



## OXIDATIVE STRESS AND SERUM FERRITIN STATUS IN RHEUMATOID ARTHRITIS PATIENTS

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### ABSTRACT

**Aim :** The study was aimed to study the serum ferritin level as a response to oxidative stress in Rheumatoid arthritis patients and to measure the degree of oxidative stress in them by estimating the level of vitamin C.

**Methods:** The study included 50 patients who were clinically diagnosed as patients with RA and 50 age and sex matched healthy volunteers. Blood samples were collected from them and serum ferritin and serum vitamin C were estimated.

**Results:** The level of Vitamin C was found to be significantly decreased in the patients than in the healthy controls. Whereas, serum ferritin level showed significant rise in the patients as compared to the healthy controls.

**Conclusions:** The significant rise in serum ferritin is highly associated with higher oxidative stress in rheumatoid arthritis patients as compared to the normal healthy controls. Serum ferritin acts as an acute phase reactant and its rise is due to inflammatory response to the rising oxidative stress in rheumatoid arthritis.

**Keywords:** Ferritin, Oxidative stress, Rheumatoid arthritis, Vitamin C

### INTRODUCTION

Rheumatoid arthritis (RA), one of the most common types of arthritis, is an inflammatory condition that primarily involves the tissues that covers the joint causing pain, swelling, stiffness and loss of functions in the joints [1]. It is a well known fact that the inflammatory reaction is not designed to cause disease but to prevent or limit it. The multiple, complex and iterating cascades that make up an inflammatory reactions are designed to prevent or inhibit infection and minimize tissue damage. Reactive oxygen species (ROS) play a critical role in all aspects of the inflammatory process, from initiation to resolution [2]. ROS, thus, produced by activated neutrophils during the inflammatory response play important role in elevating oxidative stress and thereby in the pathogenesis of RA [3].

Ferritin, an iron binding protein, by regulating the free intracellular iron pool, controls iron-catalyzed generation of ROS and has been linked to oxidative stress in many studies. Although, the relationship

between oxidant stress and ferritin synthesis exhibits considerable complexity [4], the present study supports a role of ferritin as a protectant against oxygen free radical- mediated damage. Ferritin, a key protein of iron metabolism is capable of dual function- in iron detoxification and iron storage. The synthesis and expression of ferritin in animals is adjusted by genetic DNA and mRNA sequences that selectively respond to iron or oxygen signals and hence ferritin has been linked to proteins of iron, oxygen and anti oxidant metabolism.

The present study thus suggests the rise in serum ferritin level with the oxidative stress as an inflammatory response and may be considered as a prognostic tool for RA.

### Materials and Methods:

#### Sample selection:

The subjects selected for the study were the patients attending College of Medical Science, Bharatpur

Nepal. The subjects of study were subdivided into two groups.

Group 1 consisted of 50 patients, with a mean age of  $40 \pm 20$ , who were clinically diagnosed as patients with RA. The diagnosis of RA was done based on 1988 revised American Rheumatism Association (ARA) for classification of RA.

Group 2 consisted of 50 evenly age and sex matched normal healthy individuals. Informed consent was obtained from all the subjects under study.

#### Exclusion criteria:

All subjects with clinical, biochemical or radiological evidence of any other overlapping immunological disorders and any other chronic or acute illness were excluded from the study. Also the subjects were not habituated to smoking and/ or alcohol consumption.

#### Sample collection:

Fasting blood samples were obtained from antecubital vein. The blood sample was divided into two tubes for plasma and serum sample. For the first tube which was heparinized, plasma was separated by centrifuged at 1000 rpm for 15 minutes and used for the estimation of Vitamin C. Whereas, for the next tube, the blood was allowed to clot and serum was separated by centrifugation at 3000 rpm for 10 minutes that was used for ferritin levels in the subjects.

#### Statistical analysis:

Clinical and laboratory data were expressed as mean  $\pm$ SD. Statistical analysis were done using SPSS 17.0 ,statistical software (SPSS Inc., Chicago, IL, USA) and Microsoft office access 2007 data base. The relationship between serum ferritin and other parameters was estimated by Pearson's correlation analysis. The P-values  $\leq 0.001$  were considered significant.

#### Estimation of serum ferritin:

Serum ferritin levels were estimated by Microwell Ferritin EIA, Syntron Bioresearch, Inc.

#### Estimation of vitamin c:

After protein precipitation vitamin C was estimated according to the method of Omaye *et al.* 1979 [5].

