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Research Article

STUDY OF SERUM MALONDIALDEHYDE, NITRIC OXIDE, VITAMIN E LEVELS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTACT

Background: Rheumatoid arthritis (RA) is a chronic progressive autoimmune disorder characterized by symmetric erosive synovitis and sometimes shows multisystem involvement. The long-term outcome of the disease is characterized by significant morbidity and increased mortality. Elevated free radical generations in inflamed joints and impaired antioxidant system have been implicated in RA. Nitric oxide (NO) can also induce tissue damage, especially after conversion into peroxynitrite radical ($ONOO^{\bullet}$). **Aims:** To estimate the serum levels of MDA, Nitric Oxide (NO) and Vitamin E in patients with Rheumatoid Arthritis**. Materials and Methods:** The study includes 50 RA patients who were fulfilling the American Rheumatism Association 1987 revised criteria for classification of RA and 50 age and sex matched healthy subjects without any major illness were considered as controls. MDA, NO and Vitamin E were estimated in serum. **Results:** The estimated mean levels (mean ± SD) of serum MDA, NO, Vitamin E, in control group were 3.55 ± 0.30, 36.23 ± 7.03, 14.61 ± 1.74, respectively and in patients with RA they were 5.39 ± 0.79, 78.81 ± 8.56, 10.56 ± 1.72, respectively. The statistical analysis by unpaired t-test shows that the levels of serum MDA and NO significantly increased ($p< 0.001$) and the vitamin E levels were significantly decreased ($p < 0.001$) in RA patients when compared to healthy controls. **Conclusion:** The serum values of MDA, NO and Vitamin E all together provided fairly useful index of oxidative stress in RA patients. The results of current study support the concept of oxidative stress leading to tissue damage.

Keywords: Malondialdehyde, Nitric oxide, Vitamin E**,** Rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis is a common inflammatory arthritis. It is one of the preventable disability conditions. The clinical features are symmetrical arthritis, both small and large joints are affected and associated with extra-articular manifestations.[1] It affects approximately 1 to 2% of general population world wide[2] and in India its incidence is 0.75%.[3] Women are affected three times more often than men. The onset is most frequent during the fourth and fifth decade of life. [4]

It is believed that RA is an auto-immune disease, triggered by exposure of a genetically susceptible person to an arthritogenic factor. It involves the activation of CD4 + helper cells mainly. Then the local release of inflammatory mediators occurs which ultimately damages the joint. [5] The cytokines also

stimulates endothelial cells, macrophages and polymorphonuclear leukocytes to produce NO.[6]

Although characteristic feature is persistent inflammation, the elevated generation of free radicals in inflamed joints and impaired antioxidant systems has been implicated in RA.[7] With this background this study was designed to estimate the serum MDA, NO and vitamin E in RA patients and apparently healthy controls from Davangere district of Karnataka.

MATERIALS AND METHODS

Fifty Rheumatoid arthritis patients who are fulfilling the American Rheumatism Association 1987 revised criteria for classification of RA[20], age from 20 to 70 years and both sex and age, controls (N=50) were included in the present study from Bapuji Hospital and Chigateri General Hospital, Davangere (Both these hospitals are attached to teaching institute, J.J.M Medical College, Davangere) and also from general population. The study was approved by Ethical and Research Committee of J.J.M. Medical College, Davangere to use human subjects in the research study.

Exclusion criteria: Patient with Osteoarthritis, Tubercular arthritis, Arthritis other than RA fitting into any syndromes and any other chronic systemic disorders like cardiovascular disorders, diabetes mellitus, liver diseases and kidney diseases are excluded from the study.

Venous blood from all the subjects was collected aseptically from anticubital vein; serum was separated by centrifuging at 3,000 RPM for 10 minutes and kept at 4^0C until analysis was carried out.

Estimation of Serum Malondialdehyde (MDA): Thiobarbituric acid method[8]

Auto-oxidation of unsaturated fatty acids lead to the formation of semistable peroxides which then undergo a series of reactions to form short chain aldehydes like malondialdehyde. One molecule of MDA reacts with 2 molecules of Thiobarbituric acid (TBA) with the elimination of 2 molecules of water to yield pink crystalline pigment with an absorption maximum at 530 nm. Results are expressed as μmol/L

Estimation of Serum Nitric Oxide (NO): Kinetic Cadmium-Reduction method [9]

Nitrate, the stable product of nitric oxide is reduced to nitrite by Cadmium reduction method after deprotinization with Somogyi reagent. The nitrite produced is determined by diazotization of sulphanilamide and coupling to naphthylene ethylene diamine. The color complex precipitated is measured at 540nm wavelength using colorimeter. Results are expressed as μmol/L

Estimation of Serum Vitamin E (Tocopherols): Baker and Frank method[10]

Serum tocopherol is measured by their reduction property. They reduce ferric ions to ferrous ions which are then reacts with α, α'-dipyridyl to form a red colored complex. First tocopherols and carotenes are extracted by xylene and the reading is taken at 460 nm. A correction for the carotenes is made by adding ferric chloride and then again reading is taken at 520 nm. Results are expressed as mg/L

Statistic analysis: Results are expressed as mean ± SD. Unpaired't' test was used for intergroup comparison and paired't' test for intra group comparison. $p < 0.05$ was considered as statistically significant.

