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Alterations in Physiological Parameters Osteocalcin, Osteonectin, Osteopontin, Vitamin D, Vaspin and Visfatin in Rheumatoid Arthritis and Osteoarthritis Patients

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Abstract

***Background*:** Rheumatoid arthritis (RA) and osteoarthritis (OA) are two common chronic joint diseases that greatly affect patients' quality of life, each characterized by distinct yet overlapping pathophysiological mechanisms. Both conditions present significant challenges in clinical management. The changes in the levels of Osteocalcin, Osteonectin, Osteopontin, Vitamin D, Vaspin, and Visfatin in RA and OA patients highlight the intricate interplay between bone metabolism, inflammation, and immune responses.

The study aims to elucidate the distinct and shared pathophysiological mechanisms in rheumatoid arthritis (RA) and osteoarthritis (OA). This research investigates the alterations in several physiological key biomarkers parameters levels, including specifically Osteocalcin (OC), Osteonectin (ON), Osteopontin (OPN), Vitamin D, Visceral adipose-specific serine protease inhibitor (Vaspin), and Visfatin in patients with rheumatoid arthritis (RA) and osteoarthritis (OA).

Material & Methods: A cross-sectional laboratory-based study with 90 patient's participant was conducted. Using ELISA kits and laboratory tests, various biomarkers (Physiological Inflammatory biomarkers levels OC, ON, OPN, Vitamin D, Vaspin, and Visfatin in patients with rheumatoid arthritis (RA) and osteoarthritis (OA).

***Results*:** Our findings indicate significant alterations in these biomarkers among the patient groups. Osteocalcin levels were notably elevated in both RA and OA patients, suggesting their role in bone metabolism and disease progression.

As Osteopontin levels showed a significant decrease in the male rheumatoid arthritis group, while Osteopontin and Osteonectin levels exhibited a tendency for a non-significant decrease in the other study groups, highlighting its potential regulatory role. Vitamin D deficiency was prevalent across all patient groups, underscoring its importance in disease management and the necessity for supplementation. Vaspin and Visfatin levels were significantly elevated, particularly in RA patients, indicating their involvement in inflammatory processes and metabolic dysregulation.

***Conclusion*:** These alterations in key physiological biomarkers provide valuable insights into the complex interactions between bone metabolism, inflammation, and immune responses in RA and OA. This study emphasizes the importance of developing medical strategies to effectively manage these disease states and highlights potential therapeutic targets for future research.

Keywords: Rheumatoid arthritis; Osteoarthritis, Osteocalcin, Osteonectin, Osteopontin, Vitamin D Vaspin and Visfatin; Physiological Inflammatory biomarkers.

Introduction

Arthritic disorders encompass inflammation and decreased function in the joints and connective tissue, causing serious health issues and mortality. With approximately 100 types, these disorders collectively impose a significant health and economic burden on communities. Common types include rheumatoid arthritis (RA), osteoarthritis (OA), ankylosing spondylitis (AS), gout, lupus, and psoriatic arthritis. (Mohammed, S. A., & Sarhat, E. R., 2024).

Rheumatoid arthritis (RA) is a chronic inflammatory condition marked by peripheral symmetry and can include systemic joint damage, manifesting as synovitis and joint deformity when cartilage and bone are involved. The inflammation and bone destruction associated with RA can lead to significant functional impairment, with the affected joints and surrounding tissues experiencing progressive damage and dysfunction. (Liu, L. N., et al., 2019).

Rheumatoid arthritis (RA) It is also considered a chronic inflammatory, immune-mediated disease characterized by synovitis, especially in the small joints of the hands and feet. However, the disease is highly heterogeneous, presenting with various extra-articular manifestations and comorbidities. (Alivernini, S., et al., 2022).

Articular bone erosion is a characteristic feature of rheumatoid arthritis (RA) associated with disease severity and poor functional outcomes. Even with the use of intensive synthetic and biologic disease-modifying therapies, some patients with RA experience inadequate or partial responses, indicating that other pathways are involved in disease progression. Thus, identifying new targets for RA therapy continues to be a significant challenge. (Qian, J., et al., 2018).

Osteoarthritis (OA) impacts over 500 million individuals globally, posing a significant socio-economic burden. It is primarily characterized by the degradation of articular cartilage, the formation of osteophytes, and varying degrees of synovial inflammation, all contributing to the loss of joint function. (Boutet, M. A., et al., 2024).

Osteoarthritis (OA) is a chronic, progressive condition that results in a significant socioeconomic burden and lowers individual health-related quality of life. (Zhao, T., et al., 2022).

Osteocalcin (OC) is crucial for the biological and mechanical functions of bone. As the most abundant non-collagenous protein (NCP) in bone, it is specifically expressed in osteoblasts. It is produced during the late stages of bone formation and mineralization, and it directly and

indirectly regulates bone mass, mineral size, and orientation. Additionally, OC plays a role in organizing the extracellular matrix (ECM). (Jarecki, J., et al., 2022).

The Osteocalcin is also known as the bone protein γ-carboxyglutamic acid. The hormone belongs to the family of proteins that depend on vitamin K, as this vitamin is a catalyst in the formation of (γ-carboxylation) that determines its attraction to the bone matrix and calcium, and this contributes to the formation of bones. (Al-Jubouri, S. M., et al., 2022).

Previously, OC was believed to be closely associated with osteoporosis. Its primary function is to maintain normal bone mineralization and inhibit osteoblast activity, which can lead to osteoporosis. Elevated expression of OC is mainly observed in the early stages of bone synthesis following bone injury, as well as in postmenopausal and parathyroid osteoporosis. (Obied, M. M., & Sarhat, E. R., 2024).

Osteonectin (ON) also known as secreted protein acidic and rich in cysteine (SPARC/Osteonectin) is among the most abundant non collagenous proteins in mineralized tissues. Additionally, SPARC's potential to regulate the cross-linking of extracellular matrix proteins by the transglutaminase family of enzymes is being explored. Understanding the specific biological functions of SPARC in bone formation and turnover is crucial. (Rosset, E. M., & Bradshaw, A. D., 2016).

Osteopontin (OPN) is a multifunctional phosphoprotein with a molecular weight of 44-75 kDa, secreted by various cell types including osteoclasts, chondrocytes, synoviocytes, macrophages, lymphocytes, epithelial cells, and vascular smooth muscle cells (SMCs). It is found in the extracellular matrix of mineralized tissues and bodily fluids at inflammation sites, playing a crucial role in regulating inflammation, biomineralization, bone remodelling, immune functions, chemotaxis, and cell apoptosis. (Bai, R. J., et al., 2022).

Osteopontin (OPN) interacts with receptors like integrin and CD44 to regulate various physiological and pathological processes, including proliferation, differentiation, inflammation, metabolism, and tumor metastasis in chondrocytes, osteoblasts, and osteoclasts. (Yuan, Y., et al., 2020).

Previous studies have demonstrated that OPN plays a crucial role in the occurrence, repair, and maintenance of cartilage and subchondral bone metabolic homeostasis. This suggests that OPN is a significant regulator of Osteoarthritis (OA) and Osteoporosis (OP), influencing chondrocyte

and osteocyte metabolism by regulating the extracellular matrix components of articular cartilage and subchondral bone. (Li, L., et al., 2020).

