

Association Of Cellular Communication Network Factor 3 (CCN3) With Rheumatoid Arthritis Disease And It's Severity (Case-Control Study)

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ABSTRACT

Background: An inflammatory condition that affects the joints in the limbs is rheumatoid arthritis. The most common symptom is chronic, recurrent joint inflammation. When patients are advanced, joint deformities and impairments may cause major impediments in the cardiac, skin, as well as other tissues and organs. A protein called cellular CCN3 is implicated in many biological processes such as cell adhesion, migration, as well as proliferation. Additionally, CCN3 has a role in a number of pathological processes, such as fibrosis, angiogenesis, and wound healing. CCN3 can be released from the cytoplasm. CCN3 may also be an inflammatory component in the course of RA. Future therapy approaches that focus on the activities and mechanisms of action of these proteins may result from better knowledge of how CCN proteins alter the pathophysiological processes underlying these types of arthritis and successfully reduce patients' pain (Wei et al., 2020). It was discovered that RA sera and tissues had increased levels of all CCN family members. IL6 production and CCN3 expression are correlated with disease activity (Giusti & Scotlandi, 2021).

Methods: Between November 2021 and April 2022, a study including 70 patients with RA who met four or more 2010 American College of Rheumatology and European League Against Rheumatism (ACR/EULAR) classification criteria for the condition and 70 healthy persons serving as control groups was conducted. All subjects had their disease activity score (DAS28-ESR), clinical activity index (CDAI), and rheumatoid factors (RF) detected by latex agglutination. By using an enzyme-linked immunosorbent assay, the levels of anti-cyclic citrullinated peptide (ACCP) and CCN3 in human serum samples were determined (ELISA). The age group of both the patients and the controls included in this study was 20-70. RA was found to be high in individuals of the age group >40 years, at a percentage of 71.4%. The mean age was 46.2±10.3 years for patients and 1.7±0.5 for controls. **Results:** The results clearly showed high serum CCN3 levels in patient groups with RA compared to control ($P = 0.0001$). CCN3 and ACCP have a significantly positive association at control ($P = 0.0001$). According to DAS28-ESR, there were significantly increased concentrations of CCN3 in severe patients at P values of 0.0001 in comparison to mild patients.

Conclusions: The biomarker CCN3 is a good prognostic marker for the severity of RA.

Keywords: Rheumatoid arthritis; Cellular Communication Network factor 3; Rheumatoid factor; Anti-cyclic citrullinated peptide.

Article Information

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory disease that affects roughly 1% of the global population. Three times as many women suffer from RA as males do (Maiuolo

et al., 2021). The condition finally has an impact on the skin, eyes, kidneys, heart, and lungs. Joint damage brought on by cartilage and bone loss can result in deformities and

bone deterioration (Saklani Kala *et al.*, 2021). The patient's quality of life, ability to work, and lifespan are all adversely affected as the condition advances, as is the socioeconomic burden on their relatives (Gautam *et al.*, 2021). A person's physical, mental, and social wellbeing are all significantly impacted by RA, and these effects may all have a detrimental impact on a person's general emotional wellbeing. Rheumatoid arthritis (RA) frequently exhibits extra-articular symptoms and comorbidities, which increases morbidity and hastens death (Conforti *et al.*, 2021; Figus *et al.*, 2021). The specific immune reactions might happen outside the articular region, especially in mucosal sites, such as in respiratory, oral, and intestinal mucosa (Catrina, 2016). However, joint degradation that begins at the synovial membrane and involves most RA tissues is caused by the complex interaction of immune modulators (cytokines and effector cells). The creation of new blood vessels and the infiltration and/or local activation of mononuclear cells, such as macrophages and T, B, plasma, dendritic, and mast cells, are characteristics of synovitis (Nogueira *et al.*, 2016). Understanding the mechanisms underlying a number of rheumatic illnesses led to the thorough investigation of biomarkers in rheumatology. The search for new biomarkers that play crucial roles at different stages of development is still a topic of interest for RA. Biomarkers are therefore required to aid in early diagnosis and forecast prognosis in RA (Stoel, 2020).

