

## Original Article

# Clinical significance of serum total oxidant/antioxidant status for the disease activity in active rheumatoid arthritis

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**Abstract:** *Background:* Oxidative stress has been considered as an important pathophysiologic factor involved in development of rheumatoid arthritis (RA). The purpose of this study was to evaluate total serum oxidant/antioxidant levels in patients with active RA and analyze its significance in disease activity. *Methods:* A total of 112 patients with active RA and 52 healthy controls were enrolled. Serum total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) were measured, and baseline characteristics were recorded. The DAS-28 scores were calculated as indicator of disease. Univariate analysis and multivariate logistic regression and receiver operating characteristic curve analyses were used to evaluate significance of TOS, TAS and OSI for indicator of disease of RA. *Results:* Both mean TOS ( $7.54 \pm 3.21$  VS  $5.73 \pm 2.81$ ,  $P < 0.05$ ) and OSI ( $0.37 \pm 0.21$  VS  $0.23 \pm 0.16$ ,  $P < 0.05$ ) of active RA patients were significantly higher than that of the controls whereas mean TAS of RA patients were remarkably lower than that of the controls ( $2.18 \pm 0.98$  VS  $2.52 \pm 0.81$ ,  $P = 0.031$ ). In univariate analysis, OSI, TOS, ESR, CRP, CCP and RF were significantly correlated to the disease activity of RA. Patients with high disease activity ( $\text{DAS-28} > 5.1$ ) had significantly higher mean TOS ( $8.32 \pm 3.81$  VS  $5.88 \pm 3.12$ ,  $P < 0.05$ ) and OSI ( $0.39 \pm 0.18$  VS  $0.25 \pm 0.11$ ,  $P < 0.05$ ) than that of RA with low-moderate disease activity. OSI also was confirmed as an independent predictive indicator for disease activity of RA in multivariate analysis. *Conclusions:* Serum OSI level represents a useful indicator for disease activity in active RA patients, thereby helping the clinician to plan more appropriate therapeutic strategies.

**Keywords:** Rheumatoid arthritis, disease activity, serum total oxidant status, total antioxidant status, oxidative stress index

## Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory and autoimmune disease characterized by infiltration of inflammatory cells into the synovium, synovial hyperplasia and finally irreversible destruction of bone and cartilage [1, 2]. The prevalence of the RA is around 0.2-0.4% of population in China, which brings a considerable burden on patients, their families, and society [3, 4]. Stratification of the patients with different risk and early intensive treatment is important for improving clinical efficacy of RA [5].

Oxidative stress and severe systemic inflammation has been considered as important pathophysiologic mechanisms involved in develop-

ment of RA [6, 7]. Oxidative stress occurs in response to the oxidative damage resulting from imbalance between antioxidant and scavenging ability and the active oxidants produced by a harmful stimulant [7, 8]. Reactive oxygen species (ROS), as the main active oxides, and accounts for more than 95% of total oxides. ROS has the potential to damage lipids, proteins and DNA in joint tissues [9, 10]. Previous reports showed that significant elevated ROS production in serum of RA patients comparing with healthy donors [11]. ROS are indirectly involved in joint damage as the secondary messengers in inflammatory and immunological cellular response in RA, which also can degrade directly the joint cartilage, influencing its proteoglycan and inhibiting its synthesis [9, 12]. Finding indicator inflecting oxidative stress sta-

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tus may be important for severity and disease activity evaluation in RA patients.

Therefore, the total oxidant status (TOS) is usually used to evaluate the overall oxidation state of the body while the total antioxidant status (TAS) is used to measure the overall antioxidant status [13, 14]. Furthermore, the oxidative stress index (OSI), which is calculated as the ratio of TOS to TAS, is considered as a more precise indicator of oxidative stress in the body because it can reflect imbalance between oxidation and antioxidant through comprehensive measurement TAS and TOS [15]. However, there was no data regarding the evaluation of TOS, TAS and OSI in patients with RA. In this study, we try to evaluate serum total oxidant/antioxidant status in RA patients through TOS, TAS and OSI measurement, and analyze the clinical significance of such indicator in RA.

