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Assessment of Cartilage Acidic Protein 1 and other Biomarkers in Pre- and Post-Menopausal Iraqi Women with Osteoarthritis Disease

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Abstract

Osteoarthritis (OA) is a degenerative disease that causes pain in humans' joints during movement, especially in the elderly and many women. Since cartilage plays a major destructive role during OA incidence, its chemical components can serve as a sensitive biomarker for diagnosing the onset and severity of OA disease. Cartilage acidic protein 1 (CRTAC1) is a critical component of the cartilage's extracellular matrix. We have aimed to test the sensitivity of CRTAC1 to the prognosis of OA disease in pre-and post-menopausal women with OA disease. The results showed that the levels of CRTAC1 in the serum of pre-and postmenopausal women with OA disease were significantly higher than those in control groups of the same age. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were also higher in these patients. Nonetheless, follicle-stimulating hormone (FSH), luteinizing hormones (LH), and testosterone were non-significantly changed in OA patients and the corresponding control group at both pre- and post-menopausal age. Pearson's correlation was non-significant between CRTAC1 and the rest of the biomarkers. Finally, using CRTAC1 as a biomarker to predict when OA will start and how bad it will get has shown that it works well as a very sensitive and specific biomarker for the disease in women before and after menopause.

Keywords: Cartilage acidic protein 1, follicle-stimulating hormone, luteinizing hormones, menopause, osteoarthritis.

1. Introduction

Osteoarthritis (OA) is a disease that causes joint pain as a result of mechanical damage to the cartilage lying in that joint [1], most commonly in the knee and hip joints [2]. OA is a chronic disease associated with inflammatory events, yet unlike rheumatoid arthritis, it is not an autoimmune disease [3]. Epidemiological studies have shown that OA has advanced incidence rates [4–6]. Reports also indicate that the incidence of OA is significantly higher in women than in men [7]. Furthermore, OA is considered a major health risk for the elderly [8]. Over 500 million individuals globally, or 7% of the world's population, have OA, which primarily impacts women. It has been expected an aging population and rising obesity rates to exacerbate OA, a primary

factor of disability in elderly adults [9]. Women in the postmenopausal stage have a wide range of hormonal and signal transduction system variations [10] that can make mechanical stress worse [11] and inflammatory conditions more aggressive [12]. As a result, it's critical to identify how the pathophysiology of OA differs in pre-and post-menopausal women. There is a lack of biomarkers that diagnose the onset and severity of OA disease. Researchers have dedicated a wide range of resources to identify a certain, sensitive, and specific biomarker for the diagnosis of OA [13–15]. It has been reported that inflammation is associated with the onset and progression of OA disease [16]. C-reactive protein (CRP) [17] and erythrocyte sedimentation rate (ESR) have been used to determine the inflammatory conditions associated with joint inflammation [18]. Nevertheless, these two biomarkers lack the specificity to distinguish OA from other inflammatory-related illnesses [19].

Human chondrogenic tissue's cartilage acidic protein 1 (CRTAC1), an extracellular matrix (ECM) peptide, has been implicated in several illnesses. For instance, in human primary fibroblast cells, CRTAC1 enhances cellular proliferation, emigration, ECM repair, and reorganization [20]. The main purpose of CRTAC1's discovery was to distinguish human chondrocytes from mesenchymal stem cells and osteoblasts [21]. Given that OA is a degenerative disease that causes degradation of the joint cartilage [22], and that CRTAC1 is an important ECM component of the cartilage [23], it can serve as a sensitive and specific biomarker for the development and progression of OA. We aimed to estimate the serum level of CRTAC1 in women at pre- and post-menopausal stages to investigate its relationship with OA at both stages and predict its sensitivity in the disease's prognosis. Moreover, this study aims to find the relationship with the routine inflammatory biomarkers, CRP and ESR, in OA women.

