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ORIGINAL ARTICLE

Study the Level of Collagen2-1NO2 (Coll2-1NO2) in Serum of Iraqi Patients with Osteoarthritis

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ABSTRACT

Osteoarthritis, often known as OA, is characterized by the degeneration of articular cartilage, limited intraarticular inflammation with synovitis, and peri-articular and subchondral bone changes. This study measured Coll2-1NO2, CRP, ESR, Vitamin D3, Mg, Calcium, and Lipid profiles in OA patients. The current study contained a total of 130 participants, who were divided into two groups. Group1 contained 90 OA patients, and group 2 controls 40; the samples were extracted from females and males, the levels, Coll2-1 NO2, CRP, ESR , Vitamin D3 ,Mg, Calcium, Cholesterol, TG, VLDL, LDL, and HDL were calculated. The levels of Coll2-1NO2, CRP, and Vitamin D3 were evaluated using an enzyme-linked immunosorbent assay (ELISA), while the levels of Mg, Calcium, Cholesterol, TG, VLDL, LDL, and HDL were determined using a Human Reader HS Device. Westergren method was used to analyze the level of ESR . The result of Coll2-1NO2 shows a high significant change among OA patients and control. Serum vitamin D3 and CRP value have high significant change in their levels in OA patients. Serum levels of Triglyceride, HDL, Mg, Ca, ESR and VLDL showed a significant variation among OA patient and control. Cholesterol and LDL values have non-significant variation in their levels in OA patients in comparison with control.

Conclusion: According to the presented results Coll2-1NO2, CRP, ESR, Vitamin D3, Mg, Calcium, T.G, VLDL, and HDL do affect by Osteoarthritis patients. LDL and cholesterol don't affect osteoarthritis patients.

INTRODUCTION

Osteoarthritis, more commonly referred to as OA, is a degenerative and incapacitating musculoskeletal ailment that affects millions of people all over the world. It is also a significant contributor to the cost of providing medical care. This type of arthritis, which is the most common form of the disease, is characterized by a deterioration of the articular cartilage, the production of osteophytes, subchondral sclerosis, inflammation of the synovium, and ultimately a loss of joint function. These symptoms are all hallmarks of the disease (1). Osteoarthritis, which manifests itself most frequently in the hands, hips, and knees, is a collective term for a group of conditions that are characterized by the degeneration of articular cartilage and the development of persistent pain (2). When a person has OA, the cartilage that is located within a joint will begin to disintegrate, and the bone that is located beneath will begin to slowly change, which will get worse over time. This condition is referred to as degenerative joint disease (3). The clinical evidence also demonstrates that osteoarthritis frequently co-exists with other metabolic illnesses and comorbidities such as diabetes mellitus and cardiovascular disorders. These two conditions are both prognostic of rapid deterioration of the OA joint. A recent systematic analysis identified six clinical phenotypes of osteoarthritis by using risk variables for the condition. Chronic pain (with a prominent central mechanism) is one of these phenotypes, along with inflammation, metabolic syndrome, bone and cartilage metabolism, mechanical overload, and limited joint disease (4). Osteoarthritis is the most frequent form of arthritic joint disease and a primary source of disability and joint pain around the world. It is also the most common form of arthritic joint disease in the United States. OA is a complex illness that can be caused by factors such as aging, joint trauma, the bio-tribology of the joint contact surface, hereditary predisposition, obesity, and metabolic dysregulation (5). Alterations in the structure of the subchondral bone as well as degradation of the articular cartilage are the clinical manifestations of osteoarthritis that are most noticeable to the naked eye. When cartilage in a joint is completely lost as a result of a disruption in cartilage homeostasis caused by the induction of catabolic factors as well as the down-regulation of anabolic factors, the bone and soft tissue structures that surround the joint are altered. This is because the disruption in cartilage homeostasis is caused by the induction of catabolic factors and the down-regulation of anabolic factors. This can result in discomfort and swelling in the joints, as well as deformity and incapacity (6). The primary forms of tissue that are harmed by osteoarthritis include articular cartilage, subchondral bone, and the synovial membrane that lines the joint that is afflicted with the condition. The disease has the potential to impact any and all of the tissues that are housed within a joint. Alterations in the physiological processes that are taking place in these tissues are the ultimate cause of the progression of osteoarthritis, which affects the joints. In the subchondral bone, the two basic types of cells that can be discovered are osteoblasts and osteoclasts, and the study of both of these cell types is extremely important when it comes to understanding osteoarthritis. Abnormal bone remodeling, which can lead to sclerosis and the development of osteophytes, is caused by disruptions in the normal cellular processes that occur in bones. (7). Osteoarthritis is an example of an issue that affects the musculoskeletal system. This condition causes irreparable damage to the function of the joints as a consequence of aging, trauma, wear and tear on cartilage, and persistent inflammation in the joints. An precise diagnosis of OA, which is characterized by the increasing narrowing of joint space, the formation of osteophytes, the sclerosis of the subchondral bone, and the generation of cysts, is made feasible by X-ray radiography (8) In OA, dysregulation caused by the presence of numerous bio factors results in the loss of cartilage homeostasis, which in turn causes the degradation of the collagen- and proteoglycan-rich extracellular matrix (ECM), fibrillation and erosion of the articular surface, cell death, matrix calcification, and vascular invasion (9).

