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Evaluation of CCN3 as an Inflammatory Marker of Disease Activity in Rheumatoid Arthritis Patients

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ABSTRACT

Background: Rheumatoid arthritis (RA) is an inflammatory form of arthritis that affects around 1% of adults worldwide. Symmetric polyarticular synovial inflammation is its defining feature. Nephroblastoma overexpressed protein (CCN3) proteins are important mediators of organogenesis and inflammation; CCN3 was found in synovial tissues of patients with RA and Osteoarthritis.

Objective: Consequently, goal of this research was to establish significance of serum CCN3 levels in RA patients and their relationship to disorder activity and severity. **Methods:** This case control investigation was performed on 50 subjects who were allocated into two groups; patient group: thirty patients with RA and control group: twenty healthy volunteers with ages and sexes that are matched. All patients were exposed to rheumatological and systemic examinations, and assessment of RA activity and X-ray on both hands and feet.

Results: RA patients had significantly greater levels of serum CCN3. Serum CCN3 levels and disorder activity in RA patients showed a positive, statistically significant connection. There were significant positive correlations between CCN3 concentration and length of morning stiffness, number of tender and swollen joints, Visual Analog Scale, Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein(CRP), Disease Activity Score (DAS28), Rheumatoid Factor (RF), Anti cyclic Citrullinated peptid(Anti CCp), erosion score and joint space narrowing.

Conclusions: Serum concentration of CCN3 was substantially higher in RA patients contrasted to controls. This level in these patients was positively connected with the severity and length of the disorder. With bone degradation, joint degeneration, and diminished functional status in RA patients, the serum CCN3 level was strongly associated.

Keywords: CCN3, Rheumatoid arthritis, DAS28, Inflammatory marker

INTRODUCTION

The prevalence of rheumatoid arthritis (RA), a chronic inflammatory systemic autoimmune disorder that mostly impacts elderly people, ranges from 0.4% to 1%. It is two times as prevalent in women as in men, with a ratio of 2:1. RA primarily affects the synovial joints causing early death, deteriorating health, and socioeconomic burdens [1, 2].

Although the pathophysiology of RA is not yet understood, immunological dysregulation is caused by the interaction of genetic, infections, sex hormone, and environment-related factors. The pathophysiology of the disorder involves a variety of immune cells and components, and RA patients' synovium and serum have been found to have an up-regulation of a variety of cytokines and chemokines [3].

Clinical manifestations of RA are arthritis, stiffness, swelling, tenderness, deformity of tiny hand joints, wrist, elbows, shoulder and metatarsophalangeal (MTP) joints, ankle, knee and hip joints with decreased range of motion on examination [4, 5]. Laboratory investigations of RA includes acute-phase reactants as erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor (RF) and anti-CCP [6]. CCN family of proteins includes the nephroblastoma overexpressed protein (NOV/CCN3), which controls a number of cellular processes including migration, proliferation, differentiation, survival, apoptosis, and extracellular matrix remodelling in a cell-specific way [7].

CCN proteins are important regulators of organogenesis and inflammation in living organisms. By controlling inflammatory chemicals in astrocytes and encouraging CNS regeneration, fibrotic disorders and cancer can be prevented [8]. These functions are mediated via own putative receptors, altering the impact of different cytokines and growth factors, interaction with integrin's and by the Notch pathway, which controls cell destiny during development and keeps adult tissue in a homeostatic state [9].

Nephroblastoma overexpressed protein (NOV/CCN3) could be found in synovial tissues of RA and OA [10]. Its role in RA remains unknown, may be a possible sign of disorder activity for RA [11].

The goal of this research was to establish the function of CCN3 serum concentrations in RA patients and their relationship to disease activity and severity.

PATIENTS AND METHODS

This case control research involved 30 patients who met 2010 American College of Rheumatology (ACR)/European League against Rheumatism (EULAR) classification criteria for rheumatoid arthritis [12]. They were chosen from outpatient clinic of Tanta University Hospitals' Rheumatology, Rehabilitation and Physical Medicine Department from March 2021.

