Oxidative Stress & C – Reactive Protein In Patients With Arthritis

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Summary:

Back ground*: Oxidative damage has been suggested to play a key role in accelerating inflammation and to be involved in the pathogenesis of rheumatoid arthritis (RA) and osteoarthritis (OA). Many studies had shown that those patients have low antioxidants level and are at risk of increased oxidative stress.*
Objective: *This study was designed to examine the levels of serum Total Antioxidant Status (TAS). Malondialdehyde (MDA) as index of lipid peroxidation and C–Reactive Protein (CRP) as a marker of oxidative stress in patients with RA and OA and compared them with healthy control.*
Method: *Serum TAS , MDA and CRP levels were measured in 16 RA and 24 OA patients and compare with those obtained from 25 healthy controls.*
Results: *Serum TAS were significantly lower in RA group than in the OA and control groups ($P < 0.05$). Serum MDA and CRP levels were significantly higher in patients with RA than in those with OA and healthy subjects ($P< 0.05$). There were significant negative correlations between TAS and MDA, CRP levels ($r = -0.850$; $p < 0.001$) and ($r = -0.498$; $P < 0.05$) respectively and a positive correlation between MDA and CRP levels in the RA group ($r = 0.686$; $P < 0.01$) .*
In OA group, the level of CRP was significantly increased ($P< 0.05$) and there was significant positive correlation between age and MDA level ($r = 0.553$; $P < 0.01$).
Conclusion: *Our results demonstrated that levels of lipid peroxidation are increased in patients with RA compared to controls and patients with OA , In addition serum TAS levels were decreased in RA. Serum TAS levels may be used as a routine and rapid test to verify the levels of oxidative stress in RA. Furthermore correlating TAS and MDA levels with a cute phase reactants such as CRP may give some clues about disease activity in RA .*

Introduction:

There has been great interest among researchers in the past 20 years for the role of oxidative stress in the development of arthritis[1]. Evans and Halliwell stated that the damaging oxidative species (reactive oxygen, nitrogen and others) arise as byproducts of metabolism and as a physiological mediators and singling molecules [2]. The levels of these oxidative intermediates are held in cheek by the anti- oxidant defense system, the component of this defense system are micronutrients like vitamin C and E[3]. A deficiency in these micronutrients leads to oxidative stress which leaves the body tissue open to the damaging effects of the oxidative intermediates which may accompany inflammatory and immunological process seen in RA patients[4,5]. In addition many studies have suggested the major role of these intermediates in altering chondrocyte (cartilage cells) function in OA[6].

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Oxygen free radicals have been suggested to exert their cytotoxic effect by causing oxidation of membrane phospholipids (i.e. lipid peroxidation)[7]. Many products of lipid peroxidation are not overtly toxic or are minor products of major toxicological interest one of them is malondialdehyde (MDA)[8],a major reactive aldehyde and is used as an indicator of tissue damage[9]. Elevated levels of serum MDA were observed in RA patients which suggested that there is an increase in oxidant stress in those patients[10,11,12].

It is now widely accepted that inflammation and oxidative stress are two important processes integrally involved in the development and progression of arthritis[13], C–reactive protein (CRP) is a well established marker of inflammation and is classified as an acute phase reactant[14] often showing coordinated response with interleukin 6[15], CRP measurement served mostly in diagnostic, albeit non – specific one , and in monitoring role in such fields of infections and rheumatology[16]. Kumon et. al.[17] found that serum CRP was significantly higher in RA than in OA, and correlated with erythrocyte sedimentation rate (ESR) in arthritis patients, he concluded that higher levels of CRP seems to reflect greater degree of joint inflammation in RA and OA patients.

The purpose of the present study was to examine the levels of lipid peroxidation (MDA), CRP, and TAS in patients with RA and OA and to Compared that with healthy control subjects.

Patients and Method:

Sixteen RA patients all fulfilling the American rheumatism criteria for RA[18] and, twenty four OA patients of the same age group were included . All patients had early active disease and were on non steroidal anti– inflammatory drugs as maintenance therapy. No prior treatment with second line anti rheumatic medication .

The data collected include age, sex , weight and height. Body mass index (BMI) was calculated with patient clothed without shoes and expressed in Kg/m^2 .

The control group consisted of twenty five healthy individuals of the same age group . All of the subjects in this study were non – smoker. Blood samples were collected from the antecubital vein into plain tubes. MDA levels were assayed in serum as described by Ohkawa [19]. TAS level were estimated using commercial assay kit obtained from Randox[20]. Serum CRP concentration were measured by nephlometric immunoassay[21].

Results were expressed as mean ± standard deviation (SD) . Differences in mean values of serum variables between healthy controls and arthritis patients were analyzed with the Students – t – test. P value of < 0.05 was considered significant. Pearson correlation coefficient was done to calculate the r value between the parameters analyzed in the serum.

Results:

Patients characteristics are given in table (1), the levels of antioxidant and markers of oxidative stress are given in table (2).

