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Vitamin E Supplementation Improves Oxidant-antioxidant Balance in Chronic Renal Failure Patients Treated by Hemodialysis

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Authors' contributions

This work was carried out in collaboration between all authors. Author KM designed the study, performed the statistical analysis, wrote the protocol, and first draft of the manuscript. Authors AT and OTB performed the majority of experiments. Authors HFT and NBB participated in the data collation and statistical analysis. Author AK performed the recruitment of patients with chronic renal failure and provided the samples collection. Author BM managed the literature searches. All authors read and, approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The purpose of this study was to evaluate the effect of vitamin E supplementation on oxidant-antioxidant balance in chronic renal failure patients treated by hemodialysis.
Study Design: The study utilized a randomized experimental design. The experimental intervention consisted of vitamin E supplementation
Place and Duration of Study: 40 patients on hemodialysis (M/W=22/18; 36±12 years) received nutritional councils based on the NKF K/DOQI (National Kidney Foundation-Kidney Disease Outcomes Quality Initiative) guidelines. Patients were randomized into 2 groups:one was used as control and the second group was treated by vitamin E supplementation (100mg/day=146IU/d) during 30 days.
Methodology: Pro-oxidant status was assessed by thiobarbituric acid reactive substances, hydroperoxides and carbonyls analysis. Antioxidant defence was performed

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by the analysis of Superoxide dismutase, Catalase, Glutathione reductase activities and Vitamin E amounts.
Results: At 30 days of supplementation, in treated patients compared to controls, levels of triacylglycerols and total cholesterol were unchanged. Hydroperoxides concentrations were decreased ($p<0.001$) while thiobarbituric acid reactive substances concentrations were unchanged. Carbonyls levels were decreased ($p<0.001$). High concentrations of vitamin E were noted in treated group ($p<0.01$). Similar superoxide dismutase activity was noted. However, an increase in vitamin E concentrations, catalase and glutathione reductase activities were observed in treated group ($p<0.01$).
Conclusion: In conclusion, in hemodialysis patients, vitamin E supplementation was without effect on lipid profile. However, vitamin E exerts a protective effect on cardiovascular diseases by decreasing radical attack of biological molecules and increasing antioxidant defense.

Keywords: Pro-oxidant; antioxidant defence; nutritional intervention; Vit E supplementation; hemodialysis.

1. INTRODUCTION

Cardiovascular diseases (CVD) are the major risk of mortality in chronic renal failure (CRF) patients [1,2]. In addition to classical cardiovascular (CV) risk factors (age, hypertension, diabetes, smoking, dyslipidemia, sedentarity and unbalanced diet), patients with CRF and treated by hemodialysis (HD) have others risk factors for CVD including oxidative stress (OS), endothelial dysfunction, malnutrition and chronic inflammation [3].

Oxidative stress is increased in hemodialyzed patients and is considered as the major risk factor for cardiovascular complications. Oxidative stress results from an imbalance between oxidant production and antioxidant defence mechanisms with increased levels of pro-oxidants leading to tissue damage [4]. Antioxidants can be divided into intracellular and extracellular antioxidants. Intracellular enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GSH-GR). Several extracellular antioxidants, such as albumin, bilirubin and urate, prevent free radical reaction by sequestering transition metal ions by chelation in plasma [5].

The repeated contact of blood with dialysis membranes in patients receiving hemodialysis exposes them to a detrimental OS causing a chronic deficit in antioxidant defense system [6]. Patients on HD have a loss of some low molecular weight plasma factors as vitamin A, C and E [7]. Vitamin E (Vit E) is an antioxidant that inhibits LDL oxidation, limits cellular response to oxidized LDL and is used as a food supplement in the usual medical therapy. In chronic HD patients, Vit E may be administered in the form of dietary supplementation, or using Vit E-coated dialysis membranes [8-9].

The purpose of this study was to evaluate the effect of Vit E supplementation on oxidant-antioxidant balance in CRF patients treated by hemodialysis.

2. MATERIALS AND METHODS

A prospective randomized trial study was carried out in the hospital of Oran (west of Algeria). Forty hemodialysis patients (M/W=22/18; 36±12years) were included. Subjects were

excluded on the basis that they had a diabetic nephropathy or thyroid disease or used anti-inflammatory drugs or antioxidants vitamins. Patients were dialyzed since 14 to 109 months, three times a week, and each session lasting 4h with polysulfone membrane. The etiology of CRF included; hypertension (66%), cystic kidney disease (14%), glomerulonephritis (10%) and (10%) were unknown.

