Thiol/disulphide homeostasis in patients with rheumatoid arthritis: a potential link with disease activity and preclinical atherosclerosis

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ABSTRACT

Introduction/objectives: Thiols are crucial anti-oxidant agents that contain a sulfhydryl group; they play an important role in defence against reactive oxygen species. We aimed to determine the thiol/disulphide homeostasis in rheumatoid arthritis (RA) patients in conjunction with its association with disease activity, preclinical atherosclerosis, and other disease-related indices.

Methods: We enrolled 64 RA patients without known cardiovascular (CV) disease or risk factors and 46 healthy controls. Disease activity was evaluated using the Disease Activity Score 28-erythrocyte sedimentation rate (DAS28-ESR). Thiol/disulphide homeostasis was evaluated using a novel automated method, and serum native thiol (NT), total thiol (TT), and disulphide(SS) levels were recorded. The carotid intima media thickness (CIMT) was measured using carotid ultrasound to evaluate preclinical atherosclerosis.

Results: The NT and TT levels were significantly lower in RA patients than in controls (231.7 ± 52.3 vs. 293.6 ± 74.8 µmol/L, $p < 0.001$; 271.6 ± 52.1 vs. 331.3 ± 68.2 µmol/L, $p < 0.001$, respectively). There was no difference in SS levels between both groups. The CIMT was significantly higher in RA patients than in controls (0.80 vs. 0.56 mm, $p < 0.001$). NT levels showed a significant negative correlation withCIMT in patients with RA ($r = - 0.253$, $p = 0.040$). In RA patients, NT and TT levels were significantly correlated with ESR ($r = - 0.394$, $r = -0.399$), high-sensitivity C-reactive protein ($r = -0.413$, $r = - 0.342$), DAS28-ESR ($r = - 0.279$, $r = - 0.312$), fibrinogen level ($r = - 0.302$, $r = - 0.346$), and anti-cyclic citrullinated peptide titres ($r = - 0.305$, $r = 0.322$) (, respectively). The association of thiol levels with CIMT did not arrive at a statistically significant level in multivariable linear regression analysis.

Conclusions: RA patients without known CV disease or risk factors exhibited increased CIMT values and decreased thiol levels; moreover, thiol levels were found to be correlated with disease activity. Further studies are needed to detect the value of thiol/disulphide homeostasis for CV risk stratification and risk prediction in RA patients.

Keywords: Rheumatoid arthritis; Thiol/disulphide homeostasis; Disease activity; Preclinical atherosclerosis.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterised by joint inflammation that affects approximately 1% of the population[1]. Adaptive and innate immune cells, adhesion molecules and autoantibodies, and soluble mediators contribute to the development of inflammation and structural changes of joints and internal organs[2].

Hypoxic situations encourage the increase of oxidative stress and reactive oxygen species (ROS), which are recognised as significant proinflammatory mediators in RA. Inflammation and oxidative stress co-exist in the inflamed milieu and interact synergistically. When the inflammatory cells are stimulated, a number of ROS are released at the site of inflammation, leading to the exaggeration of oxidative damage. Oxidative stress products and a number of ROS also increase the proinflammatory response[3]. Additionally, oxidative stress in the vessel wall is recognised as an important factor for atherogenesis to occur[4]. RA patients are at a significantly increased risk for cardiovascular (CV) dis-

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ease, which is independent of traditional CV risk factors[5]. Chronic inflammation and a damaged immune system are considered to play a critical role in the development of accelerated atherosclerosis in RA patients[6].

Thiols are crucial anti-oxidant agents that contain a sulfhydryl group and play an important role in the defence against ROS[7]. Thiols can undergo oxidation via oxidants, which results in the formation of disulphide bonds. Disulphide bonds can revert to thiol groups. Thus, dynamic thiol/disulphide homeostasis is sustained[8]. Different measurement methods have been developed to determine plasma thiol/disulphide levels. Most of these techniques are time-consuming, labour-intensive, expensive, and require complicated techniques. Recently, a novel and automated assay for thiol/disulphide homeostasis has been developed by Erel and Neselioglu[9].