25-hydroxy vitamin D (25-OH-D) is a fat-soluble compound with numerous essential functions, such as bone formation, neuromuscular functions, and the prevention of osteoporosis and inflammation. Recent data suggest that its metabolites are linked to the progression of rheumatoid arthritis (RA) and neuropathic pain in RA patients. (Cieślewicz, A., et al., 2024).

New studies revealed a significant link between vitamin D deficiency and knee osteoarthritis. However, the extent of this association is not yet fully understood. A higher prevalence of vitamin D deficiency has been observed in patients with knee osteoarthritis. Notably, maintaining adequate serum 25-(OH) vitamin D3 levels may help prevent knee osteoarthritis, particularly in women. (Tekeli, S. Ö., et al., 2024).

Adipokines are linked to the pathogenesis of rheumatoid arthritis (RA) and can serve as potential biomarkers of disease activity. Vaspin serum levels, in particular, are associated with the development of clinical manifestations in individuals with arthritis. (Romero-Sánchez, C., et al., 2023).

Visceral adipose tissue-derived serpin (Vaspin) is a relatively new adipokine linked to obesity and type II diabetes. Interestingly, its plasma levels have been found to be elevated in Knee Osteoarthritis (KOA). Recent studies suggest that knee joint degradation involves inflammatory components, with circulating Vaspin levels significantly associated with KOA, independently of age and sex. (Tarabeih, N., et al., 2023).

Adipokines, which act hormonally to regulate appetite and glucose metabolism, include leptin, resistin, adiponectin, and visfatin. These adipokines have been linked to the development of rheumatoid arthritis (RA). (Fatel, E. C. D. S., et al., 2019).

Visfatin, an adipokine with inflammatory and catabolic properties, is linked to various metabolic risk factors for Osteoarthritis (OA) and Osteoporosis (OP), including obesity, insulin resistance, and type II diabetes. Additionally, visfatin is associated with the innate immune receptor toll-like receptor 4 (TLR4), which is crucial in cartilage and bone inflammatory and catabolic responses. Visfatin is also connected to several pathological features of OA and OP. (Franco-Trepat, E., et al., 2019).

Materials and Methods

This study has been in Erbil International Hospital and Nawroz Hospital in a cross-sectional way on study population age ranged from (11-77) years old. The total number was 90 patients attending clinics specialized in Rheumatology and Osteoarthritis at Erbil International Hospital and Newroz Hospital, Erbil, Iraq, compared with normal people from whom control samples were taken. All of them were subjected to immunological tests which included OC, ON, OPN, VD3, Vaspin and visfatin.

Depending on the disease condition and the sex of the patient, the patients were divided into seven groups as the following table (Table 1)

Table (1): - A Table showing the division of patients and healthy into different groups depending on the disease condition and sex

No.	Disease Condition	Sex	Abbreviation Name	Total No. for the specific condition
1	Rheumatoid Arthritis	Male	MRA	10
2	Rheumatoid Arthritis	Female	FRA	25
3	Osteoarthritis	Male	MOA	14
4	Osteoarthritis	Female	FOA	22
5	Rheumatoid Arthritis-Osteoarthritis	Female	FRAOA	9
6	Healthy Control	Male	MCT	5
7	Healthy Control	Female	FCT	5

Samples Collection

Ten milliliters of venous blood was drawn from patients and healthy individuals participating in the current study while they were fasting, using medical syringes (5 ml) twice. After sterilizing the brachial vein area with 76% ethyl alcohol, blood was collected into two gel (yellow) tubes, each containing 3 ml (totaling 6 ml). The blood samples were then allowed to sit for 20 minutes before being centrifuged at 4000 rpm for 12 minutes to obtain serum. The serum was used to conduct immunological tests using the Automatic ELISA 96 Mindray.)

Determination of Osteocalcin (OC, ON, OPN, Vitamin D, Vaspin, and Visfatin) conc.

Principle

This ELISA kit employs the Sandwich-ELISA method. Within the kit, the Microelisa stripplate has already been coated with an antibody specific to OC, ON, OPN, Vitamin D, Vaspin, and

Visfatin. To conduct the test, you introduce either standards or samples into the corresponding wells of the Microelisa stripplate, where they bind to the specific antibody. Next, a Horseradish Peroxidase (HRP) conjugated antibody that targets OC, ON, OPN, Vitamin D, Vaspin, and Visfatin is added to each well of the Microelisa stripplate and left to incubate. Any unbound components are subsequently washed away. Then, a TMB substrate solution is introduced into each well. Only those wells containing OC, ON, OPN, Vitamin D, Vaspin, and Visfatin and the HRP-conjugated OC, ON, OPN, Vitamin D, Vaspin, and Visfatin antibody will initially appear blue in color, which changes to yellow upon the addition of the stop solution. The optical density (OD) is quantitatively measured using a spectrophotometer set to a wavelength of 450 nm. The OD value is directly proportional to the concentration of OC, ON, OPN, Vitamin D, Vaspin, and Visfatin in the sample. By comparing the OD of the samples to a standard curve, you can calculate the concentration of OC, ON, OPN, Vitamin D, Vaspin, and Visfatin in your samples.

Table (2): - Materials provided with the kit

No.	Materials provided with the kit	kit 96 determinations	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T.
4	Microelisa stripplate	1	2-8℃
5	Standard：45 ng/ml	0.5ml×1 bottle	2-8℃
6	Standard diluent	1.5ml×1 bottle	2-8℃
7	HRP-Conjugate reagent	6ml×1 bottle	2-8℃
8	Sample diluent	6ml×1 bottle	2-8℃
9	Chromogen Solution A	6ml×1 bottle	2-8℃
10	Chromogen Solution B	6ml×1 bottle	2-8℃
11	Stop Solution	6ml×1 bottle	2-8℃
12	wash solution	20ml (30X) ×1bottle	2-8℃

Sample preparation (Serum preparation)

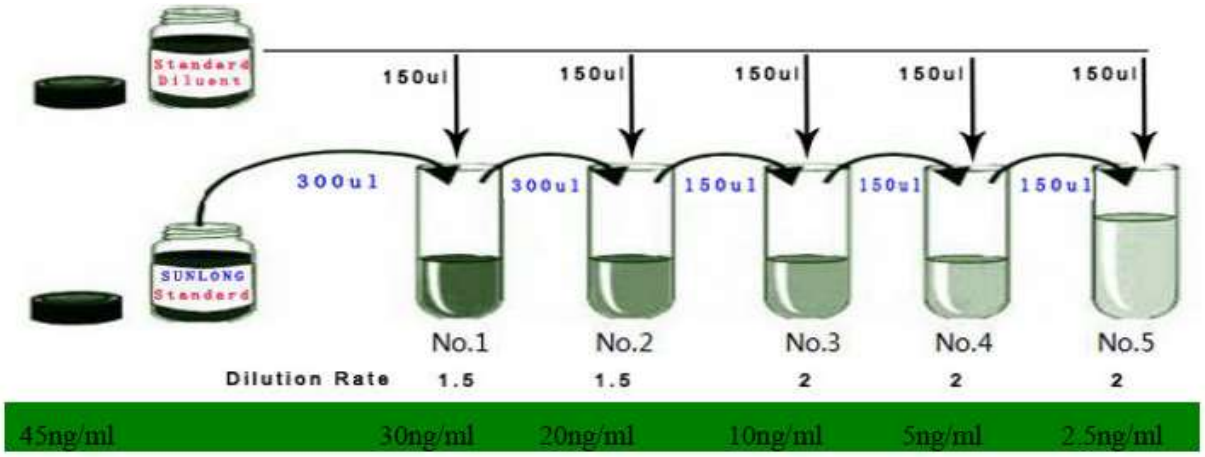
After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again.

