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Original Article

Assessment of Serum level of Cellular Communication Network Factor 3 in Rheumatoid Arthritis Patients and its Correlation to Disease Activity

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Abstract

Background: Rheumatoid arthritis [RA] is an autoimmune disease manifested by non-articular manifestations and articular ones that mostly affects synovial joints in a chronic manner.

Aim of the work: The objective of this research was to evaluate the serum level of Cellular Communication Network Factor 3 [CCN3] in patients with RA and its association with activity of the disease.

Patients and method: This case-control study involved ninety randomly selected cases over the age of eighteen, categorized into three groups: Group A consisted of thirty patients with rheumatoid arthritis in activity, Group B included thirty rheumatoid arthritis patients in remission, and Group C comprised thirty healthy individuals [Control]. All participants were collected from the Rheumatology & Rehabilitation outpatient clinic and inpatient at Bab El Sharia Al-Azhar University Hospital, Cairo.

Results: CCN3 serum levels demonstrated a strong association with inflammatory markers and the Disease Activity Score [DAS28] in addition to with the Health Assessment Questionnaire [HAQ] score. The ROC curve analysis demonstrated a good discriminating power between active, remission and control groups with sensibility of 96.7% and an accuracy of 100.0% for distinguishing active from control groups.

Conclusion: CCN3 serum levels show significant potential in differentiating between the active, remission, and control groups, indicating its promise as a biomarker for assessing activity of rheumatoid arthritis.

Keywords: Rheumatoid Arthritis; Cellular Communication Network; Disease Activity Score; Health Assessment Questionnaire; ESR.

INTRODUCTION

Rheumatoid arthritis [RA] is a debilitating autoimmune condition primarily marked by chronic inflammatory arthritis affecting the small joints of the hands and feet, along with systemic manifestations [1]. The precise cause of RA is unknown, but one theory suggests that it may involve the secretion of anti-citrullinated protein antibodies [ACPA] due to defective citrullination. Additionally, rheumatoid factor [RF] plays a role in RA pathology. Risk factors for RA include genetic predispositions, environmental factors such as smoking, exposure to silica dust and ultraviolet rays, as well as infections like Porphyromonas gingivalis and Borrelia burgdorferi [2].

Synovial macrophages in rheumatoid arthritis secrete various cytokines. This leads to activation of fibroblast-like synoviocytes [FLS] and osteoclasts. The net result is formation of matrix metallo-proteinases [MMPs] and an increase in receptor-activating nuclear factor-kB ligand [RANKL]. Consequently, cartilage and bone destruction occur, accompanied by increased angiogenesis [3]. RA is typically characterized by sustained inflammatory synovitis; previous studies has shown that the Cellular Communication Network Factor 3[CCN3] gene was highly expressed in RA synovial samples compared with normal joint tissues [4].

CCN3, which is member of the CCN group, is a matricellular protein that facilitates cellular interactions with the extracellular matrix [ECM]. CCN is present in in smooth muscle, endothelial cells, chondrocytes, and fibroblasts. So, it promotes fibroblast cell division, migration, and adhesion by binding to receptors such as α6β1, αvβ3, and αvβ5. CCN3 is involved in wound healing, angiogenesis, and fibrosis [5]. The CCN3 gene is widely expressed in cells of endoderm, mesoderm, and ectoderm layers [6].

Regulatory T cells can produce CCN3, which has a pro-inflammatory effect and contributes to myelin regeneration [7]. In human alveolar epithelial cells, blocking CCN3 reduces cell apoptosis and inflammation by interfering with pathways involving nuclear factor kappa B [NF-κB][8].

AIM OF THE WORK

This research aimed to evaluate the serum levels of Cellular Communication Network Factor 3 [CCN3] in patients with RA and its association with activity of the disease.

PATIENTS AND METHODS

This investigation, a case control study, was conducted on 90 randomly selected subjects, above 18 years, separated into three groups, Group A included thirty cases with RA in activity [according to DAS28], Group B included 30 cases with RA in remission and Group C included 30 patients free of RA [Control]. All cases were collected from the Rheumatology & Rehabilitation inpatient and outpatient clinic at Bab El Sharia Al-Azhar University Hospitals, in Cairo.

Ethical Consent:

All members were conducted with the informed written consent of the participants and adhered to the ethical guidelines established by the Al-Azhar University Ethics Committee.

Inclusion criteria:

Eligible cases age is from 18 to 60 years and was diagnosed as rheumatoid arthritis using the ACR 2010 criteria for group A and B. By using DAS-28 ESR/CRP score, Group A is in activity while group B is in remission. Group C is age-matched and has no rheumatoid arthritis by clinical examination.

