Iraqi Journal of Biotechnology, 2025, Vol. 24, Special Issue 2, 450-458

Association of C-Terminall Cross –Linked Telopeptide of Type Collagen with C-Reactive Protein and Rheumatoid Factor in Rheumatoid Arthritis Patients

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Received: February 20, 2025 / Accepted: June 22, 2025 / Published: November 16, 2025

Abstract : Rheumatoid arthritis is a chronic inflammatory disease .The condition can damage a wide variety of body systems in some people, including the skin,lungs, eyes, heart and blood vessels. The study was conducted in Al Yarmouk Teaching Hospital and Al Kadhimiya Teaching Hospital, Iraq and evaluated the biomarker of C – Terminal Cross – Linked Telopeptides of Type II Collagen (CTX II) in 80 rheumatoid arthritis (RA) patients and 40 control men and women. Our study aimed of to investigate serum levels of CTX-II in patients with RA and possible relations with joint damage.Serum levels of CTX-II, CRP, and RF were significantly elevated in rheumatoid arthritis patients compared to control , precisely: (CTX-II : 5628.93±530.27 vs. 3382.79 ±522.89; RF : 102.28±14.47 vs. 35.66 ±3.67 u/ml ; CRP : 10.98±2.38 vs. 4.82± 0.65 mg/L).Conclusion: serum CTX-II is a credible diagnostic biomarker that effectively distinguishes active RA patients from healthy control individuals.

Keywords: Rheumatoid arthritis, Rheumatoid factor, C-reactive protein, C – Terminal Cross – Linked Telopeptides of Type II Collagen.

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by synovial hyperplasia, cartilage damage, and bone erosions. Rheumatoid arthritis occurs in about 5 per 1000 people and can lead to severe joint damage and disability.(1)Women are estimated to be three times more likely to develop the disease than men, with the overall prevalence remaining less than 1%. For patients newly diagnosed with rheumatoid arthritis, nonsteroidal anti-inflammatory drugs (NSAIDs) have traditionally been recommended to relieve symptoms and decrease joint swelling.(2). It affects approximately 0.5–1% of the global population and is more common in females, especially those over the age of 50 [3) .Postmenopausal women had a 2-fold increased risk of seronegative RA compared with premenopausal women which is often compounded by overweight and obesity, conditions that exacerbate disease severity (4),(5).

Patients with RA frequently have stiffness, tender, swollen joints, generalized symptoms, and abnormal laboratory tests in up to 90% of cases, early examination can prevent or reduce the development of the disease symptoms and prevent irreversible disability and joint damage. Therefore,

early diagnosis is essential for effective treatment(6). As an autoimmune disorder, immune cells mainly B-cells, T-cells and macrophages play critical roles in RA pathogenesis. These cells can either reside in synovium or circulate in peripheral blood. B-cells secrete physiologically important proteins such as rheumatoid factors (RFs), anti-citrullinated protein antibodies (ACPA) and pro-inflammatory cytokines in supporting RA. B-cells also mediate T-cell activation through expression of costimulatory molecules. (7),(8).Biomarkers for RA , including Rheumatoid factor (RF) ,C – Reactive Protein(CRP), C – Terminal Cross – Linked Telopeptides of Type II Collagen (CTX II) , Erythrocyte sedimentation rate (ESR), Lipid profile and CDAI .

C-terminal cross-linked telopeptides of type II collagen (CTX-II), a byproduct of articular cartilage breakdown, is one of the most assessable biomarkers. CTX-II has been detected in both urine and synovial fluid. An advantage of urine evaluation is that urine can be obtained more easily than synovial fluid, which facilitates a stronger study design because samples can be collected from a comparable control group. Several previous studies have already shown that the levels of urinary CTX-II are significantly elevated in patients with knee OA as compared with controls, and the concentrations increase with disease severity. However, a single study cannot conclusively confirm the usefulness of urinary CTX-II for diagnosing knee OA.(9)

Rheumatoid factor (RF) is the autoantibody that was first found in rheumatoid arthritis which is physiologically important protein, it is secrete by B-cells(10)(7). It is defined as an antibody against the Fc portion of IgG and different RFs can recognize different parts of the IgG-Fc (10). RF and IgG join to form immune complexes that contribute to the disease process such as chronic inflammation and joint destruction at the synovium and cartilage(11).C-reactive protein (CRP) is frequently utilized as a biomarker for assessing systemic inflammation in individuals diagnosed with rheumatoid arthritis (RA). However, it also functions as an immunological regulator that plays a substantial role in the inflammatory pathways associated with rheumatoid arthritis (RA) and contributes to the development of atherogenic outcomes(12).SoRF , CRP and ESRhave the therapeutic, diagnostic, potential for prognostic , and predictive applications. These immunological markers are considered by The American College of Rheumatology and EULAR in their classification criteria(13).

