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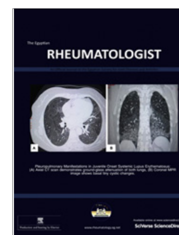

ORIGINAL ARTICLE

Assessment of lipid peroxidation and antioxidant status in rheumatoid arthritis and osteoarthritis patients

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KEYWORDS

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Abstract *Aim of the work:* The aim of the present study was to assess the lipid peroxidation (LPO) and antioxidant status of patients with rheumatoid arthritis compared with osteoarthritis.

Patients and methods: This study included 30 RA, 30 OA patients and 15 healthy subjects. Parameters of activity of RA patients and clinical parameters of OA patients were assessed. Erythrocyte sedimentation rate (ESR), C reactive protein (CRP), serum malondialdehyde (MDA), the activities of erythrocyte superoxide dismutase (SOD) and catalase (CAT), glutathione (GSH) level, plasma glutathione-S-transferase (GST) activity and ceruloplasmin (Cp) level were measured.

Results: Increased MDA, plasma GST activity and Cp levels with reduction of the activities of SOD, CAT and GSH levels were demonstrated in RA and OA patients. A positive correlation was detected between the clinical and laboratory parameters in both RA and OA patients with GST and Cp. A direct correlation was found between previous parameters and serum MDA in RA. Meanwhile, a negative correlation was observed between these parameters with erythrocyte SOD, CAT activities and GSH level. Direct correlation also existed between MDA and GST with

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Cp, between erythrocyte SOD with CAT activities and negative correlations of GST activity with GSH level.

Conclusion: Increased oxidative stress in RA and OA patients have led to compensatory changes in the levels of antioxidants. These changes provide additional protection against LPO. These findings confirm the role of oxidative stress in the pathogenesis of RA and OA, and that LPO markers and antioxidants can serve as surrogate markers for disease activity

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1. Introduction

Reactive oxygen species (ROS) including superoxide, hydrogen peroxide, and hydroxyl radicals, and their reactive products were classically described as harmful products of aerobic metabolism which could be capable of causing DNA mutations, lipid peroxidation (LPO) and protein oxidation [1]. Among ROS, the superoxide anion (O_2^-) plays a pivotal role in inflammation, particularly in patients with inflammatory joint disease [2]. The enzyme superoxide dismutase (SOD) neutralizes O_2^- by transforming it into hydrogen peroxide (H_2O_2) thereby preventing the formation of highly aggressive compounds such as peroxynitrite ($ONOO^-$) and hydroxyl radical (HO) [3].

Overproduction of ROS results in oxidative stress, a deleterious process that can be an important mediator of damage to cell structures, including lipids and membranes, proteins, and DNA [4]. Prime targets of ROS attack are the polyunsaturated fatty acids in the membrane lipids causing LPO which may lead to disorganization of cell structure and function. Further decomposition of peroxidized lipids yields a wide variety of end-products, including malondialdehyde (MDA) [5]. Measurement of MDA is widely used as an indicator of LPO [6]. Elevated levels of MDA have been reported in the serum and synovial fluid of RA patients [7].

Multiple defense systems against free radical-induced oxidative stress, collectively called antioxidants are present in human serum, plasma as well as erythrocytes. Most of the antioxidant ability of serum has been attributed to the presence of ascorbate, transferrin and ceruloplasmin (CP). Erythrocytes are excellently equipped to handle intracellular oxidative stress through the combined activities of catalase (Cat), superoxide dismutases (SODs), glutathione peroxidase (GPx), glutathione S-transferase (GST) and glutathione (GSH) [4,8].

Osteoarthritis (OA) is one of the most common chronic diseases that cause pain and physical disability in patients. Although OA is considered as a global disease affecting all joint tissues, cartilage degradation is the end point. The degradation of cartilage results from the combination of mechanical stress and biochemical factors, mainly metalloproteinases and ROS. The activity of ROS is balanced by enzymatic and non-enzymatic antioxidants, that act by inhibiting oxidative enzymes, scavenging free radicals or chelating ion metals [9].