Exclusion criteria:

Participants with other rheumatic disorders [such as systemic sclerosis and osteoarthritis] that could affect CCN3 levels, as well as those with infections, diabetes mellitus, glomerulonephritis, or malignancies, were excluded from the study.

Assessment of Disease activity by DAS28–ESR/CRP:

The Disease Activity Score for 28 swollen and tender joints [DAS28] assesses disease activity on a scale ranging from 0 to 9.4. Disease activity is categorized as high if the score is above 5.1, moderate between 5.1 and 3.2, low below 3.2, and indicative of remission if below 2.6. DAS28 is determined using measurements of erythrocyte sedimentation rate [ESR] and C-reactive protein [CRP][9].

Assessment of physical function activity using the HAQ questionnaire:

The Health Assessment Questionnaire [HAQ] assesses functional ability using 20 questions grouped into eight categories: dressing and grooming, rising, eating, walking, hygiene, reaching, gripping, and performing activities. Responses are scored based on the level of difficulty: 3 for inability, 2 for severe difficulty, 1 for some difficulty, and 0 for no difficulty. If assistance is required, a score of 2 is assigned. The HAQ disability index is calculated by taking the highest score from each category and averaging them across all categories. Overall, HAQ scores range from 0 to 3, where scores of 2 to 3 indicate severe to very severe inability, scores of 1 to 2 indicate moderate to severe inability, and scores from 0 to 1 indicate mild to moderate inability [10].

Assessment of Serum level of CCN3:

The Human Cellular Communication Network Factor 3 ELISA Kit [Cat. No. E6774Hu] of Shanghai Korian Biotech Company, has a sensitivity of 10.11 ng/L. To prepare serum samples, allow them to clot for 10-20 minutes at room temperature before centrifugation at 2000-3000 RPM for 20 minutes. Reagents should be stored at 2-8°C. The kit features a pre-coated plate with antibodies specific to Human CCN3. In the assay, Human CCN3 in the serum samples binds to these antibodies. Next, a biotinylated Human CCN3 antibody is introduced, which binds to the CCN3 in the sample. Streptavidin-HRP is then added to bind to the biotinylated antibody. Any free Streptavidin-HRP is washed. A substrate solution is added to develop a color proportional to the amount of Human CCN3 present. The reaction is finished with an acidic stop solution, and absorbance is assessed at 450 nm.

Analytical Interpretation:

Data were entered into SPSS [version 23.0, SPSS Inc., Chicago, Illinois, USA] for analysis. Quantitative results were presented as average ± Dispersion and ranges for normally spread data, and as

median with inter-quartile range [IQR] for non-normally spread ones. Nominal elements were reported as rates and ratios. The data's normality was assessed using both the Kolmogorov-Smirnov and Shapiro-Wilk tests.

RESULTS

Table [1] reveals that there are no significant statistical differences in demographic factors such as Age [years], Sex, BMI [weight/[height]^2], and Disease Duration [years] between the groups. The p-values for these comparisons are all above 0.05, suggesting that the groups are similar in these respects.

According to **Table [2]**, the median serum level of CCN3 is highest in the Active Group at 527.7 ng/L [range: 437.4-716.9], less high in the Remission Group at 297.9 ng/L [range: 166.9-424.8], and lowest levels are in the Control Group at 97.5 ng/L [range: 79.0-142.0]. The differences in CCN3 levels among these groups are analytically considerable, where a p-value below 0.05.

Additionally, there is a statistically significant positive correlation between serum level of CCN3 with ESR [mm/h], CRP [mg/dl], DAS-ESR, DAS-CRP and HAQ in patients' group, with p-value [p<0.05]; while has insignificant relation to diagnostic markers [RF and anti-CCP], with p-value [p>0.05] as illustrated in **Table [3]**.

Finally, Receiver operating characteristic [ROC] curve in review for serum CCN3 indicates high diagnostic utility. For the active versus control groups, the Area under the curve [AUC] of 0.996 [CI: 0.932 to 1.000] demonstrates excellent discriminatory ability, with a sensitivity of 96.7% and a specificity of 100.0% at a demarcation point >261.13. For the active versus remission groups, the AUC of 0.842 [CI: 0.725 to 0.924] with a cutoff of >416.77 shows good performance, achieving a sensibility of 83.3% and an accuracy of 76.7%. For distinguishing between the remission control groups, the AUC of 0.873 [CI: 0.762 to 0.945] with a cutoff of >202.4 provides a sensitivity of 70.0% and specificity of 93.3% as mentioned in **Table [4]** and **figure [1].**

Table [1]: Demographic Data Comparison across groups.

