Original Article

Oxidative Stress and Total Antioxidant Capacity in Rheumatoid Arthritis

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Summary:

Back ground:- Rheumatoid arthritis (RA) is a systemic disease characterized by progressive, erosive and chronic poly arthritis, where cellular proliferation of the synoviocytes and neo-angiogenesis leads to formation of pannus which destroys the articular cartilage and the bone .

Objective:- to evaluate theOxidative Stress index(OSI)by measuring malondialdehide(MDA) and Total Antioxidant Capacity(TAC) in serum of RA patients in addition to uric acid and albumin and to investigate the alteration in these parameters compared to healthy individuals.

Patients and Methods:- twenty five patients with rheumatoid arithritis and twenty five apparently healthy subjects matched for age and weight have been included in this study,uricacid and Serum malondialdehyde were measured by enzymatic methods. serum total antioxidant capacity was measured according to Miller et al method.

Results:- The data obtained showed that the serum levels of Albumin, the total antioxidant capacity were significantly lower in patients with RA than in healthy controls.While the serum levels of MDA,uric acid were significantly higher in patients with RA than in healthy controls.

Conclusion:- there is acontribution of antioxidant to the pathogenesis of RA.

Key word:- Albumin,oxidative stress, total antioxidant capacity,MDA,uric acid.

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Introduction:-

Rheumatoid arthritis (RA) is a systemic disease characterized by progressive, erosive and chronic poly arthritis, where cellular proliferation of the synoviocytes and neo-angiogenesis leads to formation of pannus which destroys the articular cartilage and the bone (1). The etiology of RA is unknown but some evidences suggest the involvement of free radicals (FR) and reactive oxygen species (ROS) in the pathogenesis of the disease, by causing oxidative damage to cell through destroying membrane lipids, proteins, hyaluronic acid and cartilage (2). Free radicals and reactive oxygen species can be defined as chemical species, an atom or molecule that has one or more unpaired electrons in its valance shell and is capable of existing independently. Free radical contains an odd number of electrons which makes it unstable, short lived and highly reactive, therefore it reacts quickly with other molecules in order to capture the needed electron to gain stability (3,4). FR-associated damage is an important factor in many pathological processes and a wide spectrum of human disease. However living organisms have developed complex antioxidant system to counteract reactive oxygen species and to reduce their damage (5).Antioxidant has been defined by Halliwell and Whiteman "as any substance that when present at low concentration compared with those of an oxidizablesubstrate, significantly delays or prevents oxidation of that substrate". The term "Oxidizable substrate" corresponds to every type of molecule found in vivo (6).Total antioxidant capacity (TAC) is the primary defense against oxidative stress in extracellular fluids results from a number of low molecular weight antioxidant molecules. These antioxidants either generated during normal metabolism or introduced into the body by consumption of diet rich in antioxidant. The sum of endogenous and exogenous antioxidant represents the total antioxidant capacity of the extracellular fluids (7,8,9).Uric acid (UA) is the end product of purine metabolism, which is a major protective antioxidant against some FRs, and it has the potential for chelating iron and copper rendering them unreactive and thus inhibiting lipid peroxidation (10).Albumin is the major plasma protein with molecular weight of 66 KDa, performing several important physiological and pharmacological functions. It transports metals, fatty acids, cholesterol, bile pigments and drugs. Its plasma concentration represents equilibrium not only between its synthesis in the liver and its catabolism, but also

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its transcapillary escape. Albumin represents the major and predominant antioxidant in plasma, a body compartment known to be exposed to continuous oxidative stress, So a large proportion of total serum antioxidant properties can be attributed to albumin. However it has been reported that more than 70% of the free radical trapping activity of serum is due to human serum albumin (11, 12).The relations between total antioxidant capacity and serum oxidant in patients with RA have not been elucidated in previous studies. Further, there are no studies depicting the relation between oxidative stress index (OSI) and some non-enzymatic endogenous antioxidant in RA. So the objective of the present study is to evaluate the OSI by measuringmalondialdehyde(MDA) andthe total antioxidant capacity(TCA) in serum of RA patients in addition to uric acid and albumin and to investigate the alteration in these parameters compared to healthy individuals, which could provide explanation for the contribution of such antioxidant to the pathogenesis of RA.