Ethical considerations:

The Ethical Committee, Tanta University Hospitals endorsed our research in 3/2021 with approval code 34486/2/21. The patient or their family members signed a consent form. This work has been carried out in accordance with The Code of

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Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Exclusion criteria were patients with other causes of arthritis (e.g.: osteoarthritis, septic arthritis, psoriatic arthritis, and reactive arthritis), malignant diseases, history of central nervous system or cardiovascular diseases, obesity with BMI ≥30 kg/m^2.

Two groups of participants were assigned to; patient group: thirty patients with RA and control group: twenty healthy volunteers with ages and sexes that are matched.

All patients were exposed to: taking a complete medical history as personal history, complaint, present history as morning stiffness duration, joint pain and swelling, synovial joints examination (Inspection, palpation and movement), pain assessment by visual analogue scale (VAS).

Disease Activity Score (DAS 28) at a particular point in time was used to measure disease activity. Utilizing 28-joint counts, number of swollen and sore joints need to be determined (28 tender and swollen joints) [13]. The unit of measurement for the ESR is (mm/hour). The formula below can be employed to calculate DAS28: DAS28 = 0.56 √ (tender28) + 0.28 √ (swollen28) + 0.70 Ln (ESR) + 0.014 (general health). A DAS28 value higher than 5.1 is viewed as having high disease activity. DAS28 values greater than 3.2 but equal or less than 5.1 are considered to indicate moderate disease activity. DAS28 less than or equal 3.2 and larger than 2.6 is regarded as low disease activity. A DAS28 score below 2.6 is deemed to be in remission.

ELISA: As stated by the instructions provided by the manufacturer, ELISA kits were applied to measure the concentrations of CCN3, IL-6, and TNF-α (R&D, Minneapolis, MN).

R&D (Minneapolis, MN) provided the anti-CCN3 antibody for use. After deparaffinization and rehydration, sections received a 3% H_2O_2 treatment before being blocked with 10% goat serum in PBS. Following an overnight staining procedure with an anti-CCN3 antibody at 4°C, the sections were then subjected to a two-step hypersensitive immunohistochemical detection procedure, which allowed for the identification of CCN3 expression under a microscope [14].

Radiographic assessment: Plain X-rays of hands and feet were taken, and they got a score utilizing van der Heijde modified Sharp score [15]. The score technique contains 16 places for joint space erosion in each hand, 15 sites for joint space narrowing in each foot, and 6 regions for joint space erosion in each hand.

Scoring the Hands: Each hand joint's erosion score might be between 0 and 5. According to the surface area of the joint concerned, erosions are given a score of 1 if they are discrete but clearly noticeable and a score of 2 or 3 if they are larger. If the erosion is substantial and crosses the hypothetical bone's middle, it receives a score of 3. If the joint completely collapses or is damaged across its entire surface, a score of 5 is awarded. Individual erosions are added up in each joint to a maximum of 5. Taking into account the 16 erosion-prone locations in each hand, the maximum erosion score is 80. A single score with a scale of 0 to 4 is utilized to integrate joint space narrowing and joint subluxation or luxation. A normal joint space is scored 0. One point is awarded for a focused narrowing of the original joint space. A generalized constriction that retains more than half of the original joint space is given a score of 2. Scores of 3 are assigned to subluxations of joints and generalised narrowings that leave less than half of the original joint space present. Score 4 refers to a bony ankylosis or a full laxation of the joint. Thus, each hand's maximum narrowing/subluxation score is 60.

Scoring the Feet: Each side of the joint is given a separate erosion score ranging from 0 to 5, with a range of 0 to 10 for the erosion score per joint. Thus, 60 is the maximum erosion score per foot. Utilizing a scale from 0 to 4, the joint space narrowing and joint subluxation are merged into one score. The same standards are employed for grading the hands as well. Thus, 24 is the maximum narrowing/subluxation score per foot.

Summing of Scores: The hands' maximum overall erosion score is 160 as a result. The feet's maximum overall erosion score is 120. Total erosion score (hands and feet) can go up to 280. The hands' maximum total narrowing/subluxation score is 120. The feet can score up to 48 in total narrowing/subluxation. The maximum combined narrowing/subluxation score for the hands and feet is 168. 448 is the highest possible Sharp/van der Heijde score.

