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Effect of Antioxidant Vitamins C and E Supplementation on its Plasma Levels and on Lipid Profile in Pulmonary Tuberculosis Patients

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Abstract: Problem statement: Patients with active pulmonary Tuberculosis (TB) are malnourished as indicated by reductions in lean mass, anthropometric indices and micronutrient status. Supplementation with vitamins may prove to be beneficial. Limited information is available on the supplementation of vitamin C and E in pulmonary TB patients. Hence, the present study was undertaken to address the question whether any benefit could be demonstrated with supplementation of antioxidant vitamins C and E and in combination in pulmonary TB patients. **Approach:** A five arm study was carried out for a period of 6 months in which the normal healthy volunteers served as control group and the sputum positive category I pulmonary TB patients served as the treatment group. Three out of the four patient groups received the antioxidant vitamin supplementation of either vitamin C, vitamin E or in combination along with ATT, whereas fourth group received ATT alone. Plasma concentrations of vitamin C and E were analyzed pre, during and post Anti-Tuberculosis Therapy (ATT) to establish the role of oral supplementation of these vitamins. Sputum culture was also done at all the three times points for *Mycobacterium tuberculosis*. To study the possible interaction and influence of the supplemented vitamins on LDL-C and HDL-C and other lipid parameters a lipid profile was carried out. **Results:** (1) All the patients in the treatment groups turned sputum at the end of 2 months of treatment. (2) There was a significant improvement in the body weights of the patients upon supplementation with antioxidant vitamins. (3) There was a significant increase in the of plasma ascorbic acid and α-tocopherol levels after 6 months of treatment in the vitamin C and vitamin E supplemented groups. (4) The HDL-C levels increased significantly in the antioxidant vitamin supplemented groups. (5) Lower levels of LDL-C were observed in the antioxidant vitamin supplemented groups when compared to the un-supplemented group. **Conclusion:** The plasma concentrations of ascorbic acid and α-tocopherol increased significantly after their dietary supplementation. The increase could also be due to the synergy exhibited by both the vitamins. Vitamin C and vitamin E supplementation influenced the lipid profile by increasing the serum HDL-C, improving the total cholesterol levels and decreasing the LDL-C concentration in the antioxidant vitamin supplemented groups.

Key words: Pulmonary TB, vitamin C, vitamin E, antioxidants, mycobacterium tuberculosis

INTRODUCTION

Pulmonary Tuberculosis (TB) is an infectious and contagious disease which apparently develops under conditions of a deficient immunologic response. The immune system requires a wide variety of nutrients to function adequately and some studies suggest that nutritional supplementation may represent a novel approach for fast recovery[1].

Free radicals are thought to play a major role in the etiology of a wide variety of diseases including atherosclerosis, respiratory tract, neurodegenerative disease, inflammatory bowel disease, cancer and in aging[2,3]. Human tissues are protected from oxidative damage by a variety of mechanisms including small molecular weight antioxidants like vitamins C and E[4]. Of all essential nutrients, vitamin C has generated the greatest interest for its potential influence on immune function and host defense. Vitamin C supplements have been shown to alter many different indexes of human immune responses and the concentration of vitamin C is high in activated neutrophils and macrophages[5,6].

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Vitamin E (α-tocopherol) is one of the major, antioxidant found in membranes and is found to improve the immune functions[7]. Several studies have shown that the association of ascorbic acid and their derivatives with α-tocopherol enhances the antioxidant capability of both the two vitamins. In studies with human subjects, vitamin C supplementation increased plasma lipid standardized α-tocopherol[8]. Vitamin C supplementation also led to a higher level of vitamin E in plasma of the participants who were administered 800 mg day^{-1} of vitamin E than in participants administered vitamin E alone[9]. Vitamin E and vitamin C act as antioxidants independent of each other and protect cells when compared to cells lacking both vitamins C and E. Vitamin C can also regenerate oxidized Vitamin E by reducing it back to its active form. The key step is the reaction between the tocopheroxyl radical and vitamin C[10]. Vitamin C regenerates active vitamin E and increases cholesterol excretion[11].

