGACTCATCCCT	22	141	63
	Reverse	CCAAGGAGTAGGTGTGTTGCC	21	141	
GAPDH	Forward	CCACCCATGGCAAATTCCATGGCA	24	124	68
	Reverse	TCTAGACGGCAGGTCAGGTCCAC	23	124	

2.3.2. *Osterix* (*SP7*) Gene Expression

Gene expression was analyzed using the comparative Ct method (ΔΔCt), with normalization to GAPDH as the reference gene and calibration to the control group. The analysis was performed according to the recommendations of (Al-Shakarjy., 2025).

2.3.3. RNA Extraction, cDNA Synthesis, and RT-qPCR

Total RNA was extracted from 10–20 mg of human bone tissue using the TRIzol method. The procedure included tissue homogenization, phase separation with chloroform, RNA precipitation, purification using spin columns, and storage at −20 °C until further use. Complementary DNA (cDNA) was synthesized immediately after RNA extraction using a reverse transcription master mix. The reaction was carried out under the following conditions: 25 °C for 10 min, 42 °C for 10 min, and 82 °C for 5 s, followed by storage at 4 °C. RT-qPCR was performed using SYBR Green master mix (total volume 21 µL), including cDNA, primers, and other reaction components, according to the manufacturer's instructions (Abbexa Ltd., 2023).

3. Results

The demographic data are presented in Table2 highly significant difference in age between premenopausal and postmenopausal women, the mean age was 39.50 ± 1.00 years in premenopausal women and 55.87 ± 0.89 years in postmenopausal women $p < 0.0001$.

Table2: Demographic Characteristics of Premenopausal and Postmenopausal Women

Groups Parameter	Premenopausal *n*=40	Postmenopausal *n*=50	*p*-value
Age (years)	39.50 ± 1.00	55.87 ± 0.89	< 0.0001

3.1. Serum Estrogen Levels in Relation to Bone Metabolism

Table3 shows a highly significant reduction in serum estrogen levels in postmenopausal women compared with premenopausal women. The mean estrogen level was 22.86 ± 0.16 in premenopausal women, whereas it decreased significantly to 16.22 ± 0.34 in postmenopausal women $p < 0.0001$, The results are shown in Fig.1.

Table3: Comparison of Serum Estrogen Levels Between Premenopausal and Postmenopausal Women

Groups Parameter	Premenopausal *n*=40	Postmenopausal *n*=50	*p*-value
Estrogen (pg/m)	22.86 ± 0.16	16.22 ± 0.34	<0.0001

