

Evaluating Coll2-1NO2 Level in Pre- and Post-menopausal Iraqi Women with Osteoarthritis

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Abstract

Osteoarthritis (OA) is not an autoimmune but a chronic inflammatory disease affecting the joints of humans due to mechanical stress mostly in the knees and the hip. In OA a modification of the cartilage is encountered causing a pain in the joints upon movement. Coll2-1NO2 is a specific sequence of amino acids produced from the degradation of type II collagen, which is abundantly found in the cartilage, followed by a nitration in the tyrosine residue as a consequence of stress-inflammatory events. In this study, Coll2-1NO2 is used as a biomarker of OA to predict the inflammatory stress condition as well as the cartilage degradation in pre- and post-menopausal women. The results showed a significant increase of serum Coll2-1NO2 in pre- and post-menopausal women with OA compared to pre- and post-menopausal health women, at similar body mass index category (overweight). Pearson's correlation analysis showed non-significant association between Coll2-1NO2 and other studied parameters. Moreover, ROC analysis showed a very excellent sensitivity of Coll2-1NO2 as a prognostic biomarker for OA disease in pre- and post-menopausal women.

Keywords: Coll2-1NO2, C-reactive protein, FSH, lipid profile, osteoarthritis.

Introduction

Osteoarthritis (OA) is not an autoimmune but a chronic inflammatory disease affecting the joints of humans due to mechanical stress mostly in the knees and the hip¹. OA is also known as degenerative arthritis and considered as a major health problems that faces the elder people². The global prevalence rate of OA has reached a serious point, where the major incidence rates were among women, elders, people with overweight and obesity, and certain ethnics³. OA was once thought to be only a "wear and tear" disease. Prolonged stress and poor biomechanics on the joints were thought to be

the root causes of the breakdown of cartilage in the joint and the ensuing inflammation. Stiffness, edema, and impaired movement were the results. It is now understood that OA is a much more complex condition made up of inflammatory and metabolic factors⁴⁻⁶. Women at postmenopausal stage exhibit wide spectrum of fluctuations in their gonadal hormones, and signal transduction system⁷ that can increase the mechanical stress⁸ and inflammatory events⁹. Therefore, it is important to determine the differences in the pathophysiology of OA between pre- and post-menopausal women.

The complex structure of the cartilage enables a smooth, free of pain movement for humans. In OA condition, this structure is disrupted and its function declined, causing a pain in the joints during the movement¹⁰⁻¹². It has been reported that age is a crucial factor for the modification of the cartilage leading to the development of OA, through several mechanisms including oxidative stress and oxidative damage¹³. The extracellular matrix of the cartilage contains high amount of type II collagen which gives a unique and specific sequence of amino acid residues (His-Arg-Gly-Tyr-Pro-Leu-Asp-Gly) upon denaturation called Coll2-1^{14, 15}. Hence, Coll2-1 can be used as specific biomarker for the degradation of collagen type II, and consequently for OA since it degraded during this degenerative arthritis¹⁶. More recently, a modified form of Coll2-1 has been introduced, known as

Coll2-1NO₂, which has a similar sequence but with a nitrated tyrosine residue at the position 4 of the sequence. Coll2-1NO₂ can be used as an indicator for inflammatory and oxidative/nitrative damage of the cartilage¹⁷. Since the recent researches on OA have shown a link the inflammatory events in the development and/or the progressiveness of the disease, Coll2-1NO₂ can give a better indication on the condition of OA. We have aimed to assess Coll2-1NO₂ in the sera of pre- and post-menopausal women with OA disease to predict the age-related effect on this biomarker as well as its correlation with Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), Testosterone, and inflammatory biomarker including Erythrocyte Sedimentation Rate (ESR), and C-Reactive Protein (CRP).

Materials and Methods

One hundred and fifty female subjects were enrolled in this work, 80 of which were diagnosed with OA by the specialized medical doctors at Orthopedic Consultancy Department of Baghdad Hospital, at Baghdad Medical City. The rest of the female subjects were healthy and used as control in the study. Moreover, the OA patients were divided into two subgroups based on the age of the participants, namely, OA I and OA II. The first

subgroup contained 40 pre-menopausal women with OA disease while the second subgroup contained 40 post-menopausal women with OA disease. Also, control group was divided into similar groups based on their age (Control I and Control II) where each group contained 35 females. From each female, the venous blood was collected and the serum was obtained from the blood sample and stored at -20 °C to be analyzed later.

