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EFFECT OF VITAMIN E ON SERUM MALODIALDEHYDE LEVELS IN RHEUMATOID ARTHRITIS

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ABSTRACT

Our primary objective was to demonstrate the reduction of serum malondialdehyde (MDA) in patients with Rheumatoid Arthritis (RA) when administered Vitamin E. Our secondary objective was to evaluate any corresponding reduction in disease activity by measuring erythrocyte sedimentation rate (ESR) and C reactive protein (CRP). This was a prospective, open labeled, parallel group study. 85 patients were randomized into two groups - "Control Group" (43 patients) and "Vitamin E Group" (42 patients). The Control Group received Indomethacin 25 mg BD &Chloroquine 250 mg OD, whilst the Vitamin E Group received Vitamin E 400 mg BD in addition to the above drugs for a period of 3 months. ESR, CRP and MDA were measured before and after drug administration. There was no statistically significant difference in average ESR, CRP or MDA between both groups at the start of the study. At the end of the study, there was a statistically significant decrease of all parameters in the Vitamin E group, which was not seen in the Control group. This study shows that administration of Vitamin E to patients with RA lowers serum MDA due to its antioxidant action. This study also shows that the addition of Vitamin E to the existing therapeutic regimen decreases disease activity as evidenced by reduction in ESR & CRP, which are markers of acute inflammation.

Key words: Vitamin E, Rheumatoid Arthritis, Malondialdehyde, Lipid Peroxidation.

INTRODUCTION

Rheumatoid arthritis (RA) is a common disease affecting around 1% of the population. It affects twice as many women as men, at any age. Its peak onset is in the fourth and fifth decades of life, increasing sharply with age thereafter. Although the cause of RA remains unknown, autoimmunity plays a pivotal role in its chronicity and progression [1]. Mortality is higher in RA patients, highest in those with the most severe form of the disease. Average life span is reduced by 8 – 15 years and five year survival for patients with advanced disease is only 50% [2].

Free Radicals and Immunoglobulins

Free radicals and other Reactive Oxygen Species (ROS) are derived either from metabolic processes or from external sources. Enzymatic reactions which serve as sources of free radicals include those in the mitochondria involved in the respiratory chain, in phagocytosis and in reactions involving iron and other transition metals. Other internally generated sources of free radicals are exercise, inflammation, ischemia / reperfusion. Some externally generated sources of free radicals are smoking, environmental pollutants, radiation, ultraviolet light, ozone and some chemicals [3]. ROS include superoxide (O2·-), hydrogen peroxide (H2O2), hydroxyl (OH**.**) and peroxynitrite radicals (ONOO-) and nitric oxide (NO). Superoxide, H2O2 and hydroxyl radical are produced by xanthine oxidase and are also generated by activated macrophages and neutrophils as a result of respiratory chain activity, known as the "oxidative burst", responsible for killing microbial pathogens [4]. The cytotoxic effect of free radicals and ROS is protective when directed by inflammatory cells against invading micro-organisms and

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tumor cells. Free radicals can alter Ig G to form complexes, which can stimulate the release of superoxide from normal neutrophils [5].

An imbalance between free radical generating and radical scavenging system results in oxidative stress, a condition that has been associated with cell injury in many pathological conditions. The effects of these reactive species are wide ranging, but three reactions are particularly relevant to cell injury.

(i) Lipid peroxidation of membrane
(ii) Oxidative modification of protein
(iii) Lesions in DNA [6]

Lipid peroxidation is a process of oxidative decomposition of omega-3 and omega-6 polyunsaturated fatty acids of membrane phospholipids, leading to formation of lipid hydroperoxides and aldehydic end products like malondialdehyde (MDA) and hydroxynonenol. This process may cause disruption of cell structure and function and play an important role in the etiology of many diseases. Initiation and propagation of lipid peroxidation are mediated by free radicals [6]. This reaction is auto-catalytic and can be self perpetuating. Many studies showed raised levels of malondialdehyde, a by-product of lipid peroxidation and injury, and low levels of endogenous antioxidants in patients with RA [7].