2. Materials and Methods

The consulting orthopedic department at Baghdad Hospital in Baghdad Medical City conducted the study on 80 women who had previously received a diagnosis of OA disease. These 80 women were divided into two groups based on their menopausal stage, namely, OA I and OA II. The first contained the premenopausal women (40 women, age range 25–42 years; 35.93±4.36 years), and the last contained the postmenopausal women (40 women, age range 48–67 years; 56.05±4.64 years). Accordingly, the study was controlled with two sets of healthy women. The first group was termed control I, which contained the premenopausal women (35 women, age range 25–43 years; 34.77±5.00 years), and the second group was termed control II, which contained the postmenopausal women (35 women, age range 47–63 years; 55.20±4.35 years).

This study calculated each woman's weight and height to determine her body mass index (BMI), collected her blood from the vein, extracted the serum at 1500 x g for 10 minutes, and stored it until analysis. At the time of blood collection, we determined the ESR by analyzing a whole blood sample against gravity force for one hour after mixing it with an anticoagulant (sodium citrate). We analyzed CRTAC1 in an ELISA microplate reader (BioTech, USA) using an ELISA kit from MyBioSource (USA). We used Cobas commercial kits on the Cobas E411 auto-analyzer (Roche, Germany) to analyze all of the follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone, and the Cobas B101 auto-analyzer (Roche, Germany) to analyze CRP. Finally, linear kits detected lipid profile parameters, including triglycerides (TGs), total cholesterol, and high-density lipoprotein cholesterol (HDL-cholesterol). Finally, we statistically analyzed the data using mean comparisons (ANOVA and LSD post-hoc test), correlation (Pearson’s coefficient), and receiver operating characteristics (ROC) in the SPSS software program (version 26.0).

3. Results

The participants' ages showed no significant difference between the correspondence groups of OA patients and control women. Also, the differences in BMI were non-significant between the OA patients (28.92±2.97 kg/m^2 for OA I and 29.22±2.77 kg/m^2 for OA II) and the corresponding control groups (27.74±3.73 kg/m^2 for control I and 28.01±2.06 kg/m^2 for control II). The OA II group had the highest level of CRTAC1 in their serum (16.48±3.48 ng/mL), compared to the other three groups. This difference was most noticeable when compared to control II (5.34±1.86 ng/mL) and OA I patients (12.23±3.02 ng/mL). Furthermore, CRTAC1 serum levels were significantly higher in OA I patients compared to control I (4.97±1.64 ng/mL), as shown in **Figure 1**.

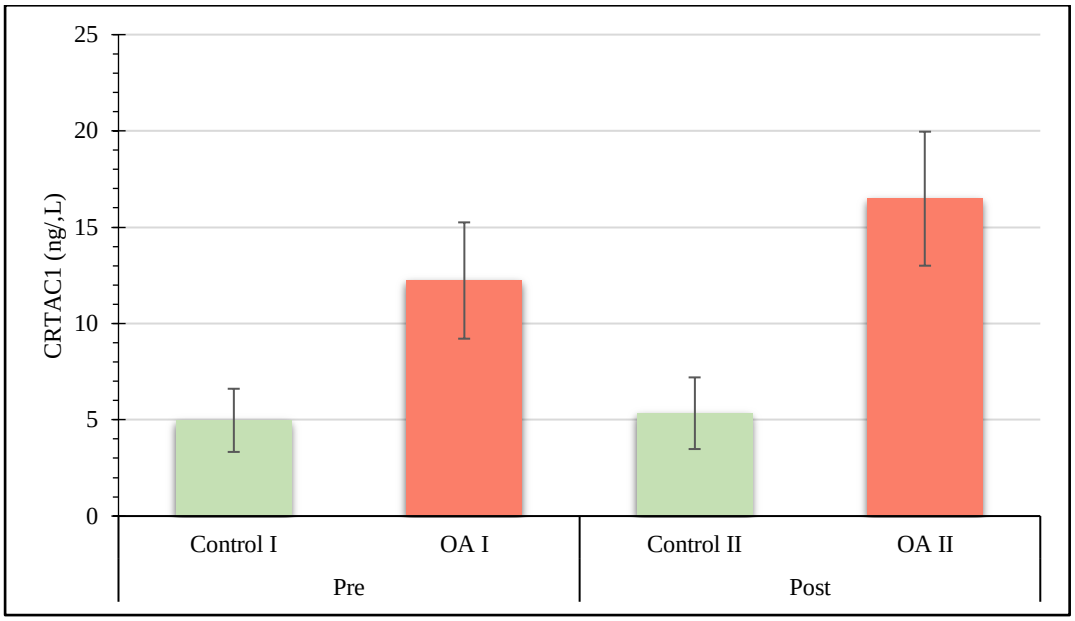

Figure 1. CRTAC1 level in OA and control for pre- and post-menopausal women.

