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Assessment of Some Bone Matrix Biochemical Parameters in Iraqi Postmenopausal Women with Osteoporosis

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ABSTRACT
Objectives: *Osteoporosis (OP) is a systemic skeletal disorder characterized with bone mass loss and microstructure, resulting in fragility fractures. Continued secretion of Osteopontin (OPN), osteonectin (ON), osteocalcin (OCN), Parathyroid hormone (PTH) and Ca^{+2} lead to bone remodeling disorders, followed by bone loss and osteoporosis (OP). The current study aims to investigate the biochemical proteins OPN, OCN, and ON in postmenopausal women with osteoporosis and determine whether we could use them as good indicators for OP diagnostics.* ***Materials and Methods:*** *Case- control study carried out between December 2022 and July 2023. OP disease was confirmed among 108 Iraqi postmenopausal women randomly selected from different Iraqi hospitals, Baghdad, Iraq. Their ages ranged between 45 and 70 years. According to DEXA scan results 70 samples were OP +ve results, while 40 samples were -ve (healthy control). Blood samples collected from all participants in order to assess the levels of Ca^{+2}, PTH, OPN, OCN, and ON by employing the ELISA technique.* ***Results:*** *High significant increase ($P \leq 0.01$) detected in PTH, OPN, and OCN serum levels, whereas, a significant decrease in Ca^{+2} and ON, in OP patients as a comparison to control group.* ***Conclusion:*** *OPN, OCN, and ON measurements are accessible, inexpensive, and easy to use and could be considered a good indicators for OP diagnostics; beside to a DEXA scan as a sensitive monitoring indicator for early detection of osteoporosis.*

Keywords: *Osteoporosis, Ca^{+2}, PTH, OPN, OCN, ON.*

Introduction Osteoporosis (OP) is a systemic skeletal disorder defined with low bone mineral density (BMD) and deterioration of bone architecture, which reduces bone strength and increases the risk of fractures [1, 2]. Vertebra, hip, spine, and forearm are the main parts of sustain fracture. All of age adventure, sex, smoke, alcohol, low vitamin D and calcium, weight loss, family history, parathyroid hormone (PTH), glucocorticoids, estrogen, early oophorectomy, immune status alteration, etc. are main factors in OP disease development. In postmenopausal women, osteoporosis is associated with estrogen deficiency, several diseases, or long-term drug uptake such as glucocorticoid [1, 3, 4, 5]. Moreover, parathyroid hormone (PTH) is a bone anabolic agent that contributes in osteoprogenitors development into mature osteoblasts and draws mesenchymal stromal cells into the osteoblast lineage, which both contribute to the expansion of bone mass [6]. The balance between bone resorption and bone formation is crucial for preserving skeletal integrity [7, 8]. However, bone strength and stiffness derive from the deposition of OPN, OCN, ON, small crystals of hydroxyapatite $Ca_{10}(PO4)_6(OH)_2$, carbonate, magnesium, and acid phosphate onto the collagen fibers, in osteoporosis case, bones become weak and brittle, making them more prone to fractures. [9].
Osteopontin (OPN), bone sialoprotein (BSP-1), secreted phosphoprotein-1 (SPP1), or early T-lymphocyte activation-1 (Eta-1), is a phosphoglycoprotein that is soluble in human physiological tissues and immobilized in the bone matrix as a component of the extracellular matrix (ECM) [10]. OPN is provided by osteoblasts (OBs), osteoclasts (OCs), osteocytes (OYs), T cells, B cells, natural killer cells (NK), dendritic cell (DC) macrophages, neural, epithelial, fibroblasts, smooth muscle, and endothelial cells [11]. Vascularization, immunological responses, inflammation, tissue remodeling, cell adhesion, cancer, and metastasis are all influenced by OPN [12]. Bone-related diseases such as osteoporosis, rheumatoid arthritis, and osteosarcoma are shown to arise and progress in OPN, and the migration, adhesion, and activation of osteoclasts (OCs) occur in an OPN-dependent manner [13, 14]. However, OCN is the most prevalent non-collagenous protein in bone that is expressed in osteoblasts [15]. OCN is regarded as a biochemical marker for bone remodeling and is involved in a number of physiological processes, including the maintenance of normal bone mineralization and the slowing down of growth-cartilage mineralization [16].