As an antioxidant vitamin C protects lipids, particularly LDL-C from oxidation. It is the only antioxidant that has been shown to prevent the initiation of the oxidative chain reaction in lipids trapping free radicals in the aqueous phase before they can diffuse into lipids such as LDL[12]. It affects serum cholesterol concentration, possibly through its role in the production of enzymes involved in the biosynthesis or hydroxylation of cholesterol[13].Vitamin E has been shown to slow the rate of LDL oxidation[12,14].

Several studies have reported that patients with active pulmonary TB are malnourished as indicated by reductions in lean mass, anthropometric indexes and micronutrient status. Malnutrition compromises barrier function, allowing easier access by pathogens and compromises immune function thereby, decreasing the ability of the host to eliminate pathogens once they enter the body. Thus, malnutrition predisposes to infections[15].

Therefore supplementation with vitamins may prove to be beneficial. Limited information is available on the supplementation of vitamin C and E in pulmonary TB patients. Hence the present study was undertaken to address the question of whether any benefit could be demonstrated with supplementation of antioxidant vitamin C and vitamin E and in their combination in pulmonary TB patients and also to evaluate their effect on the serum concentrations of ascorbic acid, α-tocopherol and lipid profile.

MATERIALS AND METHODS

Subjects: Sputum positive pulmonary TB patients in the age group of 25-60 years of either sex, registered in four corporation dispensaries in Chennai were approached to participate in the study. The patients were grouped as follows:

Group I: Controls (normal healthy volunteers, n = 30)
Group II: Patients undergoing Anti-Tuberculosis Treatment (ATT) alone, (n = 5)
Group III: Patients receiving vitamin C in addition to ATT, (n = 5)
Group IV: Patients receiving vitamin E in addition to ATT, (n = 5)
Group V: Patients receiving vitamin C and vitamin E in addition to ATT, (n = 5)

Patients were treated with category I regimen according to RNTCP (Revised National Tuberculosis Control Program) guidelines and were given short course chemotherapy DOTS (Directly observed treatment short course). The regimen consisted of Isoniazid 600 mg, Rifampicin 450 mg, Pyrazinamide 1500 mg and Ethambutol 1200 mg thrice weekly for the first 2 months followed by Rifampicin and Isoniazid for the next 4 months. Patients in groups III to V received vitamin C or vitamin E or both on days they received ATT. All the drugs including vitamin supplementation were administered under supervision.

Sputum samples of all the TB patients were tested for mycobacterium tubercle at 2and 6 month after the start of the treatment.

Exclusion criteria:

- Presence of causes of secondary immunodeficiency such as HIV, renal transplant patients, diabetes mellitus or malignancy
- Hepatitis B and C positive patients
- Patients with extra pulmonary TB and/or patients requiring surgical intervention.
- Currently receiving cytotoxic therapy, or have received it within last 3 months.

The purpose of the study was explained in detail to all the participants and only those who were willing to participate were recruited. Informed consent was obtained from all the participants. The study was conducted after obtaining approval from the Ethical committee of Madras Medical College, Chennai.

Design and conduct of study: The study was conducted at four corporation dispensaries in Chennai. All the patients were investigated before initiation of the treatment (0 month) and at 2 and 6 months after the start of treatment. The control subjects were

investigated at only one time point. At all three time points blood samples were collected in heparinised (3 mL) and plain vacutainer tubes (2 mL) after an overnight fast of 12 h. The heparinised plasma was used for vitamin C and vitamin E estimations and serum was used for measurements of lipids. Plasma and serum samples were stored at-80°C until analysis.

Estimation of vitamin C (Ascorbic acid): Vitamin C was estimated as ascorbic acid in plasma. Ascorbic acid was measured spectrophotometrically by 2,4 dinitrophenylhydrazine method[16]. In brief, plasma was mixed with meta phosphoric acid, thiourea, copper sulphate and 2,4 dinitrophenylhydrazine and incubated in a 37°C water bath for 3 hrs, to all the tubes, 2 mL of cold H_2SO_4 (conc.) was added and optical density recorded at 520 nm.