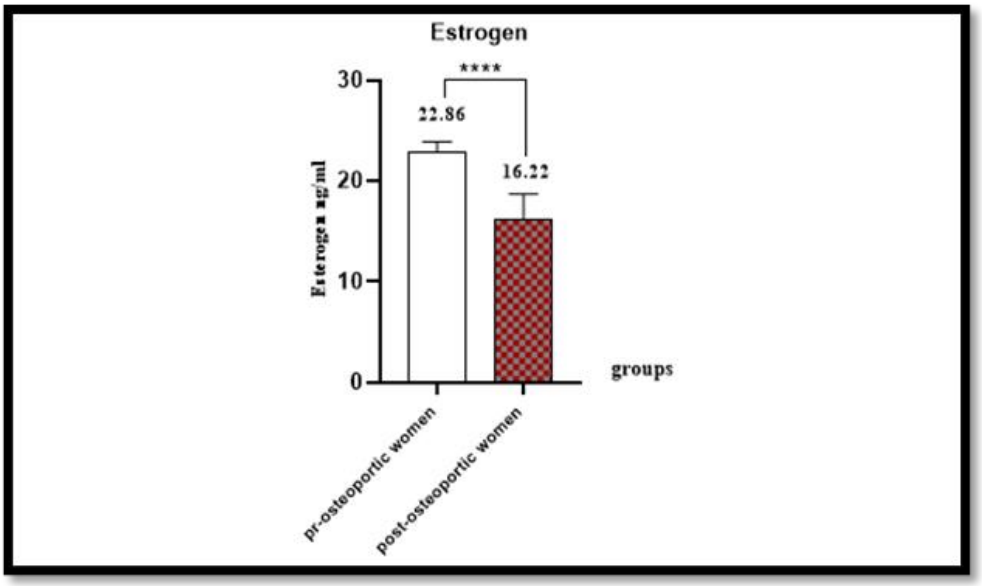

Figure1: Serum Estrogen levels in Pre- and Postmenopausal Osteoporotic Women

3.2. Alterations in vitamin D and Parathyroid Hormone in Premenopausal and Postmenopausal Women

Table4 shows a highly significant reduction in serum vit.D and PTH levels between premenopausal and postmenopausal women. Vitamin D levels were significantly higher in premenopausal women 17.28 ± 0.17 compared with postmenopausal women 11.14 ± 0.09, $p < 0.0001$. In contrast, PTH levels were significantly elevated in postmenopausal women 57.80 ± 11.82 compared with premenopausal women 30.45 ± 5.34, $p \leq 0.05$.

Table4. Comparison of Hormonal and Bone Remodeling Parameters Between Premenopausal and Postmenopausal Osteoporotic Women

Groups Parameters	Premenopausal *n*=40	Postmenopausal *n*=50	*P* value
Vitamin D	17.28 ± 0.17	11.14 ± 0.09	<0.0001
PTH (pg/mL)	30 .45± 5.34	57.80 ± 11.82	≤ 0.05

3.3. Alterations in Serum Calcium, phosphorus and Alkaline Phosphatase Levels in Relation to Menopausal Status

The results in Table5 showed statistically significant differences and revealed a significant decrease in calcium levels among postmenopausal women 7.94 ± 0.18 compared to premenopausal women 8.51 ± 0.18, with a *p*-value of 0.0016. The results also showed a highly significant decrease in phosphorus levels in postmenopausal women 3.13 ± 0.39 compared to premenopausal women 4.12 ± 0.39, with a statistical significance value of $p < 0.0001$. The results also showed a statistically significant increase in alkaline phosphatase (ALP) levels in postmenopausal women 75.12 ± 3.07 compared to premenopausal women 67.00 ± 3.07, with a statistical significance of $p = 0.0096$.

Table5: Serum Calcium, Phosphorus, and Alkaline Phosphatase Levels in Premenopausal and Postmenopausal Women

Groups Parameter	Premenopausal *n*=40	Postmenopausal *n*=50	*P* value
Ca	8.51 ± 0.18	7.94 ± 0.18	0.0016
P	4.12 ± 0.39	3.13 ± 0.39	<0.0001
ALP	67.00 ± 3.07	75.12 ± 3.07	0.0096

3.4. Osterix Gene Expression Analysis

The current study showed a significant decrease in gene expression of the Osterix protein in postmenopausal women compared to premenopausal women, as shown in Table6, the mean fold change of Osterix expression was 1.03 ± 0.04 in premenopausal women, whereas it markedly decreased to 0.24 ± 0.03 in postmenopausal women, with a highly significant difference $p < 0.001$, these findings indicate a substantial downregulation of Osterix gene expression associated with the postmenopausal state, as shown in Fig.2. and Fig.3.

Table6. Comparison of *Osterix* Gene Expression (Fold Change) Between Premenopausal and Postmenopausal Women

Groups Parameter	Premenopausal n=40	Postmenopausal n=50	*P* value
Osterix Gene Expression (Fold Change)	1.03 ± 0.04	0.24 ± 0.03	< 0.001

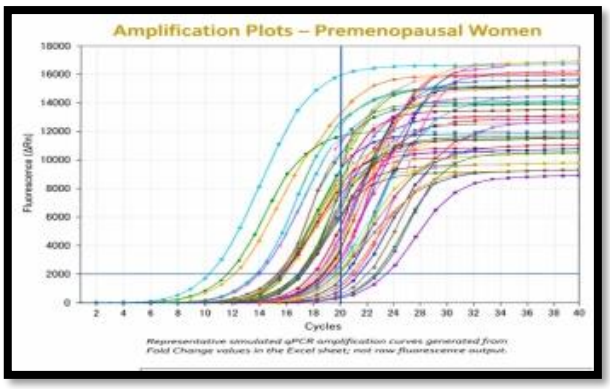

Figure2: Representative qRT-PCR Amplification Curves of Osterix (SP7) Expression in Premenopausal Osteoporotic Women.

