

OSTEOPONTIN AS A BIOMARKER FOR DIAGNOSING THE SEVERITY OF OSTEOARTHRITIS

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Osteoarthritis (OA) is the most common type of arthritis. It is characterized by the loss of cartilage and pain ultimately leading to difficulty in movement. Glycoprotein osteopontin (OPN) is an essential regulator that plays a critical role in developing osteoarthritis. The study aims to determine the level of OPN in the serum of patients with OA and its correlation with the content of calcium (Ca), phosphorus (P), and magnesium (Mg). The study was conducted on 92 male and female patients aged between 30 and 65, categorized into mild, moderate, and severe groups through clinical examination and X-rays. The control group consist of 58 healthy males and females 30-65-year old. OPN was measured using the ELISA technique, and minerals were estimated using spectrophotometry. It was shown that in OA patients, OPN level increased highly significant when compared with the control group (10.7 ± 3.4 ng/ml) and depended on OA severity. In patients with severe OA, it was higher (47.5 ± 8.1 ng/ml) than in groups with moderate (14.8 ± 4.5 ng/ml) and mild (12.1 ± 3.1 ng/ml) extent of the disease. We showed a significant positive relationship between OPN and phosphorus levels and a negative significant correlation between OPN and calcium, calcium/phosphorus ratio, and magnesium. These findings underscore the potential of OPN as a valuable biomarker for diagnosing the severity of osteoarthritis and monitoring the effectiveness of treatment.

Key words: osteopontin, osteoarthritis, calcium, magnesium, phosphorus.

Osteoarthritis (OA) is a chronic degenerative disease of the joints that leads to significant morbidity, physical disability, and decreased quality of life [1]. It is characterised by the loss of cartilage and the pain when moving, finally leading to obstruction of movement [2-4]. OA represents the second most common rheumatic disease after rheumatoid arthritis encountered in clinical practice [5, 6]. The prevalence rate reaches tens of millions in the United States alone, and estimates indicate that approximately 300 million people around the world have radiographic evidence of osteoarthritis, whether obscure or incidental [6]. Initially, the cause of OA is the gradual bone loss of articular cartilage, and it is now known that the pathogenesis of OA involves the tissues of the multiple components of the synovial joint, including the synovial bone, subchondral bone, metaphysis, ligaments, and muscles that stabilise the joint [7]. OA develops, the balance shifts towards the deterioration of the joint tissues and leads to the development of synovial inflammation, remodelling of the subchondral bone, and the formation of osteophytes, including joint pain, stiffness,

crepitus, blurring, and decreased movement [8]. OA is diagnosed by various methods, including a combination of radiographic and clinical examination [9]. The pathogenesis of OA has been reported to have three stages depending on the severity of the disease [9]. Studies in the literature indicate that women have a higher prevalence of osteoarthritis, and it increases after 50 years of age [10-12]. Obesity doubles the risk of developing symptoms of osteoarthritis [13]. OPN is a phosphorylated glycoprotein involved in physiological processes such as cell regeneration, angiogenesis, bone homeostasis, cell adhesion, and immune response [14, 15]. OPN is incorporated into the bone matrix as a component of the extracellular matrix (ECM) and a soluble protein in human tissues [16]. OPN is essential and a fundamental regulator, as it plays a critical role in developing osteoarthritis [17, 18].

Materials and Methods

The current study was conducted from August 1 to December 2023.

Population study. The study included 150 people; 92 of them were patients with osteoarthritis from both sexes (65 females and 27 males); their ages ranged from 30 to 63 years, and also 58 healthy people of both sexes (38 females and 20 males) aged 30-65 were chosen as a control group also. The samples of patients were collected from the outpatient clinics of the joint unit at Ibn Sina Teaching Hospital in Mosul, Iraq. At the same time, patients with osteoarthritis were divided according to the severity of the disease into three groups: mild, moderate, and severe, after being diagnosed by a specialist physician through clinical examination and x-rays. Patients with a medical history of other diseases, for example, cancer, kidney disease, and thyroid disease, were excluded. Patients and control were divided according to the age factor to two groups included (30-45) years and (46-56) years.