Methods

The analyses were involved the assessment of ESR by using the whole blood sample at the moment of blood sample collecting. Serum samples were used to analyze CRP, LH, FSH, and testosterone by using cobas commercial kits on cobas b101 of CRP and cobas E411 autoanalyzer for the rest (Roche, Germany). Coll2-1NO₂ was analyzed in the serum by using Sandwich based ELISA MyBioSource research kit in BioTech (USA) microplate reader. At last, lipid profile parameters were detected by

spectrophotometric kits (Biolabo, France) for triglycerides (TGs), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C). Finally, the data were analyzed statistically for mean comparisons (ANOVA, and LSD post-Hoc test) where the results were expressed as mean± standard deviation (SD), correlation (Pearson's coefficient), and receiver operating characteristics (ROC) in SPSS software program (version 26.0).

Results and discussion

In Table 1 comparisons among the subgroups are demonstrated by using the mean value and the standard deviation, encountered by the *P*-value that obtained from ANOVA analysis. According to the

design of the work the age of control I and OA I were under 45 years old and significantly differed from control II and OA II who were above 45 years old. Body mass index (BMI) of the participants lies

at the overweight category for all subgroups. The serum levels of Coll2-1NO₂ were at their highest value in OA II patients (14.44±2.40 ng/mL), where OA I patients exhibited a high level of the biomarker as well (10.96±2.32 ng/mL). This increase of Coll2-1NO₂ was significant compared to the corresponding control groups (OA I higher than control I, and OA II higher than control II). Moreover, OA II showed significant higher levels

of Coll2-1NO₂ compared to OA I. The obtained results of CRP and ESR were similar to that of Coll2-1NO₂ in which OA II patients showed the highest values. Calcium on the other hand decreased in OA I and II subgroups compared to the corresponding control groups. TGs was the only parameter in the lipid profile which was significantly higher in OA I and OA II subgroups compared to the corresponding control groups.

Table 1. Outcomes of controls and OA patients

Parameter	Control I	Control II	OA I	OA II	P-value
Age (year)	34.77±5.00	55.20±4.35	35.93±4.36 ^c	56.05±4.64	<0.001
BMI (Kg/m ²)	27.74±3.73	28.01±2.06	28.92±2.97	29.22±2.77	0.094
Coll2-1NO ₂ (ng/mL)	5.00±1.01 ^a	4.56±0.86 ^b	10.96±2.32 ^c	14.44±2.40	<0.001
FSH (mIU/mL)	5.10±2.75	34.12±8.19	6.77±2.60 ^c	35.11±8.58	<0.001
LH (IU/L)	6.80±3.85	31.07±8.72	5.84±3.56 ^c	30.00±7.65	<0.001
Testosterone (nmol/L)	0.88±0.25	0.85±0.32	0.80±0.29	0.89±0.32	0.601
ESR (mm/h)	18.37±8.35 ^a	22.77±6.52 ^b	39.15±5.18 ^c	43.25±6.03	<0.001
CRP (mg/L)	3.72±1.49 ^a	6.67±2.20 ^b	13.18±5.26 ^c	16.90±6.22	<0.001
Calcium (mg/dL)	9.27±0.70 ^a	9.01±0.60 ^b	8.68±0.63	8.61±0.69	<0.001
TGs (mg/dL)	125.03±26.78 ^a	126.58±23.88 ^b	139.23±31.68	143.30±27.56	0.009
TC (mg/dL)	163.69±26.34	171.60±24.08	168.98±26.50	174.38±24.80	0.321
HDL-C (mg/dL)	41.04±6.58	38.09±5.42	38.80±6.92	39.30±5.32	0.216

Letters on values indicate significant differences (a: Control I vs OA I, b: Control II vs OA II, c: OA I vs OA II).

The relationship between Coll2-1NO₂ and the other studied parameters is illustrated in Table 2, which showed that there was no significant association.

