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## Original Research Article

# Assessment of Risk of Hypertension in Active Rheumatoid Arthritis Patients

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**Abstract:** Rheumatoid arthritis is a systemic inflammatory disease characterized by swelling and pain of the joints. Although, persistent inflammation mediated bone or joint destruction restricts the patient's physical function, it is conceivable that oxidative stress mediated electrolyte imbalance may have a crucial role in the development of hypertension risk in active rheumatoid arthritis (ARA). Aims & objective: The present study was designed to assess the association of oxidative stress and altered serum electrolyte levels in ARA patients and to determine their effect in predicting hypertension (HT) risk. Methods: Total antioxidant activity (TAA), lipid peroxidation (malondialdehyde; MDA) and serum mineral ( $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$) levels were estimated in 40 ARA patients by using standard methods and statistically compared it with that of 40 healthy normal individuals of same age group (30-50 years). Result: Plasma TAA, serum potassium, magnesium and calcium levels were significantly low in patient group ($p<0.05$) as compared to healthy controls whereas erythrocyte MDA levels were significantly high in ARA subjects. However, serum sodium levels were increased insignificantly ($p<0.1$) in ARA subjects. Conclusion: Our findings indicate that oxidative stress plays a significant role in shaping the ARA patient to develop hypertension, characterized by altered serum minerals levels and enhanced MDA levels. Therefore, regular monitoring of blood pressure and consumption of balance diet rich in antioxidants and minerals should be increased to reduce the risk of HT with ARA progression.
**Keywords:** Total antioxidant activity, Malondialdehyde, Calcium, Magnesium, Potassium.

## INTRODUCTION:

The belief that infectious agents are a cause of chronic inflammatory diseases of unknown etiology and of active rheumatoid arthritis (ARA) is not new. Last few decades, doctors noted a connection between arthritis, inflammation, oxidative stress and cardiovascular complications that transcended mere coincidence [1]. Reactive oxygen species derived from molecular oxygen (Superoxide anion, hydrogen peroxide and hydroxyl radical) contribute to the tissue injury, which accompanies inflammatory and age associated disorders including rheumatoid arthritis, osteoarthritis, psoriasis and cardiac diseases [2, 3, 4, 5].

ARA is the most common arthritic condition and the leading cause of chronic disability in arthritic population, has been found to be associated with variety of common risk factors of hypertension such as age, sex, increase in body weight and smoking etc. [6, 7]. In this context, the role of oxidative stress has now been receiving much attention towards solving the unanswered question related to ARA pathophysiology with future risk of hypertension (HT). Oxidative stress mediated by free radicals can evade or overwhelm the antioxidant protective mechanism of cells and may cause cell membrane and cartilage destruction, DNA strand breakage, rises in intracellular free $Ca^{2+}$, damage to membrane ion transporters and other specific proteins leading to cell death followed by disease development [8, 9].

Physiologically important elements responsible for electrolyte balance include sodium, potassium, magnesium and calcium, and their optimal concentration for proper biochemical and physiological activities of the cell is maintained by ion channels. Interestingly, the role of various ion channels in CVD, CNS, taste sensation, skeletal muscle, renal, respiratory, pancreatic, and erectile and platelet function have well elucidated [10]. Inactivation of these ion channels may

produce various sorts of age related disorders including cartilage degradation in ARA patients and thereby making them physical and functional disable may be attributed to altered ionic homeostasis.

Free radicals attack on polyunsaturated fatty acid in the membrane lipids and thereby causing lipid peroxidation, a major event in the development of HT and ARA as well. Malondialdehyde (MDA), the most abundant product of lipid peroxidation, reacts with membrane proteins and ion channels, affecting their normal function and thereby may cause electrolyte imbalance [6, 9]. In order to overcome the load of free radicals, antioxidant defense system of the body (including enzymic and non-enzymic antioxidants) as determined by estimating total antioxidant activity (TAA), plays a dynamic role by regulating free radical production and thereby prevents the development of various sorts of disorders with advancing age such as hypertension and arthritis etc. Amusingly, alteration in antioxidant defense system in knee osteoarthritis patients and in older population has been well documented [11]. However to best of our knowledge, this is the first study addressing the relation of oxidative stress mediated electrolyte imbalance in active rheumatoid arthritis patients to predict future HT risk. Therefore, the overall objectives of present study were to ascertain the marker of oxidative stress and serum minerals ($Na^+$, $K^+$, $Mg^{2+}$ & $Ca^{2+}$) levels in ARA patients and their role in prediction of HT risk.

## MATERIAL AND METHODS:

In the present study 40 patients of either sex with active rheumatoid arthritis belonged to age group 30-50 years and 40 age matched healthy individuals, served as control, were taken. A general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination including blood pressure measurement was completed from all the subjects after taking their informed consent and approval of protocol by ethics committee of college.