Estimation of vitamin E (α-tocopherol): Vitamin E was estimated as α-tocopherol in plasma. Plasma levels of α-tocopherol was estimated by HPLC according to a Elisabeth Teissier[17]. The HPLC system used was Shimadzu Vp Series (Shimadzu Corporation, Japan) equipped with two pumps (LC-10 AT Vp), fluorescence detector (RF-10AXL) and system controller (SCL-10 AVp). In brief, plasma sample were suitably diluted in methanol and centrifuged and an aliquot of the supernatant was injected directly into a reverse phase, C_{18} column (LiChroCART 5 µm, 250×4.0 mm, Merck KgaA, Germany); α-Tocopherol was measured by its native fluorescence (350 nm excitation, 440 nm emission) wavelength.

Lipid profile: Total cholesterol, triglycerides, Very Low Density Lipoprotein-C (VLDL-C), Low Density Lipoprotein-C (LDL-C) and High Density Lipoprotein-C (HDL-C) were measured using the enzymatic Kits supplied by ERBA-TEST, (TransAsia Bio-Medicals Ltd, Mumbai, India) following the manufacturer's instructions VLDL-C was calculated by subtracting the sum of HDL-C and LDL-C from total cholesterol.

Statistical analysis: Statistical evaluation was carried using SPSS (version 14.0). All the values were expressed as mean and standard deviation (mean ± SD). The mean values obtained in the different study groups was compared by Student's t test and ANOVA Post-Hoc (Tukey).Test was performed with 95% confidence interval or 5% level of significance.

RESULTS

Plasma levels of α-tocopherol, ascorbic acid and lipid profile were determined in a total of 20 pulmonary tuberculosis patients before treatment i.e., at 0, 2 and 6 months of treatment. Thirty healthy volunteers served as control. Demographic data and clinical profile of controls and pulmonary tuberculosis patients are shown in Table 1. The patients of all the treatment groups were sputum negative at 2 and 6 months of ATT. The baseline hemoglobin (Hb) levels in patient groups when compared with the control group showed a significant decrease ($p<0.001$). However, at the end of 6 months of ATT there was a significant increase in the Hb levels ($p<0.001$) in all the supplemented groups. The Hb levels increased from baseline level of 10.00 gm % to 15.35 gm % at 6 months of treatment in the vitamin C supplemented group, (group III). A significant increase ($p<0.01$) was also observed in the ATT group, (group II) between the baseline level hemoglobin of 10.2 gm % to 13.5 gm % at end of 6 months of treatment. At the end of 6 months of treatment the Hb level in group III (15.35 gm %) and group V (15.22 gm %) showed a significant increase ($p<0.01$) compared the group II (13.5 gm %).

Table 1: Demographic data and clinical profile of controls and pulmonary tuberculosis patients

Particulars	Time interval (months)	Control group (Group I)	ATT alone (Group II)	ATT + Vit C (Group III)	ATT + Vit E (Group IV)	ATT + C + E (Group V)
No of subjects		30	5	5	5	5
Males		15	3	3	4	2
Females		15	2	2	1	3
Age		25-60	26-51	25-58	27-56	26-60
Sputum	0	Negative	Positive	Positive	Positive	Positive
	2nd		Negative	Negative	Negative	Negative
	6th		Negative	Negative	Negative	Negative
Hb%§	0	14.5±2.01	10.20±1.02 [a]***	10.00±0.82 [a]***	10.02±1.01[a]***	10.43±0.54[a]***
	2nd		11.16±1.1	12.14±1.27	11.35±1.12	11.96±1.07
	6th		13.50±0.60 [c]**	15.35±1.15 [d]***, [g]**	14.10±1.35[h]*	15.22±0.89[f]***, [i]**
Body weight in Kgs §	0	63.5±13.0	45.00±15.0 [a]***	45.50±9.5 [a]***	45.00±7.0 [a]***	44.50±10.0[a]***
	2nd		45.50±10.0	48.00±10.0	48.00±7.0	47.50±9.0
	6th		47.00±10.0	51.50±9.0 [d]***, [g]*	52.50±7.0 [e]***, [h]*	52.00±10.0[f]***, [i]*

§: Results are expressed as mean ± SD; Control Vs 0 month of group II, III, IV and V-a; Control Vs 6th month of group II, III, IV and V-b; Group II 0 month Vs group II 6th month-c; Group III 0 month Vs group III 6th month-d; Group IV 0 month Vs group IV 6th month-e; Group V 0 month Vs group V 6th month-f; Group II 6th month Vs group III 6th month-g; Group II 6th month Vs group IV 6th month-h; Group II 6th month Vs group V 6th month-i; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

