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Estimate levels of osteocalcin (OC) and bone-specific alkaline phosphatase (BAP) with the Genetic polymorphism of FTO in osteoporosis patients

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ABSTRACT

Background: Osteoporosis is a systemic skeletal disorder marked by the degradation of bone microarchitecture, resulting in diminished bone mineral density (BMD) and an elevated risk of fractures. **Aims of the study:** the study aimed to evalute levels of osteocalcin and bone-specific alkaline phosphatase and the Genetic polymorphism of FTO in patients with osteoporosis. **Methods:** A study was conducted on 40 postmenopausal women with osteoporosis and 40 age-matched healthy controls from Salah al-Din Governorate, Iraq. Bone mineral density (BMD) was measured using DEXA, and osteoporosis was defined as a T-score ≤ –2.5. Serum levels of BAP and osteocalcin were quantified using ELISA. Genomic DNA was extracted and genotyped for FTO and MC4R polymorphisms using ARMS-PCR, with DNA quality confirmed by agarose gel electrophoresis and Nanodrop spectrophotometry.

Results: The results of the study showed a significant difference in mean level of osteocalcin (26.15 ± 5.42 ng/ml) and BAP levels (37.63 ± 10.36 U/L) in patient group compared with control (21.87 ± 4.95 ng/ml, 30.23 ± 7.21 U/L), respectively. While the study showed no significant differences in mean age and Body mass Index (BMI) in patients' group (55.48 ± 11.53, 29.16 ± 5.31 kg/m^2) compared with control (53.82 ± 9.59, 27.91 ± 6.47 kg/m^2). The results of the analysis of the rs9939609 allele in the FTO gene indicate no significant differences when comparing the genotypes (TT, TA, and AA) between osteoporosis patients and healthy controls. In contrast, the allele analysis showed that the presence of the A allele was associated with a significantly increased risk of osteoporosis (OR=1.985, 95% CI=1.017–3.809, p=0.0414), suggesting that the A allele may represent a genetic factor contributing to susceptibility to this disease. the results of the analysis of the rs17817449 allele in the FTO gene indicate no significant differences when comparing the genotypes (TT, TA, and AA) between osteoporosis patients and healthy controls. The odds ratio (OR) and relative risk (RR) values were not statistically significant (p>0.05), indicating that the TA and AA genotypes do not constitute an independent risk factor compared to the reference TT type. the allele analysis also showed no significant differences suggesting that the G allele may not represent a genetic factor contributing to susceptibility to this disease.

Conclusion: Osteoporosis patients had higher osteocalcin and BAP levels than controls, despite identical age and BMI. The A allele of the FTO gene's rs9939609 variant enhanced osteoporosis risk, while genotypes (TT, TA, AA) and the rs17817449 variant did not. These findings indicate that the A allele may be a genetic risk factor for osteoporosis.

Keywords: Osteocalcin, BAP, Osteoporosis, Allele.

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Introduction

Osteoporosis is a systemic skeletal disorder marked by the degradation of bone tissue microarchitecture, resulting in diminished bone mineral density (BMD) and an elevated risk of fractures (Bae et al., 2025). Osteoporosis is characterised by bone fragility, influenced by various factors such as bone microarchitecture, microdamage, and the rate of bone remodelling. The rate of bone remodelling, affected by bone resorption and formation, can be evaluated using various bone turnover markers (BTMs) (Falaschi et al., 2021). Serum bone-specific alkaline phosphatase (BsALP), osteocalcin (OC), and procollagen type 1 N-terminal propeptide (P1NP) are biomarkers that denote bone formation, while carboxyl-terminal crosslinked telopeptide of type 1 collagen (CTx) and amino-terminal crosslinked telopeptides of type 1 collagen (NTx) reflect bone resorption. The amalgamation of BTMs with BMD analysis may enable the prompt detection of osteoporosis (Baik et al., 2024).