Biomarkers are biological traits that may be objectively assessed and used to indicate or measure whether a process is normal, pathological, or how well a treatment is working. RA biomarkers are often assessments of disease activity, such as acute phase reactants and diagnostic or prognostic markers such as rheumatoid factor (RF). "A property that is objectively examined and analyzed as a

signal of normal biological functioning, pathological processes, or pharmacological reactions to a therapeutic intervention" is how the National Institutes of Health defines biomarkers (Taylor *et al.*, 2016). One of the CCN family members, CCN3 is also known as nephroblastoma overexpressed protein (NOV), and it has been linked to a number of important cellular activities, including the expansion of cells, migration, and differentiation. In the last several years, CCN3 has emerged as an important regulator in a wide range of disorders, including rheumatic arthritis, systemic sclerosis, and osteoarthritis (Peng *et al.*, 2021a). CCN3 preserves the whole CCN family's prototypical structure. Functional motifs may be found in four of the protein's structural modules. The convention of referring to CCN modules by the names of the specific motifs they include has gained traction over time. As a result, they have an insulin-like growth factor binding protein-like (IGFBP) module, a von Willebrand factor type C repeat (VWC) module, a thrombospondin type I repeat (TSP1) module, and a C-terminal cystine knot (CT) module from their carboxy proximal ends (Kubota *et al.*, 2021; Chammas *et al.*, 2021). CCN3 raised the synthesis of proteoglycans and the expression of the tenascin-C gene, which is a gene produced by articular chondrocytes and is linked to cell differentiation. If CCN3 is able to inhibit cell growth, it implies that it functions locally in the epiphysis to differentiate future articular chondrocytes from those that will be replaced by bone during secondary ossification. Prior to these discoveries, we hypothesized that CCN3's role in the maintenance of the articular cartilage throughout life would lead to osteoarthritis, and that its disturbance would cause this disease (Janune *et al.*, 2017).

The purpose of this study was to measure serum levels of CCN3 in RA Iraqi patients and to investigate correlations with the activity or

severity of rheumatoid arthritis in a try to forecast the viability of using CCN3 as a disease severity indicator.

MATERIALS AND METHODS

Patients

Participants in this study were Najaf residents in good health as well as Iraqis who had RA and visited the rheumatology division of Al-Sadr Medical City. A total of 140 participants—70 patients with rheumatoid arthritis and another 70 healthy controls—were included in this study. Patients' diagnoses were made by rheumatologists using the ACR/EULAR 2010 Criteria and serological testing. There were 55 female patients and 15 male patients, with ages ranging from 20 to 70. There were 60 women and 10 men in the controls, with ages ranging from 20 to 70. Name, age, gender, diabetes, high blood pressure, smoking, RA family history, and any other questions on the questionnaire were asked of each patient and control subject. Patients enrolled in this study had RA for a minimum of months and a maximum of 30 years, and according to the data in rheumatologist questions from RA patients, so we have done the DAS28-ESR, and CDAI for patients to classify RA patients depending on the DAS-28-ESR to mild, moderate, and severe by use of the equation in the web site (<https://www.mdcalc.com/disease-activity-score-28-rheumatoid-arthritis-esr-das28-esr>).

Patients who will be recruited to the study are carefully evaluated to confirm they meet the specific diagnostic criteria required for RA. All patients that are diagnosed by a rheumatologist according to ACR/EULAR Criteria and get a $>$ or $=6$ score on this criteria, aged between 20 and 70 years, are excluded from the study if they are suffering from other autoimmune diseases, central nervous system or cardiovascular diseases, have recently had surgery or wound or acute local inflammation,

or are aged older than 70 year and age less than 20 year. All healthy controls with any inflammation and people with ESR Titer Positive who have recently undergone surgery, had a wound, or experienced acute local inflammation will be excluded from the study. Age-matched healthy controls who appear to be free of joint pain or other joint problems and have no family history of RA were also included.

All laboratory tests analysis was performed in the Clinical Microbiology Research Lab, College of Medicine, University of Kufa.

Material

The tools used in this investigation were disposable gloves, 1.5 and 2 ml Eppendorf tubes, 5 and 10 ml gel serum tubes, 5 and 10 ml disposable sterile syringes, disposable pipette tips, and 5 and 10 ml disposable sterile syringes. The following equipment was used in this study: a centrifuge, a deep freezer, an incubator, an ELISA machine, a refrigerator, and various-sized micropipettes. The rheumatoid factor RF-latex kit, the CCN3 ELISA kit (Bioassay kit), and the antibodies against cyclic citrullinated peptide (Anti-CCP) (IgM) kit were the kits used in this investigation (Bioassay kit).

