STATUS OF ANTIOXIDANTS IN RHEUMATOID ARTHRITIS PATIENTS USING NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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ABSTRACT

INTRODUCTION
Elevated free radical generation in inflamed joints and impaired antioxidant system have been implicated in rheumatoid arthritis (RA). The present study aimed at investigating the antioxidant system in patients with untreated RA, treated RA with NSAIDs and healthy controls. 100 RA patients, 50 untreated (gp I) and 50 treated with NSAIDs (gp II) are compared with 50 healthy controls were included in the present study. Levels of antioxidants including uric acid, caeruloplasmin, vitamin C and vitamin E were measured. Significant increase in uric acid and caeruloplasmin was observed, which on treatment with NSAIDs showed significant decrease in the respective levels indicating beneficial effect of NSAIDs. Levels of vitamin C and vitamin E in RA patients were significantly lower in RA patients than those of the controls subjects, which on treatment with NSAIDs, further reduced the levels. These results suggest that proper antioxidant nutrients intake may reduce free radical generation and improve antioxidant status in RA patients.

KEYWORDS
Antioxidant, Uric acid, Caeruloplasmin, NSAIDs.

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INTRODUCTION: Arthritis, the joint inflammation, refers to a group of diseases that cause pain, swelling, stiffness and loss of motion in the joints. Rheumatoid Arthritis (RA) is a chronic, systemic disease, in which various joints in the body are inflamed, leading to swelling, pain, stiffness, and the possible loss of function. It is an autoimmune disease in which the body's immune system attacks itself. Rheumatoid Arthritis affects approximately 1-2% of the total world's population.(1)

ROLE OF FREE RADICAL: Free radicals and Reactive Oxygen Species (ROS) are extremely reactive molecule generated as metabolic by products. Physiological role of these agents is for cellular defense against pathogenic organism. However, excessive amount of free radicals and ROS can lead to cellular damage and development of inflammatory, degenerative and neoplastic diseases. These free radicals induced injuries and consequently pathogenic processes can be prevented by antioxidants and enzymes. Large numbers of diseases are associated with an increased production of free radicals, which also include inflammatory disease like Rheumatoid Arthritis.

In rheumatoid arthritis, the role of mediators of inflammation like prostaglandins and leukotrienes has been established. In these patients neutrophils migrate into the synovium and release free radicals that attack and damage hyaluronic acid and cartilage of synovial fluid, thus contributing to the destruction of joints.(2)

Reactive Oxygen Species (ROS) plays a significant role in the pathogenesis of rheumatoid arthritis. The synovial fluid of the inflamed rheumatoid joints swarms with activated neutrophils which produce large amounts of superoxide radical (O_2), hydrogen peroxide (H_2O_2) and highly reactive hydroxyl radical (OH).(3)

ROLE OF ANTI-INFLAMMATORY DRUGS: The basic conventional treatment for rheumatoid arthritis consists of Non-Steroidal Anti-Inflammatory Drugs (NSAID's) including aspirin.

More recently we have a new generation of NSAID's which spare the stomach, but control pain and swelling. These are called COX II inhibitors. For example, celecoxib, Rofecoxib. Secondly, there are steroids. They are also anti-inflammatory drugs. They can produce almost instant relief from pain, but they invariably produce side effects if given in high doses or over-prolonged periods.

All NSAID's share a common mechanism of inhibiting the synthesis of Prostaglandins (PGs) from arachidonic acid. Recent research on the arachidonic acid pathway showed that there are 2 routes for prostaglandin production (Fig. 1)

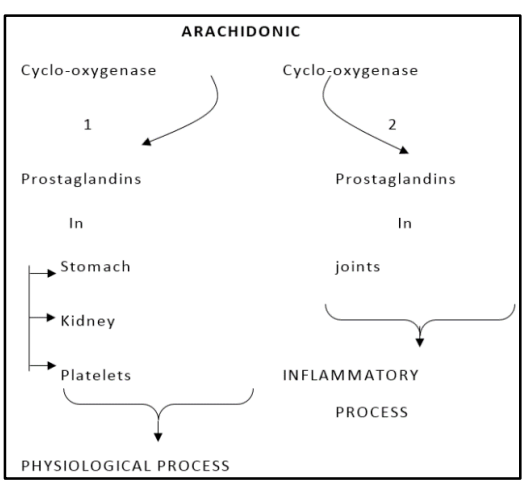

Fig. 1

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The route involving cyclo-oxygenase I (COX-1) produces prostaglandins for gastro-protection and renal perfusion. The other involving COX-2, produces pathological prostaglandins which cause inflammation of joints.[4]

ANTIOXIDANTS IN RHEUMATOID ARTHRITIS: Free radicals are produced as a normal consequences of aerobic metabolism, phagocytic activity and prostaglandin synthesis. These oxygen free radicals have been implicated as mediators of tissue damage in patients with rheumatoid arthritis (RA). Under normal circumstances, the potential damaging effects of these toxic species are limited by antioxidants.[5]

The term antioxidants have been defined by Gutteridge and Halliwell as "Any substance that delays or inhibits oxidative damage to a target molecules".[6]

A range of antioxidant defenses are present in cells and body fluids which protect cellular components from free radical damage. The most widely recognized ones are uric acid, vitamin C, vitamin E and ceruloplasmin.

