Vitamin E-coated dialyzer alleviates erythrocyte deformability dysfunction in patients with end-stage renal disease undergoing hemodialysis

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Abstract. Patients with end-stage renal disease (ESRD) are characterized by augmented oxidative stress (OS) due to the imbalance between the generation of increased concentrations of oxidative molecules and decreased antioxidant capacity. Vitamin E-coated dialyzer membranes (VEMs) have previously been reported to alleviate the imbalance of redox metabolism in patients with ESRD undergoing hemodialysis (HD); however, their effect on the deformability of red blood cells (RBCs) remains unknown. In the present study, 48 patients with ESRD undergoing HD were enrolled and randomly assigned into two groups: HD with VEMs (VEM group; n=24) and HD with polysulfone dialyzer membranes (PM group; n=24), and another 24 healthy volunteers served as the control group. The present study investigated the morphological changes and deformability of RBCs in patients with ESRD and healthy volunteers. The concentration of serum vitamin E, the parameters of antioxidant stress and OS, and the degree of oxidative phosphorylation and clustering of anion exchanger 1 (Band 3) in RBCs were measured. The results obtained suggested that VEM treatment markedly ameliorated the abnormalities of RBC morphology and deformability in patients with ESRD undergoing HD. Mechanistic studies showed that VEM treatment led to a marked improvement in the concentration of serum vitamin E, which was positively associated with the restored antioxidant capacity, and decreased oxidative phosphorylation and clustering of Band 3 in RBCs of patients with ESRD undergoing HD. Taken together, the results of the present study have demonstrated that VEM treatment effectively restored the imbalance of redox metabolism, and improved the oxidative phosphorylation and clustering of Band 3 in RBCs of patients with ESRD undergoing HD via delivering vitamin E, which may alleviate the abnormal morphological and mechanical properties of RBCs. These findings are anticipated to be useful with respect to improving the nursing care and cure rate of patients with ESRD.

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Abbreviations: ESRD, end-stage renal disease; VEM, vitamin E-coated dialyzer membrane; RBCs, red blood cells; HD, hemodialysis; EI, elongation index; OS, oxidative stress; SOD, superoxide dismutase; FRAP, ferric-reducing ability of plasma; CAT, catalase; ROS, reactive oxygen species; MDA, malondialdehyde; Hct, hematocrit; MetHb, methemoglobin; BUN, blood urea nitrogen; UA, uric acid; SCr, serum creatinine; CRP, C-reactive protein

Key words: end-stage renal disease, red blood cells, deformability, vitamin E-coated dialyzer membranes, oxidative stress

Introduction

Excessive oxidative stress (OS) is a common complication in patients with chronic kidney disease and end-stage renal disease (ESRD) (1,2). The imbalance between oxidation and antioxidation induced by ESRD is the main cause of OS, which may be further exacerbated during hemodialysis (HD) treatment (3,4). Cell components of patients with ESRD undergoing HD, such as proteins, lipids and nucleic acids, are vulnerable to oxidative damage after continuous exposure to free radicals, potentially leading to an increased risk of cardiovascular disease (5). Therefore, either treatment with antioxidant supplements or modification of the dialyzer (e.g. coated with vitamin E) that can effectively alleviate the disordered redox metabolism has become a promising therapeutic strategy for the treatment of OS in patients with ESRD undergoing HD (6,7).

Renal dysfunction, chronic inflammation and other complications are considered to be the main source of free radicals in patients with ESRD, which results in increased levels of OS biomarkers, including malondialdehyde (MDA), oxidized low-density lipoprotein and deoxyguanosine in different tissues and/or plasma (8). In addition, the interaction of blood with materials of the dialyzer and hemolysis has been considered to be the main source of free radicals in patients with ESRD undergoing hemodialysis (HD) (4,9). During this process, the antioxidant resources of red blood cells (RBCs) that are permanently exposed to high-level OS will be exhausted, resulting in a decrease in membrane lipid fluidity and oxidative damage of membrane proteins, which

further leads to an abnormal deformability of the RBCs and rheological properties (10,11).

Vitamin E is a powerful hydrophobic antioxidant that protects RBCs from lipid peroxidation (12). It has been reported that serum levels of vitamin E in patients undergoing HD were significantly decreased, and this decrease was accompanied by a decrease in antioxidant capacity and an increase in lipid peroxidation (13,14). According to previous reports, treatment with vitamin E-coated dialyzer membranes (VEMs) may restore the blood antioxidant capacity and suppress the lipoperoxidation process *in vitro* and *in vivo* (15,16). Bargnoux *et al* (17) reported that VEMs were able to markedly increase superoxide dismutase (SOD) activity and reduce OS injury in RBCs of patients undergoing HD. However, whether VEM treatment is able to ameliorate abnormal deformability and rheological properties in RBCs of patients with ESRD undergoing HD via regulating the redox metabolism remained to be clarified.

