Association of Oxidative Stress and Disease Activity in Rheumatoid Arthritis Patients in Babylon Province

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Abstract

Background: Several lines of evidence suggest a role for oxidative stress in the pathogenesis of rheumatoid arthritis (RA). Both reactive oxygen species (ROS) and reactive nitrogen species (RNS) damage cartilage. Tissue injury in inflammation results in $NO^{\bullet}$ production by articular chondrocytes and synovial fibroblasts and elevated levels of $NO^{\cdot}$ are observed in the serum and synovial fluid of RA patients. The free radicals, particularly $NO^{\bullet\bullet}$ and $O_2^{\bullet-}$, inhibit the synthesis of matrix components like proteoglycans by chondrocytes and also damage the extracellular matrix through activation and up regulation of matrix metalloproteinases.

Aim of the Study: To study the possible association between oxidative stress and RA.

Patients and Method: The present case control study was conducted on sixty one patients (18 males and 43 females) with RA patients admitted to Rheumatoid Unit in Merjan Teaching Medical City, Babylon Province, Iraq, duration the period September 2018 to July 2019, as well as 127 apparently healthy control subjects (41 males, 86 females) as control group. Malondialdehyde (MDA) and total antioxidant capacity (TAC) using ELISA technique.

Results: The level of MDA was higher in patients with RA in comparison with control group, with median of 2.35 (1.93) versus 0.86 (0.52); the difference was highly significant ($P< 0.001$). On the other hand, the level of TAC was lower in patients with RA in comparison with control subjects, with median of 0.10 (0.21) versus 0.53 (1.02), respectively; the difference was highly significant ($P< 0.001$).

Conclusion: The current study documented that RA is significantly associated increased oxidative stress.

Keywords: *Oxidative stress, Rheumatoid Arthritis, Babylon.*

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by inflammatory arthritis and extra-articular involvement. RA with symptom duration of fewer than six months is defined as early, and when the symptoms have been present for more than six months, it is defined as established[1-3]. Several lines of evidence suggest a role for oxidative stress in the pathogenesis of RA. Both ROS and RNS damage cartilage. Tissue injury in inflammation results in $NO^{\bullet}$ production by articular chondrocytes and synovial fibroblasts and elevated levels of $NO^{\bullet}$ are observed in the serum and synovial fluid of RA patients. The free radicals, particularly $NO^{\bullet}$ and $O_2^{\bullet-}$, inhibit the synthesis of matrix components like proteoglycans by chondrocytes and also damage the extracellular matrix through activation and up regulation of matrix metalloproteinases. The HOCl, produced by myeloperoxidase (MPO) in neutrophils, chlorinate the tyrosine residues to form 3-chlorotyrosine and damage

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the collagen, thus implicated in arthritogensis. RA patients have increased plasma MPO concentrations. Elevated levels of MDA, $NO^{\bullet}$, protein carbonyls, oxidized hyaluronic acid and oxidized LDL have been reported in RA patients [4]. Posttranslational protein oxidative modifications, in particular cysteine modifications, have been implicated in ischemic tolerance or preconditioning. Ischemic tolerance constitutes a positive stress that reporgrams cellular defense systems to prevent subsequent lethal injuries [5]. ROS can induce lipid peroxidation and disrupt the membrane lipid bilayer arrangement that may inactivate membrane-bound receptors and enzymes and increase tissue permeability. Products of lipid peroxidation, such as MDA and unsaturated aldehydes, are capable of inactivating many cellular proteins by forming protein cross-linkages [6].ROS can lead to DNA modifications in several ways, which involves degradation of bases, single- or double-stranded DNA breaks, purine, pyrimidine or sugar-bound modifications, mutations, deletions or translocations, and cross-linking with proteins [6]. The current study, therefore, was aiming at assessing possible association between oxidative stress and RA.

Patients and Method

The present case control study was conducted in Department of Biochemistry, College of Medicine, University of Babylon, and Rheumatoid Unit, MerjanTeaching Medical City, Hilla City, Babylon Province, Iraq. The duration of current study was extended from September 2018 to July 2019. Sample size was determined according to sample size equation. Sixty one patients (18 male and 43 female) with RA clinically diagnosis by specialist physician attended to out clinic of Merjan Teaching Medical City, Hilla City with mean age of (47.43 ±11.34 years), as well as 127 apparently healthy control subjects (41 males, 86 females) with mean age of (48.94 ±12.36 years). Disease severity score of RA patients was determined by use DAS-28[2]. Oxidative stress was evaluated by measuring MDA and TAC using ELISA technique according to providing company instructions.