		Active Group [n=30]	Remission Group [n=30]	Control Group [n=30]	Test	p
Age "years"	Mean ±SD	38.23±10.4	39.93±11.87	35.80±9.5	1.891	0.124
Sex [n,%]	Male	2 [6.7%]	6 [20.0%]	8 [26.7%]	4.257	0.119
	Female	28 [93.3%]	24 [80.0%]	22 [73.3%]		
BMI [weight/[height]^2]	Mean ±SD	30.40±6.70	28.41±7.08	30.46±6.6	0.880	0.418
Disease duration [years]	Median[IQR]	9.0 [2.8-13.5]	8.0 [2.0-12.3]	--	0.097	0.756

Using: One way Analysis of Variance test was performed for Mean ± SD; Kruskal–Wallis was performed for Median [IQR]; Using: x^2: Chi-square test for Number [%] or Fisher's exact test, when appropriate; NS: Non-significant; S: Significant; HS: Highly significant

Table [2]: Comparison between groups according to s. CCN3**.**

s. CCN3	Active Group [n=30]	Remission Group [n=30]	Control Group [n=30]	Test value	p-value	P1	P2	P3
Median [IQR]	527.7 [437.4-716.9]	297.9 [166.9-424.8]	97.5 [79.0-142.0]	57.672	<0.001	<0.001	<0.001	0.015

Kruskal–Wallis was performed for Median [IQR] & Multiple comparison between groups through Mann-Whitney test; P1: Comparison between Active Group and Remission Group; P2: Comparison between Active Group and Control Group; P3: Comparison between Remission Group and Control Group

Table [3]: Correlation between serum level CCN3 with different parameters in patients Group, using Spearman's rank correlation coefficient [r].

Parameters	Serum Level of CCN3	
	r	p-value
Age "years"	0.185	0.080
Disease duration [years]	0.175	0.181
BMI [wt/[ht]^2]	-0.013	0.902
ESR [mm/h]	0.621	<0.001**
CRP [mg/dl]	0.413	<0.001**
DAS-ESR	0.474	<0.001**
DAS-CRP	0.470	<0.001**
HAQ	0.295	0.044*
RF	-0.125	0.343
Anti-ccp	0.027	0.837

Using: Spearman's rank correlation coefficient [r]; p-value >0.05 NS; *p-value <0.05 S; **p-value <0.001 HS.

Table [4]: Diagnostic Performance of s. CCN3 in Discrimination of all groups using ROC curve.

Items	Cut-off	Sen.	Spe.	PPV	NPV	AUC [C.I.95%]	p-value
Active vs. Control	>261.13	96.7%	100.0%	100.0%	96.8%	0.996 [0.932 to 1.000]	<0.001**
Active vs. Remission	>416.77	83.3%	76.7%	78.1%	82.1%	0.842 [0.725 to 0.924]	<0.001**
Remission vs. Control	>202.4	70.0%	93.3%	91.3%	75.7%	0.873 [0.762 to 0.945]	<0.001**

ROC curve analysis for Diagnostic Performance of s. CCN3 in Discrimination of Active group, remission group and control group.

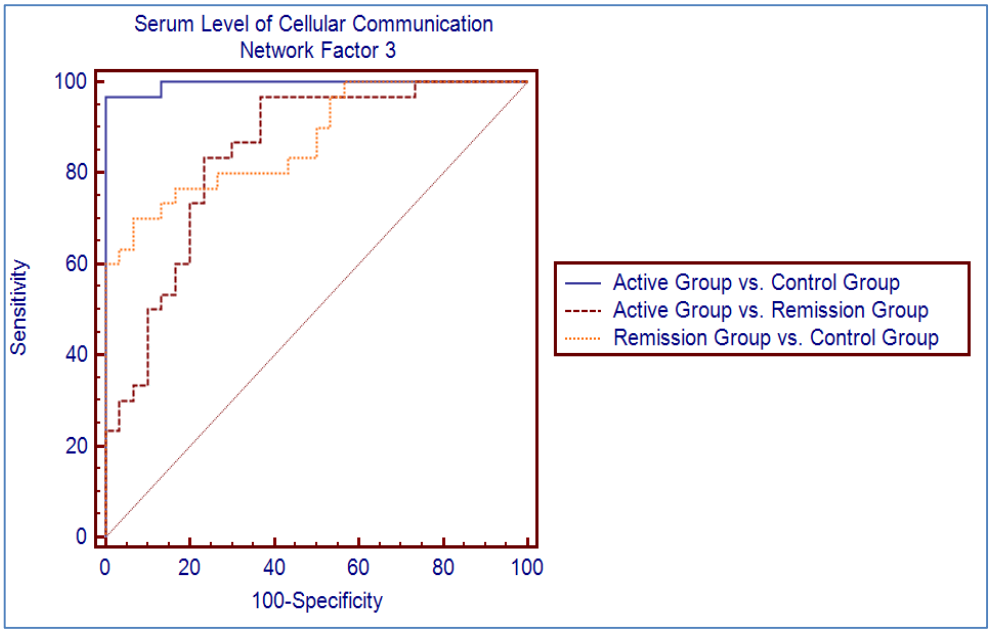

Figure [1]: ROC curve analysis for Diagnostic Performance of s.CCN3 in Discrimination of Active group, remission group and control group