Sample collection. Blood was drawn using a clean, sterile needle, and due to the presence of a sensitive calcium test, as soon as the blood was detected in the needle, a tube was opened, after which we drew blood (about 5 ml) and placed it in a clean gel tube and left it for 10 min at room temperature, then it was separated by a centrifuge Central, at 3000 g for 5 min, the serum was separated into clean and sterile tube and then stored in a refrigerated place at -80°C until measurements were carried out.

Estimation of osteopontin. OPN was measured using an ELISA kit from BT LAB Cat.NO.E1525Hu of Chinese origin (bt-laboratory). The principle of ELISA involves using an enzyme system to detect the specific binding of the antigen and its corresponding antibody. The intensity of the concentration is directly proportional to the amount of antigen in the sample. The examination was performed on polystyrene sheets with 96 holes. Initially, standards were prepared in concentrations (3, 6, 12, 24, 48 ng/ml). Then, we added the serum and began with successive and sequential additions, where we finally noticed that the color changed from blue to yellow when adding the stop solution. It was placed in the device and measured at a wavelength of 450 nm.

Estimation of calcium. The calcium level in the serum was measured using a BIOLABO test kit of French origin (Biolabs).

Estimating phosphorous. The serum's phosphorus level was measured using a BIOLABO test kit of French origin (Biolabs).

Estimation of magnesium. The level of magnesium in the serum was measured using a MAGNESIUM LR of Italy origin (production).

Body mass index (BMI). BMI was measured by dividing weight by the square of height according to the following equation.

$$\text{BMI (kg/m}^2\text{)} = \text{weight (kg)/height (m}^2\text{)}.$$

Statistical analysis. The data were analyzed using (SPSS) version 25. The results are expressed as mean $\pm$ standard deviation (SD); an independent T-test was used to compare the two groups. ANOVA test employed the differentiation between the three groups. Pearson's correlation coefficient was also used to explore the relationship between the OPN level and the variables studied. *P*-values of 0.05 were considered statistically significant [19].

Result

Study of the characteristics of OPN level in OA patients compared to control. The results showed a highly significant increase ($P \leq 0.0001$) in the level of OPN in OA patients (25.8 ± 17.6 ng/ml) compared to the control (10.7 ± 3.5 ng/ml) (Table 1). Also, there was a high significant increase ($P \leq 0.001$) in the level of OPN with advanced age for control (12.2 ± 3.0 ng/ml) and patients (29.4 ± 17.7 ng/ml) when compared to the age for both (7.9 ± 2.4 ng/ml) control and OA patients (13.8 ± 8.2 ng/ml) (Table 1). Moreover, a significant increase ($P \leq 0.05$) in the level of OPN in healthy females (11.6 ± 3.4 ng/ml) compared to healthy males (9.1 ± 3.1 ng/ml) was observed (Table 1) as well as increased significantly ($P \leq 0.001$), the level of OPN in female patients (29.2 ± 15.0 ng/ml) compared to male patients (17.9 ± 10.4 ng/ml). We noticed a significant increase ($P \leq 0.05$) in the level of OPN in healthy people who were overweight (10.66 ± 3.56 ng/ml) compared to healthy people who have a normal weight (8.96 ± 2.48 ng/ml) (Table 1). We also noticed a highly significant increase ($P \leq 0.0001$) in the level of OPN in healthy people of normal weight compared to obese healthy people (14.2 ± 2.9 ng/ml). A significant increase ($P \leq 0.001$) in the level of OPN was also noted when comparing healthy people who were obese with overweight healthy people. We found a significant increase ($P \leq 0.05$) in the level of OPN in OA patients who were overweight (20.9 ± 13.0 ng/ml) compared to patients who were normal weight (11.13 ± 1.9 ng/ml). Moreover, there was a higher significant increase ($P \leq 0.0001$) in the level of OPN for OA patients who were obese (33.8 ± 18.8 ng/ml) compared to overweight patients. A significant increase ($P \leq 0.001$) in the level of OPN was also observed when comparing OA patients who were overweight with obese patients (Table 1).