Procedure

1. Dilution of Standards. Dilute the standard by small tubes first, then pipette the volume of 50ul from each tube to microplate well, each tube uses two wells, total ten wells.

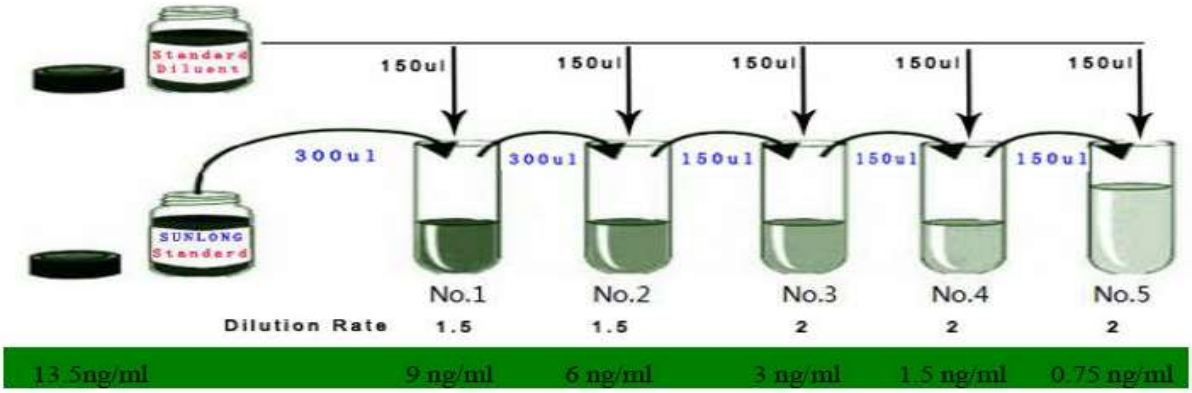
OCN and Vit D3

30ng/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
20ng/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
10ng/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
5ng/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
2.5ng/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent



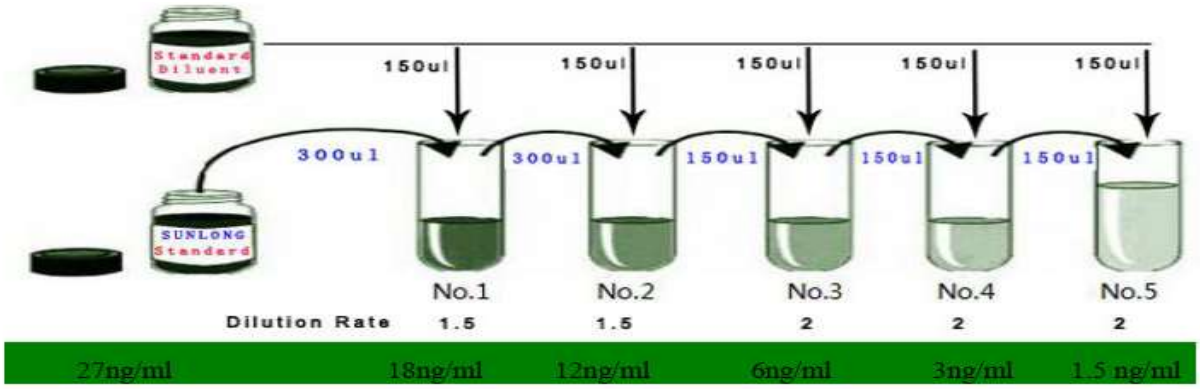
ON AND OPN

9ng/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
6ng/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
3ng/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
1.5ng/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
0.75ng/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent



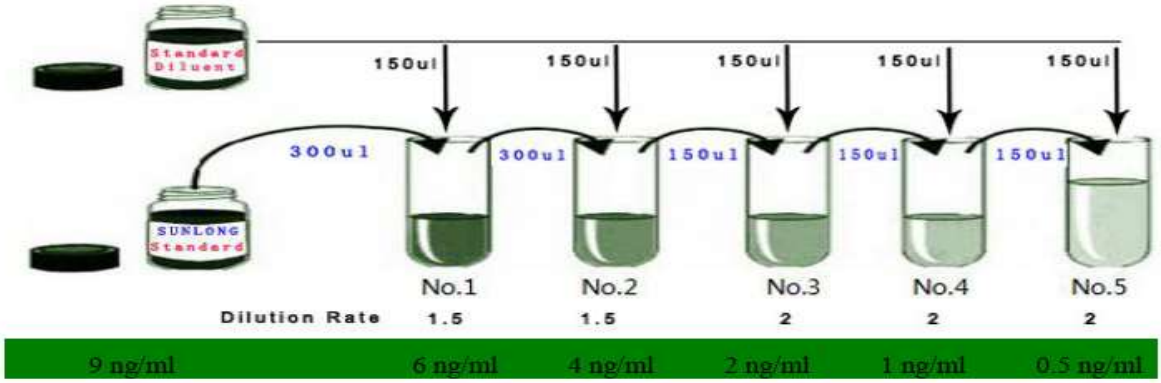
Vaspin

18ng/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
12ng/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
6ng/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
3ng/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
1.5ng/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent



Visfatin

6 ng/ml	Standard No.1	300µl Original Standard + 150µl Standard diluents
4 ng/ml	Standard No.2	300µl Standard No.1 + 150µl Standard diluents
2 ng/ml	Standard No.3	150µl Standard No.2 + 150µl Standard diluent
1 ng/ml	Standard No.4	150µl Standard No.3 + 150µl Standard diluent
0.5 ng/ml	Standard No.5	150µl Standard No.4 + 150µl Standard diluent



2. In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.

3. Incubation: incubate 30 min at 37°C after sealed with Closure plate membrane.

4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).

5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.

6. Add 50 µl HRP-Conjugate reagent to each well except the blank control well.

7. Incubation as described in Step 3.

8. Washing as described in Step 5.

9. Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37°C for 15 minutes. Please avoid light during coloring.

10. Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.

11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

Notes:

1. Store the kit at 4 ° C upon receipt. The kit should be equilibrated to room temperature before the assay. Remove any unneeded strips from Human OC, ON, OPN, Vitamin D, Vaspin, and Visfatin Antibody-Coated plate, reseal them in zip-lock foil and keep at 4°C.

2. Precipitates may appear in concentrated washing buffer. Please heat the buffer to dissolve all the precipitates, which will not affect the results.
3. Accurate pipette should be used to avoid experimental error. Samples should be added to the Microplate in less than 5 minutes. If a large number of samples are included, multiple channel pipette is recommended.
4. Standard curve should be included in every assay. Replicate wells are recommended. If the OD value of the sample is greater than the first well of standards, please dilute the 6 sample (n times) before test. When calculating the original OC, ON, OPN, Vitamin D, Vaspin, and Visfatin concentrations, please multiply the total dilution factor (XnX5).
5. In order to avoid cross-contamination, Microplate sealers are for one-time use only.
6. Please keep Substrate away from light.
7. All the operation should be accordance with the manufacturer's instructions strictly. The results determined by the Microtiter Plate Reader.
8. All the samples, washing buffer and wastes should be treated as infectious agents.
9. Reagents from different lots should not be mixed.