Table 2. Correlation of Coll2-1NO₂ with the other variables in OA patients

Variables	Pre-menopausal		Post-menopausal	
	r	P	r	P
Age	0.200	0.216	0.077	0.637
BMI	-0.141	0.384	-0.042	0.798
ESR	0.153	0.346	0.137	0.401
CRP	0.092	0.574	-0.196	0.225
FSH	0.132	0.418	-0.131	0.421
LH	0.101	0.534	-0.039	0.810
Testosterone	-0.074	0.648	0.006	0.970
Calcium	-0.194	0.231	0.306	0.055
TGs	-0.009	0.956	-0.075	0.646
TC	-0.144	0.375	-0.156	0.336
HDL-C	0.279	0.082	-0.033	0.840

The ROC analysis indicated very excellent sensitivity (the area under the curve is 1.000) for Coll2-1NO₂ in the prognosis of OA disease in women at both pre- and post-menopausal age. In pre-menopausal women with OA disease, a cut-off value of 7.29 ng/mL can be used with 100%

sensitivity and 100% specificity as showed in Fig. 1A, while in post-menopausal women with OA disease, a cut-off value of 8.38 ng/mL, can be used with 100% sensitivity and 100% specificity as shown in Fig. 1B.

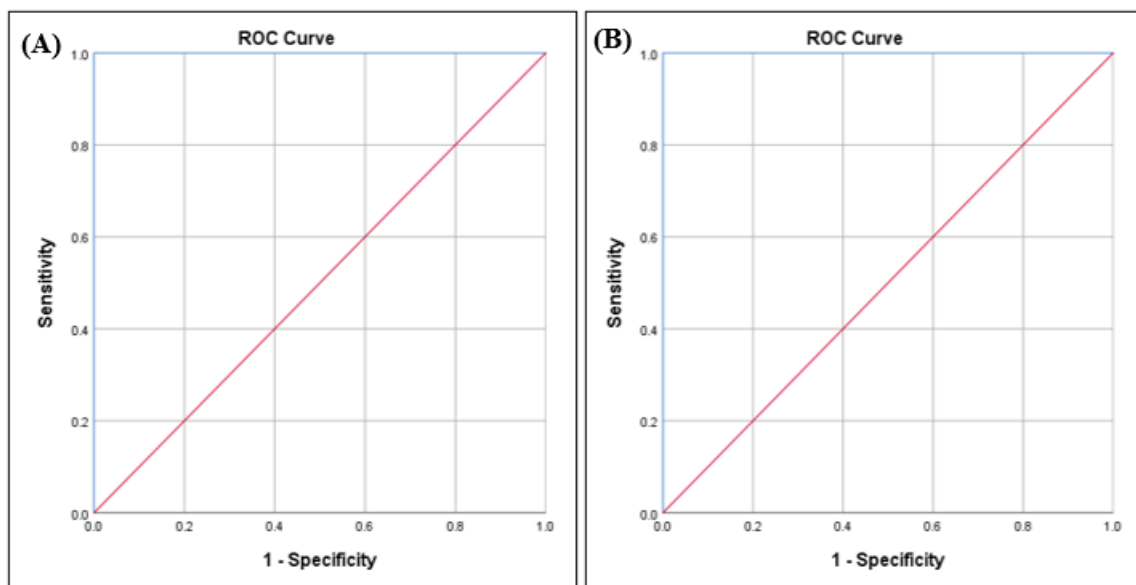


Figure 1. ROC curve of Coll2-1NO₂ in the prognosis of OA disease for (A) pre-menopausal women, (B) post-menopausal women.

Pre- and post-menopausal women with OA disease were submitted to Coll2-1NO₂ analysis in their serum. As shown in Table 1, all of Coll2-1NO₂, CRP, and ESR were elevated significantly in OA patients at both pre- and post-menopausal age scale. Nevertheless, CRP and ESR cannot be used as specific biomarkers for OA disease considering the wide spectrum affecters of these two inflammatory biomarkers, in which they are increased in other conditions such as rheumatoid arthritis¹⁸. Yet, Coll2-1NO₂ can be used as specific biomarkers for OA considering its production from the degradation of type II collagen that was found predominantly in the cartilage. Also, the nitration of its sequence that arises from inflammatory gives an impression of the stress-induced inflammation that accompanied OA disease. Hick *et al.* reported that both Coll2-1 and Coll2-1NO₂ were linked to knee OA and can be used to predict the knee cartilage features in patients. Also, they showed quite engagement between Coll2-1NO₂ serum level and pain in OA patients¹⁴. Mutar *et al.* showed that serum Coll2-1NO₂ level increased significantly in both males

and females with OA disease. Additionally, their reports agreed with our findings regarding the use of Coll2-1NO₂ as predicative biomarker of OA disease¹⁹.

