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Assessment of Lipid Peroxidation and Non-Enzymatic Antioxidants in Patients of Rheumatoid Arthritis

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a systemic disease which affects approximately 1-2% of the total world's population. Recent findings indicate that increased oxidative stress and/or defective antioxidant status contribute to the etiology of RA. Objective: The objective of the present study was to assess oxidative stress by measuring malondialdehyde and antioxidant status by estimating vitamins C and E levels in rheumatoid arthritis patients. Methods: 30 patients with RA and 30 normal healthy individuals were included in this study. MDA was measured by the method described by Kei Satoh. Vitamin C was measured by the method decribed by Roe and Kuther's method. Vitamin E was measured by Baker and Frank Method Results: The mean MDA level was significantly increased in RA patients as compared to controls where as mean Vitamins C and E levels were significantly decreased in RA patients compared to control group. Conclusion: From our study, it is possible to conclude that increased oxidative stress in RA patients evidenced by increased serum MDA, have led to compensatory changes in the levels of some non enzymatic antioxidants, such as vitamins C and E. These changes, in turn, may due to the increase turn over, for preventing oxidative damage in RA.

KEYWORDS Rheumatoid arthritis, Oxidative stress, Antioxidants, Vitamin C, Vitamin E.

Introduction:

Rheumatoid arthritis (RA) is a systemic disease characterized by progressive, erosive, and chronic polyarthritis. Cellular proliferation of the synoviocytes and neo-angiogenesis leads to formation of pannus which destroys the articular cartilage and the bone[1]. Rheumatoid Arthritis (RA) affects approximately 1-2% of the total world's population[2]. Annual incidence rate of rheumatoid arthritis between 0.5%-1% of total population is reported every year in both developed and developing countries[3]. Lower incidences of rheumatoid arthritis are reported every year in East Asia[4]. Rheumatoid Arthritis affects around 1 in 50 people and is more common in women than men. It is most common after the age of 40, but can happen at any age[5].

Free radicals, the highly reactive entity and very short-lived molecules, are constantly produced in a wide variety of normal physiological functions. Although free radicals perform some useful functions, they are toxic when generated in excess. The most important characteristic of toxic free radicals either ***in vivo*** or ***in vitro*** is peroxidation of lipids resulting in tissue damage and death of affected cells[6]. The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several pathophysiologies[5]. Alteration in the oxidant-antioxidant profile is known to occur in rheumatic diseases[7]. Moreover the body's defense mechanisms would play an important role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated. They exist in both the aqueous and membrane compartment of cells and can be enzymes or non-enzymes[5].

In recent years, it has been shown that oxidative stress and antioxidants play an important role in the disease process of RA. So, the present study was undertaken to assess oxidative stress by measuring malondialdehyde and antioxidant status by estimating vitamins C and E levels in rheumatoid arthritis patients.

Materials and Methods:

The present work was carried out in the Department of Biochemistry, Muzaffarnagar Medical College, Muzaffarnagar, India. The study was undertaken between October 2014 to September 2015. The investigations were carried out in Biochemistry Laboratory, Muzaffarnagar Medical College and Hospital, Muzaffarnagar, India**.**

Subjects:

A total 60 subjects of both sex groups, aged between 40-60 years were enrolled for the present study. Out of 60 subjects, 30 were patients of rheumatoid arthritis and 30 were normal healthy individuals. Patients were diagnosed by the criteria's recommended by the American Rheumatism Association[8]. Normal healthy individuals serve as controls for the study and were selected from general population of the same region. Participants were informed about the study. Informed consent was taken from each subject.

Exclusion criteria:

The individuals suffering from osteoarthritis, tubercular arthritis, infective arthritis, rheumatic fever, pulmonary tuberculosis, pneumonia, costochondritis (Tietze's disease), arthritis other

than RA fitting into any syndrome, chronic smokers & alcoholics and any other systemic disease where free radical damage has been incriminated were excluded from the study.

Collection of Blood Sample:
Fasting venous blood samples were collected in plain vacutainers without any anticoagulant from both the groups. Blood collected without anticoagulant was centrifuged at 3000 rpm for 5 minutes. Serum was collected carefully and used for estimation of MDA, Vitamin E and Vitamin C.

Parameters Measured:
- The following parameters were measured in the present study-
- MDA was measured by the method described by Kei Satoh[9].
- Vitamin C was measured by the method described by Roe and Kuther's method[10].
- Vitamin E was measured by Baker and Frank Method[11].

Statistical analysis:
Results were statistically analyzed by 'GraphPad QuickCals t-test calculator'. Student's t-test was used to assess the significance of difference between the groups. All results are presented as mean ± S.D. A ***'p'*** value of less than 0.05 was considered significant.

Results:

Parameters	Patients with Rheumatoid Arthritis	Normal Healthy Individuals (Controls)	p-value
MDA (nmol/ml)	3.76±0.15	1.46±0.14	<0.0001
Vitamin C (mg/dl)	0.81±0.04	1.38±0.09	<0.0001
Vitamin E (mg/L)	7.37±0.91	9.80±0.50	<0.0001

Table 1: Showing mean and standard deviation of MDA, Vitamin C and Vitamin E in patients with rheumatoid arthritis and normal healthy control group.

The mean MDA level in patients with rheumatoid arthritis was increased compared to control group and was statistically significant where as there were significant decrease in the mean levels of vitamins C and E in rheumatoid arthritis patients compared to normal healthy controls.

