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Serum Connective Tissue Growth Factor (CTGF) As a Potential Biomarker for Diagnosis of Rheumatoid Arthritis

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Abstract

Background: The most prevalent inflammatory arthropathy in the world is rheumatoid arthritis (RA), a chronic inflammatory autoimmune (AI) disease. As rheumatoid factor (RF) and anti-CCP are unable to diagnose correctly all the patients, recent research raised the possibility that serum connective tissue growth factor (CTGF) could function as a highly sensitive and specific diagnostic biomarker for RA.

Objectives: To evaluation of diagnostic value of serum CTGF in RA patients, investigation of the relationships between serum CTGF and RF and anti-CCP, and investigation of the relationships between serum CTGF and disease activity. Subjects and Methods: Four groups were involved in this case-control study: 45 RA cases, 45 OA cases, 45 SLE cases, and 45 healthy controls. ESR, CRP, RF, and anti-CCP laboratory tests were performed on all RA cases. Physical examination for painful and swollen joints, the DAS 28 CRP test, and a history-taking procedure were performed. All subjects underwent testing to determine their serum CTGF levels. Results: The present study found that serum CTGF levels were higher in RA patients than in SLE patients, OA patients and healthy control individuals. The correlation between serum CTGF and rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and DAS-28-CRP was positive. Serum CTGF had a high specificity and sensitivity for RA cases. Conclusion: CTGF could be a possible biomarker for diagnosing RA as well as monitoring disease activity.

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Keywords: *Rheumatoid arthritis, Connective tissue growth factor, Cellular communication network factor 2, Systemic lupus erythematosus, Osteoarthritis.*

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune illness that causes synovial membrane breakdown in both the small and large joints and is characterized by chronic inflammation. Due to this, life expectancy and quality of life both decline [1].

Cellular communication network factor 2 (CCN2) also known as connective tissue growth factor (CTGF), is one of the CCN family members that has been the most thoroughly researched [2], a group of extracellular matrix (ECM) regulating proteins with a similar structural make-up of their functional domains [3].

CTGF has been demonstrated to be involved in a number of biological processes, which include fibrosis, cancer, angiogenesis, the control of cell growth, tissue modelling, and endochondral ossification [4]. It has been suggested that the autocrine system may help chondrocytes maintain the homeostasis of cartilage tissue [5].

According to some earlier investigations, CTGF fuels the inflammatory condition in RA patients. One of the researchers discovered higher levels of CTGF expression in RA patients' serum as compared to OA patients. Additionally, it was determined that CTGF could increase osteoclast activity by activating integrin protein V3 to worsen bone degradation [6].

Another study found that CTGF, by inhibiting apoptosis, promotes the growth of fibroblast-like synoviocytes (FLS) in RA, which may lead to synovial lining cell hyperplasia and finally joint degeneration [7]. According to certain research, people with RA had significantly greater serum CTGF concentrations than those with other rheumatic disorders. Additionally, serum CTGF is higher in RA that is active compared to RA that is inert [8].

Objectives of this study were evaluation of diagnostic value of serum CTGF in RA patients, investigation of the relationship between serum CTGF and RF and anti-CCP, and investigation of the relationship between serum CTGF and disease activity.

Subjects And Methods

During their clinical visits from April 2022 to August 2022, participants in this case-control study were chosen from the outpatient clinics of the Department of Rheumatology, Rehabilitation, and Physical Medicine at Makkah Hospitals, Saudi Arabia.

The participants in this study were divided into 4 groups:

Group 1 consisted of 45 RA patients who were diagnosed with the disease using the ACR/EULAR 2010 criteria [9], group 2 consisted of 45 SLE cases whom their diagnosis was done using the ACR/EULAR 2019 revised classification criteria for SLE, group 3 consisted of 45 OA cases of OA whom diagnosis was done using the ACR criteria, and group 4 consisted of 45 seemingly healthy hospital staff or blood donors.

The patients who were included were at least 18 years old. Patients with other types of arthritis, such as septic arthritis, any other AI diseases, as well as those with cancer, chronic renal, and liver diseases and those who refused to participate were not included in the study.

Methods

All RA patients underwent a thorough history- taking and general examination. For

musculoskeletal examination, the number of affected joints was determined, and the range of motion, abnormalities, and rheumatoid nodules were also checked. For measuring pain severity, (VAS pain) was employed. Disease activity was evaluated by using the Disease Activity Score-28-CRP calculation [10].