Nutritional patient's intervention: All patients received nutritional advices based on the NKF K/DOQI (National Kidney Foundation Kidney Disease Outcomes Quality Initiative) guidelines [10]. Patients were randomized into two groups; 20 patients received a supplementation with Vit E ((100mg/day=146IU/d) during 30 days, 20 patients were used as controls

All patients were treated at the Nephrology ward of the University Hospital of Oran. The purpose of this study was explained to the subjects and the investigation was carried out with their consent. The experimental protocol was approved by the Committee for Research on Human Subjects of Oran.

2.1 Assays

In all patients, blood samples were drawn after a 12-hours overnight fast by dialysis fistule, at the beginning (T0) and 30 days (T1) after initiating treatment. Samples were collected by low speed centrifugation at 3000xg at 5°C, for 15min, and were preserved with 0.1% Na_2 EDTA and 0.02% sodium azide. The characteristics of the study population are shown in (Table 1).

Table 1. Characteristics of the study populations

	T0		T1	
	Control group	**Vit E group**	**Control group**	**Vit E group**
Age(years)	37±13	45±15	55±11	45±15
Weight (kg)	66±12.74	67±13.96	67±16.12	67±13.96
BMI(Kg/m2)	24.07±7.16	24.91±3.54	25.35±5.63	24.91±3.54
Sex ratio(M/F)	10/10N=20	08/12N=20	10/10N=20	08/12N=20
Urea(g/l)	0.24±0.25	1.25±0.11	0.53±0.20	1.05±0.34
Creatinin(mg/l)	17.20±2.89	42.15±11.36	18.54±5.26	41.75±11.46
Uric Acid(g/l)	62.63±11.11	82.111±12.10	64.10±13.55	80.85±14.11
Total proteins(g/l)	72.10±5.11	72.10±10.10	74.29±7.52	71.16±11.15

Data are presented as the mean±SD

2.2 Lipids Analysis

Triacylglycerols (TG) and total cholesterol (TC) were determined by colorimetric methods (BioMérieux Kits, France). Serum high density lipoprotein-cholesterol (HDL-C) was determined enzymatically using the CHOD-PAP kit (BioMérieux Kits, SA-France) after precipitation of the chylomicrons, very low density lipoprotein cholesterol and Low density lipoprotein cholesterol (LDL-C) were precipitated with phosphotungstic acid and Mg^{++} (BioMérieux Kits, France). Serum LDL-C was determined enzymatically using the CHOD-PAP kit (France) after precipitation of LDL.

2.3 Lipid and Protein Peroxidation

Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) and hydroperoxides (LPO). TBARS concentrations were analyzed according to the method of Quintanilha et al. [11], using tetramethoxypropane (Prolabo) as precursor of malondialdehyde. One milliliter of diluted sample (protein concentration about 2mg/ml) was added to 2ml of thiobarbituric acid (final concentration, 0.017mmol/L), butylated hydroxytoluene (concentration, 3.36mmol/L) and incubated for 15min at 100°C. After cooling and centrifugation, the absorbance of supernatant was measured at 535nm. Data were expressed as mmol of TBARS produced/ml of serum. The plasma was also assayed to determine LPO with an assay kit from Cayman Chemical (cat #705003, USA), this kit allows the measurement directly hydroperoxides using redox reactions with ferrous ions. LPO are very unstable and react with the ferrous ions to generate ferric ion. The resulting ferric ions are detected using chromogenic as thiocyanate ion; the rate of increase in the absorbance at 500nm is directly proportional to the LPO produced. Oxidized proteins were estimated by measuring carbonyls concentrations according to the method of Levine et al. [12] using the 2.4-dinitrophenylhydrazine (DNPH).

2.4 Antioxidant Measurements

Activity of antioxidant enzymes was measured in serum. Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined with Sigma Chemical kits (cat # 19160; USA); at 450nm by measuring the dismutation of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit was defined as the amount of enzyme necessary to produce 50% inhibition in the rate of p-iodonitrotetrazolium reduction.

Catalase (CAT; EC 1.11.1.6; 2H2O2 oxidoreductase) activity was measured with an assay kit from Cayman Chemical (cat #707002, USA). CAT is involved in the detoxification of hydrogen peroxide (H_2O_2). CAT enzyme activity could be determined by using the peroxidatic function of CAT at 540nm. Glutathione reductase (GSH-GR; EC 1.6.4.2) enzyme activity was determined with an assay kit from Sigma Chemical (cat # GRSA, USA). GSH-GR catalyzed the reduction of GSSG to reduced glutathione (GSH). GSH-GR activity can be measured either the increase in absorbance caused by the reduction of DTNB at 412nm. For the Vit E we measured it in plasma with an assay kit from Cayman Chemical (cat # 10010621, USA). The absorbance of samples was measured between 405-420nm by a plate reader.