Osteocalcin is a protein synthesised by osteoblasts that governs bone mineralisation and acts as an endocrine hormone originating from osteoblasts. Osteocalcin, the primary non-collagenous protein in bone, operates as a hormone and is clinically essential for bone integrity, acting as a biomarker for bone formation (Wang et al., 2021). Osteocalcin can activate both osteoblasts and osteoclasts in the early stages of bone development. Elevated serum osteocalcin concentrations correlate with expedited bone resorption. Osteoporosis leads to a decrease in hydroxyapatite crystal synthesis, hence increasing blood osteocalcin concentrations (Komori, 2020). The elevation of serum osteocalcin levels signifies its crucial involvement in the formation of early osteoporosis in postmenopausal women, indicating that serum osteocalcin measurement may function as a diagnostic marker for early osteoporosis (Bhadricha et al., 2019).

Serum osteocalcin serves as a specific marker of osteoblast function, with its levels correlating with bone formation rates (Zoch *et al.,* 2016) Traditionally, Predicting fracture risk and bone strength have relied on densitometry. Recently, bone turnover biomarkers, including osteocalcin—also known as bone gamma-carboxyglutamic acid-containing protein (BGLAP)—have gained prominence for assessing bone turnover rates and monitoring osteoporosis treatment (Ali, 2020). Secreted by osteoblasts during bone formation, osteocalcin is calcium-dependent and binds strongly to the bone matrix. In osteoporosis, reduced hydroxyapatite crystal formation leads to elevated serum osteocalcin levels due to calcium and phosphorus deficiencies (Jagtap *et al.,* 2011).

Bone-specific alkaline phosphatase (BAP) is a reliable marker for osteoblast activity related to bone formation. Moderate elevations in BAP levels are seen in osteomalacia and return to normal with vitamin D therapy. In osteoporosis, BAP activity is typically normal, whereas in rickets, BAP levels can increase 2 to 4 times, normalizing with treatment. A transient increase in BAP activity is often observed during the healing of bone fractures (Bansal *et al.,* 2020).

The FTO gene's role in osteoporosis is complex and appears to involve influencing the differentiation of bone-forming cells (osteoblasts) and fat-forming cells (adipocytes). FTO is an RNA demethylase that, when dysregulated, can promote the shift from osteoblast to adipocyte differentiation, leading to increased fat and decreased bone mass seen in osteoporosis(Shen *et al.,* 2018).

Aims of the study: the study aimed to evaluate levels of osteocalcin and bone-specific alkaline phosphatase and the Genetic polymorphism of FTO in patients with osteoporosis.

Methods

Population Study

A random sample of women from the Salah al-Din Governorate in Iraq who had been diagnosed with osteoporosis was chosen for this study. There were 40 women in total in the sick group and 40 in the control group. A systematic collection of blood samples was conducted from November 2023 to February

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2024. Furthermore, healthy controls with a family history of osteoporosis were not included in the study, which was limited to postmenopausal women. We used a dual-energy X-ray absorptiometry (DEXA) scanning device to test the bone mineral density (BMD), which produced the best results; a T score of -2.5 or below is a clinical indicator for the presence and severity of osteoporosis.

Bone biomarkers

Blood samples were collected from patients and healthy volunteers. The serum from the cellular components was used to estimate the BAP and OC using an enzyme-linked immunosorbent assay (ELISA) kit.

Genomic DNA Extraction

The study samples' entire genomic DNA was extracted using the method described by Gaaib et al. (2011). Electrophoresis on a 1% agarose gel was used to evaluate the purity of the DNA extraction. A spectrophotometer called a Nanodrop 2000 (made by Thermo Scientific in the USA) was used for the quantification and purity evaluation.

Genotyping

The polymorphism of the FTO genes was analysed using ARMS-PCR. The table below (1) delineates the primers.

Table 1. Primers sequence, PCR yield, and optimal annealing temperature for FTO genes.