The levels of FSH, LH, and Testosterone non-significantly changed between OA patients and the age corresponding control groups. Yet, it was reported that FSH in post-menopausal women with OA disease can enhance the pain worsening in these patients through decreasing the type II collagen concentration in the cartilage of the knee²⁰. Nevertheless, we did not find a correlation between Coll2-1NO₂ and FSH in post-menopausal women with OA disease. But it can explain the significant high level of OA II compared to OA I (Table 1). Like FSH, high levels of LH in post-menopausal women with OA were reported to enhance the progression of OA disease²¹. Furthermore, TGs level in OA patients was clearly increased compared to healthy women at both pre- and post-menopausal age. Hypertriglyceridemia is linked to the lifestyle of individuals predominantly²². Since

all of the enrolled women were overweight, high TGs is expected, as it was reported by previous studies²³⁻²⁵. Increased TGs level in OA patients

enhances the inflammatory condition²⁶ of these patients and can lead to increase the pain accompanying this disease.

Conclusion

In conclusion, high level of Coll2-1NO₂ in serum of OA patients can predict high cartilage damage with the presence of stress-inflammatory condition in these patients. It is safe to presume that Coll2-1NO₂

can be used as a specific and sensitive biomarker for OA disease based on the collected data in this study and previously mentioned studies.

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Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for

- re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Authors' Contributions

N. B. G. collected the specimens, carried out the research experiment, analyzed, and discussed the

results. B F. H. put the design of the work and supervised the project.

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تقييم مستوى $CoII2-1NO2$ لدى النساء العراقيات المصابات بمرض الفصال العظمي قبل وبعد انقطاع الطمث

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الخلاصة

يعد مرض الفصال العظمي (OA) ليس بمرض مناعة ذاتية ولكنه مرض التهابي مزمن يؤثر على مفاصل الأنسان بسبب جهد ميكانيكي وخصوصاً مفاصل الركبة و الحوض. وفي هذا المرض تحدث تغييرات في الغضروف مسببةً آلام في المفاصل خلال الحركة. $CoII2-1NO2$ هو مصطلح يشير الى سلسلة من الأحماض الأمينية تتكون خلال تحطيم النوع الثاني من الكولاجين والذي يتواجد بشكل وافر في الغضروف، حيث يتم إضافة مجموعة نترات الى الحامض الاميني التايروسين نتيجة لحالة التهابية ناتجة عن الجهد. في هذه الدراسة تم استخدام $CoII2-1NO2$ كمؤشر حيوي لمرض هشاشة العظام للتنبؤ بحالة الاجهاد الالتهابي وكذلك تحطم الغضروف في النساء قبل وبعد انقطاع الطمث. أظهرت النتائج زيادة ملحوظة في مستوى $CoII2-1NO2$ في امصال النساء المصابات بمرض هشاشة العظام قبل وبعد انقطاع الطمث بالمقارنة مع النساء السليمات بمستوى العمر ذاته واللاتي يمتلكن مؤشر كتلة جسم مماثل (زيادة في الوزن). وأظهر تحليل ارتباط بيرسون عدم وجود علاقة بين $CoII2-1NO2$ وباقي متغيرات الدراسة. إضافة لذلك، أظهر تحليل ROC حساسية ممتازة جداً لـ $CoII2-1NO2$ كمؤشر حيوي تشخيصي لمرض هشاشة العظام في كل من النساء قبل وبعد انقطاع الطمث.

الكلمات المفتاحية: نايتروكولاجين 1-2، البروتين الفعال C، الهرمون المنشط للحوصلة، ملف الدهون، الفصال العظمي.