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EFFECTS OF VITAMIN E-MODIFIED DIALYSIS MEMBRANE ON LIPIDS IN PATIENTS ON HEMODIALYSIS

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Abstract: We evaluated the effects of a vitamin E-modified dialysis membrane on lipids in patients on hemodialysis (HD). Forty patients on maintenance HD were divided into two groups. Patients in group A (n=20) were subjected to HD with a vitamin E-modified membrane; patients in group B (n=20) were subjected to HD only with conventional dialyzers for a period of 4 weeks. Blood samples were collected prior to the first and thirteenth dialysis sessions to determine the concentrations of malonylaldehyde (MDA), vitamin E, red blood cells (RBC), triglycerides (TG), total cholesterol (TCH), low density lipoprotein (LDL), high density lipoprotein (HDL), lipoprotein A [Lp(a)], apolipoprotein B (ApoB) and apolipoprotein A1 (ApoA)1) in plasma. In group A we observed a significant increase in vitamin E in both plasma (P=0.003) and RBC (P=0.032), and a decline in MDA in both plasma (P=0.043) and RBC (P=0.033). There were no marked changes in TC, TG, LDL and HDL, but a decline in Lp (a) (P=0.049) and ApoB (P=0.040) and a significant increase in ApoA1(P=0.027). In group B, no significant changes of any of the relevant parameters were detected upon conclusion of the study. Vitamin E-modified membranes supply anti-oxidant effects on patients on HD and likely improve lipoprotein metabolism. These effects reduce cardiovascular risk in HD patients.

Key words: Hemodialysis (HD); oxidative stress; Vitamin E-modified dialysis membrane; lipid

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INTRODUCTION

For chronic kidney disease (CKD), cardiovascular diseases account for more than 40% of the deaths in hemodialysis (HD) patients. Extensive investigations have been carried out in order to identify the risk factors of the accelerated atherosclerosis detected in HD patients, where oxidative stress (OxSt) and dyslipidemia have suggested their susceptibility to cardiovascular disease complications (Jurek et al., 2006; Kwan et al., 2007).

OxSt, which has been defined as an imbalances between formation of reactive oxygen species (ROS) and antioxidant defense mechanisms, was frequently found in HD patients (Del Vecchio et al., 2011). Increased oxidative stress has been postulated to be responsible for oxidative modification of low-density lipoprotein (LDL), which is a critical step in the development and

progression of atherosclerosis in patients with chronic renal disease undergoing HD (Giray et al., 2003). Decreased antioxidants, especially vitamin E concentration, in LDL and increased susceptibility to oxidation has been reported in a prolonged dialytic treatment (Maggi et al., 1994). Further evidence also suggests that a decrease in HDL content and higher susceptibility of LDL to peroxidation are caused by oxidative modification (Montazerifar et al., 2010). This has led to the formulation of the "oxidative theory of atherosclerosis", in which oxidative modification of lipoproteins is considered as a key step in the pathogenesis of atherosclerosis. The modification of lipoprotein profile and susceptibility of LDL to oxidation have been previously demonstrated in HD patients (Baldi et al., 2013).

Since lipid peroxidation results from the balance between the pro-oxidant stimuli on the one hand and antioxidant defenses on the other, it was theoretically possible to interfere with the oxidation process by increasing the antioxidant defenses (Calo et al., 2004). The ability of the body to battle oxidative changes depends on the levels of enzymatic antioxidants, such as superoxide dismutase, glutathione peroxidase and catalase, as well as of nonenzymatic antioxidants (e.g., vitamins A, E, and C). As a lipophilic antioxidant, vitamin E has been proposed against oxidative stress and atherosclerosis due to its ability to inhibit lipid peroxidation by interacting with scavengers (Calo et al., 2004). On the basis of this assumption, new dialysis strategies based on the use of vitamin E has been developed for antioxidant therapy in HD (Galli and Azzi, 2010; Kirmizis et al., 2011), with ameliorated oxidative stress and lower lipid peroxidation demonstrated in HD patients (Ardalan et al., 2007).

It has been proposed that in uremia and dialysis, oxidative stress and lipid peroxidation accompany abnormalities of the plasma lipid composition in HD patients (Cristol et al., 1998), which possibly contribute to acceleration of the atherosclerotic process in these subjects (Tetta et al., 1999), and lipid abnormalities are presented as complications in HD patients. The aim of this cross-sectional study was to investigate the effect of a vitamin E-modified dialysis membrane on lipid and apolipoprotein profile, including malondialdehyde (MDA) in plasma and red blood cells (RBC), triglyceride (TG), total cholesterol (TCH), low density lipoprotein (LDL), high density lipoprotein (HDL), lipoprotein A [Lp(a)], apolipoprotein B (ApoB) and apolipoprotein A1 (ApoA1) in plasma. The possible variations in lipid and apolipoprotein profiles and the levels of vitamins E were analyzed in HD patients.