Primer Sequence for FTO gene- rs9939609 OF GCTGCTATGGTTCTACAGTTCCA OR TGTTCAAGTCACACTCAGCCTC IF CCTTGCGACTGCTGTGAATATA IR CAGAGACTATCCAAGTGCATCTCA	PCR Product	Annealing Temperature	Source
	Product size for A allele: 211 Product size for T allele: 296	58 C	(Mozafarizadeh *et al.,* 2019)
Primer Sequence for FTO gene - rs17817449 OF TGAGGCAGCAATTAACTGATCATG OR TCCATGTAACAAAACTGCACTGGTAC IF TCAGCTTGGCACACAGAAGCT IR AGGAGCTGGACTGTTAAATTAAAGCC	Product size for G allele: 286 Product size for T allele: 225	59 C	Current study

Statistical analysis

We used GraphPad Prism 10.4.0 from GraphPad Software, Inc., USA, to do the statistical analysis. To determine if the data was normally distributed, the Student's T-test was used. For the FTO gene genotyping, odds ratios (ORs) with 95% CIs were calculated.

Results

The results of the study showed a significant difference in mean level of osteocalcin (26.15 ± 5.42 ng/ml) and BAP levels (37.63 ± 10.36 U/L) in patient group compared with control (21.87 ± 4.95 ng/ml, 30.23 ± 7.21 U/L), respectively. While the study showed no significant differences in mean age and Body mass Index (BMI) in patients' group (55.48 ± 11.53, 29.16 ± 5.31 kg/m^2) compared with control (53.82 ± 9.59, 27.91 ± 6.47 kg/m^2), respectively as shown in table (2).

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Table 2. Demographic and biochemical data in the current study.

Categories	Osteoporosis group	Control group	*p* value
Age (years)	55.48 ± 11.53	53.82 ± 9.59	0.0749
BMI (kg/m^2)	29.16 ± 5.31	27.91 ± 6.47	0.0878
BAP (U/L)	37.63 ± 10.36	30.23 ± 7.21	0.0003
OC (ng/ml)	26.15 ± 5.42	21.87 ± 4.95	0.0042
BMD	-2.103 ± 0.45		

The results of the analysis of the rs9939609 allele in the FTO gene indicate no significant differences when comparing the genotypes (TT, TA, and AA) between osteoporosis patients and healthy controls. The odds ratio (OR) and relative risk (RR) values were not statistically significant (p>0.05), indicating that the TA and AA genotypes do not constitute an independent risk factor compared to the reference TT type. In contrast, the allele analysis showed that the presence of the A allele was associated with a significantly increased risk of osteoporosis (OR=1.985, 95% CI=1.017–3.809, p=0.0414), suggesting that the A allele may represent a genetic factor contributing to susceptibility to this disease. Therefore, the results support the role of the A allele as a potential risk factor for osteoporosis, while the different genotypes did not demonstrate a statistically significant association.

Table 3. allele, genotype analysis of the FTO gene-rs9939609.

rs9939609	Study participants						
	Osteoporosis No.	Healthy No.	Relative risk		Odds ratio		p-value
			OR	95% CI	OR	95% CI	
TT	9	12	1				
TA	14	15	1.105	0.6446 to 1.839	1.244	0.3908 to 3.626	0.7044
AA	17	13	1.319	0.7464 to 2.297	1.744	0.5605 to 4.944	0.3316
T	32	39	1				
A	48	41	1.365	1.012 to 1.838	1.985	1.017 to 3.809	0.0414

Also, the results of the analysis of the rs17817449 allele in the FTO gene indicate no significant differences when comparing the genotypes (TT, TA, and AA) between osteoporosis patients and healthy controls. The odds ratio (OR) and relative risk (RR) values were not statistically significant (p>0.05), indicating that the TA and AA genotypes do not constitute an independent risk factor compared to the reference TT type. the allele analysis also showed no significant differences suggesting that the G allele may not represent a genetic factor contributing to susceptibility to this disease.