Patients and methods:

Twenty five subjects male and female who were newly diagnosed as RA bythe physician were included .They were selected from Baghdad Teaching Hospital; . The patients were in the mean age of the patients was52.76±4.82.

The control group consisted of 25 healthy volunteers who were selected from the national centre for blood transfution with mean age of 51.48±4.34. Patients with history of chronic diseases such as liver disease, diabetes mellitus, respiratory disorder, cardiovascular disease, also arthritis other than RA, chronic smokers, chronic alcoholics, and patients treated with any immunosuppressant drugs were excluded. Five ml of blood were collected from the patients and controls using a disposable syringe in plain tube from which serum was separated by centrifuging at 3500 rpm for ten minutes and kept frozen. Serum malondialdehyde was measured quantitatively by the reaction with thiobarbituric acid according to the method of Buege and Aust (13). Serum total antioxidant capacity was measured according to Miller et al method (14), where the capacity of serum to inhibit the oxidized ABTS [2,2-Azino-bis (3-ethylbenzthiazoline sulphonate] formed under influence of peroxidase (met myoglobin) and H_2O_2. The oxidized ABTS has a relatively stable blue-green color, which is measured at 600nm. Antioxidant in the added serum causes suppression of this color production to a degree which is proportional to their concentration.Serum uric acid was measured by enzymatic colorimetric assay using commercial kit, according to the method of Fossati et al (15). Serum albumin was measured based on its quantitative binding to bromocresol green (BCG) according to the method of Doumas et al (16).

Statistical analysis

Student's t-test was applied to compute the significance of the difference in the mean values of RA and control groups, and ($p< 0.001$) was considered statistically significant. The correlation coefficient (r) test is used to describe the association between the different studied parameters.

Results:

Results obtained in the present study showed that serum levels of albumin,TCAwere significantly lower in patient with RA than in control, While the serum levels of MDA, MDA\TCA and uric acid were significantly higher in patient with RA than in control.as shown in table 1.

Table (1): Mean±SD of serum Albumin (g/dL), MDA (µmol/L), TAC (mmol/L), MDA/TAC and Uric Acid (mg/dL)levels in serum of Control and RA patients

Parameter	mean±SD		P value
	Control group	(RA) group	
Albumin (g/dL)	5.16±0.52	3.31±0.35	p< 0.001
MDA (µmol/L)	1.46±0.54	3.24±1.14	p< 0.001
TAC (mmol/L)	1.79±0.66	0.36±0.08	p< 0.001
MDA/TAC	0.90±0.35	9.22±3.73	p< 0.001
Uric Acid (mg/dL)	5.10±0.69	9.34±1.11	p< 0.001

The correlations between Total Antioxidant Capacity and Albumin,Total Antioxidant Capacity and malondialdehydie,Total Antioxidant Capacity and uric acid are shown in figure 1,2,3.