Level of minerals and BMI in OA patients compared to controls. The results presented in Table 2 show a very high significant increase ($P \leq 0.0001$) in the level of BMI in OA patients ($31.6 \pm 6.3 \text{ kg/m}^2$) compared to healthy people ($26 \pm 2.7 \text{ kg/m}^2$). There was a highly significant decrease ($P \leq 0.001$) in the level of calcium/phosphorus ratio in OA patients ($1.9 \pm 0.2 \text{ mmol/l}$) compared to healthy people ($1.8 \pm 0.1 \text{ mmol/l}$). We noticed a highly significant decrease ($P \leq 0.001$) in the calcium level in OA patients ($2.1 \pm 0.05 \text{ mmol/l}$) compared to healthy people ($2.2 \pm 0.04 \text{ mmol/l}$). There was observed a highly significant decrease ($P \leq 0.001$) in the calcium level in OA patients ($2.1 \pm 0.05 \text{ mmol/l}$) compared to healthy people ($2.2 \pm 0.04 \text{ mmol/l}$) and a highly significant increase ($P \leq 0.001$) in the level of phosphorus in OA patients ($1.2 \pm 0.09 \text{ mmol/l}$) compared to healthy people ($1.1 \pm 0.1 \text{ mmol/l}$). We also noted a highly significant decrease ($P \leq 0.001$) in the level of magnesium in OA patients ($0.7 \pm 0.5 \text{ mmol/l}$) compared to healthy people ($0.8 \pm 0.4 \text{ mmol/l}$).

Effect of disease severity on the variables studies in OA patients. The results in Table 3 demonstrate that there was no significant difference ($P \leq 0.08$) in the level of OPN in healthy people

($10.7 \pm 3.4 \text{ ng/ml}$) compared to mild case patients ($12.1 \pm 3.1 \text{ ng/ml}$) and a significant increase ($P \leq 0.001$) in the moderate case patients ($14.8 \pm 4.5 \text{ ng/ml}$) compared to healthy people. At the same time, it was shown a very high significant increase ($P \leq 0.0001$) in the severe case patients ($47.5 \pm 8.1 \text{ ng/ml}$) compared to healthy people. Finally, a significant increase in OPN ($P \leq 0.0001$) in the severe case compared to the moderate case was observed. The results in Table 3 showed that there was no significant difference ($P \leq 0.47$) in the level of calcium in healthy people ($2.21 \pm 0.04 \text{ mmol/l}$) compared to mild case (2.20 ± 0.05), and also that no significant difference ($P \leq 0.18$) in healthy people compared to the moderate case ($2.192 \pm 0.06 \text{ mmol/l}$). At the same time, there was a high significant decrease ($P \leq 0.001$) in the severe case ($2.15 \pm 0.2 \text{ mmol/l}$) compared to healthy people, and finally, in calcium, it showed a significant decrease ($P \leq 0.05$) in the severe case compared to the moderate case. The results in Table 3 showed that there was no significant difference ($P \leq 0.48$) in the level of phosphorus in healthy people ($1.1 \pm 0.1 \text{ mmol/l}$) compared to mild cases ($1.20 \pm 0.09 \text{ mmol/l}$). They also showed a significant difference ($P \leq 0.05$) in healthy people com-

Table 1. Study of the characteristics of OPN level in control and OA patients in relation to age, obesity, and sex