Methods

Five to ten milliliters of venous blood were drawn as a sample. The blood sample was divided into two parts: two milliliters were taken in disposable ESR tubes for an ESR test using the Westergren method; the remaining three to seven milliliters were transferred to a sterile gel tube and centrifuged to separate the serum after allowing it to clot at room temperature in the laboratory; finally, the serum sample was divided into three aliquots in an Eppendorf tube for each patient and stored at a range of -20 to 20 degrees Celsius. Prior to the start of the study, the ethical committee of

the Faculty of Medicine, University of Kufa, provided its permission. Individuals' informed agreement was also gained.

The patients were clinically evaluated and diagnosed by consultant rheumatologists. Measure the serum level of CCN3 in patients and control them by using a specific enzyme-linked immunosorbent assay kit. Measure RF by agglutination test kit, ESR in the serum of patients by the Westergren method, and serum levels of Anti-CCP antibodies in patients by ELISA kit. For RA activity, the disease activity score (DAS28) and clinical disease activity index (CDAI) will be assessed.

STATISTICAL ANALYSIS

The SPSS program, version 20, was utilized to analyze the data. When the data was presented, Continuous variables were displayed as mean, standard deviation, and error, whereas categorical variables were shown as frequencies and percentages.

All of the research variables were significant for the Shapiro-Wilk test ($p=0.001$), suggesting that the variables did not follow a normal distribution, which led to comparisons between two groups being made using the Whitney U test. This method was used to compare patients and healthy subjects, as well as to compare regularity with treatment, age, gender, and response to treatment, while comparisons between three groups were made using the Kruskal-Wallis test. such as in the comparison of CDAI and DAS28-ESR. In RF comparison, the Chi square is used. In order to evaluate the degree of correlation that existed between the study's variables, Pearson correlations and scattered plots were used. Statistical significance was regarded as having a P value must be equal to or less than 0.05.

RESULTS

Table 1 explains the association of CCN3 between patients and controls. The table revealed that there was an increase significantly in the concentration of CCN3 among patients 1.2 ± 0.09 when compared to the mean of the concentration among the control group 0.2 ± 0.02 . According to age, there is a significant increase ($p=0.0001$) in the serum biomarker concentration CCN3 among patients of various ages as compared to the control group, whereas the serum biomarker concentration CCN3 increases in patients that are aged >40 years as compared to those aged ≤ 40 years. According to gender, there is a considerable increase significantly ($p=0.0001$) in the serum concentration of CCN3 in both male and female patients compared to control groups.

Table 1: the CCN3 levels in patients and controls.

Variable		CCN3 patients		CCN3 Controls		P*
		N o.	Mean± SE	N o.	Mean± SE	
CCN3		70	1.2 ± 0.09	70	0.2 ± 0.02	0.0001
Age group	≤ 40	20	1.06 ± 1.8	20	0.3 ± 0.04	0.0001
	>40	50	1.2 ± 0.1	50	0.2 ± 0.02	0.0001
Gender	Male	15	0.9 ± 0.1	10	0.2 ± 0.07	0.0001
	Female	55	1.2 ± 0.1	60	0.2 ± 0.02	0.0001

Table 2 explains the association between CCN3 and the study parameters in each patient. The study show that CCN3 has moderate correlation with ACCP(r)= 0.505; ESR(r)= 0.584; and CDAI (r)= 0.484; however, there are strong correlation of CCN3 with DAS-28ESR (r)= 0.621; while very weak with disease duration(r)= 0.043; age(r)= 0.194. As illustrated in the figures below (1,2,3,4,5,6).

Table 2: Correlation of the CCN3 in serum with the studied parameters in patients with RA.

Parameters	CCN3	
	<i>P</i>	<i>R</i>
Age	0.108	0.194
Duration	0.722	0.043
ACCP	0.0001	0.505**
ESR	0.0001	0.584**
DAS28-ESR	0.0001	0.621**
CDAI	0.0001	0.484**

* significant at p value 0.05 or less.

** . significant at p value 0.01 or less.

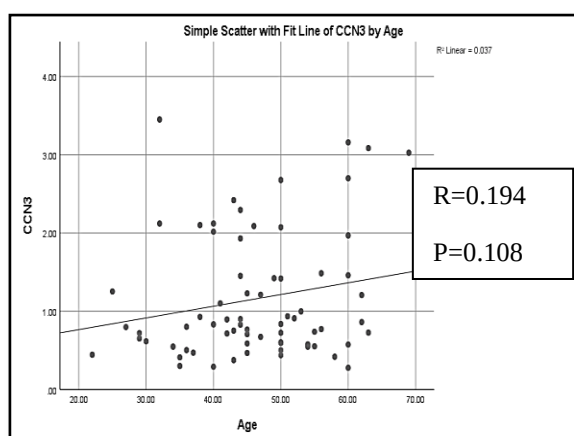


Figure (1): CCN3 & Age correlation.