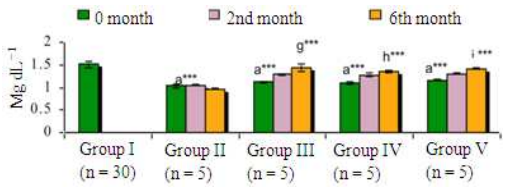

Fig. 1: Serum ascorbic acid levels in control and treatment groups. The blood samples were collected before the start of the treatment (0 month) and follow up was done at 2nd and 6th of treatment. Following comparisons were done:
Control Vs 0 month of group II, III, IV and V-a;
Group II 6th month Vs group III 6th month-g,
Group II 6th month Vs group IV 6th month-h;
Group II 6th month Vs group V 6th month-i;
*: p<0.05; **: p<0.01; ***: p<0.001

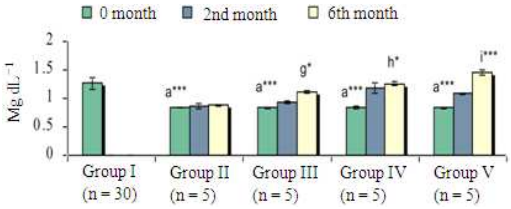

Fig. 2: Serum α-tocopherol levels in control and treatment groups. The blood samples were collected before the start of the treatment. (0 month) and follow up was done at 2nd and 6th of treatment. Following comparisons were done:
Control Vs 0 month of group II, III, IV and V-a;
Group II 6th month Vs group III 6th month-g;
Group II 6th month Vs group IV 6th month-h;
Group II 6th month Vs group V 6th month-i;
*: p<0.05; **: p<0.01; ***: p<0.001

Malnutrition is usually associated with pulmonary TB patients. The body weights of treatment groups were significantly lower (p<0.001) with an average of 45 kg when compared to the control group 63.5 kg. All the antioxidant vitamins supplemented groups (group III, V and V) showed a significant increase (p<0.01) with an average increase of 7 kg in body weight after 6 months of treatment. A notable increase of p<0.05 was observed in all the supplemented groups when compared to the 6th month values in group II.

Plasma ascorbic acid levels in pulmonary tuberculosis patients and normal healthy volunteers are shown in Fig. 1. The mean plasma ascorbic acid levels were significantly lower (p<0.001) at baseline with an average of 1.08 mg dL^{-1} in all TB patients when compared to controls 1.52±0.07 mg dL^{-1}. However, there was no significant difference in ascorbic acid levels among patient groups II, III, IV and V at baseline. At 2 months of treatment the plasma ascorbic levels showed a statistically significant increase (p<0.001) among all the three vitamin supplemented groups when compared to those on ATT alone. No significant difference in plasma ascorbic acid levels were observed between 0 and 2 months of treatment in group II (1.03±0.03 mg dL^{-1} and 1.05±0.02 mg dL^{-1}). After 6 months of treatment, groups III (1.43±0.09 mg dL^{-1}) group IV (1.34±0.03 mg dL^{-1}) and group V (1.42±0.02 mg dL^{-1}) showed a significant increase (p<0.001) in the plasma ascorbic acid levels when compared to group II (0.97±0.01 mg dL^{-1}), indicating increase in ascorbic acid levels upon supplementation.

A significant increase (p<0.001) was observed in groups III and V when compared to groups IV and II at the end of 6 months of treatment. The plasma ascorbic acid were found to be decreased from (1.03±0.03 mg dL^{-1}) to (0.97±0.01 mg/ dL^{-1}) at 0 and 6 months in the group II.

Plasma α-tocopherol levels in normal healthy volunteers and pulmonary tuberculosis patients at 0, 2 and 6 months of treatment are shown in Fig. 2. At baseline the α-tocopherol levels were found to be significantly lower (p<0.001) in patient groups with an average of 0.83 mg dL^{-1} when compared to the control group level of 1.27±0.10 mg dL^{-1}. No significant increase in plasma α-tocopherol levels was observed after 2 and 6 months of treatment in group II.