### Materials and methods

#### *Patients*

This prospective study enrolled 112 consecutive patients with active RA admitted to Department of Orthopedics and Traumatology in the Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine from June 1, 2014, to June 1, 2016. The diagnosis of RA was made by according to the criteria proposed by the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) [16]. Patients with severe comorbidity, such as other ischemic diseases, severe cardiac or neurological disorders, malignant disease, chronic inflammatory disease, acute and chronic infective disease, preexisting organ failure, chronic obstructive airways disease and immunosuppressive disorders, were excluded from the study. Fifty-two healthy volunteers were enrolled as healthy controls. Written informed consent was obtained from individual patients and healthy subjects. This study was approved by the Ethics Committee of the Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine.

#### *Clinical assessment*

Demographic characteristics of all patients was recorded by one physician and furtherly check by another physician, such as age, gen-

der, the duration of disease, medication and laboratory data including C reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) and cyclic citrullinated peptide (CCP). The Disease Activity Score based on 28 Joints (DAS-28) were obtained to determine the activity of RA [17]. All patients received appropriate medical therapy and were followed through regular outpatient visiting. Outcome was assessed as disease activity representing through DAS-28 score. In details, the DAS-28 score >5.1 defined high disease activity whereas DAS-28 score 2.6-5.1 defined Low-moderate disease activity [18].

#### *Measurement of TOS, TAS and OSI*

The TOS and TAS level were measured according to Erel's method [13, 14]. Approximately 5ml peripheral venous blood samples of all patients and controls were obtained and stored in a blood collection tube (BD Vacutainer). After centrifugation, the serums were decanted and measured immediately by a commercially available test kits (Rel Assay Diagnostics kit; Mega Tip, Gaziantep, Turkey) according to the manufacturer's instructions and using their reagents and equipment. The results of TAS are expressed as mmol Trolox Eq/L while the result of the TOS are expressed as  $\mu\text{mol H}_2\text{O}_2$  Eq/L. Oxidative Stress Index (OSI) values were calculated as the ratio of the TOS level to TAS level. Specifically, OSI (arbitrary unit) =  $\text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ Eq/L}) / \text{TAS } (\mu\text{mol Trolox Eq/L}) \times 100$ .

#### *Statistical analysis*

Analyses were performed with SPSS 20.0 (IBM, USA).  $P < 0.05$  (two sided) was considered statistically significant. Data for categorical variables are expressed as a percentage and continuous variables as mean  $\pm$  SD. The  $\chi^2$  test or Fisher's exact test was used to compare categorical variables while continuous variables were analyzed by independent student's t test. The odds ratio (OR) was determined using multivariate logistic regression analysis for variables with significant  $p$  values ( $P < 0.05$ ) on univariate analysis.

### Results

#### *Patient characteristics*

A total of 112 RA patients and 52 healthy controls were enrolled in this study. There were 14

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**Table 1.** Baseline clinicpathologic characteristics of the RA patients and the controls

Characteristics	RA patients (n=112)	Controls (n=52)	P
Male	14 (12.5%)	4 (7.7%)	0.359
Age (y)	55.41±12.62	52.31±11.41	0.133
ESR (mm/h)	32.52±17.61	15.74±11.22	<0.001
CRP (mg/L)	33.51±25.23	3.43±2.12	<0.001
CCP (RU/mL)	341.21±127.4	14.20±9.34	<0.001
RF-IgM (IU/mL)	153.63±98.2	10.33±7.11	<0.001
RF-IgG (IU/mL)	136.7±88.62	9.2±3.31	<0.001
RF-IgA (IU/mL)	134.8±89.81	12.4±8.42	<0.001
TOS (mmol Trolox Eq/L)	7.54±3.21	5.73±2.81	<0.001
TAS (μmol H <sub>2</sub> O <sub>2</sub> Eq/L)	2.18±0.98	2.52±0.81	0.031
OSI	0.37±0.21	0.23±0.16	<0.001

RA, rheumatoid arthritis; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; CCP, cyclic citrullinated peptide; RA, rheumatoid factor; TOS, total oxidant status; TAS, total antioxidant status; OSI, oxidative stress index.