Therefore, the present study aimed to explore the protective effects and underlying mechanisms of VEM treatment on the redox metabolism and deformability of RBCs in patients with ESRD undergoing HD. The results of the present study are expected to provide a theoretical basis for the formulation of a therapeutic schedule for patients with ESRD undergoing HD with erythrocyte dysfunction and associated cardiovascular disease.

Materials and methods

Subjects. Patients with ESRD undergoing maintenance HD three times per week for at least 12 months were recruited to the present study between January and June 2021 from the Department of Nephrology, Yantai Hospital of Traditional Chinese Medicine (Yantai, China). The patients were randomly assigned to two groups: HD with VEMs (Excebrane E15; Terumo Medical Corporation) (VEM group, n=24, 10 male and 14 female patients, aged 50±14 years) and HD with polysulfone dialyzer membranes (PMs) [F60; Fresenius SE & Co.) (PM group, n=24, 13 male and 11 female patients, aged 55±15 years). The inclusion criteria were as follows: i) The patient was on chronic HD; ii) the treatment was received three times weekly for ≥12 months; iii) the age of the patient was >18 years; and iv) the patient was able to give their informed consent. The exclusion criteria were as follows: The patient i) was pregnant; ii) was already involved in another study, iii) had active/chronic inflammation or a malignancy; and iv) was receiving antioxidant and anti-inflammatory therapy. In addition, 24 healthy volunteers (10 male and 14 female individuals, aged 54±12 years) were recruited as the control group. The study population characteristics are summarized in Table I.

None of the patients with ESRD or healthy volunteers took drugs with a potential oxidizing effect or undertook any exhaustive exercise; nor did they take antioxidants, such as vitamin C or E, during the 8-week experimental period. All experimental procedures were conducted according to the Guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Yantai Hospital of Traditional Chinese Medicine (approval no. 2020-06). Patients and healthy volunteers were informed of the procedures in detail, and provided written consent before the examination.

HD. For all patients with ESRD undergoing HD, the blood flow rate was set to 250-300 ml/min, the dialysate flow rate was 500 ml/min, and during the HD therapy, there were three sessions per week (each session lasting for 4 h). The HD treatment was delivered by a low-flux dialyzer (ARTIS CN; Gambro Lundia AB). The transmembrane pressure of the dialyzer was 0-500 mmHg and the system was maintained at 36˚C. No changes were made to the dialysate composition (HCO_3^-, 35.0 mmol/l; Mg^{2+}, 0.50 mmol/l; Na^+, 139.8 mmol/l; Cl^-, 106.8 mmol/l; K^+, 2.0 mmol/l and CH_3COO^-, 4.0 mmol/l) used by patients undergoing HD during this study period.

Preparation of the blood samples and detection of the hematological parameters. Fresh blood was collected by venipuncture from both the patients with ESRD undergoing HD and the healthy volunteers. After centrifugation at 900 x g at 4˚C for 10 min, the plasma and buffy coat were removed, and RBCs were isolated and washed three times in isotonic Hepes buffer. Subsequently, RBCs were resuspended in a hematocrit (Hct) of 50% in Krebs buffer containing 2 g/l glucose (pH 7.4) for further experiments. The hematological parameters of the participants were determined using an electronic hematology analyzer (ABX Micros 60; Horiba, Ltd.). Biochemical parameters of the patients were obtained from the patients' records.

Preparation and detection of erythrocytes by scanning electron microscopy. The RBCs were fixed with 2% glutaraldehyde for 24 h at 4˚C. Subsequently, the cells were washed in phosphate buffer for 30 min, and then the material was dehydrated in an ascending series of ethanol concentrations (50, 70, 80, 95 and 100%) (15 min in each concentration); finally, the material was allowed to remain in pure ethanol for 30 min. The RBCs were subsequently dried for 12 h at room temperature. Then, the samples were coated with gold using a sputter coater (cat. no. Q150RS; Quorum). Under high vacuum and accelerating voltage [acceleration voltage (EHT), 10 kV] the ultrastructure of the RBCs from patients with ESRD undergoing HD and the healthy volunteers were detected using a scanning microscope (ZEISS EVO LS15 SEM; Zeiss GmbH) with an SE1 detector. Individual forms of the erythrocytes were ascribed morphological indices according to the Bessis scale (18).

Detection of erythrocyte deformability. By using an ektacytometer (LBY-BX; Beijing Precil Instrument Co., Ltd), the deformability of RBCs from patients with ESRD undergoing HD and healthy volunteers was analyzed by laser diffraction analyses (19). The elongation index (EI) was calculated under shear stresses of 3 and 30 Pa, as based on the geometry of the elliptical diffraction pattern (where 'L' and 'W' are the length and width of the diffraction pattern): EI=(L-W)/(L+W).