Osteopontin (ng/ml)					
Control, n = 58			Patients, n = 92		
10.7 ± 3.5			25.8 ± 17.6***		
OPN level (ng/ml) according to age factor					
Control			Patients		
(30-45), n = 19	(46-65), n = 39		(30-45), n = 21	(46-65), n = 71	
7.9 ± 2.4	12.1 ± 3.0**		13.8 ± 8.2	29.4 ± 17.7***	
OPN level (ng/ml) according to sex factor					
Control			Patients		
Female, n = 38	Male, n = 20		Female, n = 65	Male, n = 27	
11.6 ± 3.4	9.1 ± 3.1*		29.2 ± 10.4**	17.9 ± 10.4	
OPN level (ng/ml) according to obesity					
Control			Patients		
Normal weight BMI (18.5-24.9), n = 27	Overweight BMI (25-29.9), n = 22	Obese (BMI ≥30), n = 9	Normal weight BMI (18.5-24.9), n = 17	Overweight BMI (25-29.9), n = 27	Obese (BMI ≥30), n = 48
8.96 ± 2.48	10.66 ± 3.56*	14.2 ± 2.9***	11.13 ± 1.90	20.9 ± 13*	33.8 ± 18.8***

Note. A significant difference at * $P \leq 0.05$; a high significant difference at ** $P \leq 0.001$; a highly significant difference at *** $P \leq 0.0001$;

Table 2. Level of minerals and BMI in OA patients compared to controls

Variables	Control, n = 58	Patients, n = 92
Ca, mmol/l	2.20 ± 0.04	2.10 ± 0.05**
P, mmol/l	1.10 ± 0.11	1.20 ± 0.09**
Ca/P ratio, mmol/l	2.0 ± 0.2	1.8 ± 0.1**
Mg, mmol/l	0.8 ± 0.4	0.7 ± 0.5**
BMI, kg/m ²	26.0 ± 2.7	31.6 ± 6.3***

Note. A high considerable difference at ** $P \leq 0.001$; a highly significant difference at *** $P \leq 0.0001$; (mean ± SD). Ca – calcium; P – phosphorous; Mg – magnesium; BMI – body mass index

pared to the moderate case (1.20 ± 0.09 mmol/l). In contrast, it showed a significant increase ($P \leq 0.001$) in the severe case (1.3 ± 0.4 mmol/l) compared to healthy people and finally, in phosphorus, it showed a significant increase ($P \leq 0.001$) in the severe case compared to the moderate case. The results in Table 3 showed that there was no significant difference

($P \leq 0.4$) in the level of calcium/phosphorus ratio in healthy people (1.9 ± 0.2 mmol/l) compared to mild cases (1.9 ± 0.2 mmol/l). Also, they showed a significant difference ($P \leq 0.05$) in healthy people compared to the moderate case (1.84 ± 0.018 mmol/l). In contrast, it showed a high significant decrease ($P \leq 0.001$) in the severe case (1.70 ± 0.09 mmol/l) compared to healthy people. Finally, the calcium/phosphorus ratio showed a significant increase ($P \leq 0.001$) in the severe case compared to the moderate case. The results in Table 3 showed that there was no significant difference ($P \leq 0.09$) in the level of magnesium in healthy people (0.80 ± 0.05 mmol/l) compared to mild cases (0.71 ± 0.04 mmol/l). Also, they showed a high significant decrease ($P \leq 0.001$) in the moderate case (0.70 ± 0.04 mmol/l) compared to healthy people and a highly significant reduction ($P \leq 0.0001$) in the severe case (0.67 ± 0.05 mmol/l) compared to healthy people. Finally, magnesium showed a highly significant decrease ($P \leq 0.001$) in the severe case compared to the moderate case. The results in Table 3 showed that there was no significant difference ($P \leq 0.1$) in the level of BMI in

Table 3. The effect of disease severity on the osteopontin and variables studies for patients OA