Inflammatory biomarkers, CRP and ESR, were at their highest levels in OA II patients (CRP 16.90±6.22 mg/L; ESR 43.25±6.03 mm/h), which were significant ($p < 0.05$) compared to control II (CRP 6.67±2.20 mg/L; ESR 22.77±6.52 mm/h), and OA I patients (CRP 13.18±5.26 mg/L; ESR 39.15±5.18 mm/h). Furthermore, OA I patients have shown significantly higher levels of CRP and ESR compared to control I (CRP 3.72 1.49 mg/L; ESR 18.37 8.35 mm/h), as shown in **Figure 2**.

The levels of FSH, LH, and testosterone were non-significantly ($p > 0.05$) different between OA patients and the corresponding control groups. Yet, according to their age, OA II patients have shown significant ($p < 0.05$) higher levels of FSH and LH compared to OA I, while the differences in testosterone levels have remained non-significant. The levels for FSH, LH, and testosterone, respectively, were 5.10±2.75 mIU/mL, 6.80±3.85 IU/L, and 0.88±0.25 nmol/L for control I, 34.12±8.19 mIU/mL, 31.07±8.72 IU/L, and 0.85±0.32 nmol/L for control II, 6.77±2.60 mIU/mL, 5.84±3.56 IU/L, and 0.80±0.29 nmol/L for OA I patients, and 35.11±8.58 mIU/mL, 30.00±7.65 IU/L, and 0.89±0.32 nmol/L for OA II patients, as shown in **Figure 3**.

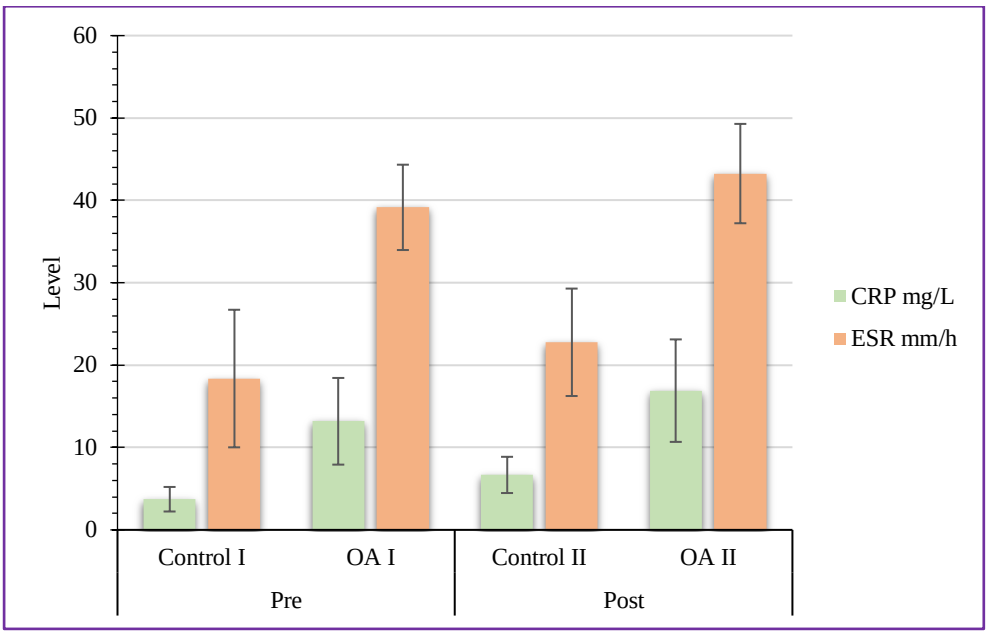

Figure 2. The levels of CRP and ESR in OA and control pre- and post-menopausal women.