There is increasing evidence demonstrating the deterioration of thiol/disulphide homeostasis in various disorders including diabetes[10], neurodegenerative disease[11], chronic kidney disease[12], CV diseases[13-15], and inflammatory arthritis[16-19]. To the best of our knowledge, thiol/disulphide homeostasis has not been evaluated previously as a biomarker for CV disease in patients with inflammatory arthritis. Therefore, in the present study, we aimed to assess the thiol/disulphide homeostasis in RA patients without known CV disease or risk factors compared to healthy controls, and to determine the association of thiol/disulphide homeostasis with disease activity, carotid intima media thickness (CIMT; an indicator of preclinical atherosclerosis), and other disease-related parameters.

MATERIALS AND METHODS

This cross-sectional study was conducted at the outpatient clinic of the Physical Medicine and Rehabilitation Department, RecepTayyip Erdogan University, Faculty of Medicine, between September 2016 and October 2017. Sixty-four RA patients (age, 48.1±8.2 years; 52 women and 12 men) who met the 2010 American College of Rheumatology (ACR)/European League against Rheumatism (EULAR) classification criteria[20] for RA were included in the study. All patients were taking conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs) including methotrexate, sulfasalazine, leflunomide, and hydroxychloroquine, either as monotherapy or in combination, and 9 patients were treated with biologicals. Forty-six (age 46.3 ± 6.1 years; 35 women and 11 men) healthy subjects without known rheumatic disease were enrolled as controls. Healthy controls were selected from hospital staff members who volunteered to participate in the study. Subjects with any reported CV disease (myocardial infarction, heart failure, coronary angina, stroke, peripheral vascular disease), current smoking, infections, diabetes, hypertension, or any other autoimmune disease were excluded from the study. Subjects who were receiving antihypertensive and/or lipid-lowering drug therapies were also excluded from the study. The study was performed in accordance with the principles stated in the Declaration of Helsinki. The local Ethics Committee of our institution approved the study protocol (No: 44/2016), and written informed consent was obtained from all participants prior to the study.

CLINICAL ASSESSMENT

The clinical and demographic characteristics of all subjects were recorded. The 28-joint disease activity score-erythrocyte sedimentation rate (DAS28-ESR) was performed to assess disease activity in RA patients[21]. Based on the DAS28, patients were subdivided into three subgroups, with low (DAS28 ≤ 3.2), moderate (3.2 < DAS28 ≤ 5.1), and high (DAS28 > 5.1) disease activity.

A single experienced cardiologist who was blinded to the clinical data of the participants performed the vascular assessment. The blood pressures of all participants were obtained in the morning at the same visit as the participants' vascular assessment. An automated sphygmomanometer was used to measure the resting blood pressure in the seated position.

LABORATORY ANALYSIS

Glucose (glu), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels obtained from overnight fasting blood samples of all subjects were determined using standard methods.

Anti-cyclic citrullinated peptide (anti-CCP) antibody was measured with enzyme-linked immunosorbent assay using a commercial kit according to the manufacturer's instructions. A result was considered positive for anti-CCP antibodies if the titre was above 5 U/mL. Serum levels of rheumatoid factor (RF)-IgM were measured by the nephelometric method, and the result was considered positive for RF when its concen-

tration was above 20 IU/mL. ESR was recorded. Serum high-sensitivity C-reactive protein (hs-CRP) level was measured with an immune turbidimetric method using an Abbott auto-analyser (Architect C1600; Abbott, USA). The normal hs-CRP range was defined as ≤ 0.5 mg/dL.

Thiol/disulphide homeostasis was evaluated using a novel automated method described by Erel and Neselioglu[9]. Venous blood samples were collected in SST tubes (BD Vacutainer SST II Advance, USA) after 12h of overnight fasting. After centrifugation, the serum sample was obtained and stored at -80° until analysis. Serum levels of native thiol (NT) and total thiol (TT) were measured. Measurements were performed using a Cobas c501 chemical analyser (Roche Diagnostics, Manheim, Germany). NT content is subtracted from the TT content, and half of the obtained difference gives the disulphide (SS) amount. After the measurement of NT and TT, the ratios of SS to NT, SS to TT, and NT to TT were calculated.