Detection of OS. The intracellular production of reactive oxygen species (ROS) in RBCs from patients with ESRD undergoing HD and healthy volunteers was assessed using 2',7'-dichlorofluorescein diacetate (H2DCF-DA; MilliporeSigma). The RBCs were collected, washed with PBS solution, and then H2DCF-DA (10 μM) was added to the cells and incubated at 37˚C for 30 min. After incubation, cells were washed and analyzed using a flow cytometer (FACSCanto II; BD Biosciences, Inc.) and analyzed using FlowJo software 8.7.1

Table I. Clinical parameters and causes of ESRD in patients and control subjects.

A, Clinical parameters

Variable	Control (n=24)	PM (n=24)	VEM (n=24)
Age (year)	54±12	55±15	50±14
Sex (M/F)	11/13	13/11	10/14
Weight (kg)	62±8	64±7	60±10
HD treatment (months)	-	26±16	33±18

B, Cause of ESRD

Variable	Control (n=24)	PM (n=24)	VEM (n=24)
Polycystic kidney disease	-	5	6
Chronic kidney failure	-	6	6
Chronic glomerulonephritis	-	4	3
Nephrosclerosis	-	1	2
Others	-	8	7

ESRD, end-stage renal disease; HD, hemodialysis; PM, polysulfone dialyzer membrane; VEM, vitamin E-coated dialyzer membrane; M, male; F, female.

(FlowJo LLC). The levels of MDA (cat. no. A003-1-2) and methemoglobin (MetHb; cat. no. A102-1-1) were assessed by colorimetry using commercial kits (all from Nanjing Jiancheng Bioengineering Institute). The manufacturer's instructions for the corresponding assay kit were precisely followed (20).

Detection of antioxidant capacity. The ferric-reducing ability of plasma (FRAP) values were assessed according to the method of Benzie and Strain (21). The activities of catalase (CAT; cat. no. A007-1-1) and SOD (cat. no. A001-3-2) were assessed by colorimetry using commercial kits (all from Nanjing Jiancheng Bioengineering Institute). The manufacturer's instructions for the corresponding assay kit were preceisely followed.

Detection of vitamin E. The serum concentration of vitamin E was detected by colorimetry using a commercial kit (cat. no. BC1420; Beijing Solarbio Science & Technology Co., Ltd). The manufacturer's instructions for the corresponding assay kit were precisely followed.

Western blot analysis. Western blot analyses were performed as described previously (22). Total protein from the RBCs was obtained using a protein extraction kit (cat. no. BC3711; Beijing Solarbio Science & Technology Co., Ltd) according to the manufacturer's instructions. The protein content of the membranes was quantified using a BCA protein assay kit (cat. no. PC0020; Beijing Solarbio Science & Technology Co., Ltd). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by heating the samples for 8 min at 100˚C and loading 10 μg membrane proteins on to a 5-15% linear acrylamide gradient gel (10 μg protein/lane).

After SDS-PAGE, proteins were electrotransferred to PVDF membranes and blocked in 5% BSA (cat. no. SW3015; Beijing Solarbio Science & Technology Co., Ltd) dissolved in TBS-Tween-20 (20%) for 2 h at room temperature. After incubating the PVDF membranes with primary antibody overnight at 4˚C, the membranes were incubated with secondary antibody for 2 h at room temperature. The primary antibodies used were anti-Band 3 (1:3,000 dilution; cat. no. ab108414), anti-phosphotyrosine (1:3,000; cat. no. ab190824), and anti-β-actin (1:3,000; cat. no. ab8227; all antibodies were purchased from Abcam). The horseradish peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. (1:5,000; cat. no. sc-2357). The protein bands were visualized using an ECL kit (cat. no. 32209; Thermo Fisher Scientific, Inc.), and semi-quantitative densitometry was performed on the identified bands using Image Quant 5.2 software (Molecular Dynamics, Inc.). The levels of the proteins of interest were normalized against those of β-actin.

Immunofluorescence and image analysis. RBCs from patients with ESRD undergoing HD and healthy volunteers were fixed with 4% paraformaldehyde and 0.05% glutaraldehyde at room temperature for 30 min, before being permeabilized in the same solution containing 0.05% Triton X-100 at 4˚C for 10 min. RBCs were treated with primary antibodies against Band 3 (1:1,000; cat. no. ab108414; Abcam) for 1 h at room temperature after being blocked with 3% BSA and 0.1% Tween-20 at 4˚C for 1 h. After being washed three times in PBS, RBCs were incubated at room temperature for 1 h with goat anti-rabbit IgG H&L secondary antibody (1:200; cat. no. ab150077; Abcam). After being washed three times in PBS, fluorescence images were captured using an Olympus IX71 fluorescence microscope (Olympus Corporation) (23).