Table (1) Base line characteristics of arthritis and control groups

Characteristics	Control N= 25	RA group N=16	OA group N= 24
Male : Female	11 : 14	7 : 9	10 : 14
Age (years)	49.12 ± 4.25	49 ± 3.72	52.08 ± 3.32
BMI kg/m^2	24.36 ± 1.32	22.24 ± 2.18	23.06 ± 2.09

N= Number of patients
Data are presented as mean ± SD.

Table (2) Serum TAS, MDA and CRP levels in RA, OA and control groups

Subject Croup	N	TAS (mmol/L)	MDA (μ mol/L)	CRP (mg/L)
Control group	25	1.44±0.19	1.08±0.21	2.875±0.52
RA group	16	1.06±0.21*	2.01±0.26**	23.61±10.43***
OA group	24	1.43±0.20	1.19±0.36	5.50±0.83*

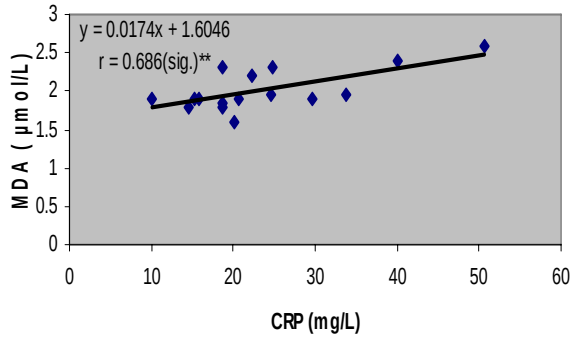
N= Number of patients
Data are presented as mean ± SD.
** p<0.05, ** p<0.01, *** p<0.001 vs. the control group.*

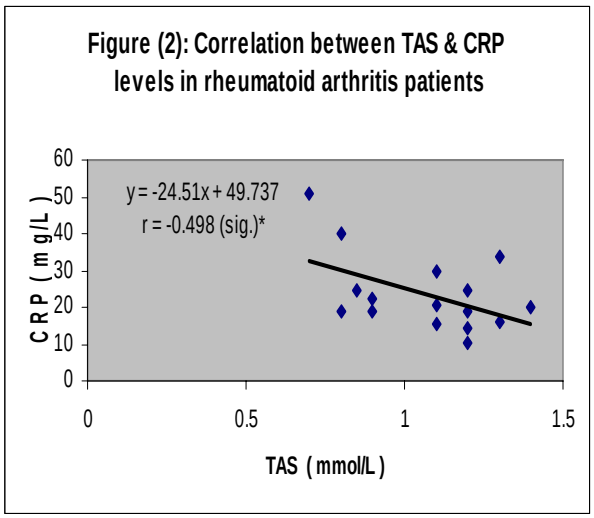
In RA patients, mean values of TAS were significantly lower by – 25.35% (P< 0.05) from the control group while MDA and CRP mean levels are significantly increased by 85.32% (P< 0.01) and 723.0 % (P< 0.001) respectively when compared with health control subject. Serum levels of MDA and CRP where significantly higher (P< 0.05) in patients with RA that those with OA while the TAS level was significantly lower (P< 0.05).

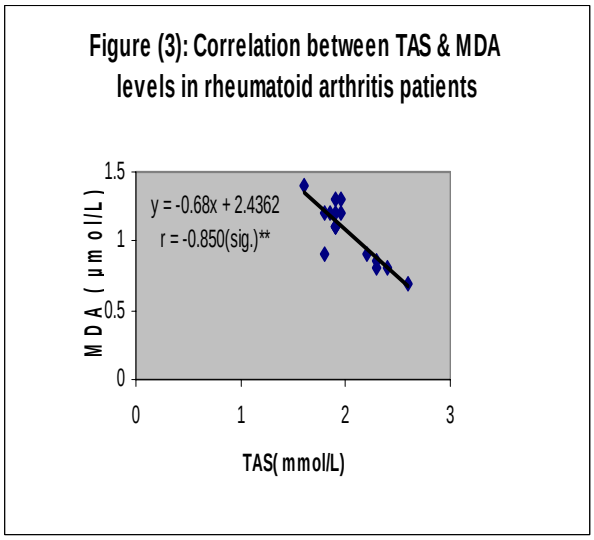
Pearson correlation showed that there were significant positive correlation between MDA and CRP level (r = 0.686 ; P< 0.01) (Fig. 1). while there were significant negative correlation between TAS and CRP (r = -0.498; P < 0.05) (Fig. 2) and between TAS and MDA levels (r = -0.850; p < 0.001) as shown in (Fig. 3).