ASSESSMENT OF CAROTID INTIMA MEDIA THICKNESS

The evaluation of the carotid intima media thickness (CIMT), a valuable indicator of preclinical atherosclerosis, was performed using a high-resolution ultrasonography scanner (VingMed Vivid 3; GE Medical Systems, Horten, Norway) with a 7-MHz linear-array transducer. Measurements were performed on the right and left carotid arteries with the subject in the supine position. The region, 1 cm proximal to the carotid bifurcation, was identified, and the CIMT of the far wall was evaluated as the distance between the lumen-intima interface and the media-adventitia interface. The CIMT measurements were obtained from four contiguous sites at 1-mm intervals on each carotid artery. The mean value of all eight measurements (in millimetres) was calculated for analysis. A CIMT equal to or higher than 1.3 mm in any measurement site was defined as a plaque.

STATISTICAL ANALYSIS

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 18.0, for Windows (SPSS, Chicago, IL, USA). Continuous variables are presented as mean ± standard deviation or median. The normality of the distribution for all variables was assessed using the Kolmogorov-Smirnov test. Inter-group comparisons were performed using Student's *t*-test for normally distributed variables and the Mann-Whitney *U* test for non-parametric variables. The subgroup analyses were performed using one-way analysis of variance in RA patients. To assess the correlation between variables, Spearman's rank or Pearson's correlation analysis was used according to the distribution of data. Multivariate linear regression analysis was performed to investigate the association between the parameters of thiol/disulphide homeostasis and CIMT. A p value of < 0.05 was considered statistically significant.

RESULTS

The demographic and clinical characteristics of RA patients and controls are shown in Table I. There were no significant differences in age, sex, or BMI between the groups ($p = 0.206$, $p= 0.675$, $p = 0.344$, respectively). The mean disease duration and DAS28-ESR were 5.8 ± 3.9 years and 3.7 ± 1.4 in RA patients. RF and anti-CCP positivity were observed in 64.1% and 57.8% of the patients with RA, respectively. Neither RA patients nor controls had carotid plaques.

The intergroup comparisons of thiol/disulphide homeostasis and other clinical parameters between RA patients and controls are shown in Table II. Systolic blood pressure (SBP), diastolic blood pressure (DBP), ESR, hs-CRP, TC, LDL-C, HDL-C, fibrinogen, and glu levels were significantly increased in patients with RA compared to those in controls ($p < 0.05$ for all). The median CIMT values were significantly higher in RA patients than in controls (0.80 vs. 0.56 mm, $p < 0.001$). While the mean NT and TT levels were significantly lower in RA patients than in controls (231.7 ± 52.3 vs. 293.6 ± 74.8 µmol/L, $p < 0.001$; 271.6 ± 52.1 vs. 331.3 ± 68.2 µmol/L, $p < 0.001$, respectively), there was no difference in the mean SS level between groups (19.6 ± 8.6 vs. 18.8 ± 9.3 µmol/L, $p = 0.672$) (Figure 1). Moreover, patients with RA showed significantly increased SS/NT and SS/TT, and decreased NT/TT ratios when compared tocontrols (8 vs. 6, $p = 0.007$; 7 vs. 5, $p = 0.006$; 86 vs. 89, $p = 0.010$, respectively).

According to the subgroup analysis of RA patients in terms of disease activity, there were no significant differences in thiol/disulphide homeostasis parameters among RA patients with low, moderate, and high disease activity (data not shown, all $p > 0.05$). SS level, SS/NT and SS/TT ratios were significantly higher in RA patients with RF positive than those in RF negative group (21.3 ± 8.4 vs. 16.4 ± 8.1 µmol/L, $p = 0.027$;