2.5 Statistical Analysis

Statistical analysis was performed using SPSS 20.0 (IBM SPSS statistics. USA). Data were expressed as the mean±SD (standard deviation). The comparison between groups was performed using way analysis of variance (ANOVA) or the Mann-Whitney U-test when results are non-parametrically distributed and a p value below .05 were considered statistically significant.

3. RESULTS

3.1 Lipids Parameters

No significant difference was noted in triacylglycerols, total cholesterol, C-HDL and C-LDL concentrations, in Vit E group compared to control group at T1 (Table 2). However, in Vit E group and control group, a significant reduction in triacylglycerols and total cholesterol was noted at T1 (P=.05) compared to T0, respectively.

3.2 Oxidative and Antioxidative Status

No significant difference was noted in thiobarbituric acid reactive substances values (Table 3) in Vit E group compared to control group at T1. However, as significant decrease in thiobarbituric acid reactive substances was noted in Vit E group at T1 compared to T0 (P=.05). In Vit E group, a significant decrease in LPO concentrations was noted at T1 compared to control group and to T0 (P=.001). Carbonyls concentrations showed a significant decrease by 43% in Vit E group at T1 compared to control group (P=.001), values were also more lower compared to T0 (P=.001).

Table 2. Changes in lipids, apolipoproteins and atherogenic indices in CRF patients supplemented with Vit E

	T0		T1	
	Control group (n=20)	**VitE (n=20)**	**Control group (n=20)**	**Vit E (n=20)**
TG (mmol.L^{-1})	3.96±0.5	3.45±0.1	2.65±0,13*	2.32±0,13*
TC (mmol.L^{-1})	6.2±0.11	5.16±0.11	3.12±1,09*	3.67±0,66*
C-HDL (mmol.L^{-1})	2.05±0.52	2.05±0.41	2.50±0.20	2.09±0.33
C-LDL (mmol.L^{-1})	2.92±0.80	3.16±0.79	2.08±0.27	3.00±0.50

*T0: the beginning of the study, T1: 30 days after initiating treatment, Control group: HD patients without treatment, Vit E group: HD patients with Vit E supplementation. *T1 vs T0. Data are presented as the mean±SD*

Table 3. Changes in pro-oxidant and antioxidant parameters in HD patients supplemented with Vit E

	T0		T1	
	Control group (n=20)	**Vit E(n=20)**	**Control group (n=20)**	**Vit E(n=20)**
TBARS(µmol.L^{-1})	7,4±0,9	8,0±0,4	7,3±0,6	6,3±1,1*
LPO(nmol/ml)	2,25±0,25	2,05±1,33	2,57±0,15	1,71±0.15**##
Carbonyls(nmol/mg)	0,44±0,12	1,20±0,20	0,54±0,14	0,31±0,12###***
SOD(U/ml)	22,15±1,9	22,12±1,98	24,65±2,9	24,94±3,19
CAT(U/ml)	40,12±11,2	23,17±10,42	40,73±13,5	58,08±14,08###***
GSH-GR(U/ml)	5,22 ±0,10	7,6±0,1	6,11±0,80	10±1###***
Vit E(ng/ml)	0.31±0.01	0.66±0.01	0.29±0.03	0.81±0.06###***

*T0: the beginning of the study, T1: 30 days after initiating treatment, Control group: HD patients without treatment, Vit E group: HD patients with Vit E supplementation. *T1 vs T0, #Vit E group vs Control group. Data are presented as the mean±SD*

There was no difference in serum SOD activity in Vit E group (Table 3) compared to control group at T1 and to beginning of nutritional intervention (T0). An increase by 43% in catalase activity was noted at T1 in Vit E group compared to control group (P=.001), furthermore, the values of catalase activity were more accentuated in Vit E group compared to T0 (P=.001). Moreover, we observed a significant increase in GSH-GR activity by 63% at T1 in vit E group compared to control group (P=0.001). Values of GSH-GR activity were also significantly increased compared to T0 (P=.001). Vit E concentrations were 2-fold higher in Vit E group compared to control group at T1 (P=.001). Values were more elevated than T0 (P=0.001).