Calculation of Results

Known concentrations of Human OC, ON, OPN, Vitamin D, Vaspin, and Visfatin Standards and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human OC, ON, OPN, Vitamin D, Vaspin, and Visfatin in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor.

Results and Discussion

Descriptive Characteristics:

A total of ninety patients, comprising both males and females, participated in this study. They were categorized into five groups: Male RA, Female RA, Male OA, Female OA, and Female RA-OA, with ten healthy individuals as the control group. The research findings reveal that, despite significant progress in rheumatoid arthritis (RA) management, the disease continues to

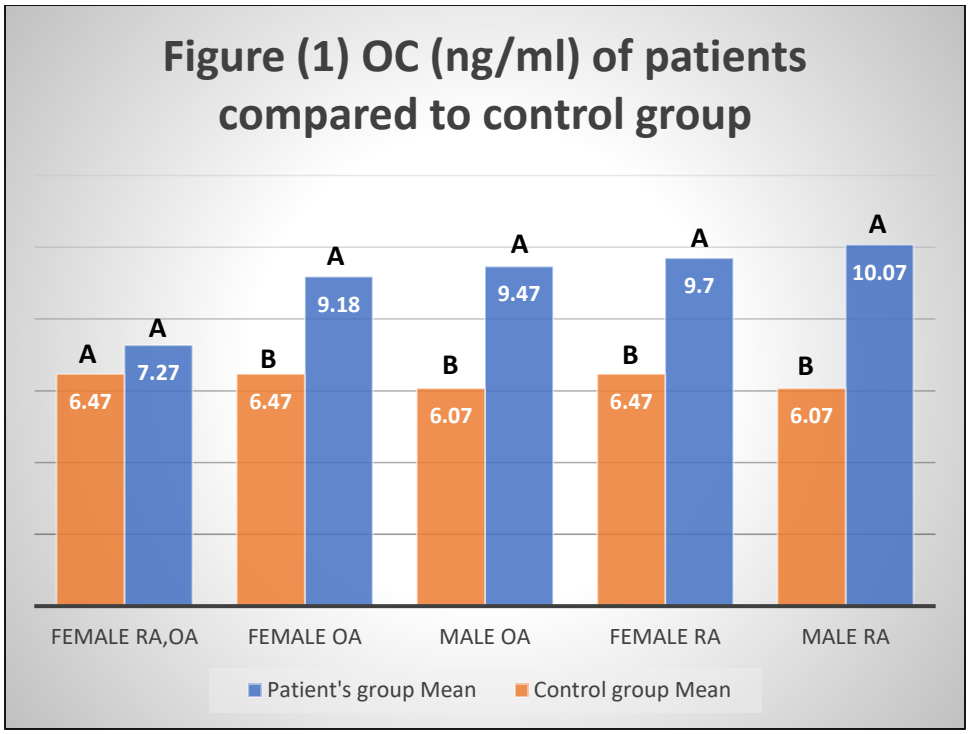
substantially impact many patients. Notably, specific factors, including certain parameter levels, are closely linked to various symptoms such as fatigue, pain, morning stiffness, and changes in functional ability. These factors persist in significantly affecting the daily lives of individuals with rheumatoid arthritis and osteoarthritis.

OC of patients compared to control group

Table (3), which included OC of patients (ng/ml) compared with the OC of control group, shows that there are significant differences appeared in all study groups **Males RA (10.07), Female RA (9.7), Males OA (9.47),** and **Female OA (9.18)**, except **Female RA-OA** group, when compared with the control groups (Male control= **6.07**, Female control= **6.47**). **At P ≤ 0.05** as shown in table (3) and figure (1).

Table (3) OC (ng/ml) of patients compared to control group

Case of study	Male RA	Female RA	Male OA	Female OA	Female RA, OA
Patient's group **Mean ± SD**	**10.07 A ± 1.32**	**9.7 A ± 1.39**	**9.47 A ± 1.23**	**9.18 A ± 1.09**	**7.27 A ± 1.11**
Control group **Mean ± SD**	**6.07 B ± 1.52**	**6.47 B ± 1.37**	**6.07 B ± 1.52**	**6.47 B ± 1.37**	**6.47 A ± 1.37**
T test	**5.47**	**5.43**	**8.49**	**4.73**	**0.94**
P value	**0.00005**	**0.00001**	**0.00001**	**0.00003**	**0.18**



The results of the present study were consistent with those of Liu's research in 2019, indicating a significant rise in osteocalcin (OC) levels across all patient groups, except for the female Rheumatoid arthritis - osteoarthritis group (FRA-OA). While there was an increase observed in this group, it was not as pronounced when compared to the control group. (Liu, L. N., et al., 2019). The findings of our present investigation align closely with those reported by Al-Jubouri and colleagues in 2022. Specifically, our study revealed a significant elevation in osteocalcin (OC) levels across all study groups, encompassing both rheumatoid arthritis groups (RA) and osteoarthritis groups (OA), with one exception being the female Rheumatoid arthritis - osteoarthritis group (FRA-OA). Although an increase was noted in this particular group, it was comparatively less pronounced in comparison to the control group. (Al-Jubouri, S. M., et al., 2022).

Conditions impacting the endocrine system, like hyperparathyroidism or hyperthyroidism, can disrupt bone metabolism, resulting in increased levels of osteocalcin. Moreover, hormonal fluctuations during stages like puberty, pregnancy, or menopause can influence bone turnover, thereby affecting osteocalcin levels. Adequate levels of vitamin K are crucial for osteocalcin activation, and research suggests a potential correlation between elevated osteocalcin levels and insulin resistance or type 2 diabetes, although the precise mechanisms remain under investigation.

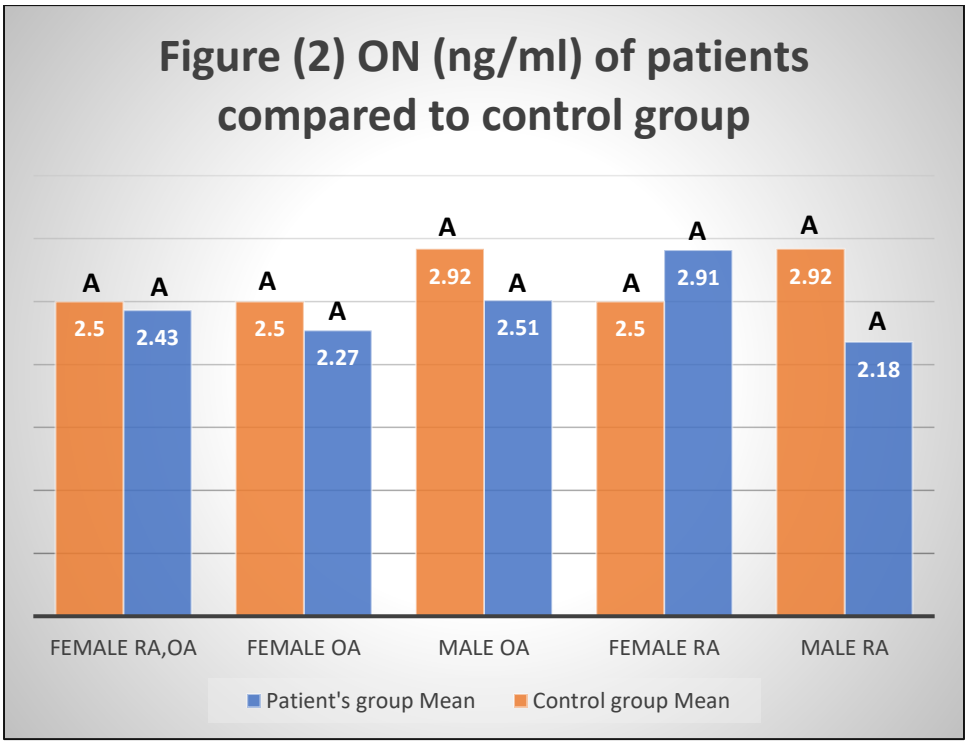
Additionally, certain medications such as glucocorticoids or antiepileptic drugs can interfere with bone metabolism, potentially leading to elevated osteocalcin levels. Lastly, genetic variations may impact an individual's susceptibility to changes in bone metabolism, contributing to variations in osteocalcin levels among different individuals.