Uric Acid is produced by the oxidation of hypoxanthine and xanthine-by-xanthine oxidase. Ames et al.[7] demonstrated that free radical attack on uric acid generates allantoin.

Ascorbic Acid (Vit. C) is a powerful water-soluble reducing agent, which is readily and reversibly oxidized to dehydroascorbic acid. Ascorbate/DHA ratios seem to be maintained very high levels in the body fluids and tissues during normal conditions, i.e. almost no DHA is present. This ratio has been reported to fall only in some diseases including rheumatoid arthritis and diabetes.[8]

Vitamin-E is widely recognized as being one of the most important lipid soluble antioxidants, as it has been shown to modulate the activity of the cyclo-oxygenase and lipo-oxygenase enzymes. These enzymes are involved in the conversion of arachidonic acid to pro-inflammatory prostaglandins and leukotrienes, respectively. According to Edmonds SE, et al. on the clinical studies using vitamin E as a treatment for rheumatoid arthritis have shown more modest improvement, however, concluded that vitamin E's small but significant pain-relieving effect justifies using this vitamin as a complement to standard anti-inflammatory treatment.[9]

CERULOPLASMIN: Recently, it has been suggested that ceruloplasmin may be the major antioxidant in serum, based on its ability to inhibit auto oxidation of lipids induced by ascorbic acid or by inorganic iron Cells and tissues are protected from injury induced by superoxide anion radicals (O_2^-) primarily by means of superoxide dismutase (SOD), a copper containing intracellular enzyme that catalyzes dismutation of the free radicals to hydrogen peroxide and oxygen. This function of cytoplasmic SOD can be mimicked by the copper containing plasma protein ceruloplasmin.[10]

AIM OF THE STUDY: The aim of our study was to investigate the antioxidants activity in RA patients as well as the use of NSAIDs on these patients with active disease.

MATERIAL AND METHOD: The study population selected was from the city of Allahabad (UP); 150 volunteers, both male and females between 20-60 yrs. of age were taken up for the proposed study. They were further classified into: Control group consisting of healthy males and females, Group I consisting of individual suffering from rheumatoid arthritis and Group II consisting of rheumatoid arthritis patients taking anti-inflammatory drugs.

Blood samples were collected from the subjects in heparinized vial, 0.5 ml. of whole blood was centrifuged for 10 min. at 3000 rpm. Determination of vitamins, i.e. Vit. C and Vit. E by Roe and Kuether method.[11] and Emmere-Engel method.[12] respectively. Uric acid is estimated by Caraways method.[13] and ceruloplasmin by Ravin's method.[14]

STATISTICAL ANALYSIS: Values are expressed as mean±S.D. The significance of mean difference between groups was analysed by the student 't' test and distribution of probability 'P'.

OBSERVATIONS: Observations tables and graph of various parameters are tabulated as follow:

Particulars	Control group	Group–I	Group–II
Sample size	50	50	50
Mean	4.7	8.9	7.6
±S.D	0.213	0.179	0.216
%INC	-	89.3% ↑	61.7% ↑
%DEC	-	-	-
t-value	-	20.65	13.8
p-value	-	<0.001	<0.001

Table 1: Uric acid (mg/dl)

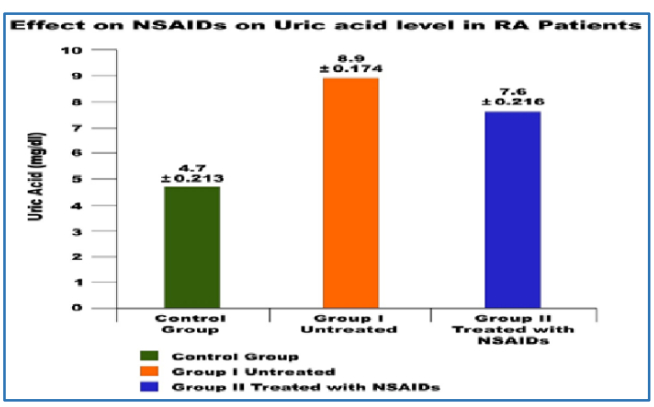

Fig. 2

Particulars	Control group	Group-I	Group-II
Sample size	50	50	50
Mean	23.6	31.5	24.1
±S.D	0.883	0.817	0.560
% INC	-	33.4% ↑	2.1% ↑
% DEC	-	-	-
t-value	-	9.1	0.66
p-value	-	<0.01	>0.05