Rheumatoid arthritis (RA) is a heterogeneous disease with a spectrum of clinical severity ranging from mild arthritis to a crippling joint disorder with internal organ involvement [10]. Although the pathophysiological basis of RA is not yet fully understood, ROS have been implicated in its pathogenesis [11]. It was suggested that free radical production is increased or antioxidant defense system is destroyed in RA [12].

The aim of the present study was to assess the lipid peroxidation (LPO) and antioxidant status of patients with RA compared with OA.

2. Patients and methods

2.1. Study population

This study included 30 RA patients (group I) diagnosed according to the revised criteria of the American College of Rheumatology (ACR) [13] and 30 OA patients (group II) diagnosed according to the ACR criteria for classification of OA knee [14].

Patients were collected consecutively from those attending the outpatient clinics of Physical Medicine, Rheumatology and Rehabilitation department, Tanta University Hospitals, EGYPT. All RA patients were under disease-modifying antirheumatic drugs (DMARDs) either as monotherapy or in combination. Almost all OA patients and the vast majority of RA patients were consuming non-steroidal anti-inflammatory drugs (NSAIDs) on irregular basis. Fifteen age and gender matched healthy subjects were enrolled in the study as a control group (group III).

Patients suffering from chronic disorders such as diabetes mellitus, thyroid dysfunction, liver or kidney disease, obesity (body mass index > 30), dyslipidemia, inflammatory disease, infection, smoking, consumption of any antioxidant supplements at the start of the study or in the previous month were excluded.

All patients were subjected to:

- Careful history taking.
- Thorough clinical examination with assessment of:

In RA patients:

1. Duration of morning stiffness (MS) in minutes.
2. Disease activity score 28 (DAS28) was determined for the evaluation of current RA activity [15]. The DAS28 considers 28-joint count for tenderness, a 28-joint count for swelling, erythrocyte sedimentation rate (ESR) and the patient's overall assessment of well-being. DAS28 was then calculated using Webculator.

In OA patients:

1. Pain at rest and pain with movement on a visual analog scale (VAS) of 0 to 100 mm, with 0 representing the absence of pain.

2. Western Ontario and McMaster Universities (WOMAC) index for knee OA, measured as total pain score (W-TPS), total stiffness score (W-TSS) and total physical function score (W-TPFS) [16,17].

2.2. Biochemical analysis

Fasting blood samples were collected from patients and controls under aseptic precautions by venepuncture; 1.6 ml in a citrated tube for ESR, 5.0 ml in a heparinized vial for glutathione and enzyme estimations and 3.0 ml in a plain vial for serum MDA and plasma CP estimations. Samples were centrifuged as soon as possible at 2000*g* for 10 min at 4 °C. Serum samples were stored at −70 °C until the time of analysis of MDA. GST, Cp, and routine biochemical analysis such as ESR, and CRP, were assessed in plasma samples. After separating the plasma, erythrocytes were washed three times in 0.9% NaCl solution and were haemolysed by dilution in water and stored at −20 °C until used for measurement of SOD, CAT activities and GSH concentration.

Specific laboratory investigations including:

1. Serum MDA as a marker for oxidative stress was determined using the thiobarbituric acid (TBA) method [18].
2. Erythrocyte SOD activity was assessed according to the method of Marklund and Marklund [19], which is based on the ability of SOD to inhibit auto-oxidation of pyrogallol. One unit of SOD is described as the amount of enzyme required to inhibit the auto-oxidation of pyrogallol by 50% in the assay mixture and the results are expressed in units/g Hb.
3. Erythrocyte CAT activity was assessed according to the method of Aebi [20] which is based on the decomposition of H202 by CAT. Enzyme activity was expressed as the first order kinetic constant (K) of the rate of disappearance of H202 for 15 s as measured by decrease in absorbance at 240 nm. Results were expressed as K/gHb.
4. Erythrocyte GSH concentration was determined according to the method of Beutler et al. [21] using 5,5′-dithiobis 2-nitrobenzoic acid as disulfide chromogen that is easily reduced by sulfydryl compounds to an intensely yellow compound. The absorbance of the reduced chromogen was measured at 412 nm and GSH concentration was expressed as mg per g hemoglobin.
5. Plasma GST activity was determined by incubating CDNB (1 chloro 2, 4 dinitro benzene) with reduced GSH in the presence of serum containing GST. 2,4-Dinitrophenylglutathione (adduct) formed was read at 340 nm [22].
6. Plasma ceruloplasmin was determined by p-phenylene diamine oxidase activity [23].