Laboratory Tests

Complete blood count (CBC), ESR, CRP, IgM RF, and anti-CCP were performed. According to the manufacturer's instructions, high-sensitivity commercially available ELISA kits were used to measure the quantities of CTGF in serum.

Sample Preparation

All blood samples (five milliliters) were taken from each person using a sterile venipuncture. After the full blood was drawn, it was left undisturbed at room temperature to allow the blood to clot. It took ten to twenty minutes. After that, the clot was eliminated by centrifuging for 20 minutes at 2,000–3,000 rpm. If precipitates developed during reservation, a second centrifugation of the material was performed. Each sample's serum was maintained frozen at -20° C until the assay. (CTGF) ELISA Kit, INNOVA BIOTECH CO., LTD., (China), was used to perform a direct high sensitivity sandwich ELISA approach to measure serum CTGF concentrations.

Ethical approval:

Ethical Committee gave its approval to this study. All participants agreed to share in the study and signed an informed consent after being briefed about its purpose. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis

The data were analysed using SPSS software, version 20 (SPSS Inc., PASW statistics for Windows version 18). The terms used for describing qualitative data were number and percentage. The median (min and max) (interquartile range) was employed for characterization of the quantitative data for non- normally distributed data. Mean±standard deviation (SD) were used to represent quantitative data with a normal distribution after the Kolmogorov-Smirnov test. The significance of the results was determined at the ($P<0.05$) level. Monte Carlo test was used for comparing qualitative data between groups as necessary. Kruskal Wallis was test was used to compare non-normally distributed quantitative data. One-way ANOVA test was used to compare normally distributed quantitative data, and the Post Hoc Tukey test was used to determine pair-wise comparisons. To assess the strength and direction of a linear link between two continuous variables that are ordinal or not normally distributed, the Spearman's rank-order correlation was used. The right cutoff value was chosen using the ROC curve, which was used to evaluate the validity (sensitivity and specificity) of continuous variables.

Results

Differences between RA patients and other groups regarding age, sex, smoking, and disease duration (matched groups) in the sociodemographic features of the examined groups showed no statistical significance (Table 1).

	1.Rheumatoid cases n=45	2.Osteoarthritis n=45	3.SLE n=45	4.Healthy n=45	Overall significance
Age/years	38.84±11.21	42.87±9.44	41.60±14.37	38.44±11.76	F=1.48 P=0.223
Sex Male Female	13(28.9) 32(71.1)	13(28.9) 32(71.1)	9(20.0) 36(80.0)	10(22.2) 35(77.8)	MC=1.51 P=0.680
Smoking No ex-smoker smoker	39(86.7) 3(6.7) 3(6.7)	37(82.2) 2(4.4) 6(13.3)	39(86.7) 4(8.9) 2(4.4)	39(86.7) 0 6(13.3)	MC=6.97 P=0.324
Disease duration / years	7(0.33-19)	4(0.08-17)	3(1-18)	Not applicable	KW=2.40 P=0.301

The length of morning stiffness and serum CTGF showed a weakly significant positive connection. Serum CTGF showed a marginally statistically significant positive connection with RF, ESR, CRP and DAS 28 CRP. Serum anti- CCP and serum CTGF showed a modestly significant positive connection (Table 2 and Figures 1–6).

Rheumatoid cases (n=45	Serum CTGF	
	Rs	p
Age/years	0.194	0.201
Disease duration / years	0.175	0.249
Morning stiffness duration	0.304	0.042*
ESR	0.333	0.025*
CRP	0.451	0.002*
DAS 28 CRP	0.391	0.008*
Serum RF	0.398	0.007*
Serum anti CCP	0.541	<0.001*

Figures 1–6

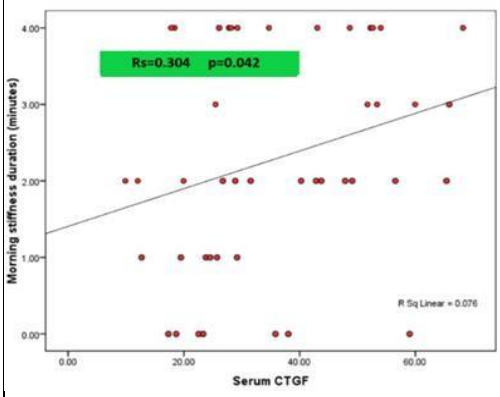


Figure (1): Scatter diagram showing correlation between serum CTGF and morning stiffness among studied cases