ON of patients compared to control group

Table (4), which included ON (ng/ml) of patients compared with ON of control group, shows that there are no significant differences in all groups when compared with the control groups. (Male control= **2.92,** Female control=**2.5**). **At P ≤ 0.05** as shown in table (4) and figure (2).

Table (4) ON (ng/ml) of patients compared to control group

Case of study	Male RA	Female RA	Male OA	Female OA	Female RA, OA
Patient's group **Mean ± SD**	**2.18 A ± 0.83**	**2.91 A ± 0.98**	**2.51 A ± 0.93**	**2.27 A ± 0.86**	**2.43 A ± 0.85**
Control group **Mean ± SD**	**2.92 A ± 0.82**	**2.5 A ± 0.83**	**2.92 A ± 0.82**	**2.5 A ± 0.83**	**2.5 A ± 0.83**
T test	**1.54**	**1.02**	**1.2**	**0.61**	**0.2**
P value	**0.07**	**0.15**	**0.12**	**0.27**	**0.42**



The findings of the present investigation contrasted with those of Nie and colleagues' study conducted in 2024. Unlike the results of Nie's study, which demonstrated a notable increase in Osteonectin levels in all groups, including patients with rheumatoid diseases like osteoarthritis, the current study did not reveal any significant elevation in Osteonectin levels across the study groups compared to control samples. (Nie, Y., et al., 2024).

Secreted protein acidic and rich in cysteine (SPARC), also known as Osteonectin or BM-40, is a matricellular protein that regulates various cellular processes such as cell adhesion, production of extracellular matrix, activity of growth factors, and the cell cycle. Despite not having a structural role, SPARC influences interactions between cells and the surrounding extracellular matrix through its anti-proliferative and anti-adhesion properties. The increased presence of SPARC in locations such as injury sites, during regeneration, in cases of obesity, cancer, and inflammation, suggests its potential as a target for therapy and as an indicator for assessing diseases. (Jiang, S., et al., 2023).

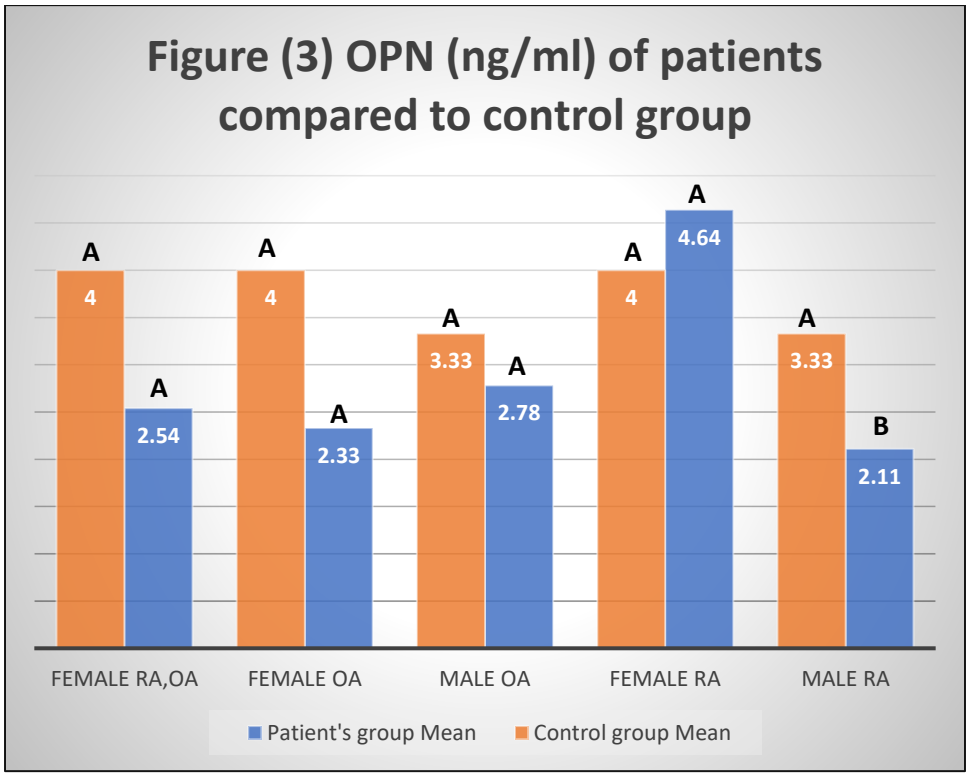
It's important to highlight the limited available information regarding Osteonectin, particularly concerning its measurement in patients with rheumatoid arthritis or osteoarthritis. Studies examining this parameter in other patient populations have been few in number, such as Davies' investigation of Osteonectin levels in scleroderma patients. Davies and colleagues' 2006 study revealed a marked increase in Osteonectin levels in scleroderma patients. Therefore, the findings of the current study contradict those of Davies and his team, as no significant differences in Osteonectin levels were observed across all study groups compared to control samples. (Davies, C. A., et al., 2006).

OPN of patients compared to control group

Table (5), which included OPN of patients (ng/ml) compared with the OPN of control group, shows that there are significant differences appeared in the **Males RA** group only, while no significant differences in all other groups when compared with the control groups (Male control= **3.33**, Female control= **4**). **At $P \leq 0.05$** as shown in table (5) and figure (3).

Table (5) OPN (ng/ml) of patients compared to control group

Case of study	Male RA	Female RA	Male OA	Female OA	Female RA, OA
Patient's group **Mean ± SD**	**2.11** ^{B} **± 0.39**	**4.64** ^{A} **± 0.41**	**2.78** ^{A} **± 1.99**	**2.33** ^{A} **± 0.7**	**2.54** ^{A} **± 0.37**
Control group **Mean ± SD**	**3.33** ^{A} **± 1.0**	**4** ^{A} **± 0.32**	**3.33** ^{A} **± 1.0**	**4** ^{A} **± 0.32**	**4** ^{A} **± 0.32**
T test	**3.11**	**0.29**	**0.55**	**1.09**	**0.62**
P value	**0.004**	**0.38**	**0.29**	**0.14**	**0.27**



The findings of the current study diverged from those of Liu and colleagues in 2019, who reported a significant increase in the level of osteopontin. In contrast, the results of the current study contradicted Liu's findings, as all groups except the female rheumatoid arthritis group (FRA) exhibited a decrease in osteopontin levels compared to the control samples, In the case of the FRA group showed a non-significant increase in osteopontin levels compared to the control sample. (Liu, L. N., et al., 2019).