PATIENTS AND METHODS

This study was approved by an Institutional Review Board of the Beijing Tiantan and MeiTan General Hospitals. The study was conducted in accordance with good clinical practice, all applicable regulatory requirements and the guiding principles of the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to admission to the study.

Subjects

Forty HD patients maintained at least 3 months (average hemodialysis period of 31.03±35.49 months) on HD were recruited into a 4-week interventional study. HD was carried out 3 times per week for 4 h per session. None of the patients enrolled in this study were afflicted by severe heart failure, liver disease, infectious, hemorrhage unstable angina pectoris, wound or operation. The etiology of CKD for the patients was as follows: chronic glomerulonephritis (32 patients), polycystic kidney

(3 patients), diabetic nephropathy (1 patient), interstitial nephritis (1 patient), hypertensive kidney lesion (2 patients) and vasculitis (1 patient).

Intervention procedure

A subgroup patients (group A, N=20; gender, 12 males and 8 females; average age 56.00±9.55 years) were subjected to HD with a vitamin E-modified membrane (Terumo Corporation, Tokyo, Japan); the control group patients (group B, N=20; gender, 13 males and 7 females; average age 52.95±13.10 years) were subjected to HD with conventional cellulose acetate dialyzers. Twenty healthy subjects with normal renal function were recruited as a reference group.

After fasting overnight, blood samples were collected prior to the 1st and 13th HD sessions. Heparin anticoagulant was used for RBC separation and MDA determination. For the remaining part, plasma was collected by centrifugation (3000 r/min, 10 min), and used to determine plasma MDA and vitamin E. The remaining RBC was dissolved in water, and determination of the RBC vitamin E concentration was carried out. Plasma levels of TG, TCH, HDL, LDL, Lp (a), ApoB and ApoA1 were evaluated using a blood sample without anticoagulant.

The concentrations of MDA and vitamin E in RBC and plasma were determined using commercial kits (Nanjing Jianchen, Co., Nanjing, China), and quantified using spectrofluorometric assay. Plasma TG, TCH, HDL, LDL, Lp (a), ApoB and ApoA1 levels were evaluated using the Hitachi 7600 automatic biochemical analyzer (Hitachi Ltd., Japan).

Statistical methods

Data were analyzed using SPSS software version 9.0 (SPSS, Inc., Chicago, IL, USA), and expressed as mean ± SD. Paired Student's t-test analysis was carried out to compare data within the same population; comparison of data between groups was performed using one-way ANOVA analysis.

RESULTS

Tables 1 and 2 show the alteration of vitamin E and MDA concentrations in RBC and plasma, respectively, and Table 3 shows the plasma lipid and apolipoprotein profile in patients undergoing HD treatment.

Vitamin E-modified dialysis membrane changes MDA level

Before HD, vitamin E levels was significantly lower, whereas MDA concentration was higher than in normal subjects, and did not differ significantly among the groups (vitamin E: P_p=0.987; P_r=0.676; MDA: P_p=0.930; P_r=0.967). After HD, significant differences were observed among the groups. In group A, there were significant increases in Vitamin E levels in both plasma

Table 1. Vitamin E levels in RBCs and plasma

Parameter	Plasma Vitamin E			RBC Vitamin E		
	Group A	Group B	Control	Group A	Group B	Control
Before HD	5.37±2.44	5.32±2.77	7±0.45	5.19±1.91	5.53±3.14	6.96±0.67
After HD	6.91±2.10**	5.56±3.27	7±0.45	6.87±2.11*	5.26±3.10	6.96±0.67

*P<0.05, **P<0.01, before versus after HD. HD -, hemodialysis; RBCs – red blood cells

(P=0.003) and RBC (P=0.032), and a decline in MDA levels in both plasma (P=0.043) and RBC (P=0.033), compared with levels detected at the initiation of the study. In group B, there was no significant change in any of the parameters mentioned above in both plasma and RBC (p>0.05).