MATERIALS AND METHODS

Patients' selection: The study was carried out at the Kindy Hospital/Baghdad for the period from November 2021- January 2022. We studied 90 OA patients and 40 controls, a total of 5 milliliters of blood from the patient's veins was extracted, and the study's parameters were then analyzed Coll2-1NO2, CRP, and Vitamin D3 levels were measured using an enzyme-linked immunosorbent test (ELISA). The levels of Mg, Calcium, Cholesterol, TG, VLDL, LDL, and HDL were measured using a Human Reader HS Device. Westergren method was used to analyze the level of ESR.

Inclusion Conditions: X-rays were used to diagnose all patients with osteoarthritis.

Exclusion Criteria: Individuals with various inflammatory disorders, diabetic patients, and patients with thyroid diseases were excluded from the study based on their medical history and clinical evaluation.

Statistical analyses: The data was analyzed using a statistical analysis program (SPSS 25). The T-test was applied to data with a normal parametric distribution and 0.05 alpha values.

RESULT AND DISCUSSION

Table (1) showed the mean ±SE of BMI and WC in OA patients and healthy control [(30.31kg/m^2 ± 0. 64) (24.93kg/m^2 ± 0.42)] [(100.71cm±1.28) (84.05cm ± 1.43)] respectively, there is a high significant alteration in BMI and WC among OA patients and controls, where the result indicates a high significant difference among two groups (P≤0.01).

According to Vasilic-Brasnjevic S et al. (10), WC was significantly different between patients with osteoarthritis and controls, and the difference between the two groups was statistically high.

In their study (11), Christiansen MB, et al. discovered that an increasing WC increases the chance of incident impaired physical function in the next year. This was one of their findings. Keeping up with your WC could help prevent you from acquiring a low physical function.

Table (1): Comparison between patients and control groups in BMI and WC.

Group	Mean ± SE	
	BMI (kg/m^2)	WC (cm)
Patients	30.31 ± 0.64	100.71 ±1.28
Control	24.93 ± 0.42	84.05 ± 1.43
T-test	1.989 **	4.256 **
P-value	0.0001	0.0001
** (P≤0.01).		

The BMI in patients was significantly (P=0.0001) high in patients than control group (30.31kg/m^2 -24.9 kg/m^2)respectively .

The results of Coll2-1NO2 showed a high significant change (P≤0.01). The mean ±SE for OA patients and control were [(8.94pg/ml ±0.32) (3.04pg/ml ±0.09)] respectively. As shown in Table (2).

A study conducted in 2021, found there is a correlation between serum cartilage biomarkers called Coll2-1NO2 and numerous knee OA characteristics that may be detected with WORMS. Also demonstrates that a positive association exists between the baseline value of Coll2-1NO2 and the escalation of pain. (12).

In the study by Henrotin Y, et al (13) also revealed that the serum levels of Coll2-1 and Coll2-1NO2 were modestly decreased after completing a marathon, indicating that intense running could minimize cartilage catabolism. In addition, there was no correlation found between Coll2-1NO2 and either the total or the active MPO, which suggests that the nitration of Coll2-1 did not come from a systemic oxidative process but rather reflects local alterations.

The result of CRP presented a high significant change (P≤0.01).The mean ±SE for OA patients and control were [(13.42ng/ml ±0.31) (4.94 ng/ml±0.23)] respectively.

Hanada M, et al (14). Researchers found that patients with knee osteoarthritis had higher levels of the inflammatory marker CRP, as well as more frequent instances of knee discomfort and swelling than people without the condition. In the early stages of knee osteoarthritis (KOA), a greater CRP concentration may be a more accurate indication of OA progression than ESR.