Osteopontin (OPN) is a phosphoglycoprotein derived from bone that plays a crucial role in both physiological and pathological processes. Its significance has increased due to its involvement in

the immune system's response to chronic degenerative diseases like rheumatoid arthritis (RA) and osteoarthritis (OA). OPN, as an extracellular matrix (ECM) glycoprotein, is pivotal in bone remodeling, thus acting as an effector molecule that contributes to the observed joint and cartilage destruction in clinical studies. However, since OPN undergoes various modifications, such as posttranslational changes, proteolytic cleavage, and binding to a wide array of receptors, the precise mechanisms underlying its effects remain unclear in some cases. Despite strong evidence suggesting OPN's significant contribution to the immunopathology of RA and OA when viewed as a common denominator molecule, some experimental trial results suggest a potential protective role for OPN in rheumatic diseases. (Martín-Márquez, B. T., et al., 2023).

The findings of the current study mirrored those of Ji and colleagues' 2014 study, wherein no significant increase was observed across all groups in the current study, except for the female rheumatoid arthritis group (FRA), where there was a slight increase in Osteopontin (OPN), although not statistically significant. Conversely, a significant decrease in OPN levels was noted in the male rheumatoid arthritis group (MRA). Among the remaining study groups, a reduction in osteopontin levels was observed, but no significant differences were found compared to the control samples. (Ji, H. I., et al., 2014).

Osteopontin (OPN) serves as a multifunctional cytokine and adhesion molecule, playing a unique role in regulating both innate and adaptive immune responses. Various immune cells, including dendritic cells (DCs), macrophages, and T lymphocytes, have the capacity to produce OPN. Its expression is noted to increase in a broad spectrum of disorders, encompassing autoimmunity, cancer, and allergy. (Xu, C., et al., 2022). However, low levels of osteopontin can stem from several factors. In some autoimmune conditions like systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), there are instances of decreased osteopontin levels. The precise mechanisms driving this decrease are not entirely elucidated, but they are believed to involve immune system dysregulation and altered cytokine profiles. Additionally, chronic inflammation, as observed in conditions such as chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), and chronic kidney disease (CKD), can suppress osteopontin expression. Hormonal factors, such as estrogen and testosterone, are also influential in osteopontin expression, as fluctuations in hormone levels during menopause or andropause can impact its production. Furthermore, osteopontin levels may diminish with age, contributing to age-related alterations in bone metabolism and heightened susceptibility to fractures and osteoporosis. Finally, certain

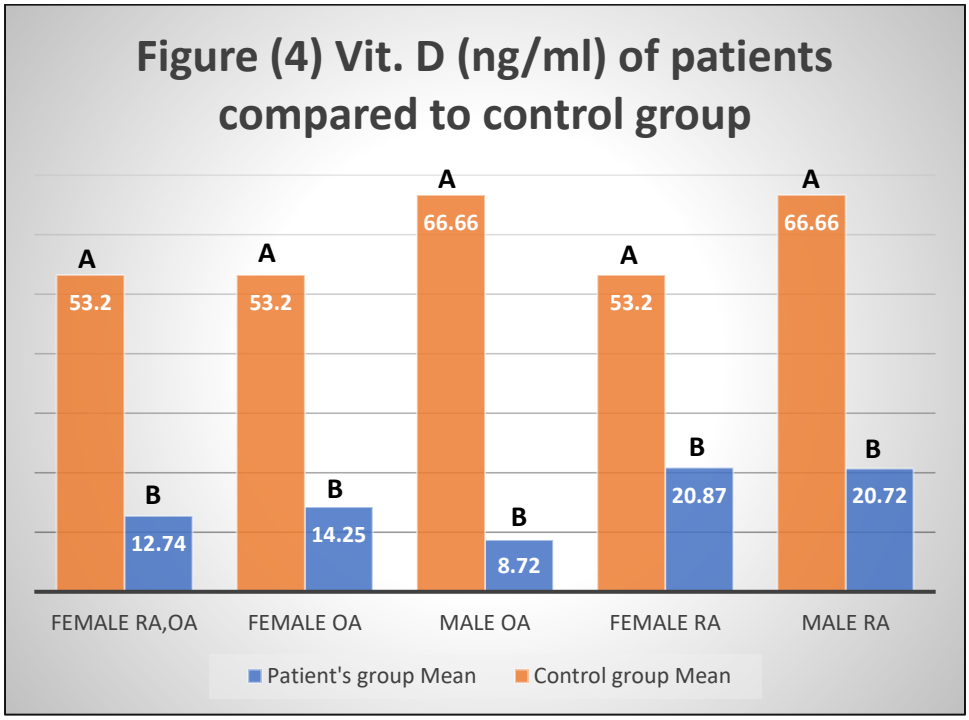
medications like glucocorticoids and certain chemotherapeutic agents have the potential to suppress osteopontin expression or interfere with its function, resulting in osteopontin deficiency.

Vit. D of patients compared to control group

Table (6), which included Vit. D of patients (ng/ml) compared with the Vit. D of control group, shows that there are significant differences appeared in all study groups, **Males RA (20.72), Female RA (20.87), Males OA (8.72), Female OA (14.25)**, and the **Female RA-OA (12.74)**, when compared with the control groups (Male control= **66.66**, Female control= **53.2**), **At P ≤ 0.05** as shown in table (6) and figure (4).

Table (6) Vit. D (ng/ml) of patients compared to control group

Case of study	Male RA	Female RA	Male OA	Female OA	Female RA, OA
Patient's group **Mean ± SD**	**20.72** ^{B} **± 5.31**	**20.87** ^{B} **± 7.17**	**8.72** ^{B} **± 1.86**	**14.25** ^{B} **± 3.8**	**12.74** ^{B} **± 2.02**
Control group **Mean ± SD**	**66.66** ^{A} **± 9.53**	**53.2** ^{A} **± 12.27**	**66.66** ^{A} **± 9.53**	**53.2** ^{A} **± 12.27**	**53.2** ^{A} **± 12.27**
T test	**11.14**	**7.73**	**20.44**	**12.01**	**8.94**
P value	**<0.00001**	**<0.00001**	**<0.00001**	**<0.00001**	**<0.00001**



The findings of the present study corroborated those of a study conducted by Gopal and colleagues in 2019, which indicated a deficiency in vitamin D levels among rheumatoid arthritis (RA) patients. This alignment with the current study's results was marked by a significant and substantial deficiency in vitamin D levels across all groups compared to the control samples. (Gopal, K., et al., 2019).

Research has demonstrated that vitamin D can alleviate both the severity of osteoarthritis and the autophagy process. Nevertheless, the impact of vitamin D on autophagy in knee osteoarthritis (KOA) remains unclear. (Saengsiwaritt, W., et al., 2023).

The findings of the present study were consistent with another significant study, namely the Amirkhizi study in 2022, which revealed a substantial decrease in vitamin D levels among patients with osteoarthritis. This correlation was mirrored in the current study, which also demonstrated a marked decline in vitamin D levels across all study groups, including those with osteoarthritis, in comparison to control samples. (Amirkhizi, F., et al., 2022).