#### Results:

Serum ferritin level showed significant rise in RA patients as compared to the healthy controls. The level of Vitamin C was found to be significantly decreased in the patients than in the healthy controls as shown in table 2,3 and graph 1and 2.

**Table 1: Demographic characteristics of the healthy controls and Rheumatoid arthritis patients**

Parameters	Controls (n=50)	RA patients(n=50)
Age (years)	$40 \pm 20$	$40 \pm 20$
Gender(M/F)	15/35	25/25
Glucose random(mg/dl)	$95 \pm 10$	$99 \pm 15$
Hb(gm/dl)	$14.8 \pm 1.5$	$10 \pm 1.5^*$

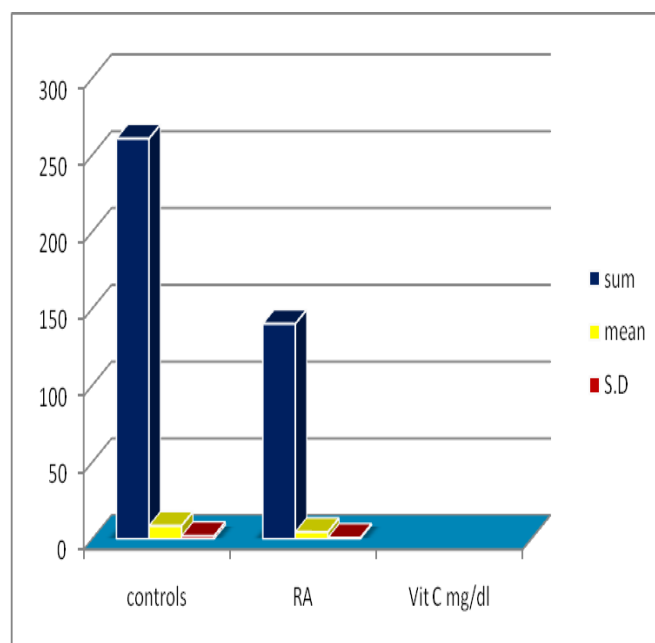
Data are expressed as mean  $\pm$  SD. \* represents  $p < 0.05$  compared to Healthy controls.

**Table 2: Levels of vitamin C and serum ferritin in healthy controls and RA patients**

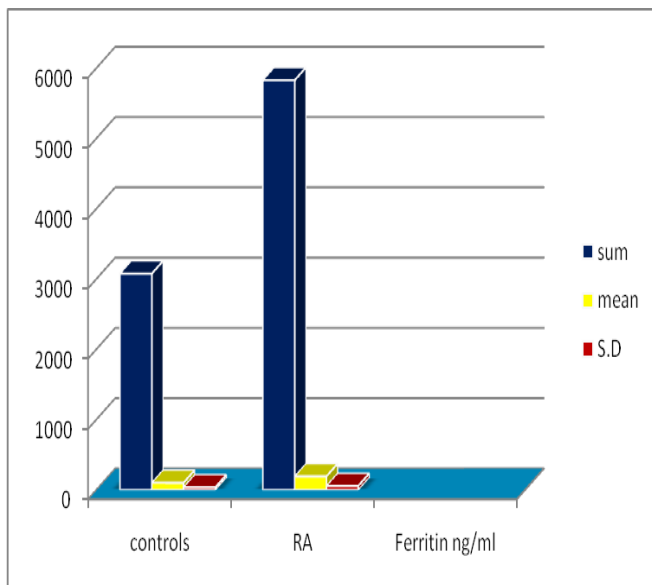
Parameters	Controls	RA patients
Vitamin C(mg/dl)	$8.78 \pm 2.0$	$4.64 \pm 0.91^*$
Ferritin(ng/ml)	$146 \pm 62.23$	$213 \pm 79.50^*$

The level of Vitamin C ( $p < 0.001$ ) was found to be significantly decreased in the patients than in the healthy controls. Whereas, serum ferritin level showed significant rise in RA patients as compared to the healthy controls.

Data are expressed as mean  $\pm$  SD. \*represents  $p < 0.001$  compared to healthy controls.



**Graph 1: levels of plasma Vitamin C in RA and healthy controls.**



Graph 2: levels of serum ferritin in RA and healthy controls.