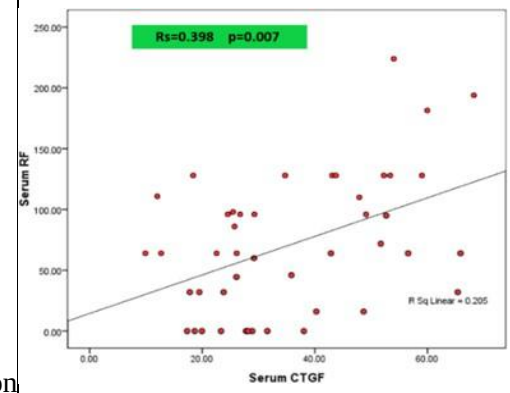


Figure (2): Correlation between serum CTGF and serum RF showed by scatter diagram

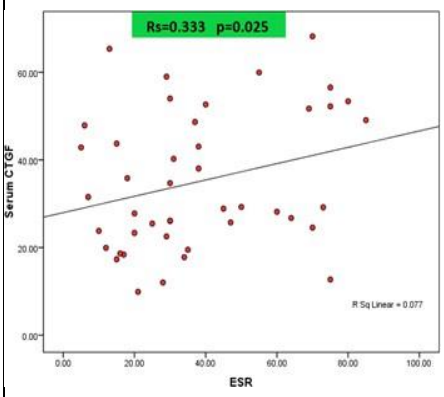


Figure (3): Correlation between serum CTGF and ESR showed by scatter diagram

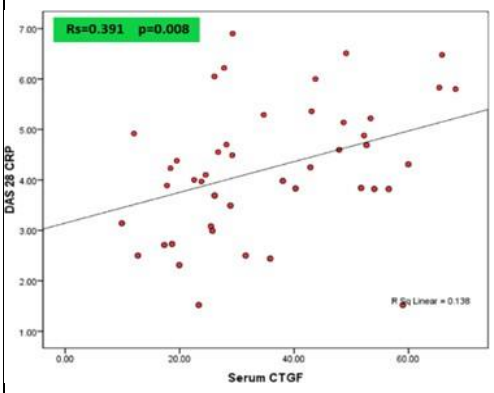


Figure (4): Correlation between serum CTGF and DAS28 CRP showed by scatter diagram

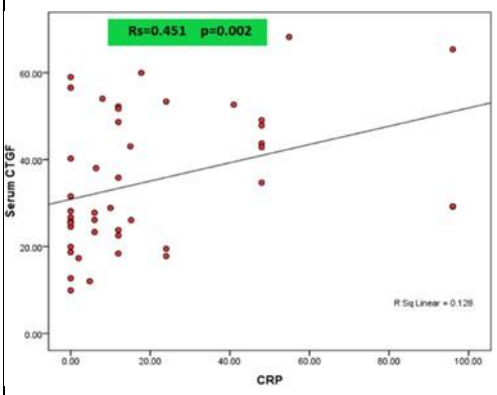


Figure (5): Correlation between serum CTGF and CRP showed by scatter diagram showing

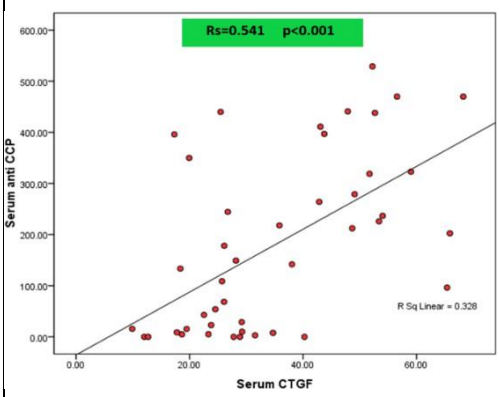


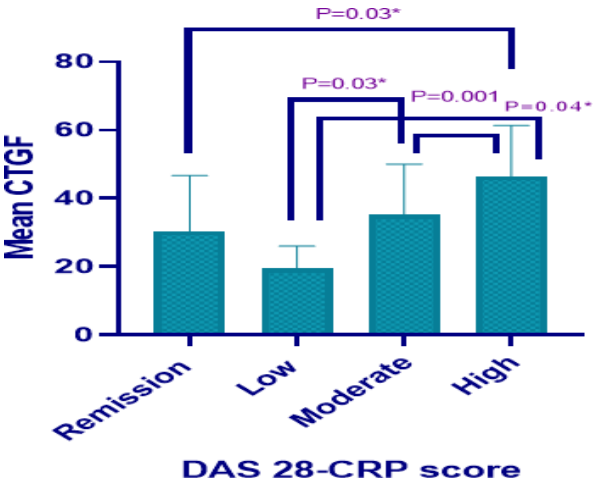
Figure (6): Correlation between serum CTGF and serum anti-CCP showed by scatter diagram

As regard the correlation of serum CTGF and different degrees of DAS 28-CRP, serum CTGF was higher in high disease activity in contrast to moderate, low disease activity and remission (Table 3). Fig. 7

showed that the p values for correlation of serum CTGF with different degrees of DAS 28-CRP was statistically significant.

