Original Research Article

Study of serum Malondialdehyde, nitric oxide and vitamin E in rheumatoid arthritis patients before and after the supplementation of vitamin E

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder that may affect many tissues and organs – skin, blood vessels, heart, lungs and muscles but principally attacks the joints, producing a non suppurative proliferative and inflammatory synovitis that often progresses to arthritis and ankylosis of the joint. The exact reason behind bone erosion and joint deformities is not fully understood. Formation of reactive oxygen species and lipid peroxides as a result of disease activity may play an important role in RA. There is a close association between bone loss and oxidative threat in patients presenting with RA. **Objectives:** To evaluate the preventive role of antioxidant therapy in RA by measuring serum Malondialdehyde (MDA), Nitric oxide (NO), and Vitamin E before and after co-administration of vitamin E 400 mg/day for 12 weeks. **Methods:** In this study50 RA patients who were fulfilling the ARA-1987 revised criteria were included and they are divided into two groups. **Results**: In RA patients, after vitamin E therapy, serum MDA, NO levels were statistically highly significantly ($p<0.001$) reduced and Vitamin E statistically highly significantly ($p<0.001$) increased as compared to RA patients who had not received vitamin E therapy **Conclusion:** There was decrease in oxidative stress in RA patients with Vitamin E therapy. The results suggest proper antioxidant nutrient intake may reduce free radical generation and improve antioxidant status and thus reducing the morbidity in RA patients.
Key Words: Malondialdehyde; Nitric oxide; Vitamin E.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder that may affect many tissues and organs – skin, blood vessels, heart, lungs and muscles but principally attacks the joints, producing a nonsuppurative proliferative and inflammatory synovitis that often progresses to arthritis and ankylosis of the joint. Pathogenesis of RA is unknown. At present some of the hypotheses of pathogenesis of RA includes:[1]

- The autoimmune reactions
- Mediators of tissue injury
- Genetic susceptibility
- Triggering antigens.

There is decreased concentrations of antioxidants in blood considerably increase the probability of occurrence of RA and it is possible that generation of reactive oxygen species may play an important role in bone resorption in inflammatory disease like RA. There is a direct relationship between bone loss and oxidative stress in RA.[2] Growing evidences implicate nitric oxide ($NO^{\bullet}$) in immune regulations, inflammation, autoimmunity and arthritis. Several studies suggest that tissue injury in inflammation involves NO production. Increased levels of NO in serum and synovial fluid have been reported in patients with RA.[2,3,4,5,6] Better recovery in patients treated with antioxidant supplemented drug regimen suggests

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that antioxidants may have an important role to play in this inflammatory disorder, as they lower the oxidative stress and resultant inflammatory damage.[7] With this background this study was designed to evaluate antioxidant role of vitamin E therapy of 400 mg/day for 12 weeks than RA patients without therapy.

MATERIALS AND METHODS

Fifty subjects who were fulfilling the American Rheumatism Association, 1987 revised criteria (ARA 1987 criteria)[8]for classification of Rheumatoid Arthritis were included in this study. All the cases are selected from Viswabharathi medical college and cancer hospital, kurnool as well from general population. This study was approved by ethical and research committee of Viswabharathi medical college and cancer hospital. Informed consent was taken from all subjects. They are grouped into two groups.

Group 1: 25 cases are supplied with vitamin E 400 mg/day (Evion) for a period of 12 weeks.

Group 2: 25 cases are not supplied with vitamin E.

Collection of blood samples: Five ml of venous blood was collected aseptically from anti-cubital vein; serum was separated and kept at 40C until analysis was carried out. Serum MDA was estimated spectrophotometrically by Thiobarbituric acid method[9], serum NO by kinetic Cadmium – Reduction method[10], Vitamin E by Baker and Frank method[11].

RESULTS AND DISCUSSIONS

Table 1: Sex-wise distribution RA patients

		Cases (n = 50) Mean ± SD	Group 1	Group 2
Gender	Male	14	5	9
	Female	36	20	16

Table 2: Serum levels (mean ± SD) of MDA, NO and Vitamin E, in RA patients treated with vitamin E therapy (400 mg/day for 12 weeks) and RA patients treated without vitamin Etherapy.

		Before	After 12 weeks	Difference	t-value*	p level
MDA (μmol/L)	With therapy (group I)	5.59 ± 0.76	3.99 ± 0.46	1.60 ± 0.63	12.71	< 0.001, HS
	Without therapy (group II)	5.18 ± 0.77	5.09 ± 0.66	0.09 ± 0.51	0.88	0.39, NS
	With V/s Without**	-	t = 6.84 p < 0.001	t = 9.31 p < 0.001	-	-
NO (μmol/L)	With therapy (group I)	78.87 ± 9.27	49.82 ± 8.55	29.05 ± 11.0	13.20	< 0.001, HS
	Without therapy (group II)	78.76 ± 7.98	78.77 ± 7.17	0.01 ± 6.02	0.01	0.99, NS
	With V/s Without**	-	t = 12.97 p < 0.001	t = 11.58 p < 0.001	-	-
Vit. E (mg/L)	With therapy (group I)	9.95 ± 1.16	13.57 ± 2.35	3.61 ± 1.72	10.48	< 0.001, HS
	Without therapy (group II)	11.17 ± 1.99	10.93 ± 1.83	0.24 ± 1.69	0.72	0.48, NS
	With V/s Without**	-	t = 4.43 p < 0.001	t = 6.99 p < 0.001	-	-