**Inclusion criteria:**

Subjects, who gave informed consent for study, having no history of any type of arthritis, don't under any medical treatment (anti-inflammatory drug) or taking antioxidant supplement for at least 1 month prior to blood collection were included. All patients had active RA, defined as the presence of at least three of the following criteria: six or more tender joints; three or more swollen joints; ≥ 30 min of morning stiffness; an erythrocyte sedimentation rate of ≥ 28 mm/h. Patients were required to have pain on more than half the days of a month and at least pain score above 20% using a 5 cm visual analogue scale (VAS) [12]. Patients already receiving anti-inflammatory drugs were not excluded if the dosage and regularity of administration was not expected to alter during last three months.

**Exclusion criteria:**

Patients with diabetes mellitus, hepatic disease, hypertension, those taking antioxidant vitamin supplements or non-steroidal anti–inflammatory drugs and with other connective tissue disease like systemic sclerosis and osteoarthritis were excluded.

Fasting blood samples were collected in plain vial (for serum minerals estimation) and in EDTA vial from anticubital veins avoiding venostasis from each patients and healthy controls. Samples were processed immediately for plasma and serum separation. Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reactive substances, after preparation of hemolysate [13]. The heat induced reaction of malondialdehyde (MDA) with thio barbituric acid (TBA) in the acid solution forms a trimethine coloured substance, which is measured spectrophotometrically at 532 nm.

Plasma total antioxidant activity was estimated spectrophotometricaly by the method involving reaction of standardized solution of iron EDTA complex with hydrogen peroxide i.e. Fenton type reaction, leading to the formation of hydroxyl radicals. This reactive oxygen species degrades benzoate, resulting in the release of thio barbituric acid reactive substances (TBARS). Antioxidants from the added plasma cause the suppression of TBARS production. The reaction was measured spectrophotometrically at 532 nm [14].

Serum electrolytes ($Na^+$ and $K^+$) levels were measured by Sinha method by using flame photometer in which test sample is aspirated followed by calculation of test sample value from calibration curve of standard solution (i.e. NaCl and KCl solution) [15]. Serum magnesium levels were estimated by Neill and Neely method in which protein free filtrate is treated with titan yellow solution. A red color complex is formed which is measured at 520 nm [16]. Serum calcium levels were estimated by Tinder's method. Calcium in an alkaline medium combines with o-Cresol phthalein Complex one to form a purple coloured complex, which is measured at 570 nm [17].

**Statistical analysis:**

The data collected from study group subjects were entered separately in Microsoft Excel sheet of windows 2007 and values were expressed as Mean ± SD. The significance of mean difference between study group subjects was compared by using Student's t test. The distribution of 't'- probability was calculated depending on 'n' and significance of test was obtained. P value <0.05 and <0.001 were considered as significant and highly significant respectively.

**RESULT:**

The demographic indexes along with clinical profile of ARA patients and controls are depicted in Table 1. All the ARA patients fulfilling inclusion criteria have characteristic pain and inflammation as revealed in Table 1. VAS score was above 20%. Markers of oxidative stress and serum minerals levels in active rheumatoid arthritis patients and control group were represented in Table 2. Marked reduction ($p<0.05$) in plasma TAA levels were observed in patients group i.e. 26.05% low as compared to healthy controls. Conversely, erythrocyte MDA levels were increased significantly ($p<0.001$; 40.0% high) in ARA subjects as compared to healthy controls. Similarly, serum $K^+$, $Mg^{2+}$ and $Ca^{2+}$ levels were decreased in ARA subjects i.e. 16.0%, 23.75% & 19.78% low (Table 2). Statistically, these levels were altered significantly ($p<0.05$) in ARA subjects as compared to controls. However, serum sodium levels were increased insignificantly ($p<0.1$; 13.14% high) in ARA subjects with respect to control.

**Table 1: Demographic profile of Active rheumatoid arthritis patient and Control groups (Mean ± SD)**

| Parameter | Control Group (n=40) | Patient group (n=40) | % Increase | % Decrease |
|---|---|---|---|---|
| Age (years) | 58.2 (5.0) | 60.7 (4.2) * | - | - |
| M:F ratio | 1:1 | 1:1 | - | - |
| Height (meter) | 1.57 (0.030) | 1.59 (0.025) | - | - |
| Weight (Kg) | 58.4 (1.7) | 62.4 (2.4) | - | - |
| BMI ($Kg/m^2$) | 22.6 (1.2) | 24.5 (1.3)* | 8.40 % | - |
| Systolic blood pressure (mm Hg) | 108.5 (3.42) | 114.30 (4.28) | | |
| Diastolic blood pressure (mm Hg) | 74.3 (2.35) | 77.4 (2.38) | | |
| VAS (mm) | 0.0 | 37.08 (4.2) | - | - |
| ESR (mm/h) | 15.2 (2.17) | 32.4 (3.74)** | 113.2% | |

Where,
* $p < 0.1$ : Non-significant;
** $p < 0.05$ : Significant,
BMI, Body mass index;
ESR, Erythrocyte sedimentation rate