Statistical analysis

IBM, Chicago, Illinois, USA's SPSS v27 was employed for the statistical study. Mean and standard deviation (SD) of quantitative parametric data were cited, and an unpaired student t-test was employed to examine them. The median and interquartile range (IQR) of quantitative non-parametric data were displayed, and Mann Whitney-test was employed to examine them. Fisher's exact test was applied to examine qualitative variables, which were provided as frequency and percentage. To determine how closely two quantitative variables are correlated, Pearson's correlation was employed. By using ROC curve analysis, overall diagnostic effectiveness of inflammatory marker was examined. Two-tailed P values < 0.05 were required in order to be considered statistically significant.

RESULTS

The two groups did not significantly vary in terms of age, sex, or occupation. ESR 1st h, CRP and CCN3 were significantly greater in study group than control group (Table 1).

Table (1): Age, sex, occupation, acute phase reactants and serum CCN3 level of the studied groups

		Study group (n=30)	Control group (n=20)	P value
Age (years) (Mean ± SD)		45.2 ± 7.47	48 ± 7.05	0.198
Sex	**Male**	1 (3.33%)	3 (15%)	0.289
	Female	29 (96.67%)	17 (85%)	
Occupation	**Worker**	4 (13.33%)	4 (20%)	0.697
	Housewife	26 (86.67%)	16 (80%)	
Acute phase reactants				
ESR 1st h (mm/h) (Mean ± SD)		16 - 73	15 – 37	<0.001*
CRP (mg/L) (Mean ± SD)		1.1 - 50.	1.1 - 5.9	<0.001*
Serum CCN3 level				
CCN3 concentration (pg/ml)		150 – 2400	215.97 - 1739.5	0.006*

ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, *: significant

Clinical data and radiological assessment of both hands and feet by van der Heijde modified sharp score of RA patients were displayed in table 2.

Table (2): Clinical data and radiological assessment of both hands and feet by van der Heijde modified sharp score of RA patients.

	RA patients (n=30)
Clinical data (Mean ± SD)	
Duration of illness (years)	6.3 ± 6.07
Duration of morning stiffness (min)	54.8 ± 26.8
Number of tender joints	6.8 ± 1
Number of swollen joints	6.4 ± 1.07
VAS	4.9 ± 1.78
Radiological assessment (Mean ± SD)	
Erosion score	1.9 ± 1.41
Joint space narrowing	2.1 ± 1.2

VAS: visual analog scale, SD: standard deviation

DAS28 score, Positive RF, positive Anti-CCP in RA patients were displayed in table 3.

Table (3): The number and percentage of DAS28 score, positive (RF), positive anti-CCP in RA patients (n=30)

DAS28 score		
Clinical remission (<2.6)	**N (%)**	2 (6.67%)
	Mean ± SD	2.1 ± 0.28
Low disease activity (2.6-3.2)	**N (%)**	2 (6.67%)
	Mean ± SD	3 ± 0.14
Moderate disease activity (3.2-5.1)	**N (%)**	12 (40%)
	Mean ± SD	4.21 ± 0.52
High disease activity (>5.1)	**N (%)**	14 (46.67%)
	Mean ± SD	5.83 ± 0.48
Total	**N (%)**	30 (100%)
	Mean ± SD	4.74 ± 1.26
Positive rheumatoid factor (RF)		
Positive	**N (%)**	25 (83.33%)
	Mean ± SD	116.44 ± 7.92
Negative	**N (%)**	5 (16.67%)
	Mean ± SD	28 ± 7.81
Positive Anti-CCP		
Positive	**N (%)**	26 (86.67%)
	Mean ± SD	329.62 ± 81.82
Negative	**N (%)**	4 (13.33%)
	Mean ± SD	9 ± 0.82

DAS28: disease activity score in 28 joints; N: number; SD: standard deviation; RF: rheumatoid factor; CCP: cyclic citrullinated peptide.

