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Could SCUBE be used as a therapeutic target in rheumatoid arthritis?

Nurce Cilesizoglu Yavuz[a,*], Erhan Capkin[b], Asim Orem[c], Ahmet Mentese[c], Diler Us Altay[d]

[a] *Giresun University, Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Giresun, Türkiye*
[b] *Karadeniz Technical University, Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Division of Rheumatology, Trabzon, Türkiye*
[c] *Karadeniz Technical University, Faculty of Medicine, Department of Medical Biochemistry, Trabzon, Türkiye*
[d] *Ordu University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Ordu, Türkiye*

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Abstract

Aim: To investigate the levels of signal peptide-CUB-EGF domain-containing protein (SCUBE 1 and 3) in rheumatoid arthritis (RA).

Materials and Methods: Twenty eight RA patients and 28 healthy volunteers were included in the study. SCUBE-1, SCUBE-3, Vascular Endothelial Growth Factor (VEGF), Matrix Metalloproteinase-9 (MMP-9), CD-40L, Interleukin-6 (IL-6) levels, which are important markers in angiogenesis, were measured twice, at baseline and after the treatment.

Results: Compared to the healthy group, only MMP-9, one of the angiogenesis markers, was elevated in the patient group ($p>0.05$). After treatment, a significant decrease was observed in VEGF levels ($p<0.05$), while SCUBE-1, SCUBE-3, IL-6, CD-40 and MMP-9 levels remained at similar levels ($p>0.05$).

Conclusion: In our study, RA patients responded to treatment with clinical improvements. Although there were differences in the levels of MMP and VEGF, there was no association with the disease in SCUBE.

Introduction

Rheumatoid arthritis (RA) progresses with cartilage destruction characterized by persistent synovial inflammation and erosions in the subchondral bone. Synovial hyperplasia, angiogenesis, pannus formation, and destruction of cartilage and bone tissue are important in its pathology [1].

The synovial membrane becomes a hypertrophic hypervascular structure as a result of angiogenesis and the new structure is called pannus. Cytokines released from the synovial pannus tissue cause permanent damage in joints and peripherial tissues [2,3]. Signal peptide-CUB-EGF domain-containing protein (SCUBE) has been shown to be associated with molecules such as bone morphogenetic protein 2 [4], transforming growth factor [5] in angiogenesis.

It has been shown in a published study that the SCUBE protein family may be responsible in the pathogenesis of RA [6]. However, the post-treatment changes of these proteins have not been studied yet. The aim was to provide new data to the literature about the location of SCUBE-1 and SCUBE-3 proteins in RA patients and their relationship with the activity of the disease.

Materials and Methods

This prospective-controlled, three month-follow-up study included 28 patients who were diagnosed as RA according to the American College of Rheumatology (ACR) 2010 diagnostic criteria and 28 healthy subjects. Ethics committee of the study received approval number of 07.09.2015\5 by Karadeniz Technical University Clinical Research Ethics Committee. All subjects filled out a demographic data form, including age, height, weight, and educational status. Disease activity score (DAS28), visual analog scale (VAS), physician global evaluation, patient global evaluation, simplified disease activity index (SDAI), clinical disease activity index (CDAI) scores, number of swollen joints, number of sensitive joints, duration of morning stiffness and night pain of the patient group were evaluated at baseline and at 3 months post-treatment.

