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ORIGINAL ARTICLE

Role of Serum Collagen Triple Helix Repeat Containing 1(CTHRC1) Protein in Rheumatoid Arthritis and Its Relation to Disease Activity

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic, progressive, autoimmune disease affecting synovial joints with periods of flares which leads to joint erosion and bone destruction and consequent disability. Due to lack of diagnostic and prognostic markers to evaluate patients, several tests have emerged such as CTHRC1 protein.

Methods: A case-control study was carried on 60 subjects ,30 healthy controls and 30 RA patients. Disease activity assessment was done for all patients using DAS28. Laboratory parameters as ESR, CRP,RF, anticcp . Serum CTHRC1 was measured in healthy individuals and RA patients using quantitative ELISA technique.

Results: 30 RA patients (24 females and 6 males) with age mean of 37.3 ± 12.2 years and disease duration median of 5 years. We found that serum CTHRC1 was higher in RA patients than healthy controls.CTHRC1 was significantly correlated with DAS28 (p=0.02), tender joint number (p=0.004), swollen joint number (p=0.03) and dry mouth (p=0.03). No association was found between serum CTHRC1 and laboratory parameters. ROC curve analysis of serum CTHRC1 could significantly discriminate between active patients and in remission with a cut off value 10.3 ng/mL with sensitivity 65.4%, specificity 100%, PPV 100%, NPV 30.8% with area under the curve 83%.

Conclusions: serum CTHRC1 is a favorable marker to evaluate RA patients. Significant association was found between serum CTHRC1 and disease activity which may serve as a valuable marker for RA patients monitoring.

Keywords: CTHRC1 protein**;** Rheumatoid arthritis; DAS28**;** biomarker

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disorder which involves the synovial tissues. Repitition of flares of disease activity may irreversibly result in joint and bone deterioration, ultimately leading to disability [1].

RA diagnosis was made as a combination of clinical, radiological and laboratory parameters including CRP and ESR, RF, and anti-CCP. But CRP and ESR are non-specific markers, and reflect only short-term inflammatory condition [2].

Collagen triple helix repeat containing-1 (CTHRC1), is a 28-kD glycoprotein. CTHRC1 is mainly expressed in adventitia of fibroblasts and smooth muscle intima of balloon-injured blood vessels [3] and enhances motility of cells by inhibiting collagen matrix precipitation and stimulating migration of cells [4]. CTHRC1 is created by activated fibroblast-like synoviocytes (FLS) which situated at the lining of intima and synovium and the bone-pannus interface and primarily promoted the polarization of FLS through the front-tail axis and enhanced the speed of migration and movement performance of cells, which resulted in much formation of pannus and subsequent arthritis damage [5].

CTHRC1 protein is expressed in neonatal and embryological tissues, which include growing bone and cartilage [6].

The purpose of our study was to measure serum CTHRC1 in RA patients and compare it with healthy individuals and to assess its relation with disease activity.

METHODS

A case-control study was carried out at the Rheumatology and Rehabilitation Department, Faculty of Medicine, Zagazig University Hospital including 30 healthy controls and 30 RA patients , their ages ≥ 18 years who diagnosed according to the 2010 ACR and EULAR classification criteria. Patients and healthy controls are matched as regard to age and sex.

Patients with malignancy, cardiovascular, liver disease, severe lung disease, infection or other autoimmune disease were excluded.

All patients were subjected to history taking, clinical examination (general & musculoskeletal), disease activity assessment .

RA activity was estimated using DAS28.

At the Clinical Pathology Department, the samples' analysis was completed. Six mL of whole blood specimens were collected by venipuncture in plain, ESR, and EDTA tubes (2ml in each) (Becton Dickinson Company, Franklin Lakes, NJ, USA). Sample in plain tubes was allowed to coagulate at room temperature for 10-20 minutes, centrifugation 15 min at the speed of 1200 x g, and supernatant serum was separated in 2 aliquots one utilized for routine tests and the other stored at -80°C until (ELISA) analysis.

Routine laboratory investigations included ESR, CRP, RF, anti-CCP antibodies and complete blood picture (CBC). A Sysmex XN 2000 Haematology analyzer (Sysmex, Kobe, Japan) was used to do the CBC. A Vision-B analyzer (YHLO Biotech Co., Ltd., Shenzhen, China) was used to measure the ESR. The Cobas 6000-c501 Modular Analyzer (Roche, Germany) was used to measure CRP and RF. On Cobas E411 (Roche, Germany), anti-CCP2 antibodies were found using the Elecsys anti-CCP kit.