However, at 2 months of supplementation the α-tocopherol levels were increased significantly in group III, 0.93±0.02 mg dL^{-1} (p<0.05), group IV 1.18±0.09 mg dL^{-1} and group V 1.08±0.02 mg dL^{-1} (p<0.001) when compared to their baseline levels of 0.83±0.01, 0.84±0.02 and 0.83±0.01 mg dL^{-1} respectively. Group II did not show any significant difference.

There was a significant increase (p<0.001) in group V (1.45±0.04 mg dL^{-1}) at the end of 6 months of treatment as compared to group II (0.88±0.01 mg dL^{-1}), group III (1.12±0.02 mg dL^{-1}) and group IV (1.26±0.03 mg dL^{-1}).

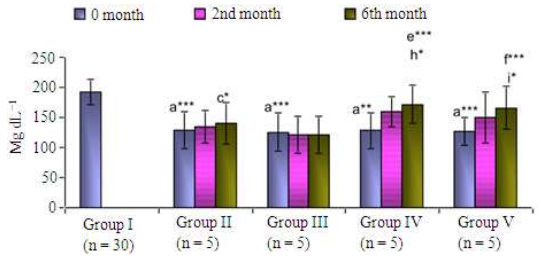

Fig. 3: Serum cholesterol levels in control and treatment groups. The blood samples were collected before the start of the treatment. (0 month) and follow up was done at 2nd and 6th of treatment. Following comparisons were done:
Control Vs 0 month of group II, III, IV and V-a;
Group II 0 month Vs group II 6th month-c;
Group IV 0 month Vs group IV 6th month-e;
Group V 0 month Vs group V 6th month-f;
Group II 6th month Vs group IV 6th month-h;
Group II 6th month Vs group V 6th month-i;
*: p<0.05; **: p<0.01; ***: p<0.001

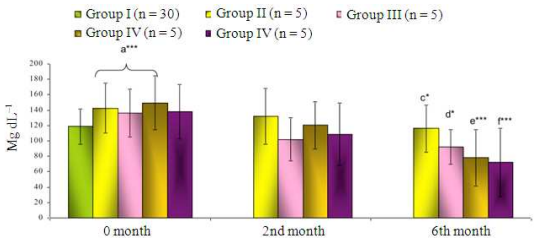

Fig. 4: Serum LDL-C levels in control and treatment groups. The blood samples were collected before the start of the treatment (0 month) and follow up was done at 2nd and 6th of treatment. Following comparisons were done:
Control Vs 0 month of group II, III, IV and V-a;
Group II 0 month Vs group II 6th month-c;
Group III 0 month Vs group III 6th month-d;
Group IV 0 month Vs group IV 6th month-e;
Group V 0 month Vs group V 6th month-f;
*: p<0.05; **: p<0.01; ***: p<0.001

Levels of α-tocopherol at the end of 6 months of treatment in group IV (1.26±0.03 mg dL^{-1}) was similar to that seen in normal healthy volunteers (1.27±0.10 mg dL^{-1}). On the other hand, we observed a significant increase of (p<0.01) in α-tocopherol levels after 6months of treatment in group V when compared to the normal healthy volunteers.

All TB patients had low levels of total cholesterol, LDL-C and HDL-C compared to the control group. However, the levels increased upon ATT treatment. A significant increase (p<0.01) in total cholesterol levels Fig. 3 were observed following 6 months of treatment in all groups with an average level from 128.21±28.0-159.67±34.2 mg dL^{-1} in all patient groups except group III where pre and post treatment cholesterol levels were 125.82±31.02 mg dL^{-1} and 122.0±30.69 mg dL^{-1} respectively. Total cholesterol levels significantly increased from 0-6 months of treatment in group II from 129.36±30.65-141.0±34.88 mg dL^{-1} (p<0.05) group IV 128.58±29.80-171.83±32.02 mg dL^{-1} (p<0.001) and group V 126.69±23.59-166.18±35.74 mg dL^{-1} (p<0.001).