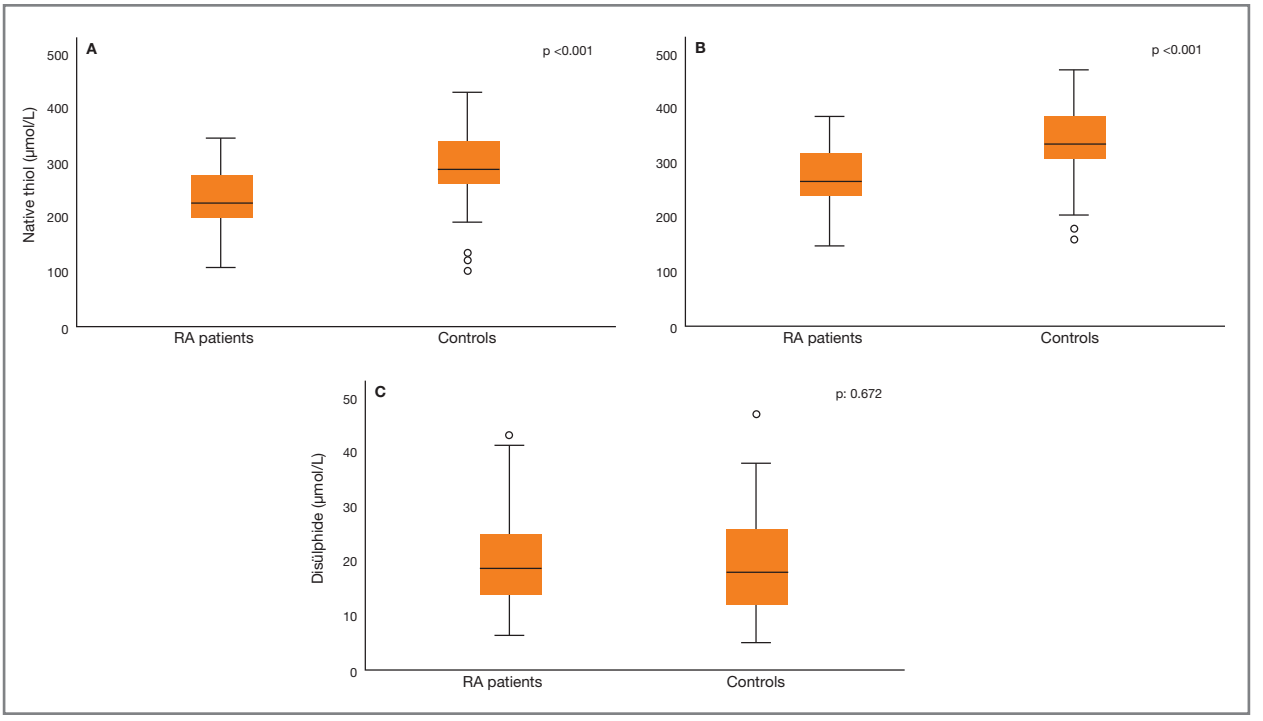

FIGURE 1. Native thiol, total thiol and disulphide levels in rheumatoid arthritis patients and control groups (A: Native thiol, B: Total thiol, C: Disulphide)

10.2 ± 5 vs. 7.6 ± 4.2, p = 0.039; 8.2 ± 3.3 vs. 6.4 ± 2.9, p = 0.037, respectively). No differences were observed between RA patients with RF positive and negative in terms of NT and TT levels (228.1 ± 47.1 vs. 238.1 ± 61.21 µmol/L, p = 0.470; 271.5 ± 44.1 vs. 271.7 ± 651 µmol/L, p = 0.987, respectively). However, the NT/TT ratio was significantly decreased in RF positive patients compared to in RF negative patients (83.6 ± 6.7 vs. 87.3 ± 5.8, p = 0.03, respectively). In addition, there were no significant differences in the thiol/disulphide homeostasis parameters between anti-CCP positive and negative RA patients (all p > 0.05, data not shown). Moreover, RA patients with csDMARD did not differ from RA patients with bDMARD in terms of thiol/disulphide homeostasis (data not shown, all p > 0.05).