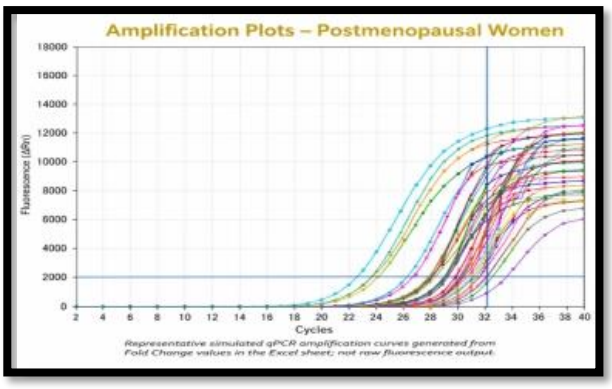

Figure3: Representative qRT-PCR Amplification Curves of *Osterix* (SP7) Expression in Postmenopausal Osteoporotic Women.

Overall, the observed decrease in *Osterix* expression in the present study suggests a molecular suppression of osteoblast differentiation in postmenopausal women. This supports the concept that *Osterix* may serve as a potential molecular biomarker for early detection of bone formation impairment in postmenopausal osteoporosis as shown in the Fig.4.

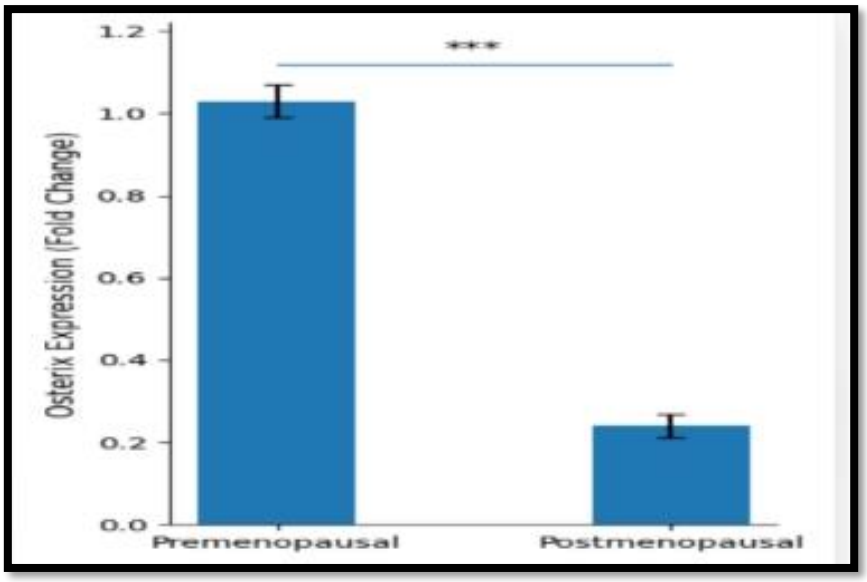

Figure4: Relative *Osterix* (*SP7*) Gene Expression (Fold Change) in Premenopausal

3.5. The Correlation Between Gene Expression of the Osteotrix Gene and Serum Estrogen Levels in Premenopausal Women with Osteoporosis

The results in Fig.5. showed a statistically significant positive correlation between the expression of the *osteotrix* gene and serum estrogen levels in premenopausal women with osteoporosis $n = 40$, $r = 0.69$, $R^2 = 0.4797$, $p = 0.0007$. The coefficient (R^2) indicated 48% of the variance in serum estrogen levels, which was attributed to the expression of the *osteotrix* gene. The linear relationship between the two variables was determined using the following regression equation: $Y = 3.311X + 19.46$. This positive correlation, $r = 0.69$, provides a direct explanation confirming the important role of estrogen in activating the gene expression of the *osteotrix* gene in premenopausal women.