The Human CTHRC1ELISA Kit (Catalogue number: 201-12-3502) was used to quantify serum CTHRC1in accordance with the instructions supplied by the manufacturer (SunRed, Shanghai, China). The optical density (OD) was measured at 450 nm within 15 minutes after the stop solution was applied. Tecan Trading AG, Männedorf, Switzerland's Sunrise absorbance reader was used to calculate the OD. Based on the standards' concentration and the corresponding OD values, the standard curve was created. The sample's OD values were applied to the curve in order to calculate the concentration of the related sample. The results for CTHRC1 were given in ng/mL units.

Ethical approval:

Ethical approval was obtained from Institutional Review Board (IRB) at the Faculty of Medicine ,Zagazig University hospitals 9883-25-9-2022.All participants signed a written informd consent before enrollment in this study. The study was done according to The code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

STATISTICAL ANALYSIS

Collected data were computerized and statistically analyzed using IBM SPSS 23.0 for windows (SPSS Inc., Chicago, IL, USA) was used for data analysis and presentation. Qualitative data: represented in the form of number and percentages (N. %) while quantitative data after testing of normality using Shapiro-wilk test: normally distributed data presented with mean ± SD and skewed data presented as median (range).

At level of significance value (P value): $P > 0.05$ (Non-significant), $P \leq 0.05$ (Significant). For qualitative data: we used Chi-square test while quantitative data that was normally distributed: t-test & ANOVA test were used. Skewed data: Mann-Whitney test & kruskal-Willais test were used. Spearman's rank correlation was used to assess correlations between two continuous variables.

RESULTS

The present study was conducted on 60 subjects, 30 RA patients 24 of them were females and 6 were males, with ages range from (23-63) years, with a mean of 37.3 ± 12.2 years, disease duration median of 5 years ranged from (1-26) years and 30 healthy volunteers, 22 of them were females and 8 males. Ages range from (25-55) years with a mean of 39.9 ± 11.5 years with no significant difference found between RA and healthy individuals as regards age or sex. . There is no significant association between serum CTHRC1 and age, sex, family history, duration of disease, and laboratory findings.

The clinical manifestations of RA patients are presented in (Figure 1). Serum CTHRC1 has significant relation with tender joint number (p=0.004), swollen joint number (p=0.03), dry mouth (p=0.03), these findings are presented in (Table 1).

The median of serum CTHRC1 in RA patients 10.67, range (5.27-33.7) was higher than in controls 7.3, range (5.22 9.81) as in (Table 2).

The increased activity level DAS28 was associated with elevated serum level of CTHRC1 being highest in high active patients and lowest in patients with remission (Table 3).

In the present study, ROC curve was conducted to detect the capability of serum CTHRC1 to differeniate between active patients and in remission with cut off value of 10.3 ng/mL, sensitivity 65.4 %, specificity 100%, PPV 100%, NPV 30.8 % with AUC 83% (Figure 2).

Table 1: Relation between CTHRC1 levels and clinical data among RA group

Variable	Clinical presentation (N=30)		P
NTJ *Median* *Range*	Median ≤15 9.8 (5.27-25.1)	Median >15 14.26 (9.3-33.7)	**0.004**
NSJ *Median* *Range*	Median ≤1 10 (5.3-25.1)	Median >1 13.8 (9.1-33.7)	**0.03**
	Absent	**Present**	
Deformity *Median* *Range*	N=17 10.9 (5.3-27.3)	N=13 10.3 (6.5-33.7)	0.06
SC nodules *Median* *Range*	N=22 11.3 (5.27-33.7)	N=8 9.7 (6.5-22.5)	0.4
Dry eye *Median* *Range*	N=23 11.1 (5.27-27.3)	N=7 9.81 (6.5-33.7)	0.4
Dry mouth *Median* *Range*	N=21 9.8 (6.5-33.7)	N=9 10.9 (5.3-27.3)	**0.03**
Raynaud *Median* *Range*	N=27 10.3 (5.3-27.3)	N=3 14.3 (12.1-33.7)	0.6
Lymphadenopathy *Median* *Range*	N=29 10.4 (5.3-15.2)	N=1 15.2 (15.2-15.2)	0.9