Osteonectin is a 32 kDa calcium-binding matricellular glycoprotein also known as basement membrane protein 40 (BM-40), or SPARC (secreted protein acidic and rich in cysteine), which is encoded by the 26.5 kb SPARC gene, which is found on chromosomes 5q31-q33. 10 exons and 9 introns, and four domains are concluded in the ON gene: 1) a cysteine-rich domain; 2) a hydrophilic area; 3) an extracellular Ca2+ (EC) domain with an E-F hand motif at the C-terminus that incorporates the collagen binding domain; and 4) an N-terminal low-affinity, high-capacity calcium-binding domain that contains the mineral binding region [17]. Reduced expression of osteonectin has been linked to bone fragility associated with certain osteopenic states, such as osteogenesis imperfecta, and it's possible that osteonectin gene mutations or polymorphisms contribute to inherited susceptibility to osteoporosis, according to a previous study using mice [18]. About 180–200 distinct molecules are involved in the non-collagenous proteins (NCPs) osteocalcin, osteonectin, and osteopontin, which play various functions in bone biology [19, 20]. Furthermore, abnormalities in the quantity and structure of one or more of these proteins can cause abnormalities in bone formation and resorption, which in turn cause defects and malformations like osteoporosis (OP). These abnormalities are caused by the coupling between osteoclasts and osteoblasts as well as non-collagenous proteins (NCPs) in the bone extracellular matrix, which together maintain normal bone metabolic regulation [21]. Furthermore, the production, assembly, and maturation of type I collagen are regulated by decorin (DCN), osteonectin (ON), and transforming growth factor beta (TGF-β), which also avoids degradation and maintains structure. Conversely, osteocalcin (OCN), osteonectin (ON), and osteopontin (OPN) are the mediators of bone mineralization, and type I collagen regulates this process [21, 22].
This study aimed to assess the biochemical proteins OPN, OCN, and ON in postmenopausal women with osteoporosis and determine if we could use them as good indicators for OP diagnostics.

1. Materials and Methods

2.1. Ethics approval and consent to participate

This study took place between December 2022 and July 2023. Written informed consent was obtained from all participants. The study was approved by the ethical committee of the Department of Biology, College of Science, University of Baghdad (reference code CSEC/0922/0092, on 26-9-2022).

2.2. Analytic Study Design

108 Iraqi postmenopausal women randomly selected from different Iraqi hospitals as; Al- Imamein Kadhimein Medical City, Al-Wasity Hospital and Medical city, Baghdad, Iraq to joining in this study according to certain conditions.

Inclusion Criteria: this study was included patients: a) only women in the postmenopausal stage with a different height and body weights, b) their ages ranged between 45 to 70 years old, c) without autoimmune disease or chronic diseases except (hypertension, Diabetes, Rheumatoid arthritis), d) without hereditary diseases, e) hormonal balanced and f) no smoker or alcoholism.

Exclusion criteria: this study was excluded patients: a) men, b) women with menopausal or premenopausal period, c) women with the ages below 45, d)women with autoimmune diseases except (hypertension, diabetes mellitus and rheumatoid arthritis), e) women with acute and infectious disease, for instant: covid-19, and ovary and uterine resection, f) women with hereditary diseases, g) hormonal imbalance, h)smoker and alcoholic and i) women refused to join to study.

2.2. Demographical and clinical cases

According to the conditions (as in inclusion criteria paragraph in above), 108 samples of Iraqi postmenopausal women selected and collected to participant in this study. In the baseline questionnaire, participants answered questions about their cities of living, age, race/ethnicity (white or other), weight, height, marital state, healthy state if they had (chronic diseases, autoimmune diseases, diabetes, infectious disease), smoking, Alcoholic, family history and if they got glucocorticoids or certain type of medicine.
DEXA scan was succumbed for OP presence investigation and bone mass index (BMI) calculation. DEXA scan performed to both lumbar spine and femoral neck for each sample to assessing bone health and osteoporosis resembled with T and Z scores. 70 samples confirmed with +ve OP disease, while, the reminder 40 samples were healthy control. The BMI (kg/ m^2) was measured by divided the weight (kg)/ height (m^2).

2.3. Collection of samples

2 ml of venous blood collected from each participant and sent to Iraqi hereditary company for analytic laboratory, each blood sample placed in a gel tube and coagulated for 10 minutes at room temperature. Subsequently, the serum was separated through centrifugation at a speed of 3000 rpm for 10 minutes. The serum was stored in a deep freezer at -20 °C until used to estimation, Ca^{+2}, PTH, OPN, OCN, and ON.