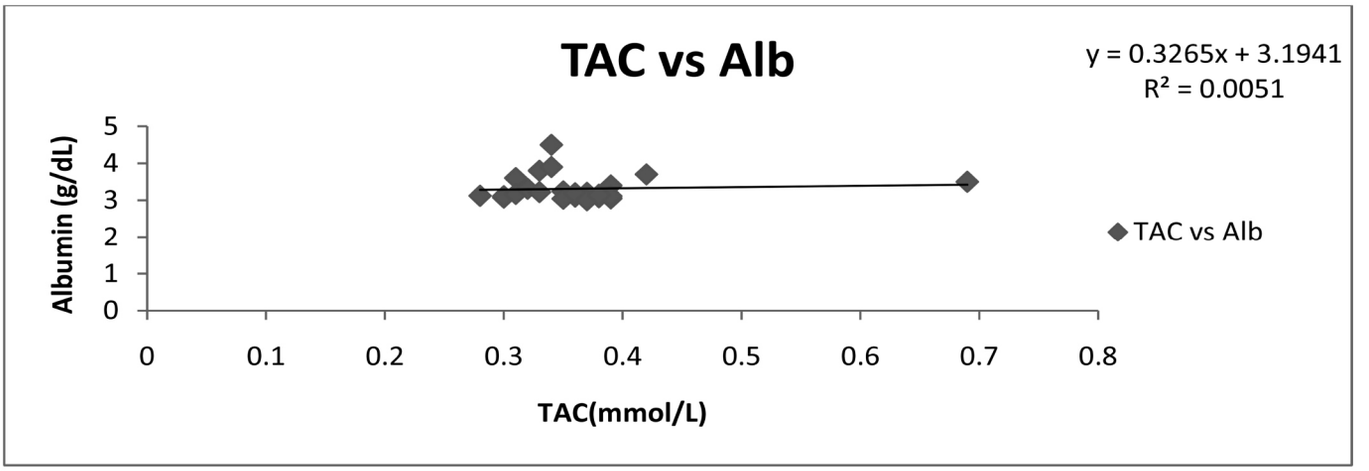

Figure (1): Correlation between Total Antioxidant Capacity and Albumin in rheumatoid arthritis group.

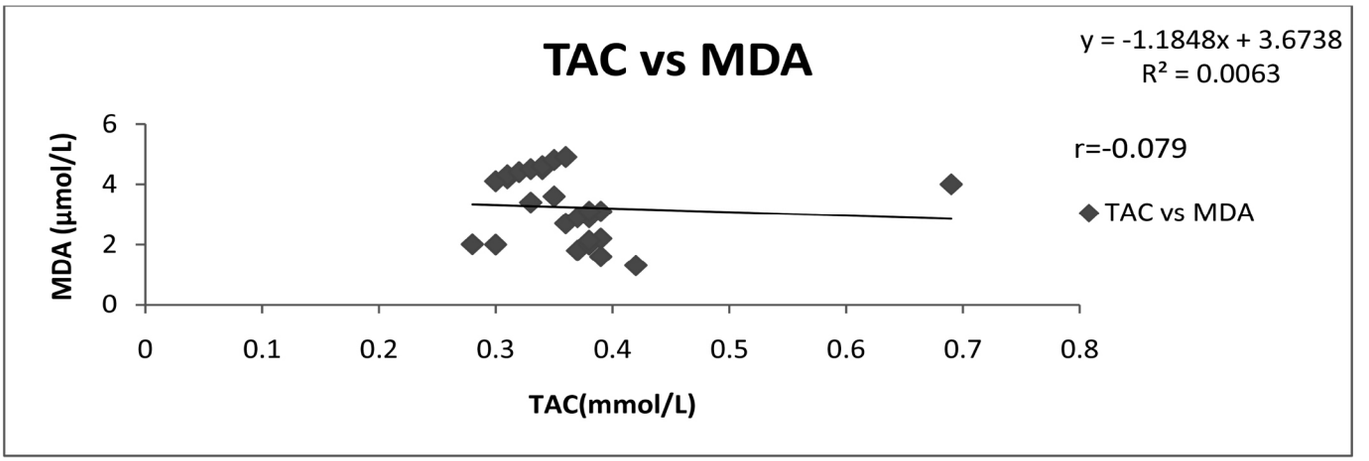

Figure (2): Correlation between Total Antioxidant Capacity and malondialdehyde in rheumatoid arthritis group.