Changed lipid profiles after vitamin E-modified dialysis membrane treatment

The plasma lipoprotein and lipid profiles were characterized by obviously declined Lp (a) (P=0.049) and ApoB (P=0.040) and significantly increased ApoA1 (P=0.027) in group A. However, no marked changes in TC, TG, LDL and HDL were observed when compared with the control group. In group B, there was no significant change detected in any of the relevant parameters upon conclusion of the study. There were differences noted when comparing the two dialysis modalities. The elevations in Lp (a), and ApoB and decreases in ApoA1 levels were marked in those subjects treated with vitamin E-bonded dialysis membranes compared with those on conventional dialyzers.

DISCUSSION

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in end-stage renal disease (ESRD), compelling investigations focused on oxidative stress, one of the most important "nonconventional" risk factors for CVD in HD patients. These studies are based on the so-called "oxidative theory" of atherosclerosis, in which the oxidative modification of lipoproteins is considered as a key pathological step. The lipoproteins, particularly for LDL, become a ligand for scavenger receptors on macrophages once oxidatively modified, leading to foam-cell formation. These processes, together with the secretion of proinflammatory cytokines, locally exacerbate the production of reactive oxygen species (ROS) and lead to an exhaustive metabolic response, cell dysfunction and death, which may ultimately further extend the lesion and promote plaque formation, influencing smooth muscle cell proliferation and fibrogenesis. Vitamin E-modified dialysis membranes have been proposed to protect patients against the complications of HD caused by OxSt, and it may protect critical cellular structures against damage caused by oxygen free radicals and reactive products of lipid peroxidation.

Earlier studies on vitamin E level in the pre-HD state are conflicting. Montazerifar and Bhogade reported there were no differences in patients' pre-HD vitamin E levels compared to the controls (Bhogade et al., 2008; Montazerifar et al., 2010), but a lower level of vitamin E was detected in our results, which is consistent with Galli's report (Galli et al., 2001). After HD, HD with Vitamin E-modified dialysis membrane led to a higher elevation of vitamin E levels in both RBC and plasma, as compared with the patients

Table 2. MDA level in RBC and plasma

Parameter	Plasma MDA			RBC MDA		
	Group A	Group B	Control	Group A	Group B	Control
Before HD	5.34±1.20	5.38±1.43	4.06±0.6	1.83±0.94	1.87±0.59	1.24±0.6
After HD	4.44±1.49*	5.70±2.08	4.06±0.6	1.33±0.57*	1.83±0.53	1.24±0.6

*P<0.05, **P<0.01, before versus after HD. HD - hemodialysis; RBC - red blood cell; MDA - malondialdehyde.

using conventional membrane. This result is partly consistent with that of Mydlik et al. (2001), with a steep increase in plasma vitamin E levels observed when shifting from the conventional dialyzer to vitamin E-modified dialyzer. However, it was in striking disagreement with the results of Mune et al. (1999), in which no obvious change in plasma vitamin E concentration throughout the study was observed.

Modification of lipoprotein profile and susceptibility of LDL to oxidation have been previously reported in HD patients (Kaysen and Eiserich, 2004). MDA, a useful marker for lipid peroxidation in biological systems, is generated by the oxidation of polyunsaturated fatty acids within LDL (Demirtas et al., 2002). The results obtained in this study provide compelling evidence that an increased oxidation of plasma LDL occurs in HD patients, and supplementing HD patients with vitamin E could ameliorate lipid peroxidation to some extent. This demonstrates that MDA levels are high in HD patients and decreased after treatment with a vitamin E-bonded dialysis membrane. Despite decreasing, the MDA level was still higher than the controls. However, a further increase in MDA concentration was observed in both RBC and plasma when the conventional HD membrane was proposed. Jackson et al. (1995) described a twofold increase of MDA in HD patients as compared with healthy controls, and a 17% decrease of MDA after HD. In our study, the concentration of MDA was not that markedly elevated, being more close to that reported by Mydlik et al. (2001)

As for the alteration in lipid metabolism, it was determined that HD patients treated with a vitamin E-modified membrane have a more favorable lipid and apolipoprotein profile than the conventional membrane-treated ones, with elevations of Lp (a), and Apo B, and a concomitant decrease of ApoA1. Nevertheless, the decreased level of ApoA1 still remained higher than the controls', whereas the levels of Lp (a) and Apo B were lower than the controls'. For HD patients with a conventional membrane, however, there was no obvious change in lipid and apolipoprotein profile before and after treatment.