No significant relationships were found between CCN3 concentrations and (age and length of illness). There were significant positive correlations between CCN3 concentration and length of morning stiffness, number of tender joints, number of swollen joints, VAS, ESR, CRP, DAS28, RF, Anti-CCP, erosion score and joint space narrowing (Table 4).

Table (4): Correlation of CCN3 concentration and different parameters

	CCN3 concentration (Pg/ml)	
	R	**P**
Age (years)	0.098	0.607
Duration of illness (years)	-0.008	0.968
Duration of morning stiffness (min)	0.308	**0.023***
Number of tender joints	0.373	**0.001***
Number of swollen joints	0.580	**<0.001***
VAS	0.312	**0.012***
ESR 1st h (mm/h)	0.628	**<0.001***
CRP (mg/L)	0.549	**<0.001***
DAS28	0.758	**<0.001***
RF	0.656	**<0.001***
Anti-CCP	0.326	**0.009***
Erosion score	0.341	**0.004***
Joint space narrowing	0.355	**0.002***

VAS: visual analog scale, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, DAS28: disease activity score in 28 joints, RF: rheumatoid factor, CCP: cyclic citrullinated peptide, *: significant.

CCN3 concentration can significantly predict RA activity. At cut offs >677.74 (pg/ml) for concentration, CCN3 has 92.86% sensitivity, 50.00% specificity, 96.3% PPV and 33.3% NPV (**Figure 1)**.

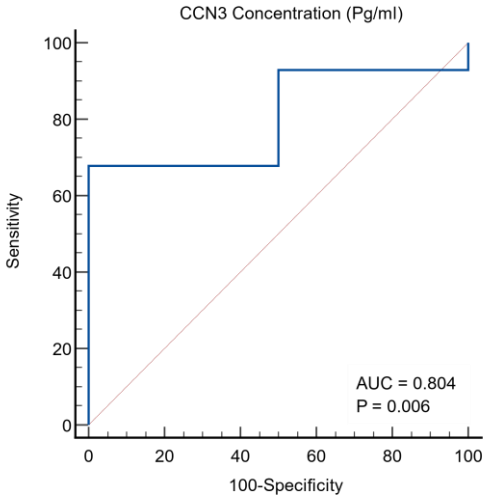

Figure (1): ROC curve of CCN3 concentration for the prediction of RA activity.

DISCUSSION

RA is a chronic, inflammatory, systemic autoimmune disorder that leads to inflammation and degeneration of articular and extra articular structures, subsequent disabling and painful condition with loss of functioning and mobility [16].

RA affects between 0.4% and 1% of the population, more frequently in females with female to male ratio is 2:1, predominantly observed in elderly [13]. Pathogenesis of RA is still unknown, interaction between genetic, infection, sex hormone, and environmental variables results in immunological dysregulation [3]. Various immune cells and components are implicated in the pathophysiology of the disorder, and RA patients' serum and synovium have been found to have an upregulated level of several cytokines and chemokines [3].

From this study, it was found that age of included patients ranged from 30 – 60 years with a mean of 45.2 ± 7.47 years while in control group the age ranged from 33 – 59 years with the mean age of 48 ± 7.05 years with insignificant variation between the both groups (P = 0.198).

This finding was consistent with that recorded by **Lin *et al*.** [17] and **Nilsson *et al*.** [18], who found that the age range of RA patients was 20 to 75 years. It was noted in the current investigation that most of patients were females (96.67%). These findings were similar to those from earlier investigations of **Aurrecoechea *et al*.**[19] and **Intriago *et al*.**[20], who recorded that most of the patients were females. However **Gossec *et al*.** [21] found no sex difference in the occurrence of RA .

As regards clinical data among the studied groups, the current investigation demonstrated that the disease duration ranged from 1 – 24 years while the mean of tender joints count was 6.8 ± 1 and the mean of swollen joints count was 6.4 ± 1.07.