Baseline serum levels of LDL-C in Fig. 4 at 0 month were higher (p<0.001) in all the patient groups when compared to the normal healthy volunteers. Upon 6 months of treatment LDL-C levels decreased significantly in from 149.40±35.0-78.26±36.81 mg dL^{-1} in groups IV and 137.90±34.96-72.02±44.60 mg dL^{-1} in group V (p<0.001), from 142.64±31.15 mg dL^{-1}-116.37±30.60 mg dL^{-1} groups II and from 136.06±31.15-92.26±22.60 mg dL^{-1} in group III with a p-value of (p<0.05).

LDL-C in group II (116.37±30.60 mg dL^{-1}) after 6 months of treatment were similar to that of normal healthy volunteers group (118.78±22.91 mg dL^{-1}). In Fig. 5 the serum HDL-C levels were low (p<0.001) at baseline in patient groups when compared to the control group.

Serum HDL-C levels increased significantly (p<0.05) from baseline to 6 months of treatment in groups II (21.46±6.61-32.25±9.98 mg dL^{-1}) where as group IV (23.33±10.62-31.38±6.53 mg dL^{-1}) and V (22.44±10.62-33.86±5.36 mg dL^{-1}). Whereas, group III showed a significant increase of (p<0.01) (21.43±6.61 - 40.93±6.99 mg dL^{-1}. However, the treatment groups had levels significantly low even after 6 months of treatment (p<0.01) compared to the control group (47.45±10.60 gm dL^{-1}).

The serum levels of VLDL-C and triglycerides did not show any significant difference between control and the treatment groups. During the course of the treatment the VLDL-C levels as shown in Fig. 6 increased significantly (p<0.05) from baseline levels in groups IV 31.20±6.84-36.58±13.50 mg dL^{-1} and group V 28.61±9.29-34.88±10.63 gm dL^{-1}. No significant

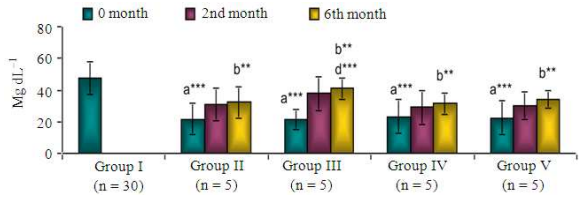

Fig. 5: Serum HDL-C levels in control and treatment groups. The blood samples were collected before the start of the treatment (0 month) and follow up was done at 2nd and 6th of treatment. Following comparisons were done:
Control Vs 0 month of group II, III, IV and V-a;
Control Vs 6th month of group II, III, IV and V-b;
Group III 0 month Vs group III 6th month-d;
*: p<0.05; **: p<0.01; ***: p<0.001

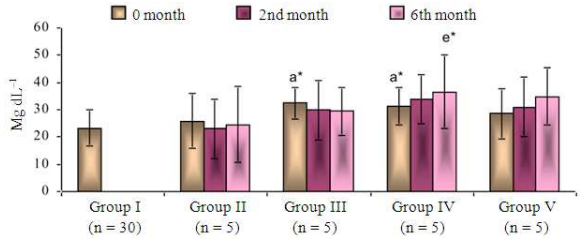

Fig. 6: Serum VLDL-C levels in control and treatment groups. The blood samples were collected before the start of the treatment. (0 month) and follow up was done at 2nd and 6th of treatment. Following comparisons were done
Control Vs 0 month of group II, III, IV and V-a;
Group IV 0 month Vs group IV 6th month-e;
*: p<0.05, **: p<0.01, ***: p<0.001

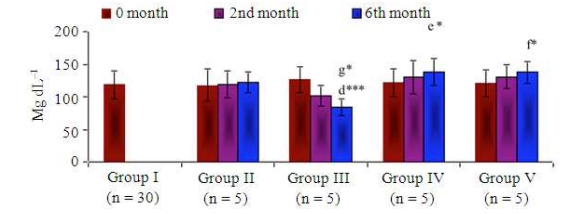

Fig. 7: Serum triglycerides levels in control and treatment groups. The blood samples were collected before the start of the treatment. (0 month) and follow up was done at 2nd and 6th of treatment. Following comparisons were done:
Group II 0 month Vs group II 6th month-c;
Group III 0 month Vs group III 6th month-d;
Group V 0 month Vs group V 6th month-f;
Group II 6th month Vs group III 6th month-g;
*: p<0.05; **: p<0.01; ***: p<0.001