	Serum CTGF	test of significance
Disease activity score 28 CRP		
Remission	30.39±16.26	KW=4.53 P=0.008*
Low	19.41±6.56	
Moderate	35.17±14.79	
High	46.27±15.03	

Fig. 7



Serum CTGF was compared between RA patients and control groups, and it showed a statistically significant distinction. No OA cases, SLE cases, or healthy control groups had serum CTGF levels that were statistically different from one another (Table 4).

	Rheumatoid cases n=45	osteoarthritis n=45	SLE n=45	Healthy n=45	Overall significance	Comparison with Group 1	Comparison within group2
Serum CTGF Ng/ml	29.26 (9.89-68.22) (23.57-50.4)	13.54(9.83-36.22) (12-20.29)	14.49(10-36.96) (1.09-23.41)	13.34(5.02-37.64) (6.65-19.98)	KW=52.98 P<0.001*	P1<0.001* P2<0.001* P3<0.001*	P4=0.825 P5=0.414 P6=0.300

Median, Range: non-parametric test.

p1: difference between Rheumatoid cases and osteoarthritis,

p2: difference between Rheumatoid cases and SLE,

p3: difference between Rheumatoid cases and healthy group, p4: difference between osteoarthritis and SLE,

p5: difference between osteoarthritis and healthy group, p6: difference between healthy and SLE.

Regarding validity of serum CTGF in differentiating between RA cases and OA controls; AUC was 0.849 and cut off point was ≥ 21.42. Regarding validity of serum CTGF in differentiating between RA cases and SLE controls; AUC was 0.847 and cut off point was ≥ 18.20. Regarding validity of serum CTGF in differentiating between RA cases and healthy controls; AUC was 0.869 and cut off point was ≥17.23 (Table 5).

	AUC (95%CI)	P value	Cut off	Sensitivity (percentage)	Specificity (percentage)	PPV (percentage)	NPV (percentage)	Accuracy (percentage)
Between rheumatoid and OA								
Serum CTGF	0.849 (0.769-0.929)	0.001*	≥21.42	80.0	77.8	78.3	79.5	78.9
Between rheumatoid and SLE								
Serum CTGF	0.847 (0.766-0.927)	0.001*	≥18.20	88.9	64.4	71.4	85.3	76.7
Between rheumatoid and healthy								
Serum CTGF	0.869 (0.797-0.941)	0.001*	≥17.23	93.3	60.0	70.0	90.0	76.7

Discussion

The most prevalent inflammatory arthropathy in the world, (RA) is a chronic inflammatory AI disease that primarily affects women, who are two times more susceptible than males. Its incidence in western countries ranges from 0.5% to 2% among the general population [11]. RA is characterized by synovial proliferation, progressive joints damaging, disability and extra-articular complications [12]. The severity of the disease is associated with the degree inflammation and joint damage [13].

CTGF has been found to be highly expressed in chondrocytes from RA hip and knee cartilage as well as in RA human hip and knee synovium tissues [14]. According to some earlier investigations, CTGF fuels the inflammatory condition in RA patients. Therefore, the objectives of the current study were evaluation of the diagnostic utility of blood CTGF in RA patients, investigation of the relationships between serum CTGF and RF and anti-CCP and investigation the relationships between serum CTGF and RA disease activity.

In the current investigation, serum CTGF levels were higher in RA cases compared to SLE cases, OA cases, and healthy controls. This result went with other authors [6-8, 15], who stated that RA patients' mean blood CTGF concentrations were significantly greater than those of people with other rheumatic diseases and healthy people .

In contrast to the current findings, Wang et al.[16] reported that blood CTGF levels were considerably higher in lupus nephritis and SLE patients without renal involvement than in the normal control group ($p<0.001$ and $p=0.035$, respectively). The lack of SLE cases with renal involvement in the current study and the small number of SLE cases may be responsible for

this contrast.

