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EFFECT OF VITAMIN E SUPPLEMENTATION ON OXIDATIVE STRESS IN HEMODIALYSIS PATIENTS

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ABSTRACT

Hemodialysis represents a chronic stress status for its recipients. Many hypotheses state that this may be associated with oxidative stress. Thus, there may be deficiency of antioxidants like erythrocytic superoxide dismutase, catalase, vitamin E or increased generation of free radicals like superoxide anions. A study was carried out to investigate oxidant and antioxidant status in chronic renal failure patients undergoing hemodialysis and effect of vitamin E supplementation on these two status. Blood samples were collected from patients before and after hemodialysis and from controls. The samples were analyzed for quantitation of MDA as index of lipid peroxide, nitric oxide, vitamin E, vitamin C and enzymatic antioxidants namely erythrocyte SOD and catalase. As compared to controls, the levels of serum MDA were significantly increased and activities of erythrocyte SOD and catalase, levels of serum nitric oxide, serum vitamin E and plasma vitamin C were significantly decreased both before and after hemodialysis. The efficiency of vitamin E therapy in hemodialysis patients was assessed by re-evaluating oxidant and antioxidant status of same patients after supplementation of vitamin E. Vitamin E supplementation caused decrease in serum MDA and increase in levels of serum nitric oxide, vitamin E, vitamin C and activities of erythrocytic SOD and catalase. Our results suggest the presence of oxidative stress and the possible preventive role of vitamin E therapy in hemodialysis patients.

KEY WORDS

Hemodialysis, Oxidative stress, Antioxidant, Vitamin E, MDA, Erythrocytic SOD.

INTRODUCTION

Development of chronic renal failure has become serious problem all over the world. Chronic renal failure (CRF) is characterized by slow and progressive decline in the kidney function. Some of the manifestations include accelerated aging, cataract, atherosclerosis, increased hemolysis, platelet dysfunction, neuropathy etc. These sequelae of chronic renal failure have been attributed to the overproduction of free radicals in these patients (1).

Reactive oxygen species (ROS) can damage proteins, lipids, carbohydrates and nucleic acids. Plasma membrane is critical target for free radicals. The malondialdehyde (MDA), a short chain aldehyde is an intermediate product of the oxidation of polyunsaturated fatty acids present in plasma membrane and has been used as indicator of lipid peroxidation reactions (2).

Nitric oxide ($NO^{\bullet}$) is a potent mediator displaying a broad spectrum of activities, including smooth muscle relaxation, impairment of myocyte proliferation, cytotoxic reactions, and neuronal transmission (3). Several investigators have focused their attention on a possible role played by $NO^{\bullet}$ in the development of uremic symptoms, but the results were controversial and the matter is still debated (4,5).

To defend themselves against the free radical attacks, cells have developed various antioxidant systems. Several enzymatic systems can detoxify free radicals: copper/zinc superoxide dismutase (Cu/Zn SOD) catalyzes conversion of superoxide anion to hydrogen peroxide and works concomitantly with hydroperoxide removing enzymes such as

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catalase and glutathione peroxidase. In addition, some micronutrients can prevent the harmful effects of free radicals by nonenzymatic modes like vitamin E (α- tocopherol) and vitamin C (ascorbic acid). α- tocopherol prevents damage to polyunsaturated fatty acids by free radicals in membranes. In this process, α- tocopherol is converted to tocopheryl radical. This radical is converted back to α- tocopherol with the help of ascorbic acid (6). An imbalance between the production of reactive oxygen species and various antioxidant defenses result in oxidative stress and may be implicated in hemodialysis related complications. The present study was planned to investigate the possible alterations of oxidant - antioxidant status in hemodialysis patients and effect of vitamin E supplementation.

MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry, Dr. V. M. G. Medical College, Solapur. This study included 20 chronic renal failure patients in the age group of 35 to 60 years undergoing hemodialysis. 20 age and sex matched controls were also included in the study. The patients were diagnosed by physicians on the basis of detailed clinical history, clinical examination, and other relevant biochemical investigations. All patients were dialyzed twice weekly, each time for four hours with polysulfone dialyzing membrane. Smokers, diabetics, alcoholics, patients with chronic inflammatory conditions, or hepatic or respiratory diseases were excluded from the study. Informed consent was obtained from each participant in the study. The study was cleared by institutional ethical committee.

10 ml blood was collected before and after hemodialysis from each patient. 5 ml of it was collected in heparinized bulb and 5 ml was collected in plain bulb. Plasma and serum were separated from respective bulbs by centrifugation at 3000 rpm for 10 minutes at room temperature. All the samples were analyzed on the same day of collection.