Table 2: Ceruloplasmin (mg/dl)

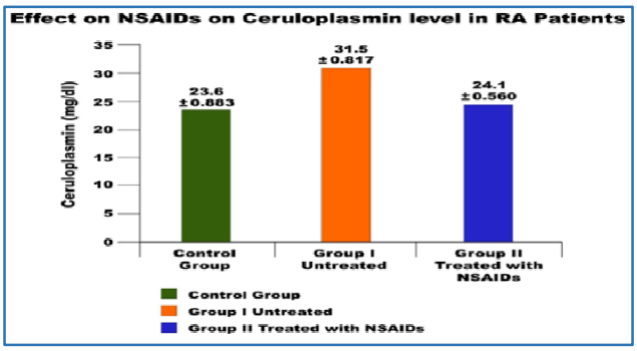

Fig. 3

Particulars	Control group	Group-I	Group-II
Sample size	50	50	50
Mean	0.9	0.31	0.28
±S.D	0.097	0.018	0.015
%INC	-	-	-
%DEC	-	65%↓	68%↓
t-value	-	8.4	8.85
p-value	-	<0.001	<0.001

Table 3: Vitamin-C/ Ascorbic acid (mg/dl)

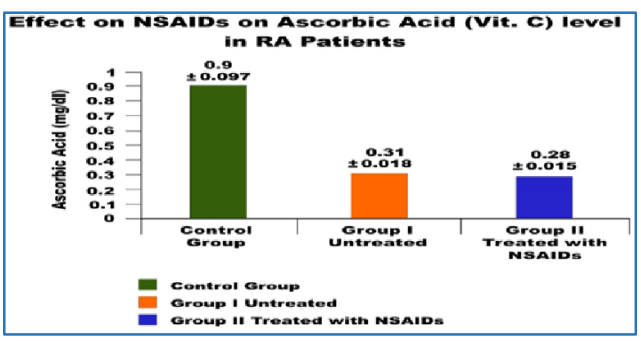

Fig. 4

Particulars	Control group	Group-I	Group-II
Sample size	50	50	50
Mean	1.2	0.24	0.17
±S.D	0.095	0.402	0.002
%INC	-	-	-
%DEC	-	80%↓	85.8%↓
t-value	-	13.71	15.14
p-value	-	<0.001	<0.001

Table 4: Vitamin -E/α-Tocopherol(mg/dl)

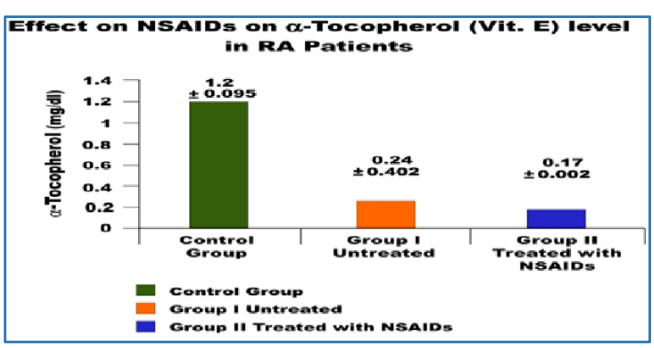

Fig. 5

RESULTS: Of the 100 RA patients enrolled in the study, 50 patients were taking medication for symptomatic relief and had been taking the same medication for at least 4 months. Of the vitamins analysed, ascorbic acid and α-tocopherol tended to be lower in RA patients as compared to the controls. The mean levels of plasma ascorbate was 0.31 mg/dl and α-tocopherol was 0.24mg/dl in rheumatoid arthritis cases. Among controls the levels of ascorbate and α-tocopherol were 0.9mg/dl and 1.2mg/dl respectively. The mean differences observed between the RA subjects and controls in serum levels of the antioxidant vitamins was statistically significant ($p<0.001$).

In the NSAIDs treated RA subjects (Group II) levels of ascorbate and α-tocopherol revealed a significant depletion ($p<0.001$) with mean levels of ascorbate and α-tocopherol being 0.28mg/dl and 0.17mg/dl respectively i.e. a depletion of almost 68 percent of ascorbate and 85.8 percent of α-tocopherol as compared to the controls.

Table 4 represents, the serum concentration of uric acid of untreated (Group I) and treated (Group II) rheumatoid arthritis patients with non-steroidal anti-inflammatory drugs (NSAIDs). The observed uric acid level of untreated was significantly increased ($p<0.001$) by 89.3% as compared to control, which on treatment with NSAIDs was reduced by 27.6 percent.