All patients gave an informed consent for a study.

Statistics: Results were expressed as mean and standard deviation (SD). Statistical analysis was carried out using the SPSS program (version 10.0 software, SPSS Inc. Chicago, Illinois, USA). For the comparison of RA, OA and control groups variance analysis (one-way ANOVA) test was used. Comparisons between each two variables were conducted using the student *t*-test. Correlation between the variables was examined using the Pearson's correlation coefficient.

3. Results

A total of 30 RA patients (group I) (25 females, 5 males), their ages ranged from 39 to 56 years (mean age 48.2 ± 7.6). The mean disease duration was 10.33 ± 2.66 years and 30 OA knee patients (group II) (23 females, 7 males), aged from 42 to 62 years (mean age 51.2 ± 6.4). The mean disease duration was 9.60 ± 2.26. Demographic data of patients and healthy subjects are shown in Table 1. There was no significant differences in age, sex and mean body mass index values between patients and controls.

Fig. 1 depicts the clinical characteristics of OA patients. In RA patients, the mean disease activity score (DAS-28) was 5.11 ± 0.74 with a mean value of the morning stiffness which was 60.00 ± 35.79 min. Comparison of the biochemical parameters of RA, OA patients and healthy controls is shown in Table 1. It demonstrates significantly high serum levels of MDA and plasma GST activity in both patient groups as compared with the healthy control group. There was insignificant difference in MDA serum levels between the RA and OA groups whereas there was statistically significant higher plasma GST activity in RA group as compared with OA group ($P < 0.001$).

We have found significantly lower erythrocyte SOD, CAT activities and GSH level in group I and II compared to the control group ($P < 0.001$). The difference between RA and OA was statistically significant. As regards plasma Cp activity, we have found significantly higher Cp activity in the RA group as compared with the OA and control groups with insignificant difference between the OA and control groups.

As regards correlation matrix (Tables 2 and 3), there was direct correlation between MS, DAS-28, ESR and CRP with serum MDA in RA patients, but not in OA patients. Meanwhile plasma GST and Cp activities showed direct correlation with parameters of activity and clinical parameters in both patient groups. A significant negative correlation was detected between activity parameters with erythrocyte SOD, CAT activities and GSH level in RA and OA patients.

As regards intra-correlation coefficients (*r* values) between chemical parameters studied in patients with RA and OA, there was positive correlation between MDA, GST with Cp, also between erythrocyte SOD with CAT activities ($P <$ 0.001) and significant negative correlations of plasma GST activities with erythrocyte GSH level ($P < 0.001$).

4. Discussion

Excessive oxidative stress is thought to have an important role in the pathogenesis of autoimmune diseases by enhancing the inflammation, inducing apoptotic cell death, and breaking down the immunological tolerance [24].

In the current study serum MDA was found in significantly high levels in RA and OA patients than in controls. Akyol et al. [25] found a remarkable elevation in MDA levels in patients with RA compared to controls; this was also observed in many published reports [5,26,27]. On the other hand, Olivieri et al. [28] reported no change in LPO in RA. Many authors sug-

Table 1 Demographic and chemical characteristics of RA, OA and controls.

Parameters	Group I RA (n = 30)	Group II OA (n = 30)	Control group (n = 15)
Age (year)	48.22 ± 7.61	51.23 ± 6.42	46.51 ± 10.05
Sex (F/M)	25/5	23/7	10/5
BMI (kg/m^2)	23.58 ± 2.45	24.24 ± 2.46	23.82 ± 2.67
Duration of disease/years	10.33 ± 2.66	9.60 ± 2.26	–
ESR (mm/h)	56.93 ± 20.81[a,b]	20.06 ± 5.53[c]	8.10 ± 3.38
CRP (mg/dl)	33.06 ± 14.13[a,b]	8.20 ± 3.09	3.05 ± 0.42
MDA (nmol/ml)	3.15 ± 0.77[a]	2.80 ± 0.19[c]	2.05 ± 0.453
SOD (U/g Hb)	1047.73 ± 126.40[a,b]	1276.06 ± 139.78[c]	1582 ± 219
CAT (k/gHb)	82.50 ± 7.08[a,b]	102.30 ± 18.40[c]	123.70 ± 4.27
GSH (mg/gHb)	2.09 ± 0.47[a,b]	3.14 ± 0.60[c]	3.98 ± 0.72
GST (IU/L)	8.54 ± 0.47[a,b]	5.00 ± 0.37[c]	4.30 ± 0.45
Cp (g/L)	2.56 ± 0.20[a,b]	0.50 ± 0.20	0.50 ± 0.03