difference was observed between 0 (baseline) and 6 month levels of VLDL-C in groups II 25.86±10.06-24.54±13.89 mg dL^{-1} and group III 32.38±5.70-29.40±8.80 mg dL^{-1}. Group III showed a significant decrease ($p<0.001$) in the levels of triglycerides from the baseline 126.20±20.33-6 mg dL^{-1} months 84.53±15.59 mg dL^{-1}. As shown in Fig. 7 a significant increase ($p<0.05$) in triglycerides level between the 0 and 6 months of treatment in the groups IV (from 121.62±21.53-138.15±20.63 mg dL^{-1}) and V (from 120.34±20.53-137.45±16.53 mg dL^{-1}) was observed.

DISCUSSION

This study investigated, whether supplementation with anti-oxidant vitamins C, vitamin E alone or in combination has a measurable effect on plasma levels of ascorbic acid and α-tocopherol. There are reports stating that low levels of vitamin A and anti-oxidant vitamins C and E have been observed patients with TB[18]. We also extended our study to correlate the changes in lipid profile with antioxidant vitamins supplementation in pulmonary TB patients. Patients with TB had low plasma levels of ascorbic acid when compared to normal healthy volunteers, however, none could be regarded as deficient in vitamin C. Vitamin C concentration of the supplemented group increased from baseline after 6 months of intervention indicating that plasma ascorbic acid takes longer to show a significant increase. A leveling-off in plasma vitamin C levels with increasing doses of vitamin supplementation has been reported by Hallfrisch *et al.*[19].

Plasma α-tocopherol levels also improved upon supplementation of vitamins E and C, this improvement in plasma α-tocopherol levels suggests synergism of vitamin C with glutathione peroxidase to revitalize vitamin E. Three antioxidants that were significantly decreased in tuberculosis patients are glutathione, ascorbic acid and α-tocopherol which forms the integral components of a regenerating redox cycle. Our results indicate this interaction between vitamins C and E *in vivo* and the extent of the interaction depends on the type of tissue. Supplementation with these vitamins maintains their plasma concentrations[20].

High correlations between plasma tocopherol and plasma lipid concentrations have been reported in the past. According to Horwitt *et al.*[21] plasma or serum tocopherol concentrations were dependent on the levels of circulating lipids and thus, tocopherol-lipid ratio could be utilized to assess the vitamin E status, as circulating vitamin E is carried with lipoprotein fractions.

We choose to supplement the TB patients with vitamin C and vitamin E as they are known to protect unsaturated fatty acids of LDL against oxidation by scavenging ROS before initiation of lipid peroxidation[22] and possibly by sparing or regenerating vitamin E[23]. Jialal *et al*.[24] showed that a minimum dose of 400 IU day^{-1} of vitamin E was required to significantly decrease the susceptibility of LDL to oxidation. In an *ex vivo* model used, LDL was exposed to Cu^{2+} ions, a potent prooxidant capable of initiating the oxidation of LDL. These results indicate d that LDL resistance to oxidation was impaired in vitamin E deficient patients but was normalized within 2 months when α-tocopherol is given in sufficient amounts[25,26]. We observed a decrease in the levels of LDL-C after supplementation with vitamin E. The same trend was observed with the other two supplemental groups also.

Our findings were similar to that of Carlos *et al*.[27] who reported decrease in levels of total cholesterol, HDL-C, VLDL-C and LDL-C in patients with diagnosis of pulmonary tuberculosis (group TB), pleural tuberculosis (group TBPL), miliary tuberculosis (group TBMI) and of patients with pulmonary tuberculosis and diabetes mellitus (group TBDM). These parameters (total cholesterol, HDL and LDL-C) can be used as an indirect marker of severity of tuberculosis[28]. This may be either due the fact that tuberculosis depletes cholesterol levels or hypocholesterolemic subjects are predisposed to develop tuberculosis. The latter association is very important in terms of cholesterol being used as a therapeutic tool for prevention and/or treatment of TB.