*Mann-Whitney test

Table 2: Serum CTHRC1 levels among RA patients in comparison to the control group

	RA group	Control group	P-value*
CTHRC1 level *Median* *Range*	10.67 (5.27-33.7)	7.3 5.22-9.81) (	**<0.001**

*Man-Whitney test

Table 3: Relation between CTHCR1 level and DAS-28 grades of RA group

Variable	Remission N=4	Mild activity N=2	Moderate activity N=9	High activity N=15	P*
CTHCR1: *Median* *Range*	8.7 (6.5-10.2)	9.5 (9.3-9.7)	10.5 (5.3-15.2)	16.7 (9.1-33.7)	**0.02**

*****Kruskal-Wallis test

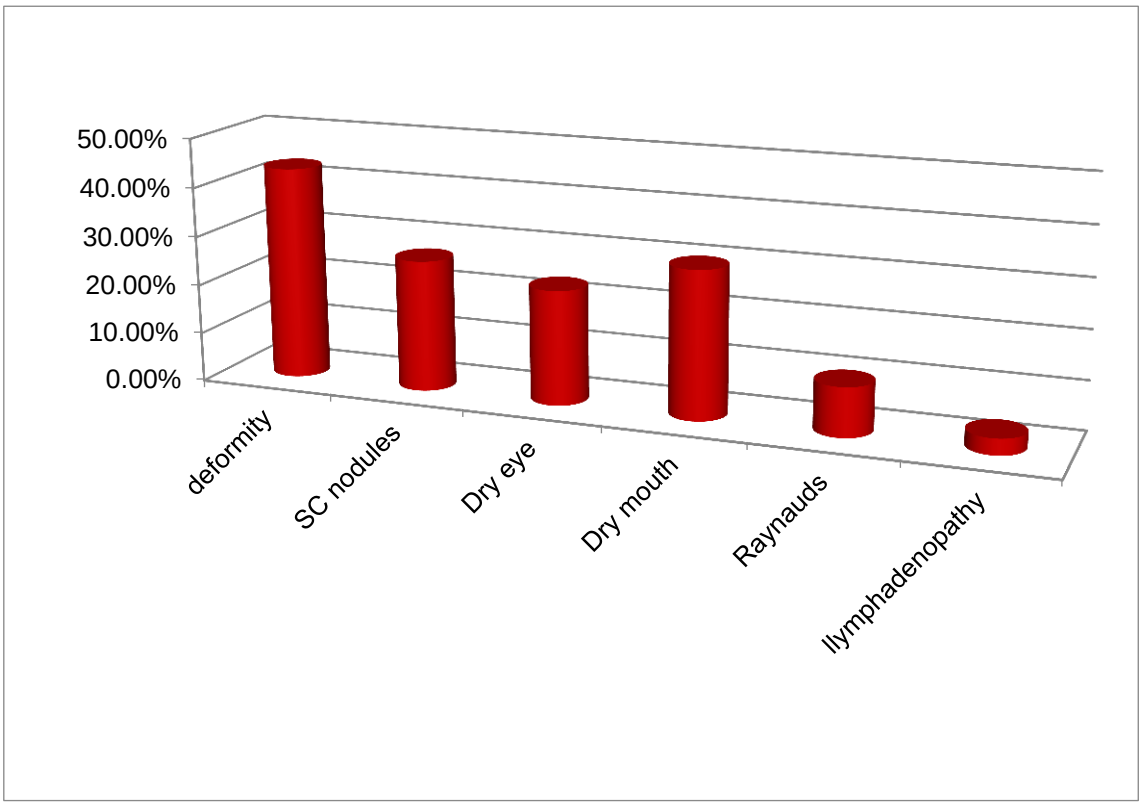

Figure 1: Clinical manifestations of rheumatoid arthritis patients

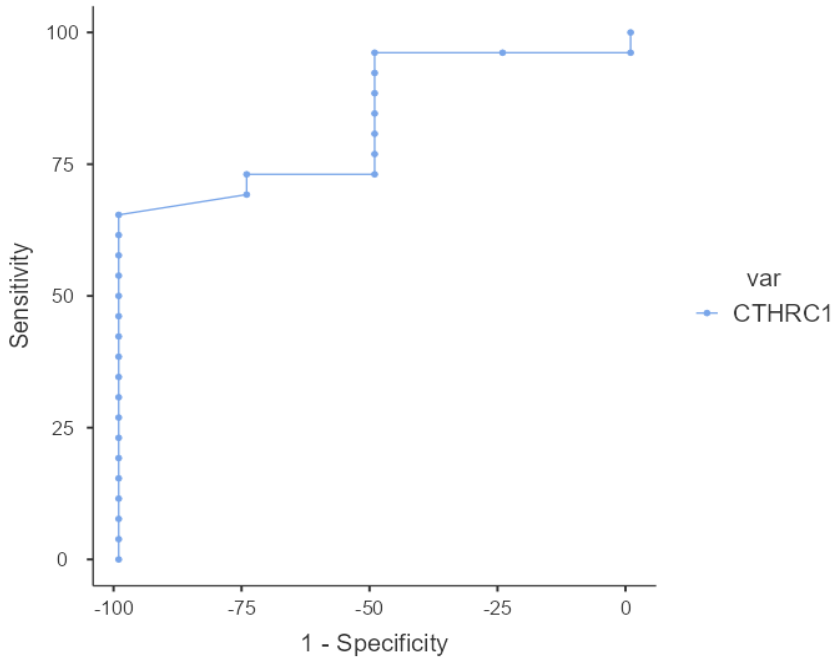

Figure 2: ROC analysis of CTHRC1 as a marker to detect disease activity in RA patients

DISCUSSION

Rheumatoid arthritis is a chronic disease involving the synovium of the joints and is associated with pannus proliferation and bone destruction which can lead to disability[7]. It is important to find new markers to monitor RA.

CTHRC1 can contribute to the remodeling of synovial tissue in RA by activation of the classic Wnt signaling cascade [7]. It stimulates cartilage

and bone erosion and production of pannus by activating FLS [5].

In our study, median of serum CTHRC1 in RA patients was higher than controls. This agrees with other studies [5,7,8,9,10,11,12,13,14].

Our study showed sensitivity 90%, specificity 96.67%, some studies showed inferior ability of CTHRC1 compared to our study for differentiation between RA patients and healthy controls as [7,8] with a sensitivity 62%, 84.5% and a specificity 86% ,75.6% respectively.

In the present study the increased activity level DAS28 was associated with elevated serum level of CTHRC1. These results were consistent with [5,8 ,9,11,12,13] but disagree with Oleiwi and zgair.,2023 who found no significant correlation between serum CTHRC1 and DAS28[14].