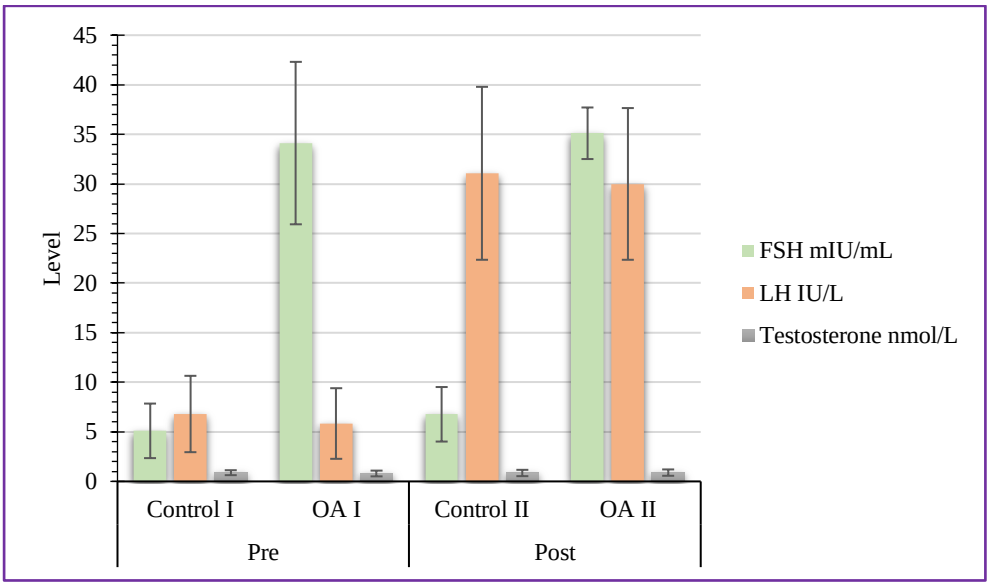

Figure 3. The levels of FSH, LH, and testosterone in OA and control pre- and post-menopausal women.

The concentration of serum TGs was observed to be significantly ($p < 0.05$) higher in OA I (139.23±31.68 mg/dL) than in control I (125.03±26.78 mg/dL) and in OA II (143.30±27.56 mg/dL) than in control II (126.58±23.88 mg/dL). On the other hand, both total cholesterol and HDL-cholesterol have shown non-significant differences in the serum of OA patients and the corresponding control groups. The levels of total cholesterol and HDL-cholesterol, respectively, were 163.69±26.34 mg/dL and 41.04±6.58 mg/dL for control I, 171.60±24.08 mg/dL and 38.09±5.42 mg/dL for control II, 168.98±26.50 mg/dL and 38.80±6.92 mg/dL for OA I, and 174.38±24.80 mg/dL and 39.30±5.32 mg/dL for OA II, as illustrated in **Figure 4**.