A significant improvement was observed in the serum cholesterol and triglyceride levels after 6 months of supplementation with vitamin E and in its combination with vitamin C. The source of blood lipids includes absorption from the intestines, mobilization from the fat depots and synthesis particularly in the liver. In tuberculosis patients decreased synthesis of lipids, either due to liver malfunction or malabsorption from the liver is reported. Almost all our study patients were malnourished, had low body weights and this has probably contributed to the lower lipid levels seen in these individuals.

Vitamin C supplementation resulted in a significant decrease in the total cholesterol levels, LDL-C and triglycerides whereas, HDL-C levels were increased significantly. Marc *et al*.[29] in a meta-analysis of randomized trials. Reported that vitamin C supplementation of 500 mg d for a minimum of 4 weeks resulted in a significant reduction in both LDL-C and triglycerides concentration which were similar to our findings. Paul *et al*.[30] observed that after vitamin C supplementation (1 g day^{-1}) for 8 months there was a significant decrease in total cholesterol and the triglyceride concentrations also decreased in first and second months of treatment. A significant ($p<0.05$) difference in HDL-C concentration between vitamin c and placebo treatment was also observed.

This study shows greatly reduced total cholesterol, LDL-C and HDL-C prior to supplementation. Six months of vitamin C supplementation was found to increase HDL-C concentrations and decrease LDL-C. The results of our study differ from the recent findings of Wolters *et al*.[20] who observed that vitamin C concentrations did not differ from baseline in healthy elderly women after 6 months of multivitamin supplementation. The observed improvement in our study could be attributed to the vitamin C's ability to intercept reactive oxygen species in the aqueous phase of plasma, thereby significantly reducing plasma lipid peroxide levels and inhibiting oxidative modification of LDLs and it also protects HDL-C from lipid oxidation and making it available for reverse cholesterol transport[31]. HDL also inhibit LDL oxidation and this free radical scavenging effect occurs via an antioxidant enzyme called HDL- associated paraoxonase and the loss of this enzyme during oxidative stress is prevented by vitamin C.[32].

Vitamin C's antioxidant protection facilitates the faster uptake of triglyceride from the plasma and promotes its removal from circulation thereby decreasing the serum triglyceride concentration[33]. Short-term vitamin E supplementation is known to improve immune responsiveness.[34]. Supplementation with vitamin E in humans decreases the susceptibility of LDL to oxidation ex vivo. It is well understood that, vitamin C preserves LDL α-tocopherol levels during oxidative stress by converting the α-tocopherol back to the reduced state so that it may function again as an antioxidant. Thus, the presence of vitamin C in the plasma may increase or maintain the content of lipid-soluble antioxidant in LDL when both are given simultaneously. The dietary supplementation of vitamin E can significantly increase the LDL content of vitamin E and in turn, confers significant protection against oxidative stress[35].

Effect of supplementation on plasma concentration of ascorbic acid and α-tocopherol showed a positive correlation as there was a significant increase in the supplemented population. It is possible, however, that tissue levels were increased more than plasma levels. Antioxidant vitamins were well tolerated and were free from toxicity.

Our study provides enough evidence that dietary antioxidant vitamins may provide an alternative to the widely applicable approach of prevention of free radical mediated tissue damage. Improve the plasma concentrations of vitamin C, vitamin E and HDL-C. It may also reduce free radical activity in plasma, free radical diffusion from plasma to lipoprotein and thus, cell membrane may be consequently be decreased of free radical-induced lipid peroxidation. We have demonstrated in this study that supplementation of antioxidant vitamins have a positive bearing on overall health and recovery of the patient. As the present study involved a small sample size, it is warranted to conduct studies in large samples to find similar encouraging results and include vitamin supplementation in the regimen of ATT.

CONCLUSION

Our results conclude that supplementation with vitamins C and E improved their levels in plasma and was helpful in improving total cholesterol and HDL-C levels and in decreasing the oxidation of LDL-C in patients with pulmonary tuberculosis. Supplementation may also be beneficial in the prevention of hypocholesterolemia in patients with tuberculosis. The results are encouraging to suggest antioxidant vitamins supplementation in the regimen of ATT for the overall well-being of the patient. However, larger cross-sectional and longitudinal studies are warranted to confirm our observations.

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