4. DISCUSSION

A randomized trial was conducted in patients with CRF on hemodialysis, in order to determine the effect of Vit E supplementation on lipid, protein peroxidation and antioxidant defence. In this study, supplementation with Vit E was without effect on triacylglycerols, total cholesterol, C-HDL and C-LDL concentrations. There are few published reports on the effect of Vit E supplementation on lipids in uremic patients. It has been showed a non significant reduction in triacylglycerols, total cholesterol and C-LDL concentrations in hemodialysed patients treated with 400mg/d of Vit E during 90 days [13].

CRF patients have high oxidative stress produced by decreasing antioxidant defences and increasing pro-oxidant factors. This oxidative stress is responsible for the peroxidation of macromolecules such as lipids and proteins causing significant damage. Several pathophysiologic explanations have been claimed; some attribute it to malnutrition and hypoalbuminemia having in these cases low availability of «thiol»; others to «uremic status» itself with solute retention that may favor their pathogenicity; and others to the association of comorbid factors such as advanced age, diabetes, and inflammatory and infectious [14-16]. The increase of oxidative stress leads to lipid and protein peroxidation. OS was increased before the dialysis and hemodialysis did not correct it [5].

The present study demonstrates a favorable effect of low dose of Vit E on antioxidant defence in hemodialyzed patients. This effect could be related to increase in catalase and glutathione reductase activities and Vit E concentrations. SOD is the first line of defence against free radical attacks. Its function is to catalyze the conversion of superoxide radicals to hydrogen peroxide (H_2O_2) [17]. In this study we did not observe significant variations of SOD activity in treated patients for four weeks. These results were in contrary to those of Giray et al. (2003) which showed an increase in SOD activity in 36 patients treated with 600mg/daily of Vit E during 14 weeks [17]. It seems that the short period of intervention can be an explanation for it. Glutathione (GSH) is a tripeptidic thiol found in the inside of all animal cells and likely is the most important cellular antioxidant. Oxidized glutathione (GSSG) is highly toxic to cells so that the organism tends to reduce GSSG to GSH through glutathione reductase. Thus, determining GSH-GR is considered a reliable estimate of the degree of cellular OS [5].

Vit E is the most important lipophilic antioxidant in humans. The diminished Vit E level could be due to increased production of free radicals. Vit E radical formed by free radical attack interact with vitamin C and regenerate Vit E. On the other hand, in the study, we found a decrease in lipid peroxidation, which is indicated by decreased production of LPO. Furthermore, we noted a significant decrease in protein oxidation from carbonyl measurement [5]. Authors found a decrease in malondialdehyde concentrations when they used 500mg of Vit E as supplement for HD patients during 6 months [18]. While others did

not find significant effects on malondialdehyde of Vit E supplementation by 400mg for 3 weeks [18].

Ours results showed that Vit E supplementation improved the OS in HD patients. The low OS was confirmed by the decrease in LPO values and the increase in the activity of GSH-GR and catalase enzymes. The amount of Vit E supplementation and its duration may have contributed to the differences noted in our results compared to those reported by other investigators. Relatively, few investigators have examined the effects of physiologic dose of Vit E. Vit E has been recognized as one of the most important antioxidants. Vit E inhibits ROS-induced generation of lipid peroxyl radicals, thereby protecting cells from peroxidation of polyunsaturated fatty acids in membrane phospholipids, from oxidative damage of plasma low-density lipoprotein, cellular proteins, DNA, and from membrane degeneration. Consequently, a dietary deficiency of Vit E reduces the activities of catalase, GSH peroxidase, and glutathione reductase, induces liver lipid peroxidation, and causes cardiovascular disorders, all of which can be treated by dietary Vit E supplementation [19].

5. CONCLUSION

Significant decrease in LPO concentrations as a marker of lipid peroxidation and carbonyls as a marker of protein oxidation, additionally to significant increases in the activities of antioxidant enzymes (GSH-GR and CAT) were observed in the hemodialysed patients treated with Vit E supplementation for four weeks reflecting an efficacy of physiological dose Vit E supplementation in reducing lipid and protein oxidation and enhancing antioxidant enzyme activities, this may contribute in the prevention of vascular complications of HD.

CONSENT

All authors declare that informed consent was obtained from the patient for undertake the study.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Schiffrin EL, Lipman ML, Mann JF. Chronic kidney disease: effects on the cardiovascular system. Circulation. 2007;116:85-97. Doi: 10.1161/CIRCULATIONAHA.106.678342.
2. Tsirpanlis G. Cellular senescence, cardiovascular risk, and CKD: a review of established and hypothetical interconnections. Am J Kidney Dis. 2008;51:131-144. Doi: 10.1053/j.ajkd.2007.07.035.