Correlation analyses of thiol/disulphide homeostasis with disease-related parameters in RA patients are shown in Table III. NT levels showed a statistically significant negative correlation with age, ESR, hs-CRP, DAS28, fibrinogen level, CIMT, and anti-CCP titres (r = -0.554, p < 0.001; r = -0.394, p = 0.001; r = -0.413, p = 0.001; r = -0.279, p = 0.026; r = -0.302, p = 0.015; r = -0.253, p = 0.040; r = -0.305, p = 0.014, respectively). TT levels showed a statistically significant negative correlation with age, DAS28, hs-CRP, ESR, anti-CCP titres, and fibrinogen (r= -0.600, p < 0.001; r = -0.312, p = 0.012; r = -0.342, p = 0.006; r = -0.399, p = 0.001; r = -0.322, p = 0.009; r = -0.346, p = 0.005, respectively). SS levels showed a statistically significant positive correlation with TC, LDL-C, TG, hs-CRP, and RF (r = 0.373, p = 0.002; r = 0.267, p = 0.033; r = 0.385, p = 0.002, r = 0.255, p = 0.042; r = 0.248, p = 0.048, respectively) and negative correlation with HDL-C, disease duration (r = -0.321, p = 0.010, r = -0.119, p = 0.035), but not with ESR, DAS28, or CIMT (p > 0.05). There was no correlation between the lipid profile and TT or NT levels (all p > 0.05). None of the parameters of thiol/disulphide homeostasis were correlated with CIMT in multivariate linear regression analysis.

DISCUSSION

In the present study, RA patients without known CV disease or risk factors showed significantly lower NT and TT levels and higher CIMT values, SS/NT, and SS/TT ratios than those in controls. Additionally, thiol

TABLE I. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF PATIENTS WITH RHEUMATOID ARTHRITIS AND CONTROL SUBJECTS

	RA patients (n = 64)	Controls (n = 46)
Age (years), mean ± SD	48.1 ± 8.2	46.3 ± 6.1
Female/male, n (%)	52/12 (81.2/18.8)	35/11 (76.1/23.9)
BMI (kg/m2), mean ± SD	28.6 ± 2.9	27.6 ± 2.7
Disease duration (years)(range), mean ± SD	5.8 ± 3.9 (1-20)	–
RF positivity, n (%)	41 (64.1)	–
Anti-CCP, positivity (%)	37 (57.8)	–
DAS28, mean ± SD	3.7 ± 1.4	–
Low disease activity, n (%)	24 (37.5)	–
Moderate disease activity, n (%)	29 (45.3)	–
High disease activity, n (%)	11 (17.2)	–
Medication, n (%)		
MTX	20 (31.3)	–
MTX+HQ	11 (17.2)	–
MTX+SS	19 (29.7)	–
LEF	5 (7.8)	–
Biologic agents + MTX	9 (14.1)	–
Anti-TNF (ADA, INF)	3 (4.7)	–
ABA	1 (1.6)	–
RTX	5 (7.8)	–

RA: rheumatoid arthritis, BMI: BodyMass Index, RF: rheumatoid factor, anti-CCP: anti-cyclic citrullinated peptide, DAS-28: Disease Activity Index, MTX: methotrexate, HQ: hydroxychloroquine, SS: sulfasalazine, LEF: leflunomide, anti-TNF: anti-tumour necrosis factor, ADA: adalimumab, INF: infliximab, ABA: Abatacept, RTX: Rituximab, SD: Standard deviation

levels showed a statistically significant association with disease activity and fibrinogen levels in RA patients.

Increased oxidative stress could be defined as a condition where in the cellular anti-oxidant system is inefficient to neutralise ROS completely because of excessive ROS formation and/or anti-oxidative defence system depletion[22]. A common feature among autoimmune disorders is the excessive formation of reactive nitrogen species and ROS in response to inflammation. Although the production of ROS is a physiological defence mechanism in the case of microbial infection, the excessive production of ROS observed autoimmune inflammation may lead to tissue damage. The main cause of this damage is the chemical reaction of certain ROS with biomolecules, including lipids, proteins, carbohydrates, and DNA. Because of the chemical modification of proteins, the immune system cannot recognise the protein as self, which results in the occurrence of an immune response against the newly formed epitope. The chemical oxidative modification of self-antigens is determined to be the cause involved in the formation of neoepitopes and stimulation of the generation of autoantibodies in autoimmune disease[23].