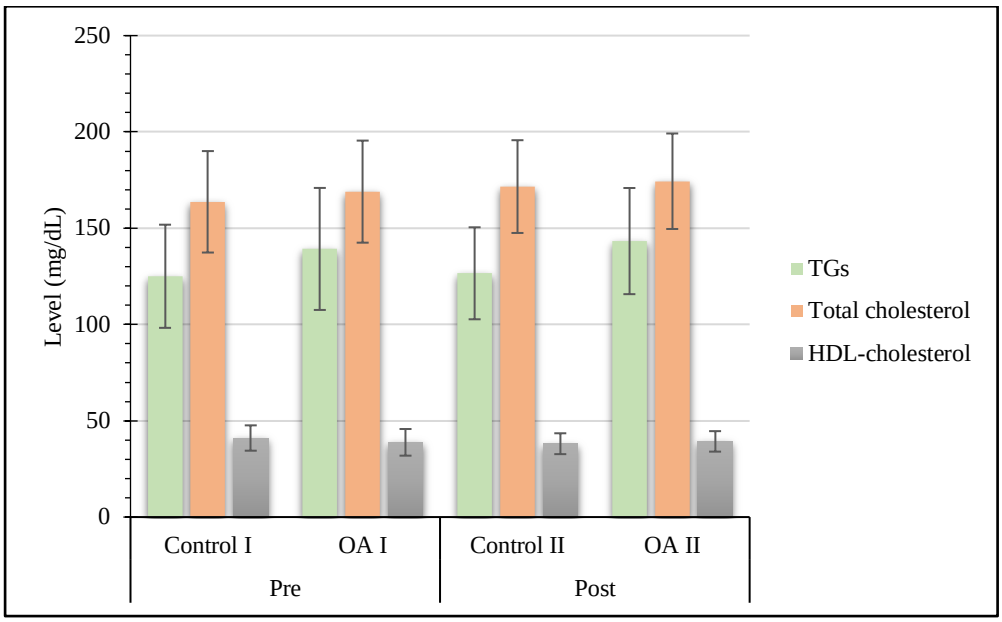

Figure 4. The levels of TGs, total cholesterol, and HDL-cholesterol in OA and control pre- and post-menopausal women.

Table 1 estimates the Pearson correlation between CRTAC1 and the other biomarkers tested in both pre- and post-menopausal OA patients. It has been observed that there is no significant correlation at all.

Table 1. Correlation of CRTAC1 with the other variables in OA patients.

Variables	Pre-menopausal		Post-menopausal	
	r	***p***	**r**	***p***
Age	0.132	0.418	0.042	0.799
BMI	-0.286	0.074	-0.184	0.256
ESR	0.100	0.541	0.260	0.105
CRP	-0.236	0.143	-0.003	0.985
FSH	-0.087	0.594	-0.157	0.333
LH	0.157	0.334	0.024	0.881
Testosterone	0.116	0.476	-0.065	0.692
TGs	-0.198	0.221	-0.215	0.183
Total cholesterol	-0.291	0.068	0.118	0.467
HDL-cholesterol	0.088	0.589	-0.217	0.178

With an area under the curve (AUC) of 0.985, the ROC analysis yielded excellent sensitivity from CRTAC1 as a biomarker for the prognosis of OA degenerative disease in pre-menopausal women. The cut-off value of the test was 7.15 ng/mL, with 97.5% sensitivity and 91.4% specificity (**Figure 5A**). Additionally, the CRTAC1 exhibits exceptional sensitivity in predicting the prognosis of OA degenerative disease in post-menopausal women, with an AUC of 1.000. The test's cut-off value was 10.05 ng/mL with 100% sensitivity and 100% specificity (**Figure 5B**).