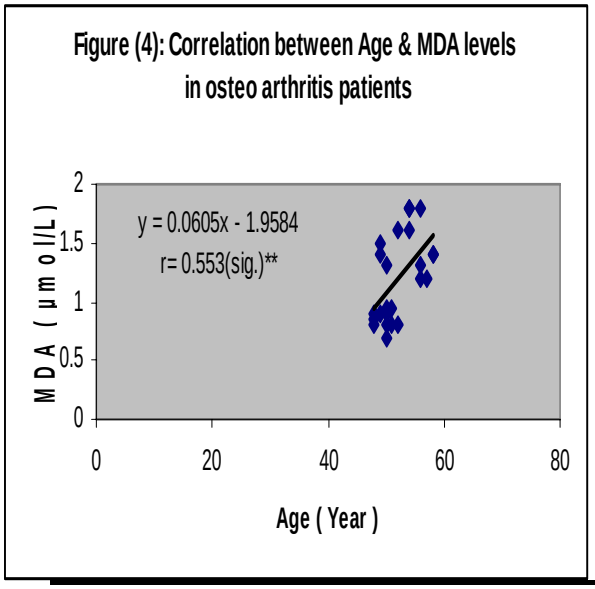
In OA group, the level of CRP was significantly increase (P< 0.05) and the percentage of increment was 91.64 % when compared with control group, no significant differences were observed between TAS and MDA levels on comparison with control. There were no significant correlation between these parameters in OA patients, the only significant correlation was between age and MDA level (r = 0.553 ; P < 0.05) as shown in (Fig. 4).

Figure (1): Correlaion between MDA &CRP levels in rheumatoid arthritis patients









Discussion:

Rheumatoid arthritis and osteoarthritis are the most common inflammatory diseases worldwide. In the present study serum TAS levels were significantly lower in the RA group than the OA and control groups, while serum MDA and CRP concentrations were significantly higher in patients with RA than those with OA and healthy subjects. These results are consisted with many studies [5,11,13], were an increased levels of lipid peroxidation reaction and lower TAS level in RA patients as compared to healthy subjects were reported.

Our previous study [12] had shown a decreased antioxidant level (glutathione) and an increase in the MDA level in erythrocyte and plasma of patients with active RA compared with healthy subjects. Antioxidants hypothesized to provide protection against RA, was 400mg of vitamin E and 100mg vitamin C given twice daily and were significantly effective in reducing duration of morning stiffness, Ritchie joint index, (patient and physician global assessment of disease activity parameters in RA patients). Wang et. al.[4] found that dietary supplementation with vitamin E alone reduces the base line inflammatory status, that is indicated by CRP concentration in healthy baboon.

A significant increase in MDA and a decrease in TAS levels in the present study reveals that there is an increase in the oxidative stress in RA. The negative correlation between MDA, CRP and TAS parameters can be explained by the impairment of the antioxidant defense mechanisms due to excess utilization by the inflamed tissue to scavenge the excessive lipid peroxide that are generated at the inflammatory site or to scavenge accumulated lipid peroxides in the plasma[22]. Increase in the *in vivo* generation of oxidants and lipid peroxidation product was demonstrated in plasma of RA patients which correlated with the antioxidant level [14].

This may indicate evidence of oxidative stress in those patients and that it plays an important role in the pathogenesis of the disease[1]. CRP was positively associated with MDA and this could give a clue the extent and rate of tissue damage due to increase oxidative stress and increased lipid peroxidation. Sukkar and Rossi [25] had supposed that oxidative stress may trigger inflammatory activity and therefore it may induce a flare of the disease.

Many studies have reported higher oxidative stress in OA [23,24] and this is in accordance with our results, where the only significant increase was in the CRP level. This increase combined with haemostatic and haemodynamic reaction to the stress task could give an indication to the disease activity in OA patients[6]. Kumon et.al. [17] found

that serum and synovial CRP levels were significantly higher in RA than OA patients, and it correlate with ESR in all arthritis patients.

In this study MDA levels were significantly correlated with age in OA patients. Our results are consisted with the hypothesis that with age the prevalence of OA increases and the efficacy of articular cartilage repair decrease[26]. It was suggested that *in vivo* chondrocyte essences contributes to the age related increase in the prevalence of OA and decrease in the efficacy of cartilage repair. Kehan and Archer[6] found that articular cartilage is susceptible to many forms of injury some of which may lead to secondary arthritis at much later time.

Numerous medical studies [22,25] concluded that oxidative stress is the root cause of arthritis when the antioxidant defense system is over whelmed, this oxidative stress within the joint causes damage to the surrounding cartilage [4]. In OA and RA, there is a focal loss of cartilage resulting from catabolic pathway in the joint over production of free radicals and lack of oxygen processing enzymes and free radical scavenging molecules can lead to inflammation. Inflammatory products like phagocytosis from neutrophils create even more free radicals [2].it is this viscious low grade inflammatory response that leads to the destruction f the joint and this may contribute to the pathogenesis of the disease [24,25].

Increasing numbers of health care recognizes the need for diagnostic laboratory tests that measure oxidative damage and the status of the individuals antioxidant defenses. Given the multiplicity of antioxidant and the influences of life style and nutritional supplements on an individuals antioxidant capacity, it is important to be able to quantitively measure the total antioxidant capacity of the antioxidant power within biological specimens and use it as a routine work to detect the level and to correlate it with the disease activity for patients at various stages of the disease.

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