Statistical analysis. Statistical analyses were performed using SPSS software (version 22.0; IBM Corp.). Data are

Table II. Hematological parameters of the patients and control subjects.

	Control (n=24)		PM (n=24)		VEM (n=24)	
Variable	Before	After 8 weeks	Before	After 8 weeks	Before	After 8 weeks
RBC, mil/μl	5.21±0.14	5.32±0.16	3.88±0.18[a]	3.45±0.16[a]	3.79±0.34[a]	4.25±0.2[b,c]
Hb, g/dl	14.11±1.43	14.42±1.55	11.51±1.64[a]	10.32±1.26[a]	11.34±1.71[a]	12.70±1.52[b,c]
RDW, %	12.51±3.71	12.92±4.13	16.52±5.41[a]	17.34±6.43[a]	15.81±4.63[a]	14.55±3.74[b,c]
Hct, %	43.54±4.43	44.24±4.65	34.58±5.45[a]	32.72±6.51[a]	33.82±5.21[a]	37.52±4.93[b,c]
MCHC, g/dl	31.51±1.94	30.81±1.74	34.55±1.71	36.21±1.93	35.61±1.85	32.61±1.53
MCH, pg	32.83±1.31	31.83±1.51	30.11±1.53	28.91±1.74	30.73±2.65	31.51±2.34
MCV, fl	98.51±3.43	99.23±3.61	89.41±5.13[a]	87.15±5.91[a]	88.71±4.73[a]	94.71±4.43[b,c]
HDL, mg/dl	38.63 ± 9.74	37.81±9.55	39.41± 9.56	40.15± 9.66	38.91± 8.26	39.91± 10.51
BUN, mg/dl	14.82±4.61	14.53±4.41	52.11±8.12[a]	58.71±8.83[a]	52.51±9.70[a]	42.51±9.12[b,c]
SCr, mg/dl	0.68±0.14	0.69±0.15	7.69±2.47[a]	8.89±3.42[a]	7.78±1.35[a]	6.78±1.44[b,c]
UA, mg/dl	3.68±1.15	3.57±1.05	5.57±3.35[a]	5.89±3.87[a]	5.44±2.92[a]	4.44±2.61[b,c]
CRP, mg/dl	2.98±0.97	3.02±0.86	14.55±9.45[a]	15.75±9.88[a]	14.78±7.73[a]	9.78±6.83[b,c]

PM, polysulfone dialyzer membrane; VEM, vitamin E-coated dialyzer membrane; RBC, red blood cells; Hb, hemoglobin; Hct, hematocrit; RDW, red blood cell distribution width; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; HDL, high-density lipoprotein; BUN, blood urea nitrogen; UA, uric acid; SCr, serum creatinine; CRP, C-reactive protein. All data are expressed as the mean ± SD. [a]P<0.05, PM or VEM vs. control group before examination; [b]P<0.05, VEM vs. control group 8 weeks after examination; [c]P<0.05, VEM vs. PM group 8 weeks after examination.

presented as the mean ± standard deviation of three independent experiments. Differences between and within groups were determined using mixed-design ANOVA followed by Bonferroni's post hoc tests. Spearman's correlation analysis was used for correlation analysis of two independent samples. P<0.05 was considered to indicate a statistically significant difference.

Results

Demographic, clinical and hematological features in healthy donors and patients with ESRD undergoing HD. As shown in Tables I and II, the patients with ESRD undergoing HD were characterized by a significantly reduced RBC count and markedly lower hemoglobin (Hb), Hct and mean corpuscular volume (MCV) values, whereas the parameters RBC distribution width (RDW), blood urea nitrogen (BUN), serum creatinine (SCr), uric acid (UA) and C-reactive protein (CRP) were markedly increased compared with the healthy volunteers 8 weeks before the examination. After 8 weeks, the levels of the RBC count, Hb, Hct and MCV were notably improved, whereas the values of RDW, BUN, SCr, UA and CRP were markedly decreased, in the VEM group compared with the PM group.

VEM alleviates the abnormal morphology and deformability in RBCs of patients with ESRD undergoing HD. The flattened biconcave disc morphology of RBCs was found to be damaged in patients with ESRD undergoing HD, and this was accompanied by the presence of dysmorphic RBCs (which featured, e.g., surface blebbing typical of acanthocytes). However, the counts of dysmorphic RBCs were markedly decreased in the VEM group compared with the PM group after 8 weeks (Fig. 1A and B).

The deformability of the RBCs in patients with ESRD undergoing HD and healthy volunteers was evaluated by measuring the value of EI under shear stresses of 3 and 30 Pa. The EI was markedly reduced in RBCs of patients with ESRD undergoing HD compared with the healthy volunteers under both 3 and 30 Pa shear stress. In addition, a notable amelioration of