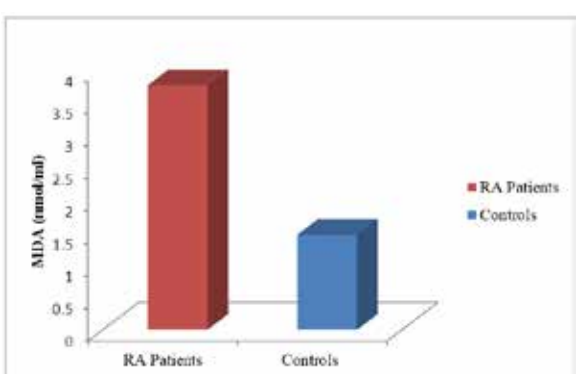

Figure 1: Showing comparison of MDA between rheumatoid arthritis patients and controls.

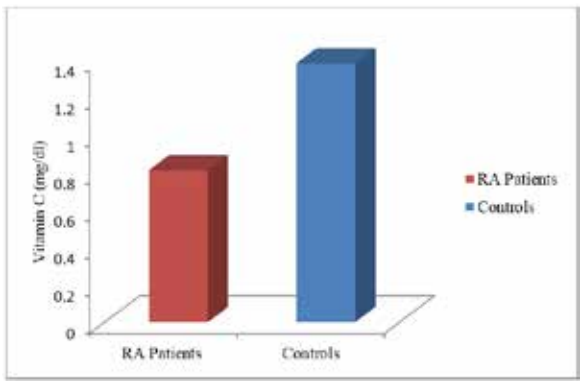

Figure 2: Showing comparison of Vitamin C between rheumatoid arthritis patients and controls.

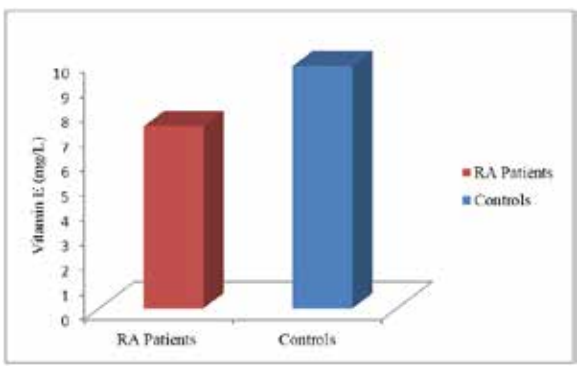

Figure 3: Showing comparison of Vitamin E between rheumatoid arthritis patients and controls.

Discussion:
Rheumatoid arthritis is a major cause of morbidity as it affects the joints, causing stiffness and loss of mobility. The cause of rheumatoid arthritis is mainly joint inflammation initiated by oxidative stress[12]. 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[13].

Lipid peroxides that are generated at the site of inflammation of tissue injury diffuse into blood and can be estimated in serum or plasma, which inturn reflect the severity of the tissue damage[14]. The main product of lipid peroxide is MDA and it is potent marker of oxidative stress in RA. ROS and other oxidants have been shown to be formed even in normal physiological process[15]. It is known that activated inflammatory cells lead to ROS production in RA, a systemic autoimmune disease[16]. Increase ROS in turn, increase lipid peroxidation products and cause tissue injury. Oxidative stress constitutes a serious pathophysiological factor for a wide variety of connective tissue disorder such as RA[17].

In the present study, mean MDA level was found to be significantly elevated in patients with RA compared to control group. This is in accordance with other studies where higher MDA levels have been reported in patients of RA[12,13,18,19,20]. MDA is a decomposition product of lipid peroxidation of polyunsaturated fatty acids which is used as an index of oxidative damage. Enhanced lipid peroxidation may occur as a result of imbalance between scavenging mechanisms and free radical generation process[12]. The mean levels of vitamins C and E were significantly decreased in cases compared to controls. These findings are in accordance with study done by Kowsalya et al[21], Karatas et al[20] and Vijaykumar et al[6]. Singh et al[17] also observed low level of vitamin C in RA patients which is in agreements with our study. Dwivedi et al[22] found that increased level of MDA and decreased levels of antioxidant vitamins (vitamins C and E) in anemic rheumatoid arthritis patients, which also supports our study.

The decrease in the levels on non-enzymatic antioxidant may be due to the increase turn over, for preventing oxidative damage in RA[22]. Vitamin E helps to trap free radicals and interrupt the chain reaction that damage the cells whereas regeneration of vitamin E depends on Vitamin C. Due to increased oxidative stress in RA there may be increased consumption of both of these vitamins[23]. There is substantial evidence indicating that a low antioxidant status is associated with a higher risk of developing RA. Furthermore, the disease process is associated with an increased generation of oxidant, which plays an important role in the inflammatory process and contributes to tissue destruction[24]. Antioxidant defenses limit the damages caused by oxidants, such as those formed during inflammation. In addition, in vitro studies and animal studies have shown that antioxidants also possess anti-inflammatory properties[25]. This implies that antioxidative defense mechanisms are of particular importance for patients with RA.

Conclusion:

From our study, it is possible to conclude that increased oxidative stress in RA patients evidenced by increased serum MDA, have led to compensatory changes in the levels of some non enzymatic antioxidants, such as vitamins C and E. These changes, in turn, may due to the increase turn over, for preventing oxidative damage in RA. These findings confirm the role of oxidative stress in the pathogenesis of RA and antioxidants can serve as surrogate markers for disease activity in RA.

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