As the principal protein found in β-lipoprotein (LDL), Apo B has been discovered to exist in lipoproteins (chylomicrons, chylomicron remnants, VLDL, intermediate density lipoprotein, LDL, and lipoprotein(a)), which are potentially atherogenic and absent from antiatherogenic proteins. Investigations demonstrated that Apo B is essential for

Table 3. Plasma lipid and apolipoprotein profiles in HD patients

Parameter	Group A		Group B	
	Before HD	After HD	Before HD	After HD
TG mmol/L	1.9170±0.7892	1.8931±0.6869	1.8899±0.7400	1.8501±0.7527
TCH mmol/L	4.8091±1.0378	4.7818±1.0017	4.7840±1.0758	4.8087±1.1863
HDL mmol/L	1.0144±0.2878	1.0239±0.3303	1.0170±0.3452	1.0093±0.3203
LDL mmol/L	2.9685±0.6852	2.8990±0.7414	2.9050±0.6612	3.0250±0.6763
Lp(a) nmol/l	46.79±8.76	41.03±7.85*	46.64±7.24	47.07±8.26
ApoB g/L	1.2620±0.2275	1.1105±0.2089*	1.2215±0.1924	1.2220±0.2357
ApoA1 g/L	1.1700±0.1704	1.2325±0.1834*	1.1713±0.2817	1.1643±0.1975

*P<0.05, before versus after HD. TG - triglyceride; TCH - total cholesterol; HD - hemodialysis; LDL - low density lipoprotein; HDL - high density lipoprotein; Lp (a), lipoprotein a; Apo B - apolipoprotein B; ApoA1 - apolipoprotein A1.

triglyceride secretion and LDL catabolism and is involved in atherogenesis intimately. Previous work indicated that oxidative modification of LDL varies from mild to severe, the minimally modified LDL, termed LDL$^-$, was proposed to be more susceptible to oxidative modification. High LDL$^-$ levels were considered potentially proatherogenic due to the high cytotoxicity and oxidizability of this LDL fraction (Sevanian et al., 1996). There was a correlation between Apo B and LDL$^-$ formation as proposed by Ziouzenkova et al. (1999). Apo B has been suggested to be a more preferable marker in atherosclerosis. Accumulation of Apo B has been suggested to be related to the arteria carotis atherosclerosis. In this study, there was a significant decline in Apo B level, indicating a lower lipid peroxidation in HD patients.

As the major apolipoprotein of HDL, ApoA-1 could protect against atherosclerosis by promoting several steps in the reverse cholesterol transport pathway, which removes cholesterol from peripheral cells and directs the cholesterol to the liver and steroidogenic organs for metabolism (Borja et al., 2013). There is strong evidence that lipoprotein(a) [Lp (a)], an LDL-like lipoprotein that consists of that is covalently bound to an LDL particle, is a risk factor for CKD and the elevation of Lp (a) in CKD is an acquired abnormality (Craig et al., 1998), corroborating the result of this study, where a steep decline in Lp (a) concentration and an increase in ApoA1 were observed. These demonstrated the efficiency of vitamin E on combating OxSt and ameliorating lipid peroxidation, with a favorable lipid and apolipoprotein profile obtained.

It was also noteworthy that no marked changes in TC, TG, LDL and HDL levels were observed in this study, consistent with the results of Mune et al. (1999), yet significantly different from the results of others, where the plasma lipid and lipoprotein profiles were characterized by elevated TC, LDL-C, TG, Lp (a), and Apo B, and decreased HDL-C and ApoA1 (Montazerifar et al., 2010). Renal dyslipidemia has been suggested to be characterized to a greater extent by abnormal apolipoprotein rather than lipid profile, including decreased levels of ApoA1 and increased levels of Apo B, which are involved in a pathologic process directly (Attman et al., 1993).

The inconsistencies our findings with previous reports are probably due to the difference in dialysis techniques: type of dialyzer membranes used (hemophane or polysulfone, cuprophane, etc.), disease duration (months to years) and process (prolonged use of catheters for vascular access aggravate oxidative stress) of dialysis treatment, dietary intake, age, malnutrition, and type of assay method. In addition, HD membrane materials have been described to play a central role in ROS production interfering with oxidative damage during HD (Naito et al., 1994).

Vitamin E is a well-known *in vitro* scavenger of lipid hydroperoxides, and has been demonstrated to regulate both enzymatic and nonenzymatic pathways of lipid oxidation. However, the role of vitamin E in protecting *in vivo* plasma lipids from peroxidation is still not clearly formulated. Vitamin E, bonded on the surface of the dialyzer membrane, is expected to compensate for its pool depletion, thereby reducing the production of ROS and providing the plasma lipid with antioxidant protection.

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Conflicts of interest: The authors have no conflicts of interest to declare in this article.

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