Punzi L, et al in their study (15), also found CRP levels are higher in erosive osteoarthritis than in non-erosive osteoarthritis patients.

In a study on CRP, Zaki NA, et al (16). There is evidence that inflammation plays a contributing role in patients who have osteoarthritis of the knee. They found that there was a difference in CRP between patients and controls, but it was not statistically significant, and our research did not find that to be the case.

In the study by Tootsi K, et al (17). It was also discovered that the OA group had a greater CRP level compared to both patients and controls.

Table (2) Comparison between patients and control groups in Coll2-1NO2 and CRP

Group	Mean ± SE	
	Coll2-1NO2 (pg/ml)	CRP (ng/ml)
Patients	8.94 ±0.32	13.42 ±0.31
Control	3.04 ±0.09	4.94 ±0.23
T-test	1.605 **	0.964 **
P-value	0.0001	0.0001
** (P≤0.01)		

The results of Vit D3 showed mean ±SE of OA patients and control [(35.20ng/ml ±1.26) (65.11ng/ml ±2.22)] respectively, there was result shows a high significant variation between two groups in Vitamin D3 (P≤0.01) as shown in Table (3).

The influence of a person's vitamin D status on the development of osteoarthritis or the rate at which it progresses OA. It is believed that a deficiency in vitamin D can have an effect on the chondrocytes, which in turn might affect the quality of the articular cartilage, which in turn can contribute to an increase in PTH as well as bone turnover. Vitamin D has the ability to alter the metabolic processes of mature articular cartilage due to the fact that it causes mature articular cartilage to create more proteoglycan (18).

In this research, Başkan BM et al. found that (18). There was found to be no connection between the levels of vitamin D and the severity of knee osteoarthritis or the functional status. Age and body mass index are the primary risk variables that influence the radiographic severity of knee osteoarthritis, whereas age, gender, BMI, and pain are the primary factors that determine a person's functional status.

In their research (19), Alabajos-Cea A, together with his colleagues, found that. Vitamin D levels are significantly lower in patients with knee EOA compared to patients with matched controls. Patients who suffer from knee EOA and have a deficiency in vitamin D have a much higher degree of pain intensity, disability, anxiety and depression symptoms, and a worse level of social involvement and physical performance. This is a significant relationship. A lower PTH level is linked to increased levels of pain severity as well as decreased levels of social interaction.

The results of Calcium (Ca) were presented in Table (3) which revealed asignificant change (P≤0.01), mean ± SE between patients OA and control [(8.24mg/dl ±0.18) (9.12mg/dl ±0.11)] respectively.

In a study that was carried out by Payment M and colleagues (20), the researchers found that the serum calcium concentration has an inverse connection with radiographic OA of the knee. This was a discovery that was made by the researchers. In the treatment of radiographic knee osteoarthritis, the presence of a high calcium level may possibly have a preventive impact OA.

Also, Musik I et al. (21), discovered that calcium levels in OA patients were not substantially different from those of the control group; however, this did not correlate with our findings.

The results of Magnesium (Mg) showed a significant variance (P≤0.05).The mean ±SE for patients and control were [(0.567mg/dl ±0.02) (0.637mg/dl ±0.02)] respectively. As shown in Table (3).

Wu Z, et al. In their study (22). In this particular research, a higher magnesium consumption was not connected with a decreased incidence of knee osteoarthritis. However, there may be the possibility of an inverse link between an increased daily magnesium intake and the risk of fracture in patients diagnosed with knee osteoarthritis.

Magnesium levels in OA patients were similarly shown not to be substantially different from those of the control group by Musik I et al. (21). In females, a considerable drop compared to the relevant control was detected, and this did not coincide with the findings of our research.

Regarding ESR the results revealed a high significant variation among two groups in ESR (P≤0.01) (30.41mmol/hr ± 1.93) (19.97mmol/hr ± 2.16) as shown in Table (4).

Hanada M, et al in their study (14), The ESR levels of individuals who had knee osteoarthritis were greater than the ESR levels of patients who did not have knee osteoarthritis.

Table (3) Comparison between patients and control groups in Ca, Mg and D3.

Group	Mean ± SE		
	Ca (mg/dl)	Mg (mg/dl)	D3 (ng/ml)
Patients	8.24 ±0.18	0.567 ±0.02	35.20 ±1.26
Control	9.12 ±0.11	0.637 ±0.02	65.11 ±2.22
T-test	0.556 **	0.059 *	4.758 **
P-value	0.0022	0.0204	0.0001

Table (4) Comparison between patients and control groups in ESR.