*Intragroup comparisons paired t-test, **Intergroup comparison unpaired t-test, p<0.001=HS (Highly significant), p>0.05=NS (Not significant)

The estimated mean levels (mean ± SD) of serum MDA, NO and vitamin E in group 1 before therapy were 5.59 ± 0.76, 78.87 ± 9.27, 9.95 ± 1.16, respectively and after 12 weeks they were 3.99 ± 0.46, 49.82 ± 8.55, 13.57 ± 2.35, respectively. In group 2, mean levels (mean ± SD) of serum MDA, NO and vitamin E before therapy were 5.18 ± 0.77, 78.76 ± 7.98, 11.17 ± 1.99, respectively and after 12 weeks they were 5.09 ± 0.66, 78.77 ± 7.17, 10.93 ± 1.83, respectively. The statistical analysis by paired t-test in group 1 shows that the levels of serum MDA, NO were high and vitamin E was low before therapy and after 12 weeks with vitamin E therapy the levels of MDA, NO were decreased and vitamin E was increased which were statistically highly significant ($p < 0.001$). The statistical analysis by unpaired t-test shows that the levels of serum MDA, NO were decreased and vitamin E was increased after 12 weeks in group 1 as compared to group 2 with statistically highly significant ($p < 0.001$). Rheumatoid arthritis (RA) is the most common inflammatory arthritis a. The typical clinical phenotype of RA is a symmetrical deforming, small and large joint polyarthritis often associated with systemic involvement.[12]Approximately 1

to 2% of general population is suffering from RA in world wide[7] and in India its incidence is 0.75%.[13]Women are three times more affected than men.[14] In rheumatoid joints activated macrophages and neutrophils release several kinds of oxidants, which in high concentration can damage to lipids, proteins, carbohydrates and DNA. Unsaturated fatty acids of cell membranes are the main targets for such oxidants. MDA is a released as lipid peroxidation product and serves as a marker of oxidative stress.[15]Increased serum MDA concentration in RA suggests the role of free radicals in pathogenesis of inflammatory arthropathy and thereis a need of assessing the therapeutic role of free radical scavengers in RA.[7] In support of the above statement, our results show highly significant decrease in concentrations of serum MDA after treatmentin RA patients who had received vitamin E 400 mg/day for 12 weeks (Group 1) as compared to RA patients without therapy (Group 2). This finding is in agreement with other studies.[7, 16,17] NO is a lipid and water soluble gas which acts as a potent inflammatory mediator because of its strong reactivity with oxygen, superoxide and iron-containing compounds.[18]NO is generated by the nitric oxide synthasase (NOS) enzyme from molecular oxygen and amino acid l-arginine.[19] Nitric oxide can induce a tissue damage especially after conversion into peroxynitrite radical ($ONOO^{\cdot}$).[25] Peroxynitrite can be directly acts as cytotoxic.[20] NO has many effects in RA, including stimulation of blood flow, inhibition of matrix production by chondrocytes, activation of metalloproteinases, modulation of immune response, suppression of osteoblast activity and enhancement of cytokine-induced osteoclastic bone resorption, Therefore, NO produced within the inflamed joint may contribute to the peri-articular bone loss in RA.[19,21] Our study also shows that highly significant decrease in levels of serum NO in RA patients who had received vitamin E therapy 400 mg/day for 12 weeks (group1) as compared to RA patients who had not received vitamin E therapy (group2). Vitamin E acts as an antioxidant by scavenging molecular oxygen and free radicals.[10] Vitamin E acts as a chain breaking free radical trapping antioxidant in cell membranes a. It reacts with the lipid peroxide radical formed by peroxidation of PUFA and fomstocopheroxyl free radical which is relatively unreactive,[22] The low value of vitamin E level may be due to the increased turnover, for preventing oxidative damage suggesting an increased defense against oxidant damage in RA.[23]In our study, the serum vitamin E level was low before vitamin E therapy and high after 12 weeks in group 1 who had received vitamin E therapy (400 mg/day for 12 weeks) which is statistically highly significant ($p<0.001$). This finding is in accordance with other studies.[17,24,25,26,27,29] Overall it is well evident that there is an increased state of oxidative stress in RA, which proposes the use of an antioxidant supplementation in such patients.[16]

CONCLUSION

The results suggest that proper antioxidant nutrient intake may reduce free radical generation and improve antioxidant status and thus reducing the morbidity in RA patients. However, due to the limited number of cases included in this study, more studies may be required to substantiate the results and arrive at a definite conclusion, in terms of safety and efficiency of adding on an antioxidant therapy for the treatment of RA.

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