The anti-oxidant defence system plays a critical role in the protection of the cellular system from the damaging effect of pro-oxidant[24]. Thiols are considered an important defence against ROS and form the majority of the total anti-oxidant pool in the body[7].

Several studies have consistently reported increased oxidative stress[25-29] as well as decreased anti-oxidative defence mechanisms[28,30,31] in patients with RA, employing various oxidative stress markers. Some of these studies also showed a positive correlation between disease activity and markers of oxidative stress in RA patients[26-29]. In the present study, the oxidative status of RA patients was investigated by measuring the parameters of thiol/disulphide homeostasis, which is defined as a novel, uncomplicated, practical, and fully automatic method. We observed that NT and TT levels (indicators of anti-oxidative defence) were significantly decreased in RA patients compared to controls. However, SS levels did not differ between the groups.

TABLE II THIOL/DISULPHIDE HOMEOSTASIS AND CARDIOVASCULAR PARAMETERS OF PATIENTS WITH RHEUMATOID ARTHRITIS AND CONTROLS

	Patients with RA (n = 64)	Controls (n = 46)	p value
NT (µmol/L), mean ± SD	231.7 ± 52.3	293.6 ±74.8	< 0.001
TT (µmol/L), mean ± SD	271.6 ± 52.1	331.3 ± 68.2	< 0.001
SS (µmol/L),mean ± SD	19.6 ± 8.6	18.8 ± 9.3	0.672
NT/TT (%), median (min-max)	86 (68-95)	89 (64-98)	0.010
SS/NT (%), median (min-max)	8 (3-23)	6 (1-28)	0.007
SS/TT (%), median (min-max)	7 (2-16)	5 (1-18)	0.006
SBP (mmHg), median (min-max)	123 (90-135)	115(90-130)	< 0.001
DBP (mmHg), median (min-max)	80 (50-90)	70 (50-80)	0.002
Glu(mg/dL), mean ± SD	94.4 ± 9.8	90.3 ± 10.1	0.024
TC (mg/dL), mean ± SD	213.8 ± 43.4	194.9 ± 33.4	0.015
TG (mg/dL), median (min-max)	108 (50-400)	100 (31-236)	0.328
HDL-C (mg/dL), mean ± SD	54.7 ± 13.8	47.4 ± 10.7	0.004
LDL-C (mg/dL), mean ± SD	131.5 ± 36.1	118.8 ± 28.1	0.040
ESR (mm/h), mean ± SD	27.8 ± 16.9	8.7 ± 4.7	< 0.001
hs-CRP (mg/dL), median (min-max)	0.74 (0.02-6.4)	0.24 (0.01-0.054)	< 0.001
CIMT (mm), median (min-max)	0.80 (0.5-1.1)	0.56 (0.40-0.75)	< 0.001
Fibrinogen (mg/dL), mean ± SD	420.3 ± 94.7	309.3 ± 65.1	< 0.001

NT: native thiol, TT: total thiol, SS: disulphide, SBP: systolic blood pressure, DBP: diastolic blood pressure, Glu: glucose, TC: cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, ESR: erythrocyte sedimentation rate, hs-CRP: high-sensitivity C-reactive protein, CIMT: carotid intima media thickness, SD: standard deviation

NT and TT levels showed a significantly negative correlation with age, ESR, hs-CRP, disease activity, and anti-CCP titres, but not with RF in the present study. SS levels showed a significantly positive association with RF and hs-CRP. In addition, RF-positive RA patients demonstrated increased SS levels, SS/NT, and SS/TT ratios when compared to RF-negative patients. Decreased NT and TT levels were also demonstrated in RA and juvenile idiopathic arthritis compared to controls by others previously[18,19]. In a study by Tuzcu et al., RA patients had increased SS levels compared to controls[18]. Although TT, NT, and SS levels were significantly associated with ESR and disease activity, there was no association between thiol/disulphide parameters and CRP values in that study. Giustarini et al. demonstrated a biochemical disturbance of plasma sulfhydryl/disulphide balance in patients with RA compared to controls[16]. The decreased TT and SS levels in patients with ankylosing spondylitis compared to healthy controls demonstrated in the study by Do ruet al[17]. However, there was no significant difference between the groups in terms of NT levels in that study. They also found a statistically significant association between NT and TT levels and disease activity in their study.