**Table 2: Markers of oxidative stress and serum minerals levels in active rheumatoid arthritis patients and control group (Mean ± SD)**

| S.No. | Particulars | Control group (n=40) | Patient Group (n=40) | % increase | % decrease |
|---|---|---|---|---|---|
| 1) | Malondialdehyde (µmol MDA/ml) | 2.40 ± 0.12 | 3.36 ± 0.11*** | 40.0% | - |
| 2) | TAA level (m mol/L) | 1.42 ± 0.16 | 1.05 ± 0.72** | | 26.05% |
| 3) | Sodium level (meq/L) | 137 ± 6.2 | 155 ± 5.4* | 13.14% | - |
| 4) | Potassium level (meq/L) | 3.75 ± 0.50 | 3.15 ± 0.40** | - | 16.0%, |
| 5) | Magnesium level (mg %) | 2.82 ±0.74 | 2.15 ±0.52** | - | 23.75% |
| 6) | Calcium level (mg %) | 9.25 ±0.68 | 7.38 ±0.43** | - | 19.78% |

Where,
. * $p<0.1$: Non-significant;
** $p<0.05$: Significant;
*** $p<0.001$: Highly significant

**DISCUSSION:**

It has been well documented that the rheumatoid arthritis progression is associated with inflammation, oxidative stress and thereby impaired cardiovascular fitness [2, 11, 18]. In this context, depletion in total antioxidant activity (TAA) indicates

the disturbance in the antioxidant defense system of the body, which could be responsible for the initiation of disease development with senescence. In the present study, plasma TAA levels were found to be significantly low ($p<0.05$) in ARA subjects which could be explained on the basis of contributory effect of reduced enzymic and non-enzymic antioxidant levels, due to augmented oxidative stress in ARA. Recently, marked reduction in TAA in inflammatory diseases including arthritis as well as elderly hypertensive subjects has been documented, which were in concordance with our findings and clarify the common culprit effect of oxidative stress in both the diseases [7, 18, 19].

Enhanced oxidative stress in ARA characterized by reduction in total antioxidant activity therefore means increased production of $H_2O_2$ or incomplete scavenging of $O_2^{\cdot-}$ radicals. These events eventually lead to further destruction related to HT risk in ARA which include membrane damage via lipid peroxidation, ion transporters and electrolyte imbalance. In addition, deleterious role of lipid peroxidation is also implicated to structural modification of complex lipid protein assemblies associated with cellular malfunction.[4] Moreover, lipid peroxidation also contributes local membrane destabilization that alters endothelial or intimal cells architecture of the blood vessels, cell signaling, proper trafficking of intracellular vesicles, phagocytosis, degranulation, antigen presentation and many other processes leading to disease complexity [20].

Moreover, biologically important elements ($Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$) have also significant role in maintenance of homeostasis by participating in various physiological activities such as neuromuscular irritability, nerve conduction, inhibition of free radical formation, in proper functioning of enzymes which include Na- K ATPase, Ca-ATPase, enzymes of carbohydrate and fatty acid metabolism; cell division, calcification of bones and teeth, in the synthesis of ATP, DNA, RNA and protein; inhibition of platelet aggregation, absorption of amino acids and in the prevention of development of various complications [21, 22, 23, 24]. Altered levels of these elements may induce series of events which in turn associated with symptoms of ARA and hypertension.

In the present study, malondialdehyde levels (marker of lipid peroxidation) were also found to be significantly high in ARA subjects ($p<0.001$, Table 2) in association with significantly altered levels of serum minerals ($Na^+$, $K^+$, $Mg^{2+}$ & $Ca^{2+}$) which indicate that ARA patients are not only closely associated with oxidative stress mediated electrolyte imbalance but also susceptible to develop future HT. Similarly, increased levels of MDA were also reported in the previous studies on rheumatoid arthritis, osteoarthritis as well as in HT subjects [7, 8]. It has been documented that lipid peroxidation initiates a complex cascade such as inhibition of NO, enhancement of cytosolic free calcium, electrolyte imbalance and leakage of lysosomal hydrolases via breakdown of lysosomal membrane which cause dystrophic changes in muscle fibers leading to weakness of muscles and difficulty in performing simple tasks. In addition, free radical mediated lipid peroxidation also causes electrolyte imbalance not only by injuring $Na^+$-$K^+$ ATPase but also by interfering with normal interaction of membrane pumps (including Na–Ka–2Cl co-transporter and $K^+$ channel) and production of protein radical in lipid membranes that effects normal ion transport, and thereby shaping ARA patients susceptible to develop HT [25, 26].

**CONCLUSION**:

On the basis of findings of present study, we conclude that the oxidative stress plays a crucial role in shaping active rheumatoid arthritis patients more susceptible to develop HT, characterized by alteration in systemic antioxidant status, lipid peroxidation and electrolyte imbalance. Furthermore, consumption of diet rich in antioxidant vitamins along with adequate mineral supplement should be increased with appearance of disease symptom, which may be effective in the prevention and management of HT in active rheumatoid arthritis patients. However, to validate the findings of the current study, multicenter study with large sample size should be carried out to support the findings of the current observation from this study.

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