It's noteworthy that certain studies have suggested a robust correlation between low vitamin D levels and elevated inflammatory biomarkers, along with heightened severity of clinical symptoms in osteoarthritis patients. (Saengsiwaritt, W., et al., 2023).

Various factors can contribute to low levels of vitamin D:

Renal Dysfunction: The kidneys are essential for converting inactive vitamin D into its active form. Dysfunction in the kidneys, as seen in conditions like chronic kidney disease, can hinder this conversion process, leading to a deficiency in vitamin D.

Medications: Certain drugs, such as anticonvulsants, glucocorticoids, and specific cholesterol-lowering medications, may disrupt the metabolism or absorption of vitamin D, thereby contributing to lower levels.

Advancing Age: Aging is linked with diminished efficiency in synthesizing vitamin D due to alterations in skin structure and decreased exposure to sunlight. Additionally, older individuals may have reduced dietary intake and impaired absorption, further exacerbating the deficiency.

Malabsorption Disorders: Disorders like celiac disease, Crohn's disease, and other gastrointestinal conditions can impair the absorption of dietary nutrients, including vitamin D, despite adequate dietary intake.

Limited Sun Exposure: Vitamin D synthesis occurs in the skin upon exposure to sunlight. Insufficient time spent outdoors, covering up with clothing, or residing in regions with minimal sunlight exposure can lead to insufficient vitamin D production.

Obesity: Vitamin D is fat-soluble, and excess adipose tissue can sequester it, reducing its availability in circulation. Consequently, obese individuals may have lower levels of circulating vitamin D despite consuming sufficient amounts.

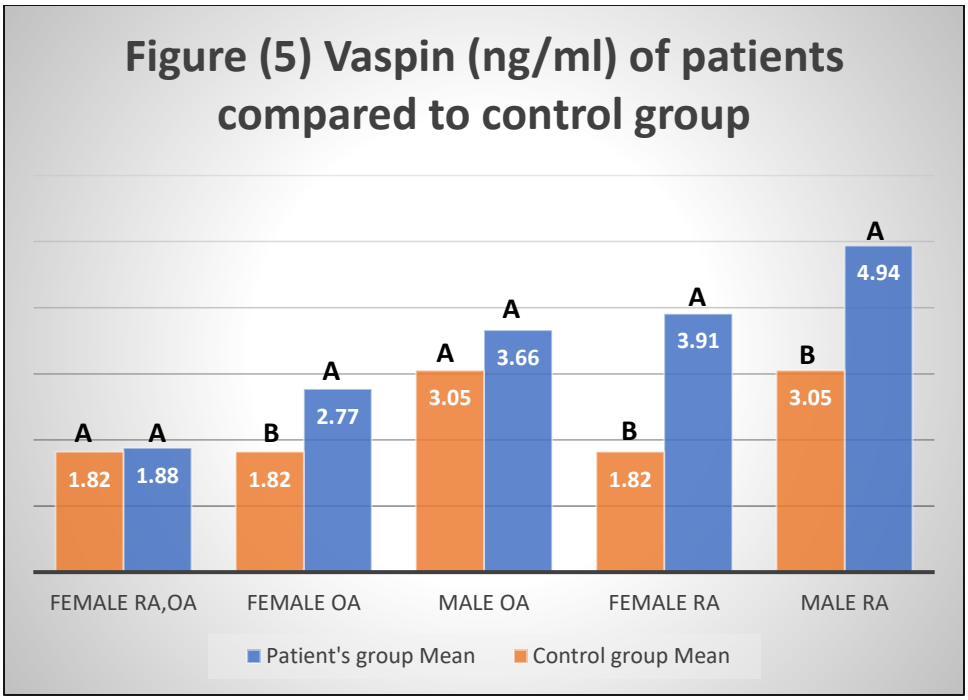
Dietary Insufficiency: Natural food sources of vitamin D are limited and include fatty fish, egg yolks, and fortified products like milk and cereal. Inadequate consumption of these foods, particularly among those with restrictive diets or specific cultural practices, can result in vitamin D deficiency.

Vaspin of patients compared to control group

Table (7), which included Vaspin of patients (ng/ml) compared with the Vaspin of control group, shows that there are significant differences appeared in the **Males RA (4.94), Female RA (3.91)** and **Female OA (2.77)**, while no significant differences in **Males OA** and **Female RA-OA** groups, when compared with the control groups (Male control= **3.05**, Female control= **1.82**). **At P ≤ 0.05** as shown in table (7) and figure (5).

Table (7) Vaspin (ng/ml) of patients compared to control group

Case of study	Male RA	Female RA	Male OA	Female OA	Female RA, OA
Patient's group **Mean ± SD**	**4.94 A ± 0.86**	**3.91 A ± 0.91**	**3.66 A ± 1.25**	**2.77 A ± 1.23**	**1.88 A ± 1.31**
Control group **Mean ± SD**	**3.05 B ± 1.40**	**1.82 B ± 1.38**	**3.05 A ± 1.40**	**1.82 B ± 1.38**	**1.82 A ± 1.38**
T test	**3.92**	**3.53**	**1.09**	**1.96**	**0.13**
P value	**0.00087**	**0.00071**	**0.14**	**0.03**	**0.44**



The findings of the present study echoed those of Rodríguez and colleagues, revealing a marked elevation in Vaspin levels within certain groups, notably the male rheumatoid arthritis (MRA), female rheumatoid arthritis (FRA), and female osteoarthritis (FOA) cohorts. Conversely, the remaining study groups showed only a marginal uptick in Vaspin levels compared to the control samples. (Rodríguez, J., et al., 2021).

Vaspin is part of a group of serine protease inhibitors and has been linked to insulin resistance, metabolic syndrome, atherosclerosis, and cardiovascular disease. Apart from being produced in subcutaneous adipose tissue, Vaspin is also expressed in other tissues like skin and skeletal muscle. It's noteworthy that Vaspin has been implicated in skeletal muscle inflammation. However, there is scarce information regarding the precise role of Vaspin in autoinflammation within the context of rheumatoid arthritis (RA). (Neumann, E., et al., 2021).

The findings of the present study contrasted with those of Wang and colleagues in 2021. Wang's study suggested a decrease in Vaspin levels in the osteoarthritis group, whereas the current study revealed the opposite. Specifically, a substantial and noteworthy increase in Vaspin levels was observed in the female osteoarthritis group (FOA), along with a slight, nonsignificant increase in both the male osteoarthritis group (MOA) and the female rheumatoid arthritis-osteoarthritis group (FRA-OA). Additionally, the rheumatoid arthritis groups exhibited a significant increase in Vaspin levels compared to control samples. (Wang, J., et al., 2021).

Elevated Vaspin levels in rheumatoid arthritis (RA) and osteoarthritis (OA) patients can be ascribed to various factors:

Inflammation: RA and OA are both inflammatory conditions, although with distinct underlying mechanisms. RA involves chronic systemic inflammation stemming from an autoimmune reaction targeting synovial joints, leading to joint damage. In OA, inflammation tends to be more localized within affected joints. The heightened inflammation can trigger Vaspin production as part of the body's response to counteract inflammatory processes.

Adipose Tissue Dysfunction: Vaspin is primarily synthesized in adipose tissue, which often shows dysfunction in RA and OA patients. This dysfunction is characterized by altered secretion of adipokines and hypertrophy of adipocytes, potentially resulting in increased Vaspin release into circulation.