*Corresponding author:
Email address: nurcecilesizoglu@yahoo.com.tr (Nurce Cilesizoglu Yavuz)

Laboratory parameters

Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), hemoglobin (Hb), hematocrit (Htc), thrombocyte, leukocyte (WBC) levels of patients were studied. A special method was used for the measurement of SCUBE and other markers of angiogenesis. Blood samples taken from the brachial vein of the patients and control group individuals were transferred to vacutainer tubes without anticoagulant. The blood samples in the tube were kept at room temperature for approximately 10 to 15 minutes to allow coagulation and then they were centrifuged at 2000g for 10 minutes to obtain serum. Serum samples were stored at −80°C for analysis. SCUBE-1, SCUBE-3, VEGF, MMP-9, CD-40 ligand and IL-6 levels of the serum samples obtained were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits in our research laboratories in accordance with the manufacturer's suggestions. Absorbances of the samples given at a specific wavelength based on their color intensities were spectrophotometrically determined in a VERSA max tunable microplate reader (Molecular Devices, USA). The concentrations of samples were calculated according to the standard graph. The results were expressed in the concentration unit of the standards.

Statistical analysis

G*Power (V3.1) software (Informer Technologies, Inc., Los Angeles, USA) was used to calculate the required sample size. Using data from a previous study, the effect size in our sample size calculation was found to be 0.74 [7]. Based on a power of 80% and a 5% one tail significance, the total sample size required was calculated as 48. The results of the research were analyzed with the SPSS version 20.0 software. Descriptive statistics were shown as mean±standard deviation, median (minimum-maximum), frequency distribution, and percentage. Pearson chi-square test, Fisher's final test and McNemar test were used to examine categorical variables. Histogram and probability graphs and Shapiro-Wilk test were used to show the compliance of the variables with normal distribution. The Mann-Whitney U test was used for statistical significance between two independent groups for the variables that did not fit the normal distribution, and the Wilcoxon signed-rank test was used for statistical significance between two dependent groups. Student's t-test was performed between two independent groups for variables with normal distribution. $p<0.05$ expressed statistical significance.

Results

A total of 56 people participated in the study, 28 of which were in the RA and 28 in the health control group. After three-month follow-up, there was no loss of live in either group. RA patients and control group individuals had the same and similar age, sex, height, weight, BMI value, smoking status and presence of any chronic diseases and presence of hypertension and diabetes mellitus ($p>0.05$) (Table 1). Pre-treatment MMP-9 levels were detected to be statistically significantly higher in the RA group ($p<0.05$). On the other hand, pretreatment SCUBE-1, SCUBE-3, VEGF, CD-40 and IL-6 values between two groups were similar ($p>0.05$) (Table 2). It is an expected result that MMPs, which increase the destruction of extracellular matrix components, are increased in RA. There was a significant decrease in the clinical findings between before and after treatment in RA patients ($p<0.05$). ESR and CRP values, platelet count and VEGF values of the patients were significantly lower after treatment. WBC, Hb, SCUBE-1, SCUBE-3, IL-6, CD-40 and MMP-9 values were similar after treatment ($p>0.05$) (Table 3). Decreases in ESR, CPR and VEGF are important in showing suppression of inflammation and angiogenesis.

Table 1. Distribution of some descriptive properties among study groups.

	RA Patient	Control (n=28)	p
Age (years), $\overline{X}$±S	54.46±14.74	50.07±9.62	0.193[a]
Sex, n (%)			
Male	9 (32.1)	9 (32.1)	1.000
Female	19 (67.9)	19 (67.9)	
Height (cm), $\overline{X}$±S	161.46±8.68	165.39±9.04	0.103[a]
Body weight (kg), $\overline{X}$±S	72.93±14.83	78.14±13.18	0.170[a]
BMI (kg/m^2), $\overline{X}$±S	27.96±5.21	28.62±4.62	0.619[a]
Smoking Status, n (%)			
Non-smoker	19 (67.9)	16 (57.1)	0.408
Smoker	9 (32.1)	12 (42.9)	
Chronic Disease Status, n (%)			
None	14 (50.0)	20 (71.4)	0.101
Available	14 (50.0)	8 (28.6)	
Chronic Ilnesses (n=22)#, n (%)			
Hypertension	13 (92.9)	7 (87.5)	1.000[b]
Diabetus Mellitus	6 (42.9)	1 (12.5)	0.193[b]

%: Column Percentage; $\overline{X}$: Average; S: Standard deviation. [a]Student's T Test; [b]Fisher's Final Test; #One patient had more than one chronic disease, calculated on the percentage of patients.