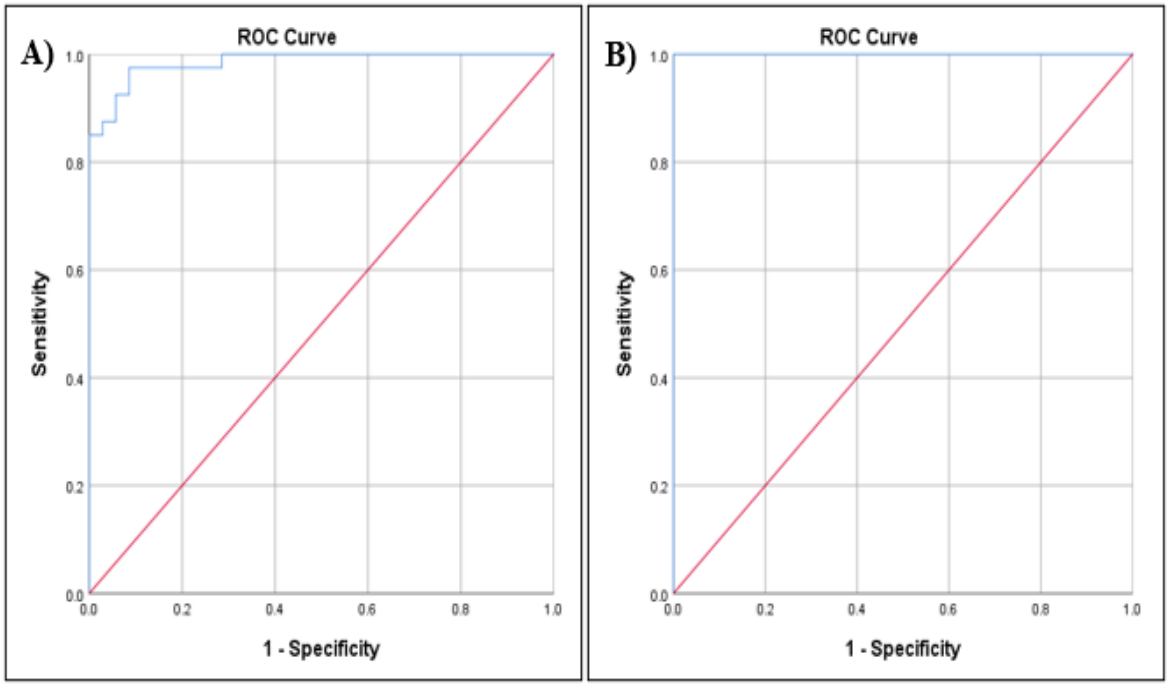

Figure 5. The ROC curve of CRTAC1 in the prognosis of OA for **A)** pre-menopausal women, and **B)** post-menopausal women.

4. Discussion

The inflammatory biomarkers CRP and ESR have shown a significant increase in women with OA disease, regardless of their age. It has been reported that the CRP indicates systemic inflammation in OA patients, in which the level of CRP increased slightly but significantly in OA patients, indicating low-grade systemic inflammation in these patients [24]. Other studies have shown similar results [25, 26]. A recent systematic review has found that inflammation and pain in knee OA patients show a moderate association, depending on CRP and other inflammatory biomarkers [27]. In Wolfe's study, ESR did not show any association with OA patients' signs and symptoms [28]. Previous study has reported that both ESR and CRP were associated with the clinical symptoms of knee OA, and their levels were significantly higher in these patients [18]. However, while they both signify systemic inflammation, they fall short of accurately diagnosing OA disease.

The CRTAC1 is a member of the cartilage's ECM [21], and its release into the blood during OA degeneration stress is sufficient to use as a biomarker for the disease. According to previous study, CRTAC1, among the 4792 proteins tested to predict a biomarker for OA, was the most sensitive and specific for OA disease. They discovered a link between CRTAC1 and the onset and progression of OA [29]. According to preceding data, CRTAC1 was the most significant in the overall OA prognosis in patients. Mutar *et al*., have reported a significant increase in CRTAC1 levels in the serum of OA patients compared to non-OA controls [30]. The severity of the disease and the need for joint replacement therapy in OA patients also showed a linear increase in CRTAC1 [31]. OA patients' cartilage degeneration, which releases CRTAC1 into the circulation, establishes a complex relationship between CRTAC1 and OA. Therefore, we can use the latter to provide a highly sensitive indication of the presence and severity of OA disease. The results of the ROC analysis proved this, as CRTAC1 demonstrated excellent sensitivity for OA disease prognosis, particularly in post-menopausal women.

Between OA patients and the age-appropriate control groups, there were no appreciable differences in the levels of FSH, LH, or testosterone. However, studies have shown that FSH in post-menopausal women with OA conditions may aggravate pain by lowering the level of type II collagen in the knee cartilage [32]. However, in post-menopausal women with OA conditions, we failed to find a relationship between CRTAC1 and FSH. This could potentially explain why the level of OA II is significantly higher than that of OA I. Similar to FSH, post-menopausal women with OA have elevated levels of LH, which may speed up the disease's severity [33]. Additionally, pre- and post-menopausal OA patients had significantly higher TG levels than healthy women. People's lifestyles are mostly associated with hypertriglyceridemia [34]. Other investigations [35,36] report that all of the included women were overweight, which predicts high TGs. Patients with OA with higher TG levels have more inflammation [37], which may increase the pain associated with the disease.

5. Conclusion

Using CRTAC1 as a biomarker to predict when OA will start and how bad it will get has shown that it works well as a very sensitive and specific biomarker for the disease in women before and after menopause. It could be linked with the clinical symptoms of OA disease, but further investigations are necessary to assure these predictions.

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Conflict of Interest

There are no conflicts of interest.

Funding

None.

Ethical Clearance

The scientific committee in the College of Science for Women/ University of Baghdad and the Medical City Hospital approved this study, and a verbal agreement was received from each person included in the study.

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