CCN3 and age have a very weak positive association, as seen in the graph above.

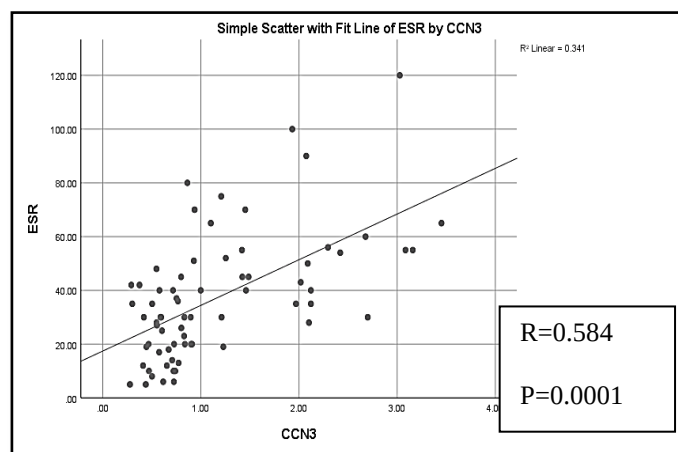


Figure (2): CCN3 & ESR correlation.

CCN3 and ESR have a moderately positive association, as seen in the graph above.

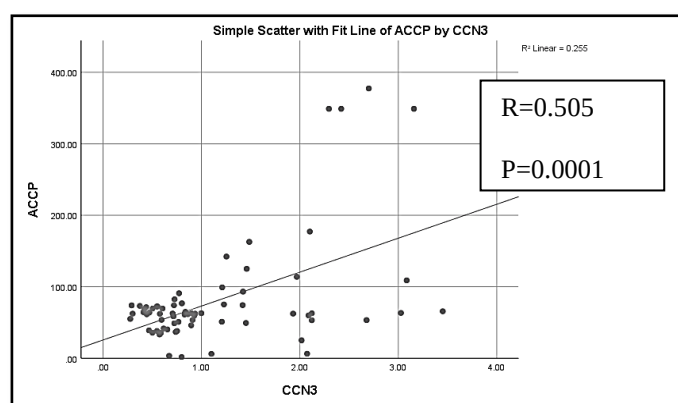


Figure (3): CCN3 & ACCP correlation.

CCN3 and ACCP have a moderately positive association, as seen in the graph above.

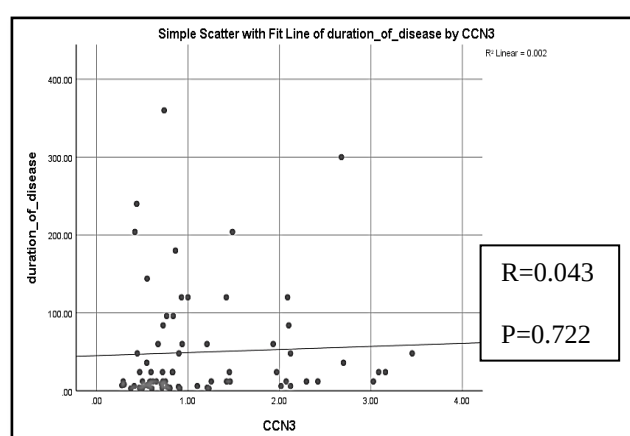


Figure (4): CCN3 & disease duration correlation.

CCN3 and illness duration have a very weak positive connection, as seen in the graph above.

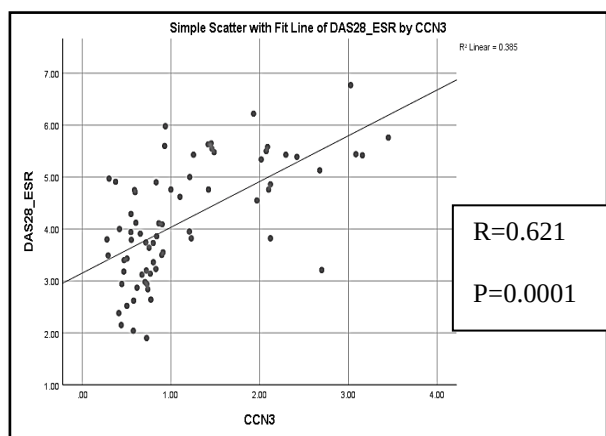


Figure (5): CCN3 & DAS28-ESR correlation

CCN3 and DAS28-ESR have a high positive association, as seen in the graph above.