Serum MDA levels were measured by reacting them with thiobarbituric acid at high temperature to form pink colored complex which was measured colorimetrically at 530 nm (7). Nitric oxide was determined by Griess reaction as in Cortas and Wakid method (8). Nitric oxide is a labile and diffusible molecule, which forms stable metabolites (nitrite / nitrate, NO_2^- & NO_3^-), which are detected by Griess reaction. In kinetic method nitrate is reduced to nitrite by copper coated cadmium granules. This nitrite produced is determined by diazotization of sulfanilamide and coupling to naphthylethylenediamine to form purple complex, which was measured at 545 nm. Serum vitamin E was measured by their reduction of ferric to ferrous ion which then forms a red colored complex with α- α'-bipyridyl as in Baker and Frank method (9). The complex was measured colorimetrically at 520 nm. Plasma vitamin C was determined by DNPH method (10) where vitamin C is oxidized to diketogulonic acid, which reacts with 2,4 dinitrophenyl-hydrazine to form diphenylhydrozone. The hydrozone dissolves in strong acid solution to form red colored comlex, which was measured at 500 nm. Erythrocyte SOD activity was measured by Kazari Das method (11) which is based on the ability of SOD to inhibit nitrite formation. Catalase activity was measured in erythrocytes by the method of Aebi H using hydrogen peroxide as substrate. The method is based on the decomposition of hydrogen peroxide, which is indicated by decrease in the absorbance at 240 nm (12).

All patients were given two months supply of 400 IU/ day of vitamin E (Bio E^{TM} 400) under medical supervision. Blood samples were collected after first and second month and processed identically. The values were expressed as mean±SD. Student's 't' test was done for comparison of data.

RESULTS

Significantly higher levels ($P<0.001$) of serum total lipid peroxide (MDA) were observed in prehemodialytic samples as compared to controls. Significantly decreased levels of serum nitric oxide ($p<0.05$), serum vitamin E ($p<0.001$) and plasma vitamin C ($p<0.001$) were observed in prehemodialytic samples as compared to controls. There was significant decrease in erythrocyte SOD activity ($p<0.001$) and blood catalase activity ($p<0.001$) in prehemodialysis samples as compared to controls.

Significant increase in serum total lipid peroxide (MDA) was observed in posthemodialytic samples as compared to prehemodialytic levels ($p<0.05$). The posthemodialytic levels of vitamin E and vitamin C were significantly decreased as compared to those of prehemodialytic levels ($p<0.05$). Nonsignificant decrease in serum nitric oxide level, erythrocyte SOD activity and blood catalase activity was observed in posthemodialytic samples when compared to prehemodialysis ($p>0.05$).

There was significant decrease in serum MDA and significant increase in serum nitric oxide, serum vitamin E, plasma vitamin C, erythrocyte SOD activity and blood catalase activity in hemodialysis patients after two months vitamin E supplementation.

Table 1 : Levels of biochemical parameters in hemodialysis (HD) patients and controls

Parameter	Controls	Hemodialysis Patients			
		Pre HD	Post HD	Vitamin E Supplementation 1 Month	Vitamin E Supplementation 2 Months
Serum MDA (μmol / l)	1.97±0.55	3.43±0.49 A1	3.78±0.30 A1, B1	3.00±0.41	2.73±0.40 B3, C1
Serum Nitrite (μmol / l)	67.41±4.07	61.58±6.75 A2	59.16±7.3 A1, B2	64.53±4.5	66.95±5.5 B1, C1
Serum Vitamin E (mg/dl)	1.25±0.20	0.89±0.08 A1	0.84±0.06 A1, B1	1.76±0.21	1.93±0.22 B3, C1
Plasma Vitamin C (mg/dl)	1.54±0.41	0.96±0.28 A1	0.79±0.08 A1, B1	1.33±0.21	1.48±0.16 B3, C1
Erythrocyte SOD (U/ mg Hb)	1.53±0.63	0.87±0.09 A1	0.81±0.10 A1, B2	1.01±0.18	1.21±0.38 B3, C1
Blood Catalase (k/gm Hb)	291.56±13.48	223.47±22.47 A1	210.09±23.78 A1, B2	233.64±19.54	244.59±21.81 B1, C1

Values are expressed as mean ± SD.
A1: Comparison with control $p<0.001$; A2: Comparison with control $p<0.05$; B1: Comparison with pre HD $p<0.05$
B2: Comparison with pre HD $p>0.05$; B3: Comparison with pre HD $p<0.001$; C1: Comparison with post HD $p<0.001$

DISCUSSION

Chronic renal failure is one of the commonest kidney diseases. It may be associated with oxidative stress. Many cells in kidney like vascular cells, glomerular and tubular cells may produce ROS in response to the inflammation process. In addition, many circulating infiltrating cells like platelets, granulocytes and macrophages may produce ROS as a part of inflammatory process (13).