Clinical disease activity index (CDAI) is a simplification from the SDAI as it does not require the measurement of an acute phase reactant. Otherwise, it uses the same clinical assessments as the SDAI. The CDAI correlates well with other disease activity scores and response criteria, as well as with progression of joint damage and functional impairment. CDAI allows immediate treatment decisions to be based entirely on clinical criteria.CDAI is based on the simple summation of the count of swollen/tender joint count of 28 joints,Patient Global Assessment of Disease Activity and Evaluator Global Assessment of Disease Activity (14), (15)

Materials and Methods

Sample collection

Eighty patients with rheumatoid arthritis were enrolled in this study (75 females and 5 males)and 40 healthy controls aged between (49.08 ±10.67)years for patients and (41.62 ±10.52) for control. The blood samples were collected from Al Yarmouk Teaching Hospital and Al Kadhimiya Teaching Hospital,Iraqbetween August 21 and November 3, 2024 .Before being a part of the study, The National Center for the Development of Humans and Medical City's Ethics Committee both gave their approval to the study.

Exclusion Criteria

congenital or acquired dysplasia, pregnancy, diabetes, anemia, elevated liver enzymes, genetic disease, cancer, and any other inflammatory disease like OA or hepatitis C, all of the above were excluded from the study

Biochemical analysis

The following laboratory examinations were done on each patient: Rheumatoid factor (RF) ,C – Reactive Protein(CRP) , C – Terminal Cross – Linked Telopeptides of Type II Collagen (CTX II) , Erythrocyte sedimentation rate (ESR) and Lipid profile. Fivemilliliters of blood were withdrawn using adisposable syringe, each sample was centrifuged for5 minutes at 3000 Xg , the blood was then placedinto gel tubes and left aside to coagulate for 30minutes at room temperature. The serum was then divided into Eppendorf tubesand kept at -20°C (for a maximum of three months) for analysis of RF, CRP and CTX-II . The quantitative sandwich enzyme immunoassay method (ELISA) was utilized toassess RF, CRP, and CTX-II in serum using commercially availablekits (PeproTech, USA) and according to themanufacturer's instructions.In a nutshell, 12x8-well plate wells were coated withan anti-RF , CRP, and CTX-II antibody (arrestingantibody). Theappropriate wells were then loadedwith serum 80µl a standard.An anti-human chemokine antibody that is bioavailable (detection antibody) was added. Horseradish peroxidase (HRP), coupled with avidin, is added after the wells have been cleaned. After washing the wells to eliminate the unstructured avidin enzyme reagent, it interacts with the substrate solution, changing color before stopping. indirectly proportional to the quantity of RF, CRP, and CTX-II bound during the first phase. (The wavelength of the tests, as mentioned in the procedure in their kits, is 450 nm.) The lipid profile was assessed using the colorimetric method with commercially available kits.

Statistical analysis

Statistical analysis was accomplished with the statistical program for social sciences (SPSS 26). The data were expressed as mean± standard error (SE) with 95% confidence intervals andCorrelations Spearman's rho test was utilized to highlight the difference between CDAI with other parameters in RA patients . A significance level of 0.05 or lower was considered as statistically significant.ROC is a statistical technique so the ROC curve methodology has been used to examine the optimal cut-off values for serum CTX-II .