1.4. Biomarker measurements

Human Ca^{+2} ELISA kit (MYBioSource, MBS2540479, USA) utilized to measurement the concentration of calcium in serum samples, Human parathyroid hormone (PTH) ELISA kit (MYBioSource, MBS263675, USA) used to human PTH serum level detection, Human osteopontin (OPN) ELISA kit (Shanghi YL Biont, YLA0342HU, China), human osteocalcin (OCN) ELISA kit (Shanghi YL Biont, YLA1183HU, China), and human osteonectin (ON) ELISA kit (Shanghi YL Biont, YLA1345HU, China) were utilized to detect OPN, OCN and ON antibodies in serum samples of participant individuals with or without OP disease. At the end, our results read at 450 nm wave length and were given as ng/ml.

2.5 Statistical Analysis

IBM SPSS 28.00 version software was used for statistical analysis, chi-square test and an independent-sample t-test was used to examine data's mean and standard deviation (SD). High significant increased ($P \leq 0.01$) was recorded in PTH, OPN, and OCN serum levels. In contrast, high significant decreased ($P \leq 0.01$) was $Ca^{+2}$and ON serum levels in OP patients as compared with the control group.

2. Results

Demographics and baseline clinical characteristics

The baseline demographic and clinical characteristics of these participants are summarized in Table.1. The gender of all participants was females and 100 % identified as White. The majority (64.81 %) of the patients population reported living in outskirts of Baghdad city, while the reminders from the center of Baghdad. No statistically significant noticed between the age of patients (63.01±1.09 years) and control group (60.43±1.14 years). Whereas, highly significant increased ($P \leq 0.01$) was observed in the BMI value of OP patients as compared with the control group.

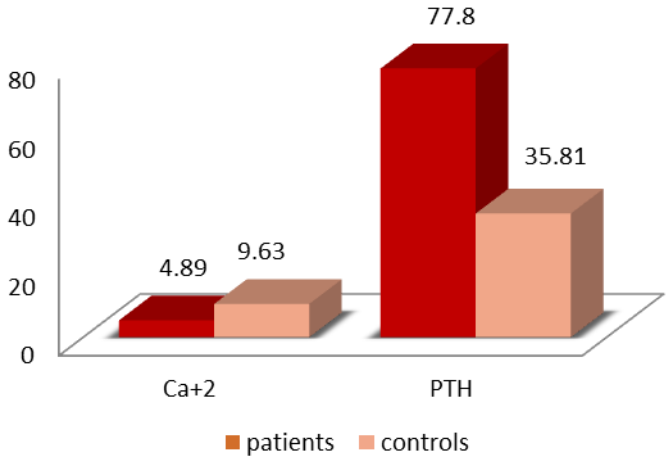
Table.1: The Baseline Demographics and Baseline Clinical Characteristics of Patients and Controls

Variables	Patients, N= 70		Controls, N=40
Race/ethnicity	White		
Gender	Females		
Age	63.01±1.09 years		60.43±1.14 years
Age groups	No. of patients	% Percentage	
>45	6	8.57	
50-59	19	27.14	
60-69	27	38.57	
≥70	18	25.71	
BMI	34.22±0.86 kg/m^2		24.83±0.43 kg/m^2
Duration of disease	No. of Patients	%Percentage	
From 1-3 years From 12-15 years From 4-7 years From 8 -11 years	43 16 8 3	61.42 22.85 17.14 4.28	
Smoking	With smoking 0 Without smoking 70		With 0 Without 40
Alcoholic	With 0 Without 70		With alcoholic 0 With alcoholic40

Glucocorticoid	With 18 (25.71%) Without 52 (74.28%)	With 0 Without 40

3.2 Ca^{+2} and PTH levels measurement

The results of the current investigation showed a highly significant (P≤0.01) increase in PTH serum level between mean ± SE of OP infected group (77.80±1.79pg/mL) and control group (35.81±2.06pg/mL), while there was a highly significant (P≤0.01) decrease in Ca^{+2} serum level as a comparison between mean ± SE of OP group (4.89±0.21mg/dL) and control group (9.63±0.13mg/dL), figure (1) and table (2).



3.3. OPN, OCN and ON measurements

High significant (P≤0.01) increase was detected in OPN (16.88±0.77 ng/ml) and OCN (29.86±2.25ng/ml) serum levels in OP patients as compared with the healthy control group, whereas OPN level was (5.95±0.41ng/ml) and OCN level was (10.73±0.86ng/ml) in the control group. Figure (2) and table (2).