One of the mechanisms of defence to counteract damage by free radicals is antioxidants. "An anti oxidant is a molecule stable enough to donate an electron to a free radical and neutralize it, thus reducing its capacity for tissue damage."Some antioxidants like ubiquinone, glutathione and uric acid are produced during normal metabolism in the body. Others like Vitamin E, Vitamin C and the carotenoids should be obtained from the diet [3].
Vitamin E is located in the within the phospholipid bilayer of cell membrane and protects polyunsaturated fats and other components of cell membrane from oxidation by free radicals. Vitamin E is particularly effective in preventing lipid peroxidation.

Hence, our primary objective was to demonstrate the reduction of serum malondialdehyde in patients with RA when administered Vitamin E. Our secondary objective was to demonstrate the corresponding reduction in disease activity by measuring serum acute phase reactants - erythrocyte sedimentation rate (ESR) and C reactive protein (CRP).

MATERIALS AND METHODS

This was a prospective, open labeled, parallel group study. A total of 85 patients were selected (out of 92 screened) from those attending the Rheumatology Out-patient Department, Stanley Medical College, Chennai. They were randomized by lots into "Control Group" (43 patients) and "Vitamin E Group" (42 patients). Sample size calculated based on previous studies [11] was estimated to be 40, for the design to have 90% power to detect a clinically significant difference between both groups. Approval was obtained from the Institutional Ethical Committee of Stanley Medical College prior to the start of the study. Information was given and written informed consent was obtained in the patient's native language.

Inclusion Criteria

 Patients of either sex between 18-40 years of age, diagnosed to have RA as per revised American College of Rheumatology criteria, who had stable disease whilst on treatment with Indomethacin &Chloroquine for at least 3 months prior to commencement of the disease
 Duration of disease more than 6 months but less than 3 years [12]

Exclusion Criteria

 History of treatment with complementary or alternative medicine
 Patients who had been treated with corticosteroids, immunosuppressants or any DMARD except chloroquine in the 3 months prior to enrollment in the study
 Patients with diabetes, hypertension, liver or renal dysfunction or any other chronic illness
 Pregnant or lactating women
 Patients with extra-articular features or severe disease

Baseline investigations done at the start of the study included complete blood counts, bleeding and clotting times, random blood sugar, serum creatinine& aminotransferases. ESR, CRP and serum MDA were measured at baseline and at 12 weeks.

The Control Group received Indomethacin 25 mg BD &Chloroquine 250 mg OD, whilst the Vitamin E Group received Vitamin E 400 mg BD in addition to the above drugs for a period of 3 months. The patient was considered to be compliant with study medication if he/she took at least 80% of the medication during the study period. Compliance was recorded by a daily drug reminder chart and confirmed by examining the number of unutilized capsules in each medication pack.We used the Draper & Hadley method for Serum Malondialdehyde Estimation [13]. 0.1 ml of serum was mixed with 0.5 ml sulphuric acid, 0.4 ml PTA and 1 ml distilled water. The tube was centrifuged for 10 minutes at room temperature. The supernatant was aspirated and the remaining pellet was mixed with 1 ml sulphuric acid and 0.15 ml PTA. This was centrifuged for 10 minutes, supernatant was discarded and the pellet was resuspended in 2 ml water. 0.5 ml TBA was added and the contents heated in a boiling water bath for 60 minutes. The tubes were cooled and 2.5 ml butanol was added. The tubes were centrifuged, the supernatant was added to the cuvette and the absorbance was measured at 533 nm. A standard calibration curve was prepared by taking various concentrations of MDA standard, treated similarly with TBA. The values are expressed in nM/mL. Normal level of serum MDA is 12 – 15 nM/mL

Statistical Analysis

The data obtained at the end of this study were analyzed using SPSS Data Editor Software. Student independent t test was used to compare differences between groups at 0 and 12 weeks. Student paired t test was used to compare pre and post test values within each group. P value ≤ 0.05 was considered significant.