Values represent the mean ± standard deviation.
BMI: body mass index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; MDA: serum malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GST: glutathione S-transferase; GSH: glutathione; Cp: ceruloplasmin.
[a] Statistically significant, compares healthy subjects and group I (P < 0.001).
[b] Statistically significant, compares group I and group II (P < 0.001).
[c] Statistically significant, compares healthy subjects and group II (P < 0.001).

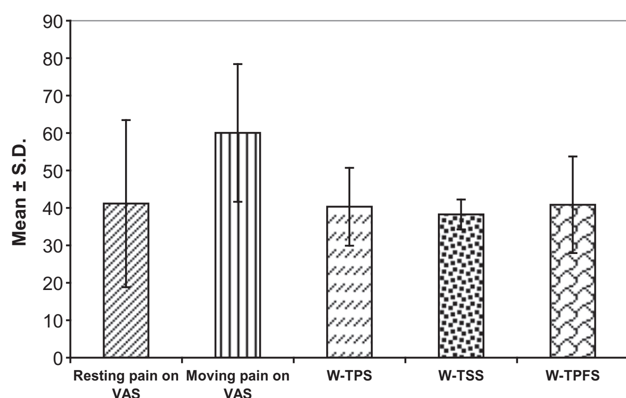

Figure 1 Clinical characteristics of OA patients (VAS = visual analog scale of 0–100 mm, W-TPS = Western Ontario and McMaster Universities (WOMAC) index for total pain score, W-TSS = WOMAC index for total stiffness score, W-TPFS = WOMAC index for total physical function score).

gested that increased ROS levels in RA may result in a pro-oxidation environment, which in turn could result in increased MDA levels. As a result, LPO may have a role in the pathogenesis of the RA. The increased MDA level in our OA patients coincides with the results of Maneesh et al. [29] and Rubyk et al. [30] who reported significantly increased serum MDA levels in OA patients compared to controls. Thus, these findings are in keeping with possible evidence of free radical production and damage in OA. The difference between MDA levels in RA versus OA group was statistically insignificant among our patients. This finding disagrees with many Refs. [11,31,32] which demonstrated that MDA levels were significantly elevated in RA patients compared to OA patients and controls. This could be attributed to less inflammation in our RA patients.

Recklies et al. [33] mentioned that SOD is the first line of defense against ROS; it catalyzes the dismutation of the superoxide anion into hydrogen peroxide. Our RA and OA patients demonstrated significantly low erythrocyte SOD activity than in controls, this agrees with Karatas et al. [34] and Banford et al. [35] who stated that antioxidant enzyme SOD activity was found to be significantly lower in patients of RA compared to controls. On the other hand, some authors [11,25] reported that SOD levels in erythrocytes of patients with RA are not different from controls. Other authors [11,25,36] found that SOD activity in erythrocytes in patients with RA is significantly decreased in comparison to patients with OA which agrees with our finding. This decreased activity of SOD in patients with RA was assumed to indicate a degradation process in which SOD is degraded by free radicals during the detoxifying processes [25]. Meanwhile, Kalpakcioglu et al. [37] suggested that there is a possibility that the lowered levels of SOD activity may be due to the inhibition of the enzyme by hydrogen peroxide, which might be an indicator of high degree of superoxide anion production. There is controversy about SOD activity in OA. Maneesh et al. [29] and Pinto et al. [38], found significant increase in SOD activity in the erythrocytes of OA patients compared to the normal healthy controls. Meanwhile our study showed decreased SOD activity in OA patients compared to controls. It is possible that differences between our results and other investigators results, regarding antioxidant status, is due to differences in the stage of the disease. Chronic joint disease may deplete antioxidant defenses whereas acute inflammation can upgrade them. Ostalowska et al. [39] have reported increased activities of SOD in synovial fluid of patients with primary and secondary knee OA. In contrast, Ivanova and Ivanova [40] found that there was no SOD, no or low CAT in synovial fluid (SF) from OA joints.