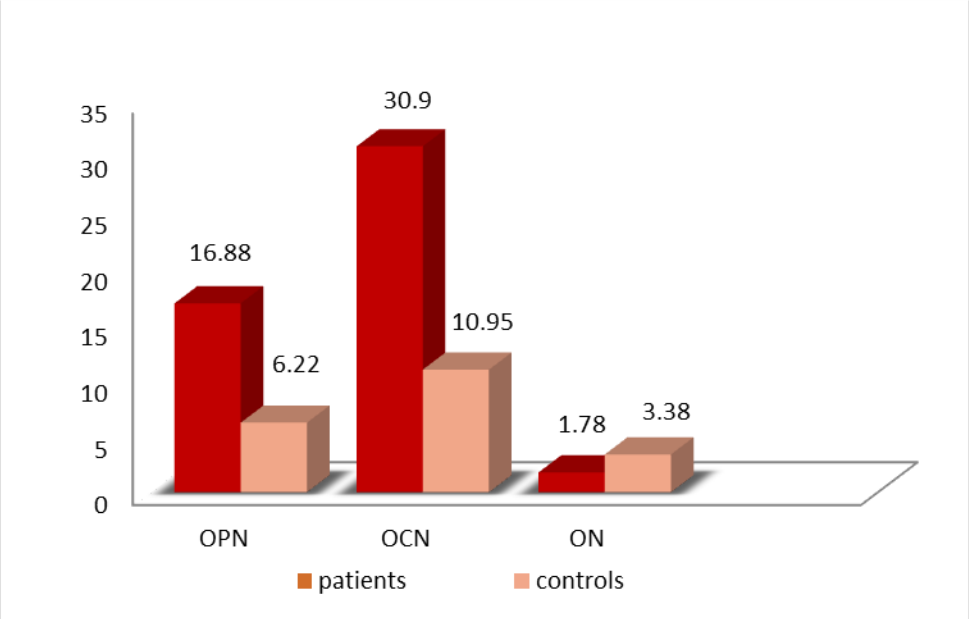


Figure (2): Levels of OPN, OCN and ON in OP patients and controls

All biomarkers results summarized in table (2).

Table (2): Serological and hematological parameters calculated in OP patients and controls.

Parameters	Patients	Controls	T- test	P- value
Ca^{+2} (mg/dL)	4.89 ± 0.2	9.63 ± 0.13	2.53**	0.0001
PTH (pg/ml)	77.80±1.7	35.81 ± 2.06	14.75**	0.0001
OPN (ng/ml)	16.88 ± 0.7	5.95 ± 0.41	5.81**	0.0001
OCN (ng/ml)	29.86 ± 2.2	10.73 ± 0.86	6.215**	0.0001
ON (ng/ml)	1.78 ± 0.97	3.42 ± 0.20	1.22**	0.0001

3.4. The correlation between biomarkers:

A positive significant correlation recorded at P-value= (0.01 and 0.05) between PTH, OPN and OCN serum levels, and a negative correlation recorded with Ca^{+2} and ON, table. 3

Table. 4: The correlation between the biomarkers.

Person Correlation		OPN	OCN	ON	PTH	Ca^{+2}
OPN	P Correlation	1	.324**	.024	.571**	.029
	Sig.		.001	.808	.000	.810
OCN	P Correlation	.324**	1	.099	.533**	.033**
	Sig.	.001		.308	.000	.360
ON	P Correlation	.024	.099	1	.027	.513**
	Sig.	.808	.308		.778	.000
	Sig.	.000	.000	.987	.000	.000

PTH	**P Correlation**	**.571****	**.533****	**.027**	**1**	**.117**
	Sig.	**.000**	**.000**	**.778**		**.240**
Ca^{+2}	**P Correlation**	**.029**	**.033****	**.513****	**.117**	**1**
	Sig.	**.810**	**.360**	**.000**	**.240**	

3. Discussion

According to the questionnaire's results and interviews, noticed that OP disease is asymptomatic up to the first fracture, joined with substantial pain, suffering, disability, and even death. When looking back to the results data found that the PTH level was greater and Ca2+ level decreased in the OP patient group, in contrast, group that is not afflicted. Bone remodeling involves a closely knit community of osteoblasts and osteoclasts that alternately produce new bone and resorb existing bone. As response to PTH activation, both calcium and phosphate are released to the blood during bone resorption, while Ca^{+2} and PO_4 deposit into bone during bone formation. This study hypothesized that continuous elevation in PTH serum level secretion and Ca^{+2} release from the bone might lead to an increase in net bone resorption, along with other factors such as RANKL increasing and OPG (decoy receptor) decreasing in osteoblasts and osteocytes. PTH works at the cellular level by indirectly stimulating osteoclasts to break down bone. PTH binds to cell receptors on osteoblasts stimulating the release of Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL). RANKL binds to its receptor on osteoclast precursors, stimulating them to fuse into mature osteoclasts, thereby increasing calcium resorption from bone [23]. Both calcium and PTH have a complementary role in bone homeostasis management and an inverse correlation with osteoporosis development. The study explanation was agreed with a study by Qu et al. [6] that found that parathyroid glands typically secrete parathyroid hormone (PTH) to maintain calcium homeostasis when the concentration of extracellular calcium drops. PTH is produced by accelerating bone resorption to release calcium and phosphorus into the circulation, increasing calcium reabsorption in the renal tubules, and encouraging the kidneys' production of active vitamin D. A studies by Jilka et al. [24] and Silva and Bilezikian [25] revealed that PTH can promote bone formation at low dosages, while it can activate bone resorption and lead to net bone loss at high dosages. A study by Zahmatkesh, [26] suggested that a high level of PTH can cause long-term bone resorption, which stimulates osteoclasts, bone-removing cells and decreases bone density. A studies by Akber and Yenzeel [27] and Marques and Moreira [28] suggested that parathyroid hormone (PTH) hypersecretion causes hyperparathyroidism, a disorder that affects calcium metabolism. Abnormal PTH elevation can increase blood calcium levels.