The importance of oxidative stress in the pathogenesis of atherosclerosis and the development of CV events has been extensively reported[32]. Oxidative impairment of the endothelium and oxidative modification of LDL are important processes for the onset and progression of atherogenesis. The endothelium is activated by oxidised LDL, resulting in the expression of adhesion molecules, which enhances the recruitment of T cells and monocytes to stimulate immune system response[33]. Patients with RA have an increased risk of CV disease and mortality. Inflammatory mediators, alterations in the composition and function of lipoproteins, post-translational modifications of peptides/proteins and subsequent immune responses, increased oxidative stress, and endothelial dysfunction are considered as significant mechanisms for the development of the CV disease in RA[34]. Because of the presence of a distinct risk profile from the general population, conventional clinical CV disease risk algorithms are not

TABLE III. CORRELATIONS OF THIOL/DISULPHIDE HOMEOSTASIS INDICES WITH VASCULAR AND DISEASE ACTIVITY PARAMETERS IN PATIENTS WITH RHEUMATOID ARTHRITIS

	NT (µmol/L)		TT (µmol/L)		SS (µmol/L)	
	r	p value	r	p value	r	p value
Age (years)	-0.554	< 0.001	-0.600	< 0.001	-0.123	0.334
Disease duration	-0.110	0.385	0.150	0.237	-0.119	0.035
DAS28-ESR	-0.279	0.026	-0.312	0.012	-0.095	0.456
ESR (mm/h)	-0.394	0.001	-0.399	0.001	-0.014	0.914
hs-CRP (mg/dL)	-0.413	0.001	-0.342	0.006	0.255	0.042
anti-CCP (U/mL)	-0.305	0.014	-0.322	0.009	0.018	0.888
RF (IU/mL)	-0.127	0.318	-0.078	0.541	0.248	0.048
CIMT (mm)	-0.253	0.040	-0.213	0.091	0.126	0.319
Fibrinogen (mg/dL)	-0.302	0.015	-0.346	0.005	-0.131	0.302
TC (mg/dL)	-0.160	0.205	-0.037	0.773	0.373	0.002
TG(mg/dL)	-0.192	0.128	-0.067	0.600	0.385	0.002
HDL-C (mg/dL)	0.055	0.605	-0.051	0.688	-0.321	0.010
LDL-C (mg/dL)	-0.161	0.203	-0.073	0.566	0.267	0.033
SBP (mmHg)	-0.042	0.743	-0.075	0.556	-0.052	0.686
DBP (mmHg)	-0.135	0.287	-0.107	0.399	0.080	0.528

NT: native thiol, TT: total thiol, SS: disulphide, DAS-28: Disease Activity Index-28, ESR: erythrocyte sedimentation rate, hs-CRP: high-sensitivity C-reactive protein, anti-CCP: anti-cyclic citrullinated peptide, RF: rheumatoid factor, CIMT: carotid intima media thickness, TC: total cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, SBP: systolic blood pressure, DBP: diastolic blood pressure

useful for the estimation of CV disease risk in the RA population. Therefore, carotid ultrasound, a non-invasive imaging modality, may represent a valuable technique for the evaluation of CV risk[35].