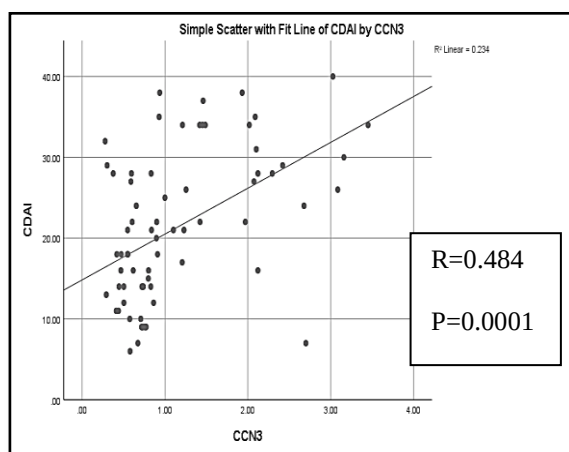


Figure (6): CCN3 & CDAI correlation.

CCN3 and CDAI have a moderately positive association, as seen in the graph above.

DISCUSSION

A matricellular protein is a major part of the CCN family of proteins and is connected to several inflammatory diseases. The CCN3 gene was first discovered to include an integrase from avian nephroblastomas brought on by viral infections. As a secreted protein, CCN3 possesses both the properties of extracellular matrix molecules and conventional cytokines. In addition to influencing the actions of several cytokines and growth factors, CCN3 also purportedly operates through its putative receptors (Huang *et al.*, 2019).

In the current study, at $p = 0.0001$, CCN3 concentrations in the serum of patients were discovered to be significantly greater than in the serum of controls. Wang *et al.*, (2021), found that the amount of CCN3 in patients was greater than in controls ($p = 0.0001$), which proved this result. Also, Peng *et al.*, (2021) found that CCN3 was highly expressed in RA synovial samples when compared with normal joint tissues.

Our study shows CCN3 had a significant positive link with disease severity in DAS28-ESR ($r = 0.621$, $p = 0.0001$) and a modestly favorable relationship with CDAI and ACCP ($r = 0.484$, $p = 0.0001$) & ($r = 0.505$, $p = 0.0001$), respectively. The current finding was consistent with Wang *et al.*, (2021), who found a significant positive liaison between CCN3 & DAS-ESR at ($r = 0.483$, $p = 0.0014$) and a moderately positive connection between CCN3 & ACCP at ($r = 0.500$, $p = 0.0009$), indicating that CCN3 may be essential for the pathophysiology and progression of RA.

Also, MacDonald *et al.*, (2021) found the serum level of CCN3 increased with DAS28-ESR and had a positive correlation with ACCP.

According to our findings, people who don't respond well to treatment had a significantly higher CCN3 level than those with a favorable response to treatment ($p = 0.02$). The current findings were consistent with Peidl, (2018) findings that patients who received treatment had lower levels of CCN3 and It may prevent articular cartilage surface deterioration in the early stages of OA, as well as increased lubricin, which appeared to be more abundant and localized to the articular cartilage surface when compared to the non-treated group.

Our study also found a very weak positive correlation between CCN3 and age in patients at $r = 0.086$, $p = 0.48$, which agrees with Li *et al.*, (2019) who discovered a weak positive relationship at $r = 0.060$, $p = 0.421$ and reported

serum CCN3 correlates positively with inflammatory indices at $p=0.01$, which supports our findings that show a moderate positive connection between CCN3 and ESR at $r = 0.58$.

According to our study, at $p = 0.0001$, CCN3 increased significantly in females compared in males. This is combatable to Pakradouni *et al.*, (2013), the average plasma concentration of NOV appeared to be significantly ($p = 0.0001$) elevated in females compared to males.

CONCLUSIONS

According to the main findings in the present study, patients have an increased concentration of CCN3 significantly in comparison to the control group. There was a significant relationship between CCN3 with disease activity score and CDAI among patients with RA. The biomarker CCN3 is a good prognostic marker for the severity of rheumatoid arthritis.

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