Insulin Resistance: Vaspin has been associated with insulin resistance, a common feature in both RA and OA. Insulin resistance contributes to the metabolic irregularities observed in these conditions, and elevated Vaspin levels might serve as a compensatory mechanism to mitigate insulin resistance.

Hormonal Factors: Hormonal imbalances or dysregulation observed in RA and OA may also impact Vaspin levels. For instance, estrogen levels in women with RA and OA could influence Vaspin production, given that estrogen has been demonstrated to modulate adipokine secretion.

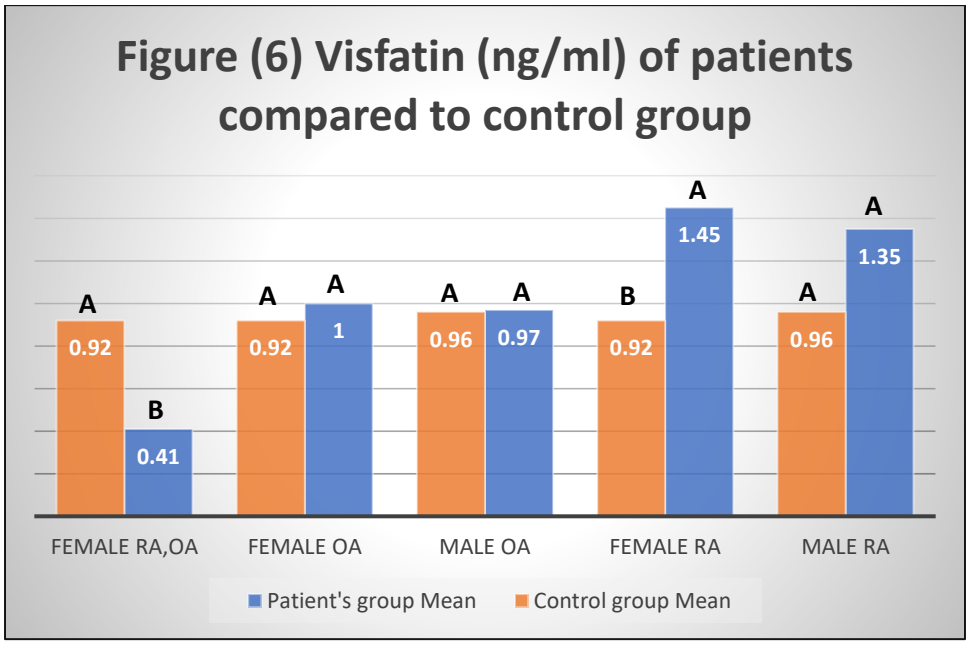
These factors collectively contribute to the heightened levels of Vaspin seen in RA and OA patients, illustrating the intricate interplay among inflammation, metabolic dysfunction, tissue remodeling, and hormonal regulation in these disorders.

Visfatin of patients compared to control group

Table (8), which included Visfatin of patients (ng/ml) compared with the Visfatin of control group, shows that there are significant differences appeared in the **Female RA** (**1.45)** and **Female RA-OA (0.41)** groups, while no significant differences in all other groups when compared with the control groups (Male control= **0.96**, Female control= **0.92**). **At $P \leq 0.05$** as shown in table (8) and figure (6).

Table (8) Visfatin (ng/ml) of patients compared to control group

Case of study	Male RA	Female RA	Male OA	Female OA	Female RA, OA
Patient's group **Mean ± SD**	**1.35** ^{A} **± 0.36**	**1.45** ^{A} **± 0.38**	**0.97** ^{A} **± 0.38**	**1.0** ^{A} **± 0.43**	**0.41** ^{B} **± 0.42**
Control group **Mean ± SD**	**0.96** ^{A} **± 0.46**	**0.92** ^{B} **± 0.47**	**0.96** ^{A} **± 0.46**	**0.92** ^{A} **± 0.47**	**0.92** ^{A} **± 0.47**
T test	**1.37**	**2.93**	**0.058**	**0.59**	**4.01**
P value	**0.096**	**0.0033**	**0.47**	**0.27**	**0.00086**



The findings of the present study mirrored those of Allam and colleagues, indicating a notable elevation in visfatin levels, particularly in the female rheumatoid arthritis group (FRA), followed by a less pronounced increase in the male rheumatoid arthritis group (MRA). Similarly, the male osteoarthritis group (MOA) and the female osteoarthritis group (FOA) exhibited a marginal rise in visfatin levels compared to the control group. In contrast, the female rheumatoid arthritis-osteoarthritis group (FRA-OA) showed a significant decrease in visfatin levels, deviating from the other groups, with levels lower than those in the control group. It's worth noting that the female rheumatoid arthritis-osteoarthritis group (FRA-OA) was not examined in Allam's study, and its

findings contradicted those of Allam and colleagues. Specifically, there was a significant decrease observed in visfatin levels in this combined group, which was noteworthy. (Allam, D., et al., 2021). The findings of our study agree those of Lee and Bae (2018) in showing a marked rise in visfatin levels within the female rheumatoid arthritis group (FRA), exhibiting a notable increase. However, our results deviated from this pattern in the remaining groups studied. Notably, the female rheumatoid arthritis - osteoarthritis group (FRA-OA) exhibited contrasting outcomes, demonstrating a significant decrease in visfatin compared to the control sample. (Lee, Y. H., & Bae, S. C., 2018).

Visfatin, a recently identified adipokine, plays significant pro-inflammatory and catabolic roles in Rheumatoid Arthritis (RA). The initiation of physiological changes characteristic of inflammation is driven by pro-inflammatory cytokines. In Osteoarthritis (OA), visfatin's activation of chondrocytes suggests a potential role. It appears that visfatin contributes to the pathogenesis of both OA and RA. Additionally, it serves as a potential indicator of disease activity and severity for both conditions. (Allam, D., et al., 2021).

Increased visfatin levels, also referred to as nicotinamide phosphoribosyl transferase (NAMPT), can be affected by a range of factors. For instance, inflammation plays a significant role: Visfatin, being a pro-inflammatory adipokine, can escalate its production in response to inflammation. Chronic inflammatory ailments like rheumatoid arthritis can consequently result in heightened visfatin levels. Furthermore, obesity contributes: Since visfatin is predominantly synthesized by adipose tissue, individuals grappling with obesity may exhibit elevated circulating levels of visfatin due to expanded adipose tissue mass. Insulin resistance is another factor: Studies suggest a correlation between visfatin levels and insulin resistance, a condition characterized by inefficient cellular response to insulin, commonly linked with obesity and type 2 diabetes. Individuals with insulin resistance often exhibit elevated visfatin levels. Additionally, metabolic syndrome can influence visfatin levels: This condition, marked by a cluster of metabolic abnormalities including obesity, hypertension, hyperglycemia, and dyslipidemia, may lead to heightened visfatin levels, contributing to the onset of cardiovascular disease and diabetes. Medications can also play a role: Certain drugs such as glucocorticoids or specific immunosuppressive agents employed in treating inflammatory disorders might impact visfatin levels. It's crucial to acknowledge that while these factors may contribute to increased visfatin levels, individual differences exist, and not all individuals with these conditions will necessarily demonstrate elevated visfatin levels. Moreover, further research is warranted to comprehensively comprehend the intricate regulation of visfatin production and its involvement in diverse health conditions.

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