Results

Clinical assessment of the severity of disease was made according to disease activity score (DAS-28) and the obtained results were demonstrated in Table 1. The mean DAS-28 score was 4.52 ± 1.42 with a range of 2 to 7.3. Cases with remission, a score of < 2.6, accounted to 6 (9.8%), cases with low disease activity, a score of 2.6 to 3.2, accounted to 9 (14.8%), cases with moderate disease activity, a score of > 3.2 – 5.1, accounted to 27 (44.3%) and cases with high disease activity accounted to 19 (31.1%), as shown in Table 1.

Table 1: Frequency distribution of patients with rheumatoid arthritis DAS-28

DAS-28 Score	Number of Cases	%
Remission (< 2.6)	6	9.8
Low activity (2.6 – 3.2)	9	14.8
Moderate activity (> 3.2 – 5.1)	27	44.3
High activity (> 5.1)	19	31.1
Mean ±SD	4.52±1.42	
Range	2–7.3	

SD: standard deviation

The oxidative stress status was assessed by measuring the serum level of the oxidative marker Malondialdehyde (MDA) and serum level of total anti-oxidant capacity (TAC). Actually, from statistical perspective, those two variables, namely MDA and TAC, are not normally distributed (non-parametric) according to kolmogorov-Smirnov test of normality distribution. For that reason, median is going to be used instead of mean as a measure of central tendency, and inter-quartile range (IQR), will be used instead of standard deviation as a measure of dispersion, as shown in Table 2.

The level of MDA was higher in patients with rheumatoid arthritis in comparison with control group, 2.35 (1.93) versus 0.86 (0.52); the difference was highly significant ($P< 0.001$), as shown in Table 2 and figure 1. On the other hand, the level of TAC was lower in patients with rheumatoid arthritis in comparison with control subjects, 0.10 (0.21) versus 0.53 (1.02), respectively; the difference was highly significant ($P< 0.001$), as shown in Table 2 and figure 1.

Table 2: Malondialdehyde (MDA) and total anti-oxidant capacity (TAC) levels in patients with rheumatoid arthritis and control group

Characteristic	Rheumatoid arthritis group n = 61	Control group n = 127	P
MDA			
Median (IQR)	2.35 (1.93)	0.86 (0.52)	< 0.001 †
Range	1.00 -3.90	0.50 -5.93	HS
TAC			
Median (IQR)	0.10 (0.21)	0.53 (1.02)	< 0.001 †
Range	0.01 -0.68	0.10 -1.90	HS

n: number of cases; IQR: inter-quartile range; MDA: malondialdehyde; TAC: total anti-oxidant capacity; †: Mann Whitney U test; HS: highly significant difference at $P \leq 0.01$

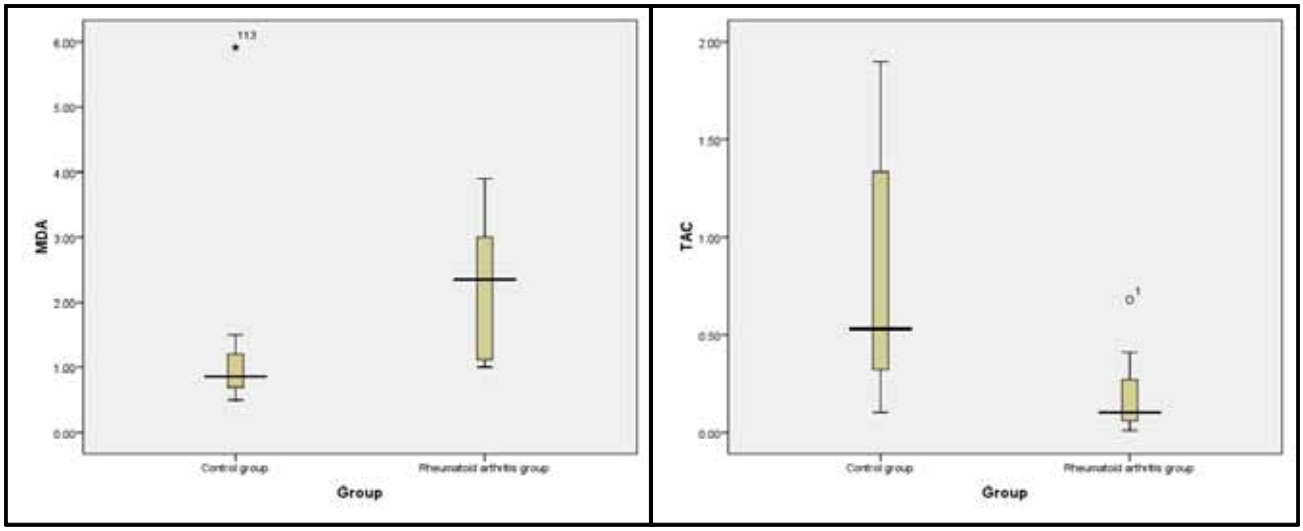

Figure 1: Box plot showing comparison of malondialdehyde (MDA) and total anti-oxidant capacity (TAC) levels in patients with rheumatoid arthritis and control group