Table 2. Distribution of pre-treatment SCUBE, VEGF, IL-6, CD-40 and MMP-9 values among study groups.

Before Treatment	RA Patient	Control (n=28)	p*
	Median (min-max)	Median (min-max)	
SCUBE-1	0.83 (0.55-7.94)	0.85 (0.57-1.87)	0.756
SCUBE-3	3.84 (0.23-13.22)	2.95 (0.16-15.06)	0.422
VEGF	720.6 (228.3-3156.2)	585.2 (173.8-1973.6)	0.112
IL-6	5.50 (1.30-47.45)	3.98 (0.97-25.32)	0.136
CD-40	4.64 (1.45-11.56)	4.04 (1.23-10.16)	0.725
MMP-9	88.46 (33.41-125.51)	32.52 (19.24-100.18)	**<0.001**

*Mann-Whitney U Test.

Discussion

In this study, we aimed to investigate the relationship between disease activity and angiogenesis markers in RA, significant improvements were detected in clinical and laboratory findings. The association of SCUBE proteins, a newly identified marker of angiogenesis, with disease ac-

Table 3. Distribution of pre- and post-treatment clinical and laboratory findings of RA patients.

(n=28)	Before Treatment	After Treatment	p*
	Median (min-max)	Median (min-max)	
Morning Stifness Time (min)	60 (0-180)	5 (0-120)	<0.001
Night Pains, n (%)			<0.001
None	3 (10.7)	21 (75.0)	<0.001
Present	25 (89.3)	7 (25.0)	
Number of Sensitive Joints	11 (0-28)	1 (0-14)	<0.001
Number of Swollen Joints	3 (0-9)	0 (0-6)	<0.001
DAS28	4.84 (2.16-7.07)	2.62 (0.68-4.24)	<0.001
SDAI	31.5 (4-50)	7 (0-37)	<0.001
CDAI	30 (4-49)	7 (0-37)	<0.001
VAS	10 (2-10)	2 (0-10)	<0.001
Patient Global Evaluation	10 (2-10)	2 (0-10)	<0.001
Physician Global Evaluation	7 (2-10)	2 (0-7)	<0.001
ESH	23 (3-98)	10 (2-55)	<0.001
CRP	1.28 (0-12.40)	0.30 (0-2.98)	<0.001
White Blood Cell	8.46 (4.7-13.8)	7.74 (4.76-15.70)	0.088
Hemoglobin	12.4 (9.2-15.3)	12.8 (8.7-15.3)	0.107
Thrombocyte	287 (181-484)	257 (183-417)	0.001
SCUBE-1	0.83 (0.55-7.94)	0.77 (0.54-13.10)	0.847
SCUBE-3	3.84 (0.23-13.22)	3.35 (0.47-18.58)	0.246
VEGF	720.6 (228.3-3156.2)	646.9 (145.6-2969.0)	<0.001
IL-6	5.50 (1.30-47.45)	2.86 (0.75-95.25)	0.246
CD-40	4.64 (1.45-11.56)	3.22 (1.16-10.77)	0.412
MMP-9	88.46 (33.41-125.51)	88.82 (41.59-120.12)	0.339

**Wilcoxon Signed-Rank Test.

tivity could not be demonstrated.

Angiogenesis causes continuous activation of immune cells in inflamed synovial tissue and persistence of inflammation [8]. There are studies showing the effectiveness of inhibition of angiogenesis in the treatment of RA. There are data that antirheumatic drugs used in the treatment of RA inhibit angiogenesis. In a study by Litinsky et al., it was shown that leflunomide significantly reduced serum MMP-1, MMP-3, IL-10, and IL-6 levels [9]. Strunk et al. showed that infliximab treatment, an anti-TNF agent, reduces serum VEGF levels in patients with active RA [10]. This finding is also supported by the decrease in VEGF values after treatment in RA patients in our study and showed that VEGF can be used as a parameter in the follow-up of treatment response. Based on these results, we aimed to research the possibility of SCUBE as a treatment target in RA, but we could not find any results to support this.