**Table 2.** Univariate analysis of risk factors associated with disease activity in RA patients

Characteristics	Low to moderate activity (2.6<DAS-28<5.1, n=39)	High activity (DAS-28>5.1, n=73)	P
ESR (mm/h)	21.32±8.71	77.81±36.45	<0.001
CRP (mg/L)	22.66±5.81	62.62±15.73	<0.001
CCP (RU/mL)	238.71±186.15	589.21±163.21	<0.001
RF-IgM (IU/mL)	118.43±36.27	201.17±132.32	<0.001
RF-IgG (IU/mL)	125.23±87.66	163.18±127.22	<0.001
RF-IgA (IU/mL)	98.61±15.26	160.86±114.15	<0.001
TOS	5.88±3.12	8.32±3.81	0.001
TAS	2.12±0.87	2.24±0.95	0.514
OSI	0.25±0.11	0.39±0.18	<0.001

RA, rheumatoid arthritis; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; CCP, cyclic citrullinated peptide; RF, rheumatoid factor; TOS, total oxidant status; TAS, total antioxidant status; OSI, oxidative stress index.

males and 98 females in RA group while female subjects account for 92.3% (48/52) of controls. The mean age of the patients with RA was 55.41±12.62 years whereas for the controls was 52.31±11.41 years. The demographic characteristics were matched between the RA patients and the controls (P>0.05, **Table 1**). Mean ESR, CRP, CCP and RF were remarkably higher in RA patients compared to control subjects (all P<0.05, **Table 1**). Moreover, both mean TOS and OSI were also significantly higher in RA patients (P<0.05, **Table 1**). However,

mean TAS of RA patients were remarkably lower than that of the controls (2.18±0.98 VS 2.52±0.81, P=0.031, **Table 1**).

### *Clinical significance of TOS, TAS and OSI for disease activity in RA patients*

All 112 patients were divided into two groups according DAS-28 score, in which, 39 patients (2.6<DAS-28<5.1) were consider as low to moderate disease activity group while 73 patients (DAS-28>5.1) were enrolled as high disease activity group. In univariate analysis, patient with high disease activity has significant higher mean ESR, CRP, CCP and RF than patients with low to moderate disease activity (All P<0.05, **Table 2**). Similarly, both mean TOS (8.32±3.81 VS 5.88±3.12, P<0.05) and OSI (0.39±0.18 VS 0.25 ±0.11, P<0.05) were remarkably higher in RA patients with high disease activity, comparing with low to moderate disease activity (P<0.05, **Table 2**). However, there was no significant different in mean TAS between low to moderate disease activity group and high disease activity group (2.12±0.87 VS 2.24±0.95, P=0.514) (**Table 2**).

A multivariate logistic regression analysis enrolled serum ESR, CRP, CCP, RF, TOS and OSI level to identify independent predictive factors for disease activity of RA. The result showed that serum OSI level (OR, 2.201; 95% CI, 0.712-6.835; P<0.05) and CRP level (OR, 3.872; 95% CI, 1.112-12.087; P<0.05) and CCP level (OR, 1.362; 95% CI, 0.981-2.514; P<0.05) and RF-IgA level (OR, 0.488; 95% CI, 0.172-1.231; P<0.05) were the independent predictive factors (**Table 3**).