In the present study, the observation of significant decrease in the activity of CAT in RA and OA patients compared to controls agrees with Surapaneni and Venkataramana [41] and Sarban et al. [11] who stated that the reduced CAT level in RA and OA is due to the its inactivation by H2O2 and suggest that these enzymes may play an important role in the rheumatic process and increased oxidative stress. Therefore, El-Sohemy et al. [42] mentioned that increasing CAT activity

Table 2 Correlation between the activity parameters and chemical parameters in the RA patients.

	DAS-28	MS/min	ESR	CRP
MDA	$r = 0.585^*$ $P < 0.001$	$r = 0.653^*$ $P < 0.001$	$r = -0.665^*$ $P < 0.001$	$r = -0.594^*$ $P < 0.001$
SOD	$r = -0.772^*$ $P < 0.001$	$r = -0.802^*$ $P < 0.001$	$r = -0.786^*$ $P < 0.001$	$r = -0.746^*$ $P < 0.001$
CAT	$r = -0.633^*$ $P < 0.001$	$r = -0.609^*$ $P < 0.001$	$r = -0.615^*$ $P < 0.001$	$r = -0.670^*$ $P < 0.001$
GSH	$r = -0.450^*$ $P < 0.001$	$r = -0.460^*$ $P < 0.001$	$r = -0.457^*$ $P < 0.001$	$r = -0.534^*$ $P < 0.001$
GST	$r = 0.516^*$ $P < 0.001$	$r = 0.509^*$ $P < 0.001$	$r = 0.513^*$ $P < 0.001$	$r = 0.460^*$ $P < 0.001$
Cp	$r = 0.556^*$ $P < 0.001$	$r = 0.599^*$ $P < 0.001$	$r = 0.450^*$ $P < 0.001$	$r = 0.460^*$ $P < 0.001$

DAS-28: disease activity for 28 joint indices score; MS: morning stiffness; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; MDA: serum malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GST: glutathione S-transferase; GSH: glutathione; Cp: ceruloplasmin.
* Values were calculated using Pearson's correlation coefficient.

Table 3 Correlation between the clinical parameters and chemical parameters in the OA patients.

	Resting pain (VAS)	Moving pain (VAS)	W-TPS	W-TSS	W-TPFS
MDA	$r = 0.397$ $P = 0.138$	$r = 0.118$ $P = 0.667$	$r = 0.406$ $P = 0.127$	$r = -0.458$ $P = 0.083$	$r = -0.238$ $P = 0.381$
SOD	$r = -0.774^*$ $P < 0.001$	$r = -0.756^*$ $P < 0.001$	$r = -0.711^*$ $P = 0.002$	$r = -0.516^*$ $P < 0.020$	$r = -0.604^*$ $P < 0.001$
CAT	$r = -0.959^*$ $P < 0.001$	$r = -0.886^*$ $P < 0.001$	$r = -0.609^*$ $P < 0.001$	$r = -0.633^*$ $P = 0.011$	$r = -0.604^*$ $P < 0.001$
GSH	$r = -0.774^*$ $P < 0.001$	$r = -0.746^*$ $P < 0.001$	$r = -0.554^*$ $P < 0.001$	$r = -0.802^*$ $P < 0.001$	$r = -0.670^*$ $P < 0.001$
GST	$r = 0.776^*$ $P < 0.001$	$r = 0.745^*$ $P < 0.001$	$r = 0.886^*$ $P < 0.001$	$r = 0.439^*$ $P < 0.005$	$r = -0.457^*$ $P < 0.043$
Cp	$r = 0.624^*$ $P < 0.001$	$r = 0.450^*$ $P < 0.001$	$r = 0.964^*$ $P < 0.001$	$r = -0.457^*$ $P < 0.043$	$r = 0.450^*$ $P < 0.046$

VAS = visual analog scale of 0–100 mm; W-TPS = Western Ontario and McMaster Universities (WOMAC) index for total pain score; W-TSS = WOMAC index for total stiffness score; W-TPFS = WOMAC index for total physical function score.
* Values were calculated using Pearson's correlation coefficient. Abbreviations as in Table 2.

can be protective against RA through limiting the production of ROS.