MMP enzymes are essential in the pathogenesis of RA. In a study investigating the relationship between MMP level and disease activity and treatment efficacy, it was shown that MMP-3 is useful as a disease activity marker in RA patients and that serial MMP-3 measurements may be useful in evaluating the effectiveness of methotrexate and infliximab treatment [11]. As a result of the study by Zhou et al., it was determined that MMP-9, which is expressed by synovites, plays an important role during angiogenesis in RA [12]. In our study, MMP-9 levels were significantly higher in RA patients, which is consistent with the literature, but the fact that no significant decrease was found in the MMP-9 levels of the patients after treatment led to the conclusion that its use in the follow-up of treatment was not appropriate. Previous studies support the strong association of the CD40 gene in the development of RA [13,14]. In the study of Chakrabarti et al., a new pathway was revealed that VEGF-CD40L interaction can regulate angiogenic and inflammatory processes depending on environmental characteristics [15]. Cho et al. showed that the CD40/CD40L interaction may be directly involved in neovascularization in rheumatoid synovitis by increasing VEGF production [16]. In our study, no significant difference was observed in CD-40 levels when compared with the control group and evaluated after treatment.

In a meta-analysis examining the relationship between RA and IL-6 and TNF-α, IL-6 and TNF-α levels were detected to be high in patients with RA [17].In our study, in which IL-6 values were the same between RA patients and the control group and no change was observed in IL-6 levels after treatment, the fact that the patients were receiving antirheumatic treatment at the beginning may have caused this result.

SCUBE is a newly identified, secreted cell surface protein that is determined during early embryogenesis and is released in conditions such as inflammation and hypoxia. The amount and function of SCUBE may change in inflammation-related diseases such as RA and cancer [6]. In the study of Bilir et al., SCUBE 1 and CD40L levels were found to be statistically higher in patients with

Hashimoto's thyroiditis compared to healthy individuals [18]. Turkmen et al. had found a statistically significant increase with a high correlation between ischemia time and the increase in SCUBE levels and suggested that SCUBE 1 may be a biomarker in the early diagnosis of acute ischemic stroke [19]. In another study, it was suggested that SCUBE 1 levels could be used in the early diagnosis of acute mesenteric ischemia and as a damage biomarker [20]. In addition to a possible role of SCUBE in ischemia and inflammation, its relationship with angiogenesis has led to the opinion that it may be a target biomarker in rheumatic diseases [6]. In our study, SCUBE-1, SCUBE-3 protein levels were found to be parallel between two groups, and no significant decrease was observed in these levels after treatment. This may be due to the progression of angiogenesis and inflammation through different pathways in RA. This result could not demonstrate the benefit of SCUBE proteins as a target molecule in the follow-up and treatment of RA.

Limitations

Since the number of patients was limited to 28 patients, no significant difference could be found in the levels of SCUBE-1 and SCUBE-3 proteins. Additionally, patients could not be grouped according to the treatments they received. The short follow-up period of 3 months may have caused no remarkable difference in the levels of SCUBE-1 and SCUBE-3 proteins after treatment. Therefore, randomized controlled studies with large sample size and longer follow-up are required.

Conclusion

Our study is a study that supports that MMP-9 and VEGF are important markers in angiogenesis in RA. However, the relationship of SCUBE proteins, which are newly defined angiogenesis proteins, with disease activity and treatment could not be demonstrated. Further studies on this subject with larger numbers of patients are needed.

Ethical approval

The study was approved by the Research and Ethics Committee of Clinical Studies linked to Karadeniz Technical University, under the 07.09.2015 \5 approval number.

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