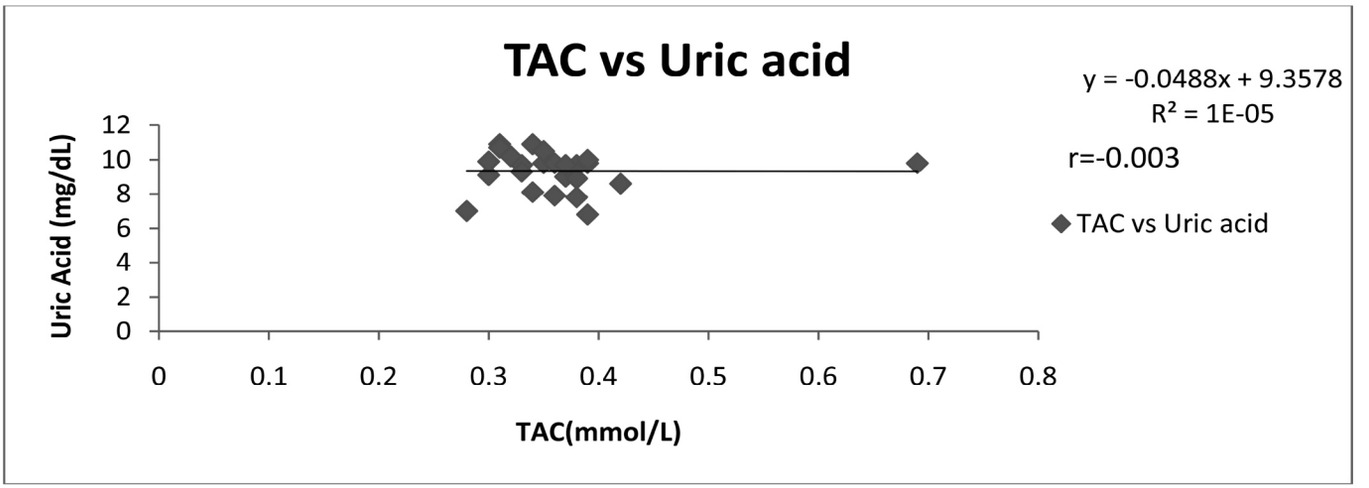

Figure (3): Correlation between Total Antioxidant Capacity and Uric acid in rheumatoid arthritis group.

Discussion:

Involvement of oxygen free radicals (OFR) in the pathophysiology of inflammation in a number of organs and tissues had been reported in literature (5). Evidence of OFR generation in patients with RA has been observed by measuring one of the final products of lipid peroxidation (i.e. malondialdehyde) which was found to be increased significantly in patients compared to control .The findings of the study are in accordance with researches of Ansari and Jaiswal (18) and Shaabani et al (17,18,19) who reported markedly an increased concentration of MDA in RA patients compared to controls.The decrease in serum TAC which was

noticed in the present study is compatible with recent study of Patil et al (20), who claimed that TAC was significantly decreased in RA patients than that found in controls while MDA was significantly increased in RA than that of healthy control. These findings led them to conclude that excessive production of ROS disturbs redox status including antioxidant and can exacerbating inflammation and affecting tissue damage in RA, as exemplified by their strong association with disease activity.

In view of animal studies, strongly suggesting anti-inflammation role of antioxidant in experimentally induced arthritis, antioxidant therapy strategies have been proposed for the prevention and treatment of RA (21).Imbalance in the human oxidative / antioxidant status leads to oxidative stress, which is involved in numbers of disorders including autoimmune diseases (i.e. rheumatoid arthritis) (22). The MDA / TAC ratio is proposed as oxidative stress index (OSI) by Suresh et al (9). The elevated values of OSI for RA in the present study are compatible with other study reported that RA is not generally recognized as a disease of oxidative stress but it has been suggested that the level of ROS in patient with RA is higher than in healthy subjects. Oxidative stress in RA is due to the fact that the antioxidant systems are impaired (23, 24).The elevated level of uric acid in the serum of RA which was noticed in this study is in agreement with a recent study concluded by Patil et al (20) on RA patients receiving ordinary dosage of non steroidal anti-inflammatory drugs. The high levels of uric acid in RA could be explained by counter balance the high rate of FR generation in such patients, because uric acid can act as scavenger in vivo, by trapping peroxy radicals in aqueous phase and contribute to the plasma antioxidant defense (25), also uric acid exerts antioxidant activity (as a chain breaking agent) by scavenging oxygen radicals once they are produced, and by forming a stable complex with iron ions (as a preventive antioxidant) (7). Also uric acid would be a particularly strong serum antioxidant, which shown that age and sex have no effect on the level of antioxidant molecules (26).The quantity of visceral protein is expressed by serum albumin. Hypoalbumineia has traditionally been considered as a classic condition of RA patients, not secondary to under nutrition. Inflammation can cause hypoalbumineia by suppressing albumin synthesis and by causing transfer of albumin from the vascular to the extravascular space. Patients with RA also have increased whole-body protein breakdown which is associated with higher rates of protein catabolism. The low levels of albumin in RA in the present study are consisted with previous studies which reported that hypoalbumineia is due to increase in catabolism rate of albumin in RA (27-29).

The results of this study confirmed the increases of oxidative stress index in RA patients which can follow the hypothesis that there isan imbalance between the generation of free radical and the antioxidant defense system in RA patient. Thus a conclusion could be drawn that enhanced oxidative stress in RA patients is due to decreased antioxidants. Hence an attention should be paid to enhance the antioxidant status of such patients by supplementation of antioxidant.

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