RESULTS

The majority of patients in this study were in the age group 30 – 50 years and more than 80% were females. There was no significant difference in sex distribution between both groups.

There was no statistically significant difference in average ESR, CRP or MDA between both groups at the start of the study, before drug administration, as evidenced by student independent t test. At the end of the study, there was a statistically significant decrease of all parameters in the Vitamin E group, which was not seen in the Control group, demonstrated by student paired t test. Comparison of mean ESR, CRP and MDA of both groups, by student independent t test, at the end of the study showed a statistically significant difference between groups in CRP and MDA (Table 1, Figure 2)

Table 1. Comparison of Mean Pre and Post Test Scores

Parameters	Group	Mean ± 2SD		Paired t test
		0 months	3 months	
ESR	Control	8.77 ± 9.26	8.54 ± 9.64	P = 0.77
	Vitamin E	10.6 ± 16.42	7.43 ± 13.16	**P = 0.001**
CRP	Control	8.31 ± 10.16	9.75 ± 10.42	P = 0.68
	Vitamin E	8.62 ± 10.22	4.5 ± 7.06	**P = 0.001**
MDA	Control	23.59 ± 8.28	24.8 ± 13.62	P = 0.15
	Vitamin E	23.18 ± 7.36	21 ± 13.76	**P = 0.04**

Table 2. Percentage of Reduction / Increase in Scores

Parameters	% of Reduction / Increase	
	Control group	Vitamin E group
ESR	-2.6	-29.9
CRP	+3.73	-53.8
MDA	-1.7	-15.12

Figure 1. Flowchart of Study Protocol

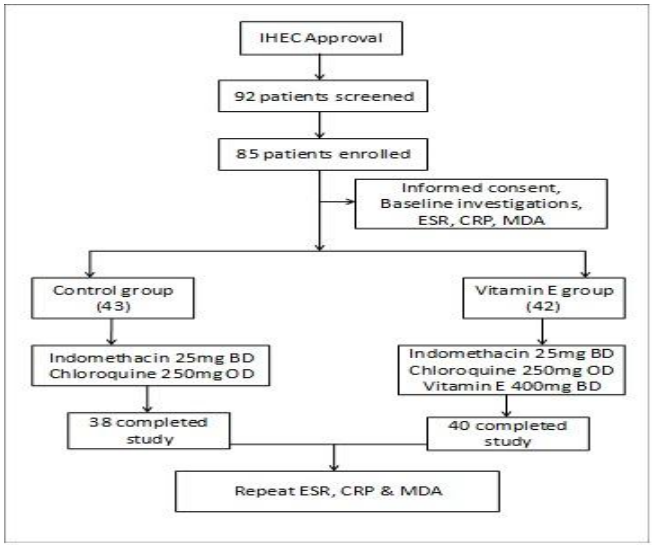
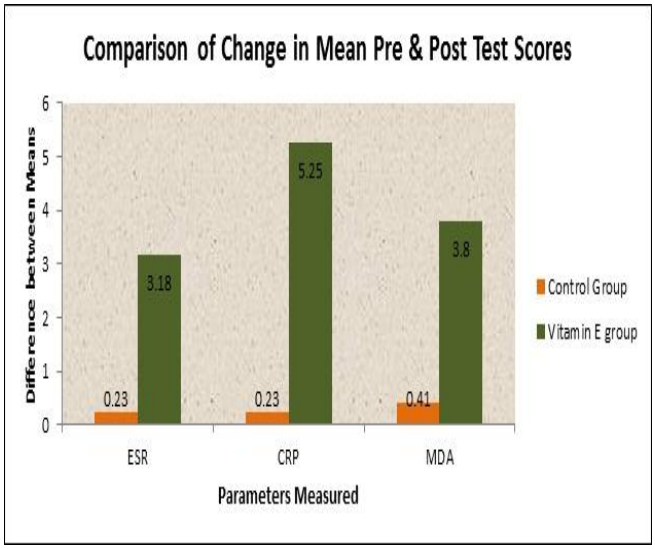


Figure 2. Comparison of Differences between Groups in Mean Pre & Post Test Scores



DISCUSSION

The treatment of rheumatoid arthritis aims to ameliorate symptoms and prevent progressive joint damage. Increasing evidence for the role played by free radicals suggests that antioxidant therapy may play an alternative approach [5].