RESULTS

Table1: Age and sex-wise distribution of controls and RA patients

		Controls Mean ± SD	Cases Mean ± SD
Age (yrs)		41.9 ± 13.6	46.2 ± 13.3
Gender	Male	18	14
	Female	32	36

Table 2: Serum levels of MDA, NO, Vitamin E in patients with RA and healthy controls.

	MDA (μmol/L)	NO (μmol/L)	Vit.E (mg/L)
Controls	3.55 ± 0.30	36.23 ± 7.03	14.61 ± 1.74
Cases	5.39 ± 0.79	78.81 ± 8.56	10.56 ± 1.72
Mean difference	1.84	42.58	4.05
t-value*	15.44	27.19	11.68
p-level	< 0.001, HS	< 0.001, HS	< 0.001, HS

*Unpaired t-test p<0.001=HS (Highly significant) p>0.05=NS (Not significant)

DISCUSSION

In the present study, the serum level of MDA is highly statistically significantly increased ($p < 0.001$) in patients with RA when compared to controls. In RA patients activated macrophages and neutrophils release oxidants in high concentrations that lead to oxidative stress. This will cause damage to lipids, proteins, carbohydrates and DNA. The unsaturated fatty acids of cell membranes undergo lipid peroxidation and MDA is released which acts as a oxidative stmarker.[11] MDA reacts with lysine residues in protein to produce immunogenic molecules, which can exacerbate inflammation. [12]

Increased serum MDA concentration in RA suggests the role of free radicals in pathogenesis of inflammatory arthropathy and supports the need for studies assessing the therapeutic role of free radical scavengers in RA.[2]

NO is a pleiotropic mediator of inflammation which was discovered as factor released from endothelial cells that caused vasodilatation by relaxing vascular smooth muscle and was therefore called endothelium–derived relaxing factor.[14] NO is a short lived radical and lipid and water soluble gas which as a potent inflammatory mediator. Because of it reacts with oxygen, superoxide and iron-containing compounds strongly.[15] NO is generated by the nitric oxide synthasase (NOS) enzyme from molecular oxygen and the terminal guanidine nitrogen of the amino acid l-arginine, yielding l-citrulline as a co-product. In our study, the serum NO level is highly statistically significantly increased ($p<0.001$) in RA patients as compared to healthy controls.There may be two possible causes for the increased serum levels of NO in RA. One is enhanced synovial inflammation, which results in increased levels of NO in synovial fluid which ultimately enters systemic circulation. Another possible cause may be production of NO by systemic vasculature and other cells.[6]

Nitric oxide can induce tissue damage, especially after conversion into peroxynitrite radical ($ONOO^-$).[25] Peroxynitrite can be directly cytotoxic and it can also decompose to give range of products, including hydroxyl radicals ($OH^\bullet$) and nitronium ion (NO_2^+). NO produced within the inflamed joint may contribute to the peri-articular bone loss in RA.[16]

For cell membrane lipids and lipoprotein vitamin E serves as a chain breaking free radical trapping antioxidant. It reacts with the lipid peroxide radical before the establishment of chains lipid peroxidation reactions occurs. In this process vitamin E produces tocopheroxyl free radical which is unreactive and results in forms nonradical compound synthesis.[17] In our study the levels of serum vitamin E is highly statistically significantly reduced ($p<0.001$) in patients with RA when compared to controls. The decrease in vitamin E level may be the for preventing oxidative stress , turnover of vitamin E can occur more in RA.[18] An epidemiological study suggested that low alpha-tocopherol status is a risk factor for RA independently of rheumatoid factor status.[19]

CONCLUSION

The enhanced oxidation plays a significant role in the tissue damage and inflammation perpetuating process in rheumatoid synovium. The oxygen free radicals lead to lipid peroxidation and bone loss. The results of current study support the concept of oxidative stress leading to tissue damage. As a consequence of the present understanding of the etiopathogenesis of RA, exogenous antioxidants i.e., Vitamins and other nutrients, appear to be potential agents for therapeutic management.

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