Positive associations between serum CTGF, serum RF, and anti-CCP were found in the current investigation. In keeping with the current findings, Ding et al. [7] discovered a weak positive connection (Rs = 0.216, p = 0.017) between the blood levels of CTGF and RF titer. Yang et al. [15] also discovered that CTGF and anti-CCP together showed greater discriminatory capacity (AUC = 0.96) than anti-CCP or RF alone (AUC = 0.80 or 0.79, respectively). The combination of CTGF, anti-CCP, and RF had the best diagnostic efficacy, with an AUC of 0.97, outperforming both single indicators and the combinations of RF and CTGF or even the indicated assay of combined RF and anti- CCP.

Ren et al. [8] also found that RF and anti-CCP antibody levels were higher in CTGF-positive patients than CTGF-negative individuals. Patients with anti- CCP antibodies and/or RF positivity mainly had positive CTGF. However, numerous people with anti- CCP antibody or/and RF negative RA nonetheless exhibited serum CTGF, suggesting that serum CTGF may be useful in diagnosing seronegative RA in addition to other markers. Ding et al. [7] research contradicted the current findings, finding no connection between anti-CCP titre and CTGF levels. This discrepancy may have been influenced by their larger sample sizes, variety of autoimmune diseases, and distinctive ethnic backgrounds.

The current results discovered that there was no statistically significant correlation between serum CTGF and disease duration in the current investigation. According to Ren et al. [8], who concurred with the current findings, CTGF-positive patients' disease duration was not significantly different from that of CTGF-negative patients.

In the current investigation, there were significant relationships between serum CTGF and morning stiffness, ESR, CRP, and DAS 28 CRP for disease activity. Serum CTGF was higher in high disease activity in contrast to moderate, low disease activity and remission.

In line with the current findings, Nozawa et al. [6] discovered that active RA had significantly higher serum CTGF levels. Following infliximab therapy, a statistically significant decrease in serum CTGF levels was seen ($P<0.05$).

Ding et al. [7] reported that there was no statistical significance discrepancy in serum CTGF levels between the intermediate group and inactive group, which is in contrast to the current finding. Additionally, they claimed that there was no connection between CTGF levels and other clinical indicators as CRP, ESR, and DAS28.

Also, in Yang et al. [15] study, the connection between serum CTGF and the duration of symptoms or the disease activity score in 28 joints (DAS28) was determined. With Rs = 0.062, $p > 0.05$, and Rs = 0.10, $p > 0.05$, respectively, no significance was seen.

Additionally, Ren et al. [8] discovered that there were no statistically significant variations in ESR and CRP values between CTGF positive and CTGF negative patients ($p > 0.05$). Regarding the following factors: gender, smoking, morning stiffness, SJC28, TJC28, and DAS28, no significant differences were seen in CTGF-positive individuals. These differences could be explained by the association between CTGF levels in the blood and synovial fluid and disease activity, the higher sample sizes used in these investigations, and the differences in ethnic groupings. In the present study, serum CTGF may have high specificity and sensitivity for differentiating RA cases from other diseases.

These results were consistent with those of Yang et al. [15], who found that the training cohort's sensitivity, specificity, and AUC were 0.86, 0.92, and 0.92, respectively. The validation cohort performed similarly, with sensitivity, specificity, positive probability, and negative likelihood of 0.12, 0.82, 0.91, 5.74, and 0.12, respectively. Positive and negative outcomes had predictive

values of 0.85 and 0.90, respectively. They demonstrated that serum CTGF outperformed both of these widely used assays, demonstrating its superiority as a serum biomarker.

In addition, Ren et al. [8] discovered that serum CTGF had a sensitivity, specificity, PPV, and NPV of 33.89 percent, 96.55 percent, 88.41percent, and 55.45 percent, respectively, for RA. This resulted in a greater specificity and lower sensitivity. The different number of enrolled patients and cases types included in each group may have an impact on the sensitivity difference.

Conclusion

According to the current study, RA patients had greater serum CTGF levels than SLE cases, OA cases, and healthy control subjects did. Positive correlations were seen between serum CTGF and RF, anti-CCP, ESR, CRP and DAS-28-CRP. Therefore, serum CTGF may be helpful for assessing the condition's severity. CTGF is a potential biomarker for RA diagnosis and tracking disease activity.

Recommendations

To obtain more substantial outcomes and longer duration, it is advised that additional studies with a large patient population and more rheumatic diseases other than OA and SLE patients be conducted. It is advised that future research correlate serum CTGF levels with the therapy (both before and after treatment).

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