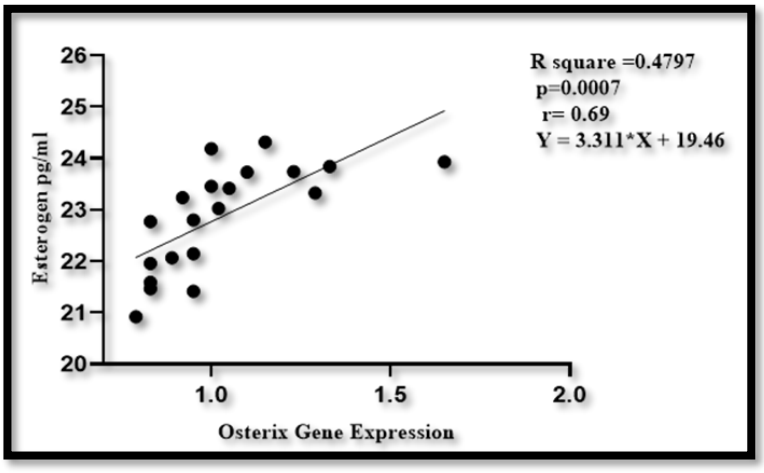

Figure5: Correlation between *Osterix* Gene Expression and Estrogen levels in Premenopausal Women with Osteoporosis

3.6. The Correlation Between Gene Expression of the Osteotrix Gene and Serum Estrogen Levels in Postmenopausal Women with Osteoporosis

The results in Fig.6. showed a statistically significant, moderate positive correlation between the expression of the *Osterix* gene and serum estrogen levels in postmenopausal women r =0.58, p = 0.0001. The coefficient R^2 = 0.3405 indicated that 34.1% of the variation in serum estrogen levels was attributable to variations in *Osterix* gene expression. Linear regression further confirmed this relationship using the following equation: Y = 9.513X + 14.09.

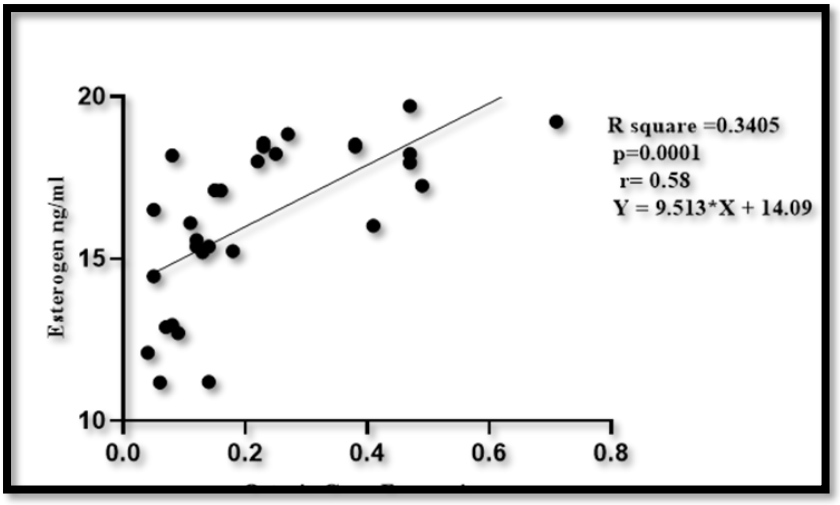

Figure6: Correlation Between *Osterix* Gene Expression And Estrogen Levels in Postmenopausal Osteoporotic Women