3. Annuk M, Zilmer M, Fellstro B. Endothelium-dependent vasodilatation and oxidative stress in chronic renal failure: Impact on cardiovascular disease. Kidney International. 2003;63:S50-S53. Doi: 10.1046/j.1523-1755.63.s84.2.x.
4. Nishizawa Y, Koyama H. Endogenous secretory receptor for advanced glycation end-products and cardiovascular disease in end-stage renal disease. J Ren Nutr vol. 2008;18:76-82. Doi: 10.1053/j.jrn.2007.10.016.
5. Morena M, Delbosc S, Dupuy AM, et al. Overproduction of reactive oxygen species in end-stage renal disease patients: a potential component of hemodialysis associated inflammation. Hemodial Int vol. 2005;9:37-46. Doi: 10.1111/j.1492-7535.2005.01116.x.
6. Tbahriti HF, Meknassi D, Moussaoui R, et al. Inflammatory status in chronic renal failure: The role of homocysteinemia and pro-inflammatory cytokines. World J Nephrol. 2013;2:31-37. Doi: 10.5527/wjn.v2.i2.31.
7. Locatelli F, Canaud B, Eckardt KU, et al. Oxidative stress in end-stage renal disease: An emerging threat to patient outcome. Nephrol Dial Transplant. 2003;18:1272-1280. Doi: 10.1093/ndt/gfg074.
8. Uzum A, Toprak O, Gumustas MK, et al. Effect of vitamin E therapy on oxidative stress and erythrocyte osmotic fragility in patients on peritoneal dialysis and hemodialysis. J Nephrol. 2006;19:739-745. Doi: 10.1093/ndt/16.11.2172.
9. Cruz DN, de Cal M, Ronco C. Oxidative stress and anemia in chronic hemodialysis: the promise of bioreactive membranes. Contrib Nephrol. 2008;161:89-98. Doi: 10.1159/000130413.
10. Kopple JD. Clinical practice for nutrition in chronic renal failure. Am J Kidney Dis. 2001;37(2):66-70. Doi: 10.1053/j.ajkd.2014.01.015.
11. Quintanilha AT, Packer L, Davies JM, et al. Membrane effects of vitamin E deficiency: Bioenergetic and surface charge density studies of skeletal muscle and liver mitochondria. Ann N Y Acad Sci. 1982;393:32-47. Doi: 10.1111/j.1749-6632.1982.tb31230.x.
12. Levine RL, Garland D, Oliver CN, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 1990;186:464-478. Doi: 10.1016/B978-0-12-800280.
13. Mortazavi Moghadam SGHR, Zarban A, Rezvani MR. The effect of vitamin E supplementation on lipid profil and oxidative stress markers in hemodialysis patients. ARYA Atherosclerosis Journal. 2007;3(3):139-142. Doi: 10.1002/ajmg.a.35214.
14. Lucchi L, Bergamini S, Iannone A, et al. Erythrocyte susceptibility to oxidative stress in chronic renal failure patients under different substitutive. Treatments Artif Organs. 2005;29:67-72. Doi: 10.1111/aor.
15. Ramos T, Martinez-Castelao A. Lipoperoxidation and hemodialysis. Metabolism: Clinical and Experimental. 2008;57:1369-1374. Doi: 10.1016/j.metabol.2008.05.004.
16. Cristol JP, Bosc JY, Badiou S, et al. Erythropoietin and oxidative stress in hemodialysis: Beneficial effects of vitamin E supplementation. Nephrol Dial Transplant. 1997;12:2312-7. Doi: 10.1093/ndt/12.11.2312.
17. Giray B, Kan E, Bali M, et al. The effect of vitamin E supplementation on antioxidant enzyme activities and lipid peroxidation levels in hemodialysis patients. Clin Chim Acta. 2003;338:91-8. Doi: 10.1016/S0009-8981(03)00003-2.

18. Mydlik M, Derzsiova K, Racz O, et al. A modified dialyzer with vitamin E and antioxidant defense parameters. Kidney Int Suppl. 2001;78:S144-S147. Doi: 10.1038/ki.2010.
19. Fang YZ, Yang S, Wu G. Free radicals, antioxidants and nutrition. Nutrition. 2002;18(10):872-9. Doi: 10.1016/j.nut.

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