The mean ceruloplasmin activity in serum was significantly raised ($p<0.01$) by 33 percent in Group I comprising of rheumatoid arthritis (RA) subjects, but there was an insignificant change ($p<0.05$) in Group II, i.e. NSAIDs dependent Rheumatoid Arthritis (RA) cases when compared to healthy controls.

DISCUSSION: In rheumatoid arthritis, large numbers of neutrophils enter the joints and become active to produce these oxygen species. The hydroxyl radical has the potential to initiate free radical chain reactions in unsaturated fatty acid molecules (lipid peroxidation) Such a process would lead to the destruction of membranes and release of hydrolytic enzymes which further amplify the damage.[15]

The body has got a well-developed endogenous antioxidant defense system like cellular enzymes and vitamins which play an important role in protecting cells from free radical induced damage.

Our study demonstrates an increased serum concentration of uric acid in patients with Rheumatoid Arthritis (RA), this is in accordance with the studies of Gudbjornsson et al.[16] on the level of Hypoxanthine, xanthine and urate in the synovial fluid of patients with inflammatory arthritide. Their findings indicate a local enhanced purine metabolism in inflamed joint tissue and diffusion of oxypurine from joint cavity to plasma.

Our findings are also well supported by those of Allen RE et al.[17] and Edmonds SE et al.[18] According to Allen RE.[17] high levels of uric acid may be due to the presence of increased concentration of xanthine oxidase. Further supported by Edmonds SE et al.[18] that inflamed rheumatoid joint upon movement and rest undergo a hypoxic reperfusion cycle which may result in ROS (Reactive Oxygen Species) generation.

Studies reveal a 27.6 percent lowering of uric acid levels in Rheumatoid Arthritis (RA) patients on treatment with NSAIDs. This could be explained by the sharing of a common

mechanism of all Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), i.e. inhibition of the synthesis of Prostaglandins (PGs) from arachidonic acid hence less inflammation in the joints.

Raised ceruloplasmin levels in untreated Rheumatoid Arthritis (RA) patients and insignificant changes in NSAIDs treated rheumatoid arthritis patients observed by us were similar to the findings reported by Linder MC et al.[19] and Gitlin JD et al.[20] They observed that ceruloplasmin synthesis in blood levels increase during inflammations and at the same time iron transport between tissues is markedly reduced.[21] Gambhir, Lali and Jain.[22] have also reported significantly raised levels of plasma ceruloplasmin and erythrocyte glutathione in patients with RA. Reactive Oxygen Species (ROS), if not scavenged lead to lipid peroxidation. Significantly elevated levels of MDA in the plasma of RA patients reported by them are indicators of increased ROS production. During health, when ROS (Reactive Oxygen Species) production is low, lipid peroxidation is inhibited by the combined activities of various antioxidants present in the plasma. However, in the event of excessive ROS production as in the case RA, this protection may be inadequate Plasma ascorbate plays a pivotal role in protecting plasma lipids from ROS attack; however, it is rapidly oxidized when challenged by oxidants released from activated Polymorphonuclear Cells (PMNs). This rapid depletion may be the reason for low plasma ascorbate values reported by us in Rheumatoid Arthritis (RA) patients. Another antioxidant, alpha- tocopherol is probably not very effective against ROS in the aqueous medium. Ceruloplasmin (CP), whose levels are consistently increasing in RA, might play a significant role. This could well support the raised levels of ceruloplasmin in RA as observed by us.

Oxygen free radicals have been implicated as mediators of tissues damage in patients with rheumatoid arthritis. Thus, it is possible that several micronutrients acting as antioxidant and free radical scavengers provide protection against RA. Elevated risks of RA were observed at low levels of alpha tocopherol, ascorbate, β- carotene and selenium.[23]

The main finding of our study was that the risk of RA was significantly elevated among those with a low antioxidant index. Depressed antioxidant levels before the onset of clinical RA, may reflect a low dietary intake or an increased demand, related for instance to the pre-illness immunological process. Aho K et al.[24] pointed out that marker antibodies of RA, such as rheumatoid factor, can also precede the manifestation of arthritis by several years, focusing attention on pre-illness events in the pathogenesis of RA.

It is also possible that low antioxidant levels could be causally connected with development of the disease, but they may also only represent a distorted shadow of the actual pathogenetic factor. If there is a causal connection, failure in antioxidant protection may be manifested at the initiation of the immunological process or at a latter phase, thereby augmenting the progression of an otherwise transient arthralgia or incipient arthritis.

CONCLUSION: In conclusion, from our above study, on the "Status of antioxidants in RA patients using non-steroidal anti-inflammatory drugs." The findings are in line with the hypothesis that low antioxidant level (uric acid, ceruloplasmin, vitamin C and vitamin E) is a risk factor for RA. Further prospective study should be carried out in larger population samples with sufficient number of incidence cases, firm evidence could be obtained by demonstrating preventive effect of the above antioxidant supplement in intervention trials.

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