DISCUSSION

CCN3 is significantly involved in regulating various diseases like osteoarthritis and systemic sclerosis, as well as rheumatoid arthritis [11]. The demographic data from our study show that the active group contains 28 females [93.3%] and 2 males [6.7%], the remission group has 24 females [80.0%] and 6 males [20.0%], and the control group is composed of 22 females [73.3%] and 8 males [26.7%]. The disease is more prevalent in females, with a ratio of females to males of 4.625:1.

Our study matches the findings of **Ali *et al*.** [12] who reported that the disease was more prevalent in females than in males, with a female-to-male ratio of 4:1.

Our study finds that the median serum CCN3 level is the highest in the active group, at 527.7 [range: 437.4-716.9], less high in the remission group with a median of 297.9 [range: 166.9-424.8], and the lowest levels are in the control group with a median of 97.5 [range: 79.0-142.0]. The differences between these groups are statistically considerable, with a p-value below 0.05.

Our study aligns with **Kular *et al.*** [13], who investigated 41 RA patients and 45 non-RA cases and found notably higher serum amount of CCN3 in rheumatoid patients. However, our study disagrees with **Komatsu *et al.*** [14] who reported CCN3 and CCN6 were unidentifiable in control OA, and RA cartilage.

The research demonstrates that CCN3 is highly correlated with inflammatory markers ESR and CRP as well as disease activity indices as DAS-28 ESR/CRP. Our study agrees with **Kular *et al.*** [13] who found that CCN3 levels were positively connected with DAS-28 ESR/CRP.

A rise in the median serum CCN3 level in our research is associated with higher HAQ score in the patient group, this association is statistically significant [p is less than 0.05]. On the other hand, there is no significant link between diagnostic markers and serum CCN3 levels in the active, remission, or overall patient groups [p > 0.05].

Consistent with our study, **Ali *et al.*** [12] reported that elevated mean RF levels were found in RA patients with high clinical activity; however, this did not correlate seriously with disease activity [p = 0.858]. The study also found that patients with moderate disease activity had increased mean anti-CCP values, but there was no important correlation between these levels and disease activity [p = 0.981]. This clashes with **MacDonald *et al.*** [15] who found that the serum level of CCN3 had a positive correlation with ACCP.

ROC curve analysis indicates that CCN3 exhibits a strong discriminative ability across all groups. Specifically, for differentiating between the active and control groups, CCN3 has a sensibility of 96.7%, accuracy of 100.0%, a True Positive Rate of 100.0%, and a True Negative Rate of 96.8%.

This aligns with the findings of **Kamel *et al.*** [16] who found that CCN3 levels significantly predict RA activity. At a limit of > 677.74pg/ml, a sensibility of 92.86%, accuracy of 50.0%, Positive predictive value [PPV] of 96.3%, and Negative predictive value [NPV] of 33.3%.

Finally, CCN3 is highly elevated in rheumatoid arthritis patients especially active group, and is correlated positively with inflammatory markers as ESR and CRP as well as with the disease activity indices such as DAS-28 ESR/CRP and HAQ score, so it may be used as a biomarker of rheumatoid activity.

The limitations of our study include a relatively small sample size, which may affect the generalizability of the findings. Additionally, the study did not account for potential confounding factors such as variations in medication adherence and lifestyle factors that could influence CCN3 levels and disease activity. Moreover, the lack of longitudinal data restricts the assessment of changes in CCN3 levels over time and their correlation with disease progression or remission.

Future studies with larger cohorts and longitudinal follow-up are needed to validate and expand upon these findings. It's recommended to study the relationship between serum CCN3 levels and extra-articular manifestations of RA, including cardiovascular, renal, and pulmonary involvement, to evaluate its potential utility in monitoring systemic complications of the disease.

In conclusion, Serum CCN3 is significantly elevated in RA patients, especially those with active disease, compared to healthy controls. We found a positive correlation between CCN3 levels and inflammatory markers [ESR, CRP] as well as disease activity scores [DAS-ESR, DAS-CRP, HAQ]. Additionally, CCN3 demonstrates strong discriminating power between active, remission, and control groups, highlighting its potential as a biomarker for assessing disease activity in RA patients.

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