From this study, our patients were active RA with morning stiffness ranged from 10 m – 120 minutes, visual analogue scale (VAS) ranged from 1 – 9, high tender joints score, high swollen joints score and high ESR, CRP in study group in contrast to control group. As stated by DAS28 score, 14 patients with RA had high DAS, 12 patients had scores reflecting moderate disease activity, and 2 had scores reflecting low disease activity. As regards RF, the RF titre of our patients varied from 12 – 130 IU with mean RF of 116.44 ± 7.92 IU. Twenty-five patients (83.33 %) had positive RF, while five patients (16.67%) had negative RF.

The anti-CCP level in our RA patients varied from 213 – 483 IU with mean anti-CCP level of 329.62 ± 81.82 IU. Twenty-six of our patients (86.67%) had positive anti-CCP and four patients (6.66 %) had negative anti-CCP with mean level of 9 ± 0.82. Also, we found radiological abnormalities in the form of bone erosions and joint space narrowing as regards Van der Heijde modified sharp score.

In the current investigation, RA patients' serum levels of CCN3 were found to be significantly higher than those of controls, CCN3 correlates with activity

parameters because DAS28 and its expression have a positive correlation, and correlate with severity parameters as it associated with anti-CCP antibody and radiographic bone erosions and joint space narrowing in RA patients. In addition, we found a connection between RF patients and serum CCN3. These findings imply that CCN3 may influence the onset of RA by controlling the inflammatory response. **Wei** [11] did their study on 41 RA patients and 45 healthy controls. They discovered that RA patients had serum CCN3 levels that were significantly greater than those of controls and that patients with higher DAS (ESR, CRP) had higher CCN3 levels than inactive patients. Additionally, the amount of the CCN3 positively linked with the concentration of the RA-specific autoantibody anti-CCP but not RF.

Our results were parallel with that recorded by **Kular L *et al.*** [22] who conducted their research on 41 RA patients and 45 healthy controls were incorporated. They found that the serum concentration of CCN3 was significantly greater in RA patients, and expression of CCN3 was positively connected with DAS28 (ESR), DAS28 (CRP), and anti-CCP antibody. According to the DAS28, they further segmented the RA patients into inactive, moderate, and extremely active groups and discovered that, in comparison to inactive patients, those with greater DAS had greater CCN3 levels. **Komatsu *et al.*** [10] showed that CCN3 could provide a biomarker for RA disease activity. Substantial positive relationships between CCN3 concentrations and 28-joint DAS were found, with greater DAS28 scores indicating deteriorating disease. CCN3 concentrations and RA-anti-CCP Ab titers have been demonstrated to have strong positive correlations, but not between CCN3 and RF. These results were consistent with **Mangnus *et al.*** [23] who discovered a substantial link between RA patients' serum CCN3 levels and joint degeneration on radiographs.

Wei *et al.* [11] observed that the serum CCN3 levels and the RA patients' age and sex were not found to be significantly correlated.

Given the link between serum CCN3 and IL-6 levels in RA patients, CCN3 may play separate functions in the disease. Common cytokines like IL-6 are important in the emergence of RA. Additionally, CCN3 is linked to the polarization of macrophages into M1 (proinflammatory) and M2 cells (anti-inflammatory). TNF-α and IL-6 are mostly made by macrophage. Additionally, there was a connection between CCN3 and TNF-α. Additionally, anti-CCP antibodies, which have been demonstrated to encourage macrophage polarization towards the M1 fraction, are positively linked with CCN3. These findings demonstrate that CCN3 has a critical function in inflammation by controlling macrophage polarization and cytokines, which has a negative impact on bone erosions and joint tissue degradation seen on radiological examination. In addition, RA patients' synovium had substantially greater serum concentrations of CCN3 than did healthy controls.

CONCLUSIONS

Serum concentration of CCN3 was substantially greater in RA patients than healthy controls. There was no correlation between CCN3 and duration of illness. With bone degradation, joint degeneration, and diminished functional status in RA patients, the serum CCN3 level was strongly associated.

DECLARATIONS

- **Consent for publication:** I attest that all authors have agreed to submit the work.
- **Availability of data and material:** Available
- **Competing interests:** None
- **Funding:** No fund
- **Conflicts of interest:** no conflicts of interest.

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