In the present study, the finding of increased serum MDA levels in CRF patients in prehemodialysis indicate that indeed there is oxidative stress. Further increase in serum MDA levels in posthemodialysis shows that the oxidative stress has increased in these patients. This could be due to the fact that hemodialysis by the application of a modified circulation and forced passage of blood through a number of filters, activates, endogenous inflammatory mechanisms and induces chronic release of molecules resulting in an increased production of reactive oxygen species (14).

Decrease in nitrite levels in the patients before and after hemodialysis may imply that either there is decreased $NO^{\bullet}$ synthesis or increased diversion towards its reaction with superoxide anion or both. It is well documented that in CRF, plasma nitrite levels increase even though $NO^{\bullet}$ synthesis is normal due to retention of nitrite in body. However in the present study, nitrite levels are decreased. This decrease cannot be attributed alone to the fact that more and more $NO^{\bullet}$ is used up in its reaction with superoxide anion. There has to be decreased synthesis of $NO^{\bullet}$ also. It could be due to inhibition of nitric oxide synthase. It is known that oxidative stress may increase the synthesis of asymmetric dimethyl arginine (ADMA), which is an endogenous inhibitor of endothelial nitric oxide synthase (15). However further studies are required to acertain this hypothesis that nitric oxide synthase is inhibited and $NO^{\bullet}$ synthesis decreases.

The most probable explanation for decreased SOD activity is a possible direct inactivation of the enzyme by its product hydrogen peroxide, or by superoxide anion itself (16,17). Decreased SOD activity could also be related to trace element deficiencies in hemodialyzed patients (18). As SOD activity is decreased in hemodialysis patients, detoxification of superoxide anion is compromised leading to an increased oxidative stress.

Significant decrease in the activity of catalase could be due to less availability of NADPH. The hexose monophsphate pathway is the principal source of NADPH in RBCs and it has been suggested that chronic acidosis in uremia leads to inhibition of glucose–6–phosphate dehydrogenase activity, the key enzyme of this pathway (19). Our study showed a significant decrease in catalase activity both before and after hemodialysis. This decrease could also be due to increase in MDA, which can cross link with amino group of protein to form intermolecular cross links thereby inactivating several membrane bound enzymes (20).

Vitamin E is the most important lipophilic antioxidant in humans. The diminished vitamin E level could be due partly to its overconsumption as an antioxidant subsequent to increased production of free radicals. Vitamin E radical formed by free radical attack interact with vitamin C and regenerate vitamin E. In the process vitamin C is consumed and vitamin E is formed (6).

In CRF patients, vitamin C levels are low before and after hemodialysis. This could be due to the fact that oxidative stress causes more and more conversion of vitamin E to vitamin E radical. In order to re-generate vitamin E, vitamin C is used up leading to decrease in its level.

Significantly decreased serum MDA levels and increased antioxidant levels after vitamin E supplementation in hemodialysis patients reported in present study suggests direct protective effect of vitamin E against lipid peroxidation.

Significant increase in vitamin E levels after two months vitamin E supplementation indicate that vitamin is properly absorbed. Vitamin E exerts its antioxidant property by preventing chain propogation as a result of its ability to transfer phenolic hydrogen to a peroxyl free radical of a peroxidized polyunsaturated fatty acid, minimizing the lipid peroxidation (21). This is reflected in terms of decreased serum MDA levels observed in the present study. Vitamin E may have additive effect on reducing ADMA level, which are reported to be increased in renal diseases, and thus improving nitric oxide availability. This might be the possible reason for elevation of nitric oxide levels in hemodialysis patients after vitamin E supplementation. The present study demonstrated significant increase in erythrocytic SOD and catalase activities after vitamin E supplementation. This is in response to decreased free radical activity and lipid peroxidation. Also, correction of impaired vitamin C status may potentiate the vitamin E effect through regenration of vitamin E from vitamin E radical formed during the antioxidant action of vitamin E.

Our findings strongly support that oxidative stress is enhanced in hemodialysis patients which may contribute to development of dialysis related complications such as cardiovascular disease, anemia. The study further points to potential usefulness of vitamin E supplementation in the management of hemodialysis related oxidative stress. Additional trials with prolonged oral supply of vitamin E will assess its usefulness and clinical relevance for prevention and treatment of other oxidative stress – related disorders.

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