Group	No	Mean ± SE of ESR (mmol/hr)
Patients	90	30.41 ± 1.93
Control	40	19.97 ± 2.16
T-test	--	6.394 **
P-value	--	0.0016
** (P≤0.01).		

In the research that Zaki NA, et al. conducted (16). The minimum ESR value was 20 mm/h, and the maximum one was 27 mm/h. There was a statistically significant difference (p = 0.028) between the mild OA group and the severe OA group in regard to ESR, but no other significant differences were observed. The mean ESR of all patients studied was 23.5 mm/h 2.04 mm/h. There was no correlation between the levels of serum ESR and the degree of OA severity. In addition, it does not provide any further diagnostic accuracy in the process of diagnosing osteoarthritis of the knee ESR.

The results of Lipid profile were presented in Table (5).The result of Triglyceride revealed a significant change (P≤0.01), in mean ± SE among OA patients and control [(123.74mg/dl ±3.28)(107.68 mg/dl ±2.85)] respectively.

The result of Cholesterol presented a non-significant variation (P>0.05). The mean ±SE for OA patients and control were [(155.61mg/dl ±4.15) (152.45mg/dl ±4.85)] respectively.

The result of VLDL showed a significant variance (P≤0.01). The mean ±SE for OA patients and control were [(25.19mg/dl ±0.78) (21.54mg/dl ±0.57)] respectively.

The HDL results shows a significant variance (p< 0.05) among the OA patients and healthy control [(48.64mg/dl ±3.09) (39.04mg/dl ±0.87)] respectively.

The result of LDL showed a non-significant alteration (P>0.05). The mean ±SE for OA patients and control healthy were [(87.44mg/dl ±3.13)(91.87mg/dl ±4.63)] respectively.

In a study by Tootsi K, et al (17), there was not a discernable difference in total cholesterol or low-density lipoprotein (LDL) cholesterol levels across the different groups that took part in the experiment. The OA group showed considerably higher levels of triglycerides and lower levels of high-density lipoprotein (HDL) cholesterol in their blood when compared to the control group.

Table (5) Comparison between difference groups in Lipid profile.

Group	Mean ± SE (mg/dl)				
	Triglyceride	Cholesterol	VLDL	HDL	LDL
Patients	123.74 ±3.28	155.61 ±4.15	25.19 ±0.78	48.64 ±3.09	87.44 ±3.13
Control	107.68 ±2.85	152.45 ±4.85	21.54 ±0.57	39.04 ±0.87	91.87 ±4.63
T-test	10.454 **	13.907 NS	2.456 **	9.273 *	11.131 NS
P-value	0.0029	0.654	0.0038	0.0425	0.431
* (P≤0.05), ** (P≤0.01).					

The receiver operating characteristics curve (ROC): The ROC analysis depicts the relationship between sensitivity and specificity to determine the optimal specificity and sensitivity for a diagnostic test. 1. Precision (23)

ROC test for Coll2_1NO2 markers showed very clear cut off value with 100% sensitivity and 100% specificity that indicates Coll2_1NO2 considered as a good diagnostic marker. The cutoff value upper than 4.6 representatives of patient. As shown in charts (1)

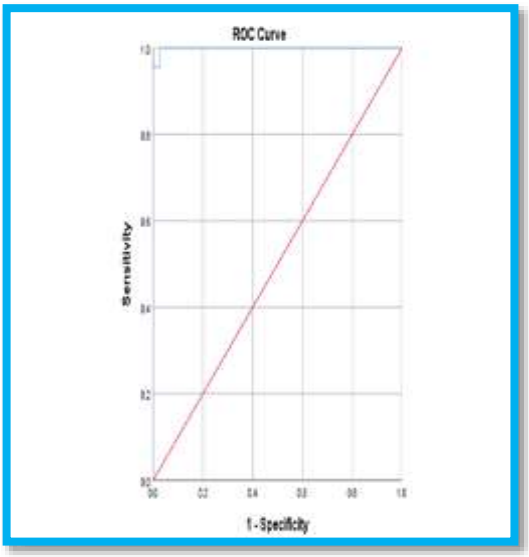

Chart 1: ROC curve for Coll2-1NO2

CONCLUSION

The result of Coll2 -1NO2 appearances a high significant alteration between patients OA and control. In our study, the high concentration of Coll2-1NO2 in patients OA compared to healthy people showed that it can be considered as a marker in the diagnosis of the disease osteoarthritis (OA). We recommend measuring Coll2-1NO2 levels with RA disease.

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