Results and Discussion

The results showed a statistically significant difference in age between the groups. Additionally, there is a slight but statistically notable difference in BMI between rheumatoid RA patients (31.47 ± 5.75) and the control group (27.72 ± 3.49).(Table 1and Fig. 1)

Table (1): The Statistical distributions between patients and control in AGE; BMI

	Group	Mean +SD	Std. Error Mean	Sig
Age	**Patients**	49.08 ±10.67	1.193	0.000
	Control	41.62 ±10.52	1.66	
BMI	**Patients**	31.47±5.75	0.64	0.000
	Control	27.72±3.49	0.55	

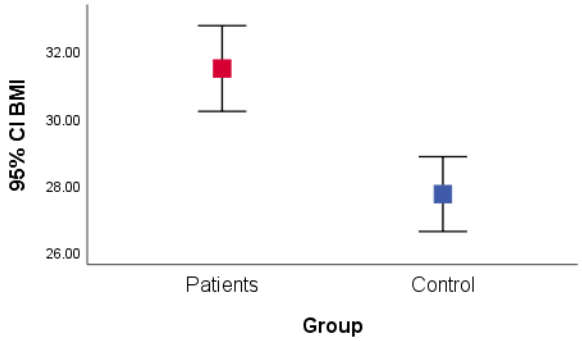

Figure (1): BMI in patients in RA and control

According to Table 2 and Figure 2, the three parameters showed that the mean serum levels in RA patients were substantially higher than those in the control group (RF: 102.28±14.47 vs. 35.66 ±3.67 u/ml); CRP: 10.98 ±2.38 vs.4.82 ±0.65 mg/L; and CTX-II: 5628.93±530.27vs.3382.79 ±522.89, respectively.

Table(2):RF;CRP and CTX-II levels in RA patients and control

	Group	Mean+ SD	Std. Error Mean	Sig.
RF	**Patients**	102.28±14.470	1.61	0.000
	Control	35.66 ±3.67	0.58	
CRP	**Patients**	10.98±2.38	0.27	0.000
	Control	4.82± .65	0.10	
CTX-II	**Patients**	5628.93±530.27	59.29	0.000
	Control	3382.79±522.89	82.68	

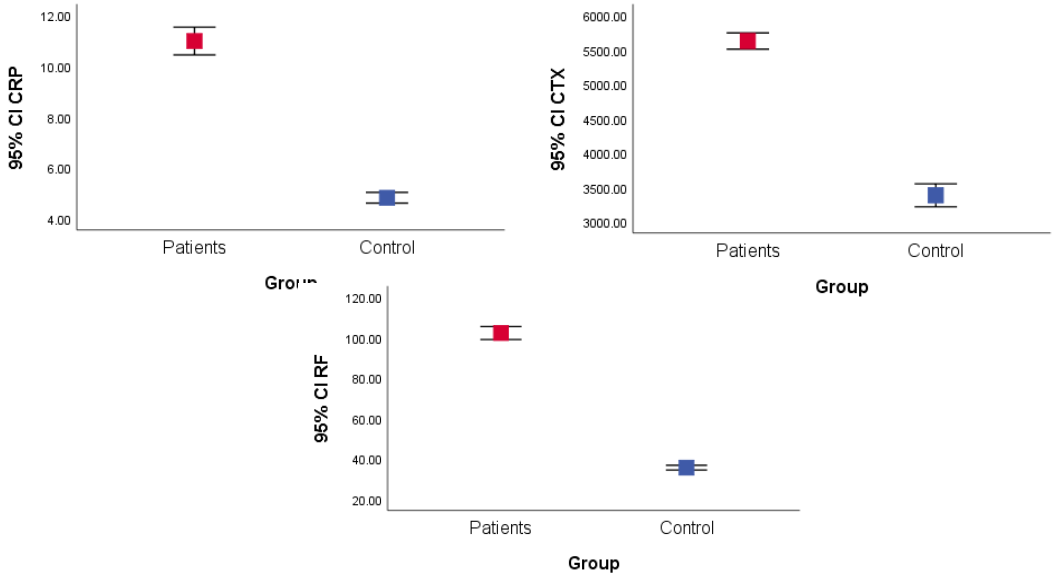

Figure (2): RF,CRP,CTX-II levels in RA patients and control group

Lipid profile results in RA patients and control were displayed asignificant increasein very low-density lipoprotein (VLDL) levels and low density lipoprotein (LDL) in patient group (36.15±3.06) ,(145.57±14.44) respectivelyversus(32.97±2.22,111.51± 11.78)in the control group whilesignificant decreased inhigh-density lipoprotein (HDL) levels in the patient group(36.52±7.32compared tocontrol group52.82±7.23).Triglyceride (TG) levels considerably increased in the patient group(180.43±15.55)compared to the control group(164.90±11.092); Cholesterol levels also were significantly higher in the patient group (217.18 ±10.65)versus(198.30 ±9.52) in the control group. Table3.