### **Discussion**

In this study, we prospectively evaluate the serum total oxidant/antioxidant status in patients with active RA though TOS, TAS and OSI measurement, and found that RA patients had a significant higher TOS and OSI level than healthy controls. Furthermore, high TOS and OSI level was significantly associated with high

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**Table 3.** Multivariate analysis of risk factors associated with disease activity in RA patients

Characteristics	OR	95% CI	P
CRP	3.872	1.112-12.087	0.031
CCP	1.362	0.981-2.514	0.043
RF-IgA	0.488	0.172-1.231	0.033
OSI	2.201	0.712-6.835	<0.001

RA, rheumatoid arthritis; CRP, C-reactive protein; CCP, cyclic citrullinated peptide; RF, rheumatoid factor; OSI, oxidative stress index.

disease activity in the univariate analysis and multivariate logistic regression analysis. Therefore, we confirmed that the serum TOS and OSI level can be examined for optimal disease activity evaluation of individual patients with active RA, which can serve as indicators for assessing disease activity of patients with RA.

Oxidative stress reactive has been implicated to play an important role in this process of RA [19, 20]. Free radicals resulted from oxidative stress are indirectly implicated in joint damage as secondary messengers in inflammatory and immunological cellular response in RA, which also can degrade directly the joint cartilage, attacking its proteoglycan and inhibiting its synthesis [12, 21]. Furthermore, it has been suggested that antioxidants systems are impaired in RA [22, 23]. In our study, interestingly, serum TOS level of RA patients were higher than that of control while TAS lever of RA was lower than controls, furtherly confirm that overproduction of oxidants and impaired antioxidants in RA. We also evaluated OSI which is expressed as the percent ratio of total TOS to TAS, and found mean OSI of RA patients also was higher than that of controls, suggesting imbalance status between antioxidant and active oxidants in RA.

ESR and CRP are the widely recognized and used biomarkers for measuring acute phase response due to their reliability, reproducibility and cost effectiveness, which also correlate closely with clinical disease activity of RA [24-26]. Our results are in accordance with these previous conclusions. The TOS, TAS and OSI, as useful biomarkers representing oxidation and antioxidant status, has been evaluated in several conditions, such as alopecia areata, chronic periodontitis, cancer [27, 28]. In present study, we evaluated the relationship of

TOS, TAS and OSI and the disease activity in patients with active RA, and found RA patients with high activity had higher TOS and OSI than low activity RA patients, suggesting severity of oxidative stress is associated with disease activity of RA. Oxidative stress can directly damage the joint cartilage, attacking its proteoglycan and inhibiting its synthesis, while DAS-28 score mainly is obtained from multi-joints status evaluation. So, Oxidative stress may be closely related to DAS-28 score, which was revealed in our study. Finally, we confirmed OSI can serve as a useful indicator evaluating disease activity of RA in univariate and multivariate analysis comparing with other predictors including CRP, CCP and RF-IgA. High OSI may predicts higher disease activity in patients with RA, so physicians should be aware of the patients with high OSI and provide early and proactive intervention to decrease OSI level. However, OSI level is not a direct contributor to disease activity, but a marker of imbalance between oxidative stress and antioxidant status. Targeting the original abnormal process might be an appropriate strategy to restore balance status between oxidative stress and antioxidant status [6].

Several limitations may influence the interpretation of the results of this study. One limitation is limited subjects. A large-scale, multi-center, prospective study should be conducted to confirm results and obtain more definite evidence. Furthermore, we measured TOS, TAS and OSI according to Erel's method, which cannot precisely reflect true level in a sample [29]. At this point, the OSI can better reflect the oxidative stress status of a subject. Thus, the results of this study may not be comparable with those of other studies. Various furtherly validation studies may be required to confirm more definite significance of OSI in RA patients.

In conclusion, the serum TAS, TOS and OSI level is increased in patients with active RA. As a biomarker of oxidative stress and antioxidants, serum OSI level may be a useful independent predictor of disease activity for patients RA.

### Disclosure of conflict of interest

None.

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