Table 3: Correlation between serum ferritin and vitamin C

Ferritin Vs	Vit C
Pearson's correlation	-0.46*
Sig. 2 tailed	0.020
No.	30

\*Correlation is significant at the 0.05 level (2-tailed).

Correlation between the serum ferritin and vitamin C. Serum ferritin showed significant negative correlation with Vitamin C level (r= -0.460) among the RA patients.

**Discussion:**

Rheumatoid arthritis is a chronic progressive autoimmune disorder characterized by symmetric erosive synovitis and also shows multisystem involvements. Reactive oxygen species are clearly involved in the pathogenesis of RA.

Age and gender distribution of the RA patients and the control groups were similar. For all the subjects random blood glucose and hemoglobin levels were also measured. The mean Hb value in the controls and RA was found to be 14.8±1.5 and 10±1.5 g/dl respectively, suggesting anemic condition in RA patients (Table 1).

In the present study the level of plasma Vitamin C (4.644±0.911), a non enzymatic antioxidant, has been significantly decreased in RA patients as compared to the healthy controls (8.786±2.055) (Table 2 and graph 2).

Vitamin C, a well known anti-oxidant, plays an important role in protecting the lipids of lipoproteins and other bio-membrane against peroxidative damage by intercepting oxidants before they can attack the tissues [6]. Lower concentration of Vitamin C has been reported in the plasma and erythrocytes of RA patients [7]. Also, an inverse relationship between lipid peroxidation and non-enzymatic antioxidants has been well documented [8]. Hence, the decrease in plasma non-enzymatic antioxidants can be correlated to impairment in anti-oxidant defense mechanism, due to excess utilization by the inflamed tissues to scavenge the excessive lipid peroxides that are generated at inflammatory sites or to scavenge accumulated lipid peroxides in plasma [9].

Further the levels of Vitamin C were found to fall with the increase in duration of the disease suggesting the consumption of Vitamin C for mopping up the free radicals neutralizing the oxidative properties of these free radicals as proposed earlier [10]. Therefore the steady state concentrations of the free radicals are determined by the balance between their rates of production and their rates of removal by various antioxidants.

Thus, the decrease in Vitamin C level in RA patients reflects declination in the antioxidant activity and implies higher oxidative stress in RA patients as compared to the healthy controls.

Further, this severity of ROS generation and its effect can be decreased and improved with the supplement of vitamins.

The mean serum ferritin level in RA patients (213±79.50) showed a moderate increment in the patient group as compared to the healthy controls (146±62.23) (Table 2, graph.2.).

With the persistence of the disease there is continuous rise in hypoxia and anemia in the site of tissue damage [2]. Regarding this complexity many studies have shown decreased in ferritin level in RA patients as ferritin is most renowned as iron storage protein, thereby indicating anemic condition in RA patients.

We found the patient group with mean Hb level of  $10\pm 1.5$  g/dl (Table 1). This showed the anemic condition in the patient group. Further the levels of Hb did not show any correlation with the ferritin level. This clearly indicated that the rise in serum ferritin level in RA patient was not related to the body iron status. Similar findings were seen in other studies. However serum ferritin level in normal healthy person does reflect the iron status of the individual.

Ferritin plays a central role in the maintenance of this delicate intracellular iron balance [11]. However, only recently a critical evaluation of the role of ferritin in protection from oxidative stress has begun. Several studies have indeed suggested that ferritin protects against the oxidative stress. And consequent induction of ferritin leads to protection against the subsequent oxidant challenge [4]. Ferritin is thus a solution to the difficult chemistry of iron and oxygen, because it stores iron and mitigates the risk for oxidation via free iron atoms [12].

In the present study, elevation in serum ferritin level with decreasing vitamin C level indicates the association between the serum ferritin and oxidant status. These findings suggest that a moderately high serum ferritin is not just a mere marker of Fe stores but more an indicator of inflammation as well as other factors like oxidative stress.

So the present study suggests the rise in serum ferritin in RA patients is solely an inflammatory response to minimize oxidative stress. The study also suggests serum ferritin as an unreliable guide to the patient's iron status in rheumatoid arthritis if diagnosed alone. Rather serum ferritin level in RA patient can serve as a tool to study the severity of the disease.

#### Conclusion:

The present study concludes the significant rise in serum ferritin is highly associated with higher oxidative stress in RA patients as compared to the normal healthy controls. Serum ferritin acts as an acute phase reactant and its rise is due to inflammatory response to the rising oxidative stress in Rheumatoid Arthritis.

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