Bothe serum MDA level and serum TAC level were correlated to characteristics of patients with rheumatoid arthritis and the results were presented in Table 3. Serum MDA was negatively correlated to age of patients (r = - 0.013); however, the correlation was statistically insignificant (P = 0.919),. Serum MDA was correlated to male gender of patients (r = - 0.008); however, the correlation was statistically insignificant (P = 0.950), Table 3. Serum MDA was positively correlated to duration of disease (r = 0.210); however, the correlation was statistically insignificant (P = 0.105), Table 3. Serum MDA was positively correlated to disease activity (r = 0.033); however, the correlation was statistically insignificant (P = 0.799), Table 3.

Serum TAC was negatively correlated to age of patients (r = - 0.160); however, the correlation was statistically insignificant (P = 0.217), table 3. Serum TAC was correlated to male gender of patients (r = - 0.181); however, the correlation was statistically insignificant (P = 0.163), table 3. Serum TAC was negatively correlated to duration of disease (r = - 0.021); however, the correlation was statistically insignificant (P = 0.874), table 3. Serum TAC was negatively correlated to disease activity (r = - 0.123); however, the correlation was statistically insignificant (P = 0.345), Table 3.

In the current study, the oxidative stress status was assessed by measuring the serum level of the oxidative marker Malondialdehyde (MDA) and serum level of total anti-oxidant capacity (TAC). It was found in this study that, the level of MDA was significantly higher in patients with rheumatoid arthritis in comparison with control group. On the other hand, the level of TAC was significantly lower in patients with rheumatoid arthritis in comparison with control subjects. These results indicate a principal role for oxidative stress in the pathogenesis of rheumatoid arthritis.

Table 3: Correlations of malondialdehyde (MDA) and total anti-oxidant capacity (TAC) levels to rheumatoid patients characteristics

Characteristics	MDA		TAC	
	r	P	r	P
Age	-0.013	0.919 NS	-0.160	0.217 NS
Gender	-0.008	0.950 NS	-0.181	0.163 NS
Duration of Disease	0.210	0.105 NS	-0.021	0.874 NS
DAS-28	0.033	0.799 NS	-0.123	0.345 NS

MDA: malondialdehyde; TAC: total anti-oxidant capacity; r: Spearman correlation coefficient; DAS: disease activity score; NS: not significant at $P > 0.05$

Discussion

A large number of studies have shown that reactive oxygen species (ROS) are implicated in the pathophysiology of many diseases including RA [7]. These are highly reactive chemical species that have the potential to damage lipids, proteins and DNA in joint tissues. Under normal conditions ROS production is controlled by a variety of antioxidant defence system present in the body. The non- enzymatic antioxidant defence includes vitamin A and C, reduced glutathione (GSH) while enzymatic antioxidant includes superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR) and glautathione-S-transferase (GST). Imbalance between oxidants and antioxidants due to increased chemical reaction or insufficient antioxidant defence system results in oxidative stress. These ROS if not scavenged properly may damage biological macromolecules [8,9].

Previous reports suggest the role of oxidative stress in inflammation and destruction in the joints of arthritic animals and RA patients. ROS formation and markers of protein and lipid oxidation has been found to be raised in arthritic animals. The oxidative status has been found to be changed in the serum of RA patients and also in the brain, liver and vascular tissues of rats with experimental arthritis [10].In the current study, MDA was elevated and this finding is in line with previous study. Lipid peroxidation was measured in terms of MDA present in blood plasma. The rise in lipid peroxidation product might be due to the increased formation of ROS which tends to increase abundantly during chronic inflammation and hence cause excessive damage to tissues. This is in line with other studies where elevated level of MDA has been found in the serum, plasma and erythrocytes of RA patients [11,12]. A significant increase in the lipid peroxidation has also been reported in the liver and brain of rats with adjuvant arthritis [13,14].In the current study, both serum MDA and TAC were not significantly correlated to age, gender, disease duration and disease activity of patients with rheumatoid arthritis. Similar results were obtained by Kardes*et al*. [15] who stated that serum MDA was not significantly correlated to any of clinical parameters of patients with rheumatoid arthritis.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicineand all experiments were carried out in accordance with approved guidelines.

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