Glutathione (GSH) is an important member of the antioxidant team as it has been shown to plays a key role in cellular resistance against oxidative damage; it destroys ROS and other free radicals by enzymatic as well as non-enzymatic mechanisms [5]. Glutathione S- transferase (GST) is an integral part of the biphasic oxidation/conjugation system that catalyzes the conjugation of GSH with several compounds produced in vivo during oxidative stress [43,44]. In our patients, GSH level in erythrocytes was found to be significantly reduced in RA and OA patients versus control group. The reduction of GSH level was significantly higher in RA compared to OA patients. On the other hand, plasma GST activity was higher in both patient groups versus controls, with RA patients having significantly higher activity than OA patients. These findings coincide with Hassan et al. [45] who demonstrated 50% reduction in GSH level and threefold increase of GST activity in RA patients. Raised GST activity in OA patients versus controls was observed by Ostalowska et al. [39] and Surapaneni et al. [41] who assumed that it could be due to its induction to counter the effect of increased oxidative stress. Pinto et al. [38] opposed all previous reports when they stated that neither erythrocytes GSH nor plasma GST were significantly altered in OA patients. Meanwhile Ostalowska et al. [39] have shown increased activity of GST in SF of OA patients.

Ceruloplasmin (Cp) is an acute phase protein that is primarily synthesized in the liver and secreted into the blood. It is a prominent antioxidant that can scavenge ROS [5,46,47]. We observed a significant increase in plasma Cp in RA than OA patients and controls, while Cp in OA patients was insignificantly higher than in control. In agreement with our findings, many authors [48,49–54] observed increased plasma Cp level in RA. On the other hand, Ashour et al. [55] stated that

the raised levels of Cp are significantly increased in RA group, but not in OA group. This outstanding agreement about high Cp level in RA was emphasized by Nagler et al. [10] as Cp is considered the principle plasma and synovial antioxidant in RA, being responsible for up to 70% of the protective capacity against superoxide free radicals. Nevertheless, Louro et al. [56] stated that although the increase in the concentration of Cp might offer an additional safeguard against oxidative stress. It does not appear to have a beneficial effect upon the activity of the illness as evaluated by means of the biological inflammation markers C-reactive protein and ESR.

We demonstrated significant positive correlation between clinical and laboratory parameters of activity in RA (DAS-28, MS, ESR, CRP) and OA (resting and movement pain, W-TPS, W-TSS and W-TPFS) with GST and Cp and negative correlation with SOD, CAT and GSH.

MDA showed direct correlation with MS, DAS-28, ESR and CRP in RA patients with no particular correlation detected for MDA in OA. Our findings agree with Sarban et al. [11] and Baskol et al. [57] who demonstrated a significant correlation between ESR and MDA levels in patients with RA, and claimed it would be useful in predicting disease activity. This correlation between MDA and antioxidants versus parameters of activity in RA makes it possible to use them as a surrogate measure of disease activity. We demonstrated a significant positive correlation between serum MDA and erythrocyte GST activity and plasma Cp concentration, another direct correlation was detected between SOD and CAT. This correlation clarifies that oxidative stress leads to increased antioxidant enzyme activities to restore the oxidant/antioxidant system balance which is shifted in favor of LPO which could lead to the tissue damage observed in OA and RA, as confirmed by Jaswal et al. [26].

In conclusion, the light of our previous findings, it is possible to conclude that increased oxidative stress in RA and OA patients evidenced by increased serum MDA, have led to compensatory changes in the levels of some antioxidants, such as SOD, CAT, GSH, GST and Cp. These changes, in turn, may provide additional protection against LPO. These findings confirm the role of oxidative stress in the pathogenesis of RA and OA, and that LPO markers such MDA and antioxidants can serve as surrogate markers for disease activity in RA and OA.

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