In this study, one group of patients received Vitamin E along with their routine NSAID and DMARD (Indomethacin & Chloroquine). They were compared with another group of patients who received only Indomethacin & Chloroquine. The study duration was for 3 months.

It was hypothesized that levels of Serum malondialdehyde (MDA), a by-product of lipid peroxidation and a marker of oxidant injury would reduce with supplementation of Vitamin E. Correlation of this reduction with a corresponding lowering of acute phase reactants, Erythrocyte Sedimentation Rate (ESR) & C-Reactive Protein (CRP), would indicate a decrease in disease activity.

Out of 92 patients screened, 85 patients were included in the study. They were randomized into Control & Vitamin E groups, 42 and 43 in number respectively. There were 6 dropouts, 4 from Control group and 2 from Vitamin E group and were excluded in statistical analysis (Figure 1).

None of the dropouts were due to adverse effects. Diarrhea, nausea, epigastric pain and rash were the adverse effects noted. Nausea was higher in the Vitamin E group, while epigastric pain was higher in the control group. Only normotensive RA patients with BP < 140/90 were included in the study. Blood pressure was monitored, as previous studies had shown hypertension to be the only major side effect of chronic vitamin E therapy [14]. There was no difference in mean pre and post test BP measurements.

The majority of patients in this study were in the age group 30 – 50 years, and more than 80% of patients were female. This was in correlation with established demographic reports [15].

There was a statistically significant lowering of serum MDA in the Vitamin E group, which was not seen in the control group. Serum MDA levels were elevated to the same extent in both groups at the start of the study. There was a statistically significant decrease in MDA in the Vitamin E group alone at the end of the trial (Tables 1 & 2, Figure 2). Similar results were seen with supplementation of Vitamin E and other antioxidants in RA by Helmy M et al and Jaswal S et al [16, 17].A recent study indicated that increased oxidative stress and/or defective antioxidant status contribute to the pathology of RA. Evidence has shown that NO, a highly reactive free radical, plays a significant role in the pathogenesis of inflammatory arthritis and inhibits proteoglycan synthesis. Inhibitors of NO synthases reduce swelling and other evidence of inflammation in animal models of arthritis [17]. Vitamin E is the most potent dietary antioxidant and it seems to uncouple joint inflammation and destruction in transgenic mouse models of arthritis, with a beneficial effect on joint destruction [18].

There was a corresponding, statistically significant, decrease of ESR and CRP in the Vitamin E group. This indicates that Vitamin E is not only an anti-oxidant that reduces a marker of oxidant tissue injury, but also lowers markers of inflammation. This is also in correlation with the results of McAlindon TE et al who showed that antioxidant property Vitamin E contributed to anti-inflammatory effect due to inhibition of formation of reactive oxygen species [19-21]. Their study also proved that high doses of Vitamin E protected people with established RA from disease progression [22,23]. In another study done by Singh U et al there was a decrease in CRP levels after therapy with Vitamin E [24]. The lowering of serum MDA by the supplementation of Vitamin E, when correlated with lowering of laboratory markers of inflammation in this group, highlights the role of free radical induced cell damage in RA, and the possible role for Vitamin E and other anti-oxidants as safer disease-modifying anti-rheumatoid agents

CONCLUSION

This study shows that administration of Vitamin E to patients with RA lowers serum MDA due to its antioxidant action. This study also shows that the addition of Vitamin E to the existing therapeutic regimen decreases disease activity as evidenced by the lowering of serum markers of inflammation.

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