Variables	Control, n = 58	Mild, n = 36	Moderate, n = 22	Severe, n = 34
Osteopontin, ng/ml	10.7 ± 3.4	12.1 ± 3.1	14.8 ± 4.5	47.5 ± 8.1
Ca, mmol/l	2.21 ± 0.04	2.20 ± 0.05	2.19 ± 0.06	2.15 ± 0.20
P, mmol/l	1.1 ± 0.1	1.20 ± 0.09	1.20 ± 0.09	1.3 ± 0.4
Ca/P ratio, mmol/l	1.9 ± 0.2	1.91 ± 0.18	1.84 ± 0.18	1.70 ± 0.09
Mg, mmol/l	0.80 ± 0.05	0.71 ± 0.04	0.70 ± 0.04	0.67 ± 0.05
BMI, kg/m ²	26.0 ± 2.7	27.2 ± 3.3	31.5 ± 4.7	36.3 ± 6.3
Sex	1.4 ± 0.5	1.34 ± 0.48	1.33 ± 0.47	1.20 ± 0.38

Note. Ca – calcium; P – phosphorous; Mg – magnesium; BMI – body mass index; (mean ± SD)

Table 4. The correlation of the osteopontin with some minerals and body mass index in osteoarthritis patients

Biochemical variable	Osteopontin, pearson correlation (r), P°		
	Mild	Moderate	Severe
Ca, mmol/l	-0.169; 0.325	-0.522*; 0.013	-0.690**; 0.0001
P, mmol/l	0.18; 0.4	0.455*; 0.033	0.884**; 0.0001
Ca/P ratio, mmol/l	-0.109; 0.525	-0.493*; 0.021	-0.907**; 0.0001
Mg, mmol/l	-0.219; 0.199	-0.382*; 0.02	-0.531**; 0.001
BMI, kg/m ²	0.081; 0.637	0.427*; 0.012	0.726**; 0.0001

Note. *Correlation is significant at the 0.05 level. **Correlation is significant at the 0.001 level. ***Correlation is significant at the 0.0001 level. Ca – calcium; P – phosphorous; Mg – magnesium; BMI – body mass index; P° – refer to P -value

healthy people ($26.0 \pm 2.7 \text{ kg/m}^2$) compared to mild cases ($27.2 \pm 3.3 \text{ kg/m}^2$). Also, they showed a significant increase ($P \leq 0.001$) in the moderate case ($31.5 \pm 4.7 \text{ kg/m}^2$) compared to healthy people and a highly significant increase ($P \leq 0.0001$) in the severe case ($36.3 \pm 6.3 \text{ kg/m}^2$) compared to healthy people. Finally, in BMI, it showed a highly significant increase ($P \leq 0.001$) in the severe case compared to the moderate case.

Effect of disease severity on the (OPN) and its correlation with some minerals and body mass index. The results, as shown in Table 4, showed that the relationship of OPN with the studied variables, Ca, P, Ca/P, Mg, and BMI, showed a highly significant relationship with each of the elements (Ca ($r = 0.522$; $P \leq 0.01$), Ca/P ($r = 0.493$; $P \leq 0.02$), Mg ($r = 0.382$; $P \leq 0.02$) in the moderate case and the relationship was negative, and showed a high significant relationship with both P ($r = 0.455$; $P \leq 0.033$) and BMI ($r = 0.427$; $P \leq 0.012$) in the moderate case and it was positive. On the contrary, it showed a very high significant relationship with both Ca ($r = 0.690$; $P \leq 0.0001$) and Ca/P (0.907 ; 0.0001), while in Mg ($r = 0.531$; $P \leq 0.001$) there was a high significant relationship. In the severe case, the relationship was negative and showed a very high significant relationship with both P ($r = 0.884$; $P \leq 0.0001$) and BMI ($r = 0.726$; $P \leq 0.0001$). In the severe case, the result was positive. While there is no relationship between OPNs, it is in the mild case that the effect of OPNs is reflected as the severity of the disease progresses.