4. Discussion

The results of the current study were similar to previous studies that confirmed the occurrence of many hormonal changes in postmenopausal age, the most important of which is the decrease in the level of estrogen. This decrease leads to an increase in the deterioration of the tissue structure of bones, which helps to increase the risk of osteoporosis in postmenopausal women (Yu *et al*., 2025). This is because a deficiency in estrogen causes an increase in the activity of osteoclasts and an increase in bone resorption, which inevitably leads gradually to osteoporosis after menopause. Therefore, women are more susceptible to fractures, and this confirms the importance of hormonal levels for bone health (Sheni *et al*., 2023). The current study showed a clear decrease in vitamin D levels accompanied by an increase in parathyroid hormone in postmenopausal women, confirming increased parathyroid gland activity, which in turn contributes to a disturbance in the balance of calcium and vitamin D. This disturbance promotes bone resorption and the development of osteoporosis (Liu *et al*., 2025). The reason for the significant decrease in calcium levels in postmenopausal women with osteoporosis compared to premenopausal women may be low estrogen levels, as a deficiency of this hormone leads to increased bone resorption by osteoclasts, which disrupts the body's calcium balance. Furthermore, low estrogen levels may impair calcium absorption in the intestines and affect its metabolism. This finding is consistent with numerous studies that have reported lower calcium levels in postmenopausal women with osteoporosis (Ito *et al*., 2024). The gene expression results of this study showed a clear decrease in the gene expression of the *Osterix* protein in postmenopausal women compared to premenopausal women. The reason for this is that the *Osterix* gene is considered an essential transcription factor important in the formation of osteoblasts and bone health; therefore, the decrease in gene expression of the ostrex gene weakens the activity of osteoblasts and leads to osteoporosis, especially in postmenopausal women. The results of the

current study are consistent with previous studies, which have shown that estrogen positively regulates *Osterix* gene expression by activating the Wnt/β-catenin and MAPK pathways, which are fundamental to skeletal homeostasis and support osteoblast differentiation (Lang *et al*., 2025). Therefore, decreased estrogen levels will disrupt these pathways, thereby inhibiting the expression of genes responsible for bone formation. As noted, Qi *et al* .(2023) reported that reduced expression of the *Osterix* gene plays a key role in impairing osteoblast maturation and reducing bone formation. Föger-Samwald *et al*. (2016) also noted that key bone formation markers, including *Osterix* and *RUNX2*, exhibit reduced activity, thereby contributing to osteoporosis. Postmenopausal bone loss due to estrogen deficiency, as estrogen is a key factor in regulating bone remodeling by promoting the activity of osteoblasts and inhibiting the activity of osteoclasts. Consequently, decreased levels lead to an imbalance in the skeletal system and reduced gene expression of genes responsible for bone formation, such as *SP7* (Yu *et al*., 2025). Estrogen acts through specific receptors called estrogen receptors (ERα and ERβ), which are located in osteoprogenitor cells and osteoblasts. Activation of these receptors leads to the stimulation of the Wnt/β-catenin and MAPK/ERK signaling pathways, which in turn regulate the transcription factors responsible for bone architecture, including *RUNX2* and *Osterix* (Hu *et al*., 2024). The results of the current study showed stable *Osterix* expression in premenopausal women were 1.03 ± 0.04, indicating normal levels, which leads to the activation of osteoblasts (Zhu *et al*., 2024). These results confirmed the protective role of estrogen in maintaining bone health. The final results confirmed a close relationship between estrogen and the gene expression of *Osterix* and that low levels of estrogen in the blood following menopause lead to reduced *Osterix* gene expression. Since the *Osterix* gene is important for bone cell differentiation, its reduced expression causes abnormalities in bone formation and accelerates bone resorption (Lang *et al*., 2025 ; Hu *et al*., 2024; Baseem *et al*., 2024).

Conclusion

Osteoporosis in women leads to specific complications, particularly after menopause, and a deterioration in bone matrix structure associated with low levels of estrogen and vit. D in the blood, elevated levels of (PTH) and certain markers of bone turnover, resulting in increased bone resorption and mineral imbalance. Additionally, there is reduced gene expression of the *Osterix* gene (*SP7*), particularly in postmenopausal women, which manifests as a marked decrease in osteoblast differentiation. Thus, the *Osterix* gene may serve as a potential molecular biomarker for detecting osteoporosis caused by genetic, environmental, or physiological factors in the future.

Ethical Approval

Scientific and ethical approval procedures were carried out by the relevant institutions at the University of Karbala, the College of Applied Medical Sciences, the Department of Medical Analysis, and the Karbala Health Directorate. Verbal consent was obtained from the participants, in compliance with health and safety requirements.

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