Table 4. allele, genotype analysis of the FTO gene- rs17817449.

rs9939609	Study participants						
	Osteoporosis No.	Healthy No.	Relative risk		Odds ratio		p-value
			OR	95% CI	OR	95% CI	
TT	7	10	1				
TG	22	14	1.513	0.824 to 1.64	2.245	0.6542 to 7.236	0.1736
GG	11	16	0.9926	0.568 to 1.62	0.982	0.3019 to 3.477	0.9772
T	36	34	1				
G	44	46	0.9503	0.6878 to 1.295	0.9034	0.488 to 1.663	0.749

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Discussion

The study revealed that Osteocalcin levels were significantly higher in osteoporosis patients than in the control group (P <0.001), likely due to low serum calcium levels reducing hydroxyapatite crystal formation, making osteocalcin more available in circulation (Zoch et al., 2016). This finding aligns with the results of other researchers, who observed elevated osteocalcin levels in osteoporotic patients, highlighting its value as a surrogate marker for osteoporosis (Mohammadi *et al.,* 2024; Singh *et al.,* 2015). Serum OC levels and the rise in bone mineral density (BMD) after osteoporosis bone-building drug treatment were reported to be strongly correlated by Martiniakova et al. (2024). In order to determine the rate of bone formation in osteoporosis, serum OC is thought to be a specific biomarker for osteoblast activity. Numerous studies have demonstrated that osteocalcin is an important biomarker for evaluating how well medications affect the development of new bone (Hasanzad et al., 2021; Xu et al., 2022).

The specific bone alkaline phosphatase enzyme (BAP) is one measurable isomer of the ALP enzyme that is produced by bone cells and is present in the bloodstream. About half of serum ALP levels are made up of ALP and BALP produced by osteoblasts. Think of a bone-production biomarker that is essential to the bone-remodeling process. BAP assessment is used to treat osteoporosis and gives an indication of osteoblastic activity (Kuo and Chen, 2017).

Ponzano *et al.,*(2023) investigated that elevated bone-specific alkaline phosphatase (BAP) levels in patients with osteoporosis reflect increased osteoblast activity as a compensatory response to increased bone loss resulting from overactive osteoclasts. In other words, in conditions of high bone turnover—a pattern common after menopause or in some cases of secondary osteoporosis—bone matrix breakdown increases. Osteoclasts respond by increasing the synthesis of enzymes involved in bone mineralization, such as BAP. Therefore, BAP is elevated in serum, although this increase does not always translate into net bone density recovery because resorption exceeds resorption capacity. Furthermore, BAP is a useful marker for monitoring treatment response (particularly to anabolic or modulatory therapies for bone turnover) and for assessing bone metabolism in patients with diseases or conditions affecting bone (Schini *et al.,* 2023).

Rapid bone loss was found to be strongly correlated with BAP levels in the Hawaii Osteoporosis Study cohort. The chance of rapid bone loss was 80% for BAP levels 2 SD above the mean, but only 20% for BAP levels 2 SD below the mean. BAP may be a useful biomarker for determining fracture risk and tracking bone health because this link is similar to that between bone mineral density (BMD) and fracture risk (Masrour Roudsari & Mahjoub, 2012).Studies show that FTO expression increases with age and osteoporosis, and its inhibition can improve bone formation in animal models, suggesting FTO could be a target for treating the condition(Haung *et al.,* 2023).

Also, Xu and Wang (2016) showed that The FTO gene exhibits diverse genetic polymorphism associations with osteoporosis and fractures. Certain studies have identified FTO variants, such as rs7206790, as potential susceptibility factors, while others associate different SNPs (e.g., rs1121980, rs1421085) with heightened hip fracture risk, even after controlling for variables such as BMD and BMI. Liu et al. (2013) demonstrated a substantial correlation between the rs9939609 mutation in the FTO gene and cardiovascular disease risk, independent of body mass index and other traditional cardiovascular risk factors.

Conclusion

The research revealed markedly increased levels of osteocalcin and bone-specific alkaline phosphatase (BAP) in osteoporosis patients relative to controls, although age and BMI exhibited no significant variations. Genetic investigation of the rs9939609 variant in the FTO gene revealed that the A allele correlates with a heightened risk of osteoporosis (OR=1.985, 95% CI=1.017–3.809, p=0.0414), while the various genotypes (TT, TA, AA) and the rs17817449 variant exhibited no significant association. These

findings emphasise the A allele's possible involvement as a genetic susceptibility factor for osteoporosis and stress the necessity for more studies to investigate gene-environment interactions affecting disease risk.

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