Nowadays, thiol/disulphide homeostasis has been defined as a novel oxidative stress marker in patients with CV disease[13]. To the best of our knowledge, this is the first study evaluating the association of thiol/disulphide homeostasis with preclinical atherosclerosis, fibrinogen levels, and lipid profiles in RA patients. In the present study, preclinical atherosclerosis was evaluated by measuring CIMT with carotid ultrasound. CIMT is a surrogate marker for atherosclerosis and a strong predictor of future CV events in the general population[36]. The results of a population-based study demonstrated that carotid ultrasound is a useful tool for CV risk stratification in RA patients[37]. Similar to the previously reported results[38,39], significantly increased CIMT values were demonstrated in RA patients compared tocontrols in the present study. However, none of the RA patients had carotid plaques. The exclusion of subjects with known CV disease or risk factors from the study might explain this result. However, it should be noted that, in the present study, plaque data were obtained only from the far wall of the common carotid artery, and we did not evaluate CIMT at the bulb and internal carotid artery. Atherosclerosis tends to form at the carotid bulb. It has been reported that measurements of intima media thickness at the carotid bulb and at the internal carotid artery are more useful than the common carotid artery, both for risk classification and risk prediction[40]. RA patients also had significantly increased fibrinogen and hs-CRP values, which are important indicators of inflammation, in the present study. Although patients with CV disease were excluded from the study, we observed a significant but weak negative correlation between NT levels and CIMT. However, we did not find any association between CIMT and NT levels in linear regression analysis. NT and TT levels were also found to be negatively correlated with hs-CRP and fibrinogen values in RA patients. CRP is considered a valuable marker and mediator for CV disease. Mechanisms concerning the role of CRP in atherogenesis include activation of lipid uptake by macrophages, complement pathway, release of proinflammatory cytokines, induction of the expression of tissue factors in monocytes, promotion of endothelial dysfunction, and inhibition of nitric oxide production[41]. It has been demonstrated

that plasma fibrinogen levels are elevated in well-controlled RA[42]. Tabakçı et al. demonstrated that plasma fibrinogen was independently associated with coronary severity and complexity in patients with coronary artery disease in their study[43]. Kotur-Stevuljevic *et al.* found that plasma levels of oxidative stress parameters were positively associated with fibrinogen and CRP values in patients with coronary artery disease[44]. In the present study, SS levels and SS/TT and SS/NT ratios showed significant positive correlation with TG, TC, and LDL-C and negatively correlated with HDL levels. It has been reported that oxidised LDL is an independent predictor of subclinical and clinical atherosclerosis[22]. Kundi et al. demonstrated that NT, TT, and SS levels were lower in acute myocardial infarction patients than in healthy controls[13]. Similar to our results, NT and TT levels showed a significant negative correlation with age in that study. In another study, decreased NT levels, NT/SS, and TT/SS ratios were also demonstrated in patients with isolated coronary artery ectasia[14].

Previous studies have demonstrated significantly reduced oxidative stress markers following administration of anti-tumour necrosis factor therapy, tocilizumab, and methotrexate[45]. We did not observe any differences in thiol/disulphide homeostasis between patients with biological and csDMARDs. However, only 9 patients treated with biological DMARDs were included in the present study. Therefore, analysis of data from a large sample of patients treated with biological agents is needed to obtain more accurate conclusive results in this issue.

Our study has some limitations. The sample size of the study population was relatively small. However, most patients with CV disease or risk factors were excluded from the study. In addition, we could not use an automated edge-tracking software program, which obviates the need to perform manual measurement and improves the reproducibility of CIMT measurement[40]. Additionally, most of the patients were on DMARDs and these treatment regimens might affect the result of oxidative, anti-oxidative status, and other disease-related parameters. Because of the cross-sectional study design, we could not analyse the effect of DMARDs on oxidative status before and after treatment. Finally, we could not evaluate the thiol/disulphide homeostasis in the inflamed tissue sample, which could be explained more accurately the value of these parameters in the pathogenesis of arthritis.

In conclusion, the results of this study demonstrated increased CIMT and decreased thiol levels in RA patients without known CV disease or risk factors compared to controls. Thiol levels were correlated with disease activity and CIMT, but the association of the thiol levels with CIMT did not reach a significant level in multivariable linear regression analysis. However, it is required the long-term follow up studies with larger sample size to clarify the value of thiol/disulphide homeostasis in CV risk prediction in RA population.

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