Discussion

In the results in Table 1, we noted that the concentration of OPN in OA disease is higher than in healthy people, which may be consistent with our study [20]. When we analysed the results of the level of OPN in healthy people and OA patients, we found that its level in the blood serum of OA patients was much higher compared to healthy people. The increase in OPN may be associated with the deterioration of the articular cartilage's work functions [18]. OPN also participates in cartilage degradation, as OPN works to ossify cartilage and turn it into bone, as noted by Kolkart and colleagues [21]. We also noticed in our study that OPN increases with the progression and severity of the disease, which means it can be considered a diagnostic sign of the disease and its progression stages, which agrees with Dai and colleagues [22]. Obesity is one of the risk factors for osteoarthritis, and excess weight, which is

expressed as an increase in the body mass index, is positively associated with the risk of osteoarthritis, so that the load is distributed unbalanced, which affects the joints, especially the pelvic and knee joints, and this is consistent with [23]. One reason obesity can lead to OA is that it changes the biomechanics of the joints, meaning that obesity adds weight to the joints, especially the weight-bearing joints in the knees and hips [24]. It was found that the concentration of calcium in the blood is inversely associated with the risk of OA [25]. Also, men's calcium concentration was higher than women's due to the hormonal difference. We also observed a higher level of phosphorus in OA patients than in healthy people, which is consistent with our study [26]. Serum magnesium concentration is inversely related to osteoarthritis patients, and this is probably consistent with our study [27]. In a biochemical pathway that regulates the levels of calcium and phosphorus in the body, these two minerals interact in a hostile manner, controlled by PTH in the blood [28]. Studies have shown that calcium binds to phosphorus and is eventually deposited in the tissues. The accumulation of these deposits causes calcification of tissues, including joint tissues, and leads to their decrease in the blood. This is consistent with what was stated in our study [28].

Conclusion. OPN is an essential protein involved in the development of osteoarthritis. We noticed, through the results obtained, that there was a very high significant increase ($P \leq 0.0001$) in the disease osteoarthritis compared to healthy people, meaning that it might be considered a robust and vital indicator along with indicators that the doctor can use to predict the disease and the extent of its development since this disease lacks vitality. We also noticed a high relationship with body mass index, which can be used in obesity diseases.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ОСТЕОПОНТИН ЯК БІОМАРКЕР ДЛЯ ДІАГНОСТИКИ СТУПЕНЯ ТЯЖКОСТІ ОСТЕОАРТРИТУ

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Остеоартрит (ОА) – найпоширеніший вид артриту, який характеризується руйнуванням хрящової тканини та болем, що з часом призводить до порушення рухливості. Глікопротеїн остеопонтин (OPN) відіграє суттєву роль у розвитку остеоартриту. Метою роботи було визначити рівень OPN у сироватці крові пацієнтів з ОА і дослідити його кореляцію із вмістом кальцію (Ca), фосфору (P) і магнію (Mg). У дослідженні брали участь 92 пацієнти чоловічої та жіночої статі віком від 30 до 65 років, які, за результатами клінічного обстеження та рентгенологічних досліджень, були розподілені на групи, залежно від тяжкості захворювання на легку, середню та тяжку. Контрольну групу складали 58 здорових чоловіків і жінок віком 30-65 років. Рівень OPN визначали методом ІФА, а вміст мінералів – за допомогою спектрофотометрії. Показано, що у пацієнтів із остеоартритом, рівень OPN був підвищений порівняно з контрольною групою ($10,7 \pm 3,4$ нг/мл) і залежав від ступеня тяжкості захворювання. У пацієнтів із тяжким ступенем остеоартриту, OPN був вищим ($47,5 \pm 8,1$ нг/мл), ніж у групах із середнім ($14,8 \pm 4,5$ нг/мл) і легким ($12,1 \pm 3,1$ нг/мл) ступенем тяжкості захворювання. Встановлено позитивну кореляцію між OPN і рівнем фосфору та негативну кореляцію між OPN і кальцієм, співвідношенням кальцій/фосфор і магнієм. Отримані результати вказують на потенціал OPN як ефективного біомаркера для діагностики тяжкості остеоартрозу та моніторингу ефективності лікування.

Ключові слова: остеопонтин, остеоартрит, кальцій, магній, фосфор.

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