However, this current study, noticed due to osteoporosis, bones become weak and easy to break. In a normal state, bone requires tissue replacement over time with bone remodeling, whereas the old or damaged bone is removed with osteoclasts and replaced with new bone formed by osteoblasts. Imbalances in bone remodeling can lead to abnormal bone formation [29]. OPN, OCN, and ON play critical roles in bone resorption and bone building. The results, revealed a high serum level of OPN and OCN in postmenopausal women, and this elevation may be responsible for osteoporosis and osteoporotic fractures. Study analysis supports the idea that serum OPN and OCN levels might be used for the early and improved detection of fracture risk, especially in postmenopausal women, with or without osteoporotic vertebral fractures. Calcium and phosphorus deficiency in osteoporotic women lowers the formation of the hydroxyapatite crystals, which make free osteocalcin circulate in the blood.

The current results were agreed with a study by Kalaiselvi et al. [30], who supported the idea of increasing the concentration of osteocalcin in the sera of osteoporotic postmenopausal women. Another study done by Si et al., [14] demonstrated that high levels of OPN are an important risk factor for OP, which positively regulates OCs and inhibits bone mineral deposition, and the lack of OPN can increase fracture sensitivity in OP patients. A study by Shamsulddin et al. [31] a weakly significant inverse connection and a substantial rise in osteopontin levels in OP patients when compared to the control group. A study by Bai et al. [33] and Al-Kenany et al. [33] suggested a link between osteoporosis and an increased risk of fracture, particularly in postmenopausal women, and low OPN activity.

The finding results were agreed with the study by Hamdi, [34], which concluded that a rise in the blood level of osteocalcin in osteoporotic postmenopausal women is a significant factor in the development of primary osteoporosis. A

study by Mayur et al. [35] suggested that osteocalcin is a highly sensitive marker of bone formation and can be used as a diagnostic or screening tool for osteoporosis in postmenopausal women. As a result, the risk of fractures may be predicted and prevented by reducing osteocalcin serum levels after menopause. Moreover, the present investigation disagreed with Liu et al. [36] study proposed that there exists no noteworthy distinction in the pooled OCN level between postmenopausal controls and PMO patients. Furthermore, OCN is a poor predictor of PMO's high bone turnover status.

Furthermore, the study findings were agreed with study by Baloglu et al. [37], osteonectin was assumed to influence the creation of collagen fibers, the development of extracellular matrix, the differentiation of osteoblasts, and the ability to regulate osteoclast activity in order to promote bone formation.

Finally, an alteration in Ca^{+2}, PTH, OPN, OCN, and ON titers at the same time due to specific reasons as immune status, estrogen deficiency and etc may be collect and work together to impact bone mass leading gradually fractures then to osteoporosis.

Conclusion

The measurements of OPN, OCN, and ON are accessible, inexpensive, and easily used. The assessment of osteoporotic fracture risks can be predicted better by calculating a combination of the biochemical markers of bone turnover, which provide a better static picture of the skeleton than the assessment by either alone. OPN, OCN, and ON blood concentrations can be used as a sensitive monitoring indicator for early detection of osteoporosis, are promising as biomarkers used for identification of female individuals at high risk for osteoporosis, and could be used with DEXA scans as complementary tools for assessing bone quality and for early prediction of fracture risk.

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Consent for publication

Not applicable

CRediT authorship contribution statement

Reem- S.S Al-Lami: samples and data collection, project work applied, formal analysis, writing original draft, Writing review, editing. Jabbar-H Yenzeel: project supervisor.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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