ROC curve analysis of CTHRC1 to discriminate active patients and in remission in RA with cut-off value of 10.3 ng/mL, sensitivity 65.4%, specificity 100%, PPV 100%, NPV 30.8% with area under the curve 83%.

The present study showed a marked relation between serum CTHRC1 levels and NTJ, NSJ and these results matched with [8,11,12] who found marked relation between serum CTHRC1 and NSJ but not with tender joints.

No significant correlation detected between serum CTHRC1 and laboratory findings among RA patients. These results matched with Alwan et al., 2021 who found that seropositivity for RF has no significant effect on CTHRC1. This agrees with a study that reported no correlation between serum CTHRC1, acute phase reactants, and inflammatory markers (10). But results disagree with Selim et al.,2022 who found that there was a marked correlation between serum CTHRC1 and ESR, CRP, RF and anti-CCP [9] and with Myngaby et al., 2019 who found that serum CTHRC1 had a positive correlation with RF, anti-CCP, CRP and also had strong correlation with IL-6, IL-1B , IL-8 and IFNɣ [8] , also disagree with Shekhani et al.,2016 and Ibrahim et al.,2023 [5, 11] respectively who found significant correlation between serum CTHRC1 and CRP and with Oleiwi and zgair.,2023 who showed that positive correlations were detected between serum CTHRC1 and CRP, ACPA , TNF-α, and IL-10 [14]. The discrepancy of results may be attributed to different sample size and disease activity status , also Rheumatoid factor is present in the sera of many inflammatory diseases and lacks specificity and acute phase reactants affected by many factors such as anemia and age

The study had limitations which include small sample size which may affect the possibility of CTHRC1 as a marker in RA.

In conclusion, serum CTHRC1 may serve as a valuable marker to evaluate RA patients and assess disease activity.

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