Table (3): The statistical distributions of the lipid profile in RA patients and control groups.

	Group	Mean ±SD	Std. Error Mean	Sig
VLDL	**Patients**	36.15±3.06	0.34	0.000
	Control	32.97±2.22	0.35	
LDL	**Patients**	145.57±14.44	1.61	0.000
	Control	111.51±11.78	1.86	
HDL	**Patients**	36.52±7.32	0.81	0.000
	Control	52.82±7.23	1.14	
TG	**Patients**	180.43±15.55	1.73	0.000
	Control	164.90±11.09	1.75	
CHOLE	**Patients**	217.18±10.65	1.19	0.000
	Control	198.30 ±9.52	1.50	

In add to thatESR value among RA patients was clearly higher compared to control , where median is 28.5000 vs. 11.0000 ; mean rank is 69.25 vs. 35.76 , Table4and Fig.3

Table(4): The statistical distributions of the ESR in RA patients and control groups

			ESR
Patients	Median		28.5000
	Mean Rank		69.25
	Percentiles	25	14.0000
		50	28.5000
		75	40.0000
Control	Median		11.0000
	Mean Rank		35.76
	Percentiles	25	9.0000
		50	11.0000
		75	14.0000

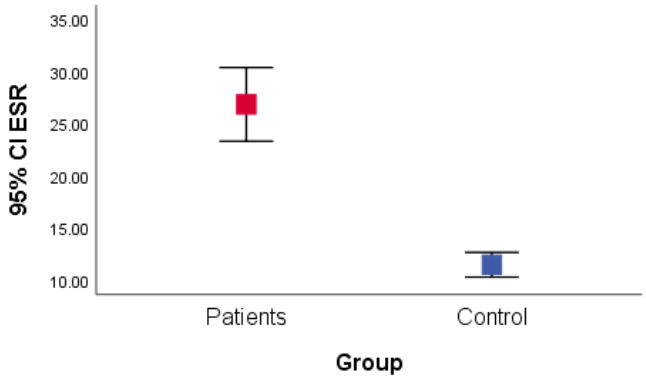

Figure (3): ESR in RA patients and control group

Table 5 presents the personal correlation of CDAI with other parameters in the patient group where CDAI levels exhibit a significant positive correlation with age ,body mass index (BMI) , rheumatoid factor (RF), C-terminal cross-linked telopeptides of type II collagen (CTX-II) and cholesterol (CHOLE)).

Table(5): Correlation between CDAI and other parameters in RA patients.

	R	P
Age	.227*	.043
BMI	.319*	.004
RF	.260*	.020
CRP	.105	.365
CTX-II	.240*	.032
ESR	-.086	.468
VLDL	.037	.743
LDL	.162	.150
HDL	-.113	.320
TG	.085	.455
CHOLE	.227*	.043
CDAI	1.00	

ROC analysis revealed that the level of CTX-II in serum could discriminate between RA with a sensitivity of 100% and a specificity of 100% (Area under curve= 1 (100%)) Fig. 4, Table 6.

Table(6): ROC analysis of CTX-II In RA patients

Parameters	Area under the curve %	Sensitivity %	Specificity %	Cutoff value
CTX-II	100	100	100	4407.6

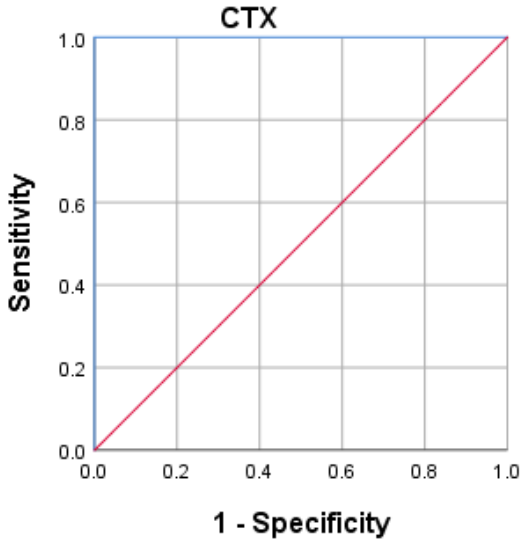

Figure (4): ROC analysis curve of CTX-II

Discussion:

CTX-II is a promising biomarker for assessing cartilage degradation in patients with RA.The current findings support its role as an indicator of disease activity and joint damage. CTX-II is a promising biomarker for assessing cartilage degradation in patients with RA.The current findings support its role as an indicator of

disease activity and joint damage. This was in agreement with other studies who reported that CTX-II and RA were proposed as feasible biomarkers for early detection and monitoring of arthritic diseases.Naglaa K. Idriss et al(16). found that CTX-II serum levels were altogether superior in RA patients contrasted with the solid gathering, which concurs that these ligament digestion variables might be tangled in the pathogenesis of RA. Furthermore, the results revealed that there was a strong correlation between serum levels of CTX-II and age, and thus it was related to the severity of articular cartilage degeneration and histopathological changes in the articular cartilage. Some serological biomarkers such as COMP, CTX-II, and DKK1 could be measure that reflect ligament and bone destruction in patientswith RA could be used to demonstrate early joint affection and disease progression, however, with low affectability and specificity. The COMP serum is a potential biomarker in RA, which indicates disease activity and articular cartilage injury as well as to monitor treatment effectiveness.

Cheng et al (9) observed a significant elevation of urinary CTX-II levels in knee OA patients . Furthermore, the levels of urinary CTX-II increased more rapidly in severe compared to moderate knee OA.

Rheumatoid Factor(RF) levels was significantly increased in the patient group compared to the control group. This aligns with the findings of Conforti A et al.(17) , who also reported elevated RF levels in RA patients. However, the study emphasizes the lack of specificity of RF in diagnosing RA and suggests that RF should be combined with other biomarkers for a more accurate diagnosis.Eman A. Al-Saffar et al (18) showed that all sixty patients with rheumatoid arthritis (RA) were RF-positive (100%), whereas only 10% of the sixty control subjects tested positive for RF. These results indicate that all RA patients were seropositive for rheumatoid factor.

CRP levels in the patient group showed a significant elevation compared to the control group, indicating active RA, which signifies the intensity of inflammation in the affected joints (19) . According to a study by Hamid et al(20) the result indicated a significant change between the two groups in the CRP ($P<0.01$). And according to another study by Qabulio et al(21) there was a significant rising in variances in the patient's group compared to the control group ($P < 0.01$).

The lipid profile data indicated a strong association in RA patients compared to the control group. Rodriguez et al.'s study (22), which revealed elevated TG levels and decreased HDL levels, supports the current study's findings. In a study conducted by Van den Oever IA et al.(23). cholesterol levels were significantly higher in RA patients compared to the control group, which supports the current study's observations. Other studies indicate that dyslipidemia, particularly elevated cholesterol, is a common feature in RA patients, potentially contributing to the increased cardiovascular risk in this population(24).A study found the results where revealed a high significant difference ($P\leq0.01$), mean±SE between Patients and control ((143.18 ±8.04) (96.37 ±3.80)) respectively. (25)

The present investigation revealed no significant changes in the mean age when comparing RA patients with the healthy control group. These findings were consistent with those of Alanzy AK et al(26) , who also found no

significant difference in age between the two groups. This suggested that rheumatoid arthritis can develop at any age and is influenced more by genetic, environmental, and immunological factors than by age alone.The ROC analysis and Statistical distributions demonstrated that CTX-II, RF and CRP may be used for this with good sensitivity and specificity. This demonstrated that CTX-II was related to disease severity and joint pain in addition to reflecting cartilage damage. It is possible to use this biomarker to diagnose, prognosticate, and predict joint cartilage breakdown in RA patients. This can help doctors figure out which patients may respond well to a certain treatment, which can lower drug side effects and costs.

Conclusion

Biomarker CTX-II has special diagnostic performance and investigates a highlevel of accuracy in distinguishing individuals with RA from control subjects. This observation suggests the existence of a potential biomarkerthat may be useful in the diagnosis of RA.

Acknowledgment

We would like to express our appreciation to the Department of Chemistry at the College of Science for Women, University of Baghdad, for their valuable assistance in this project. We also would like to thank the Al Yarmouk Teaching Hospital and Al Kadhimiya Teaching Hospital staff for their help in specimens' collection.

Authors' contributions statement.

Bushra Faris Hasan was responsible for the design and oversight of the project.

Amani Khudhair Abbas has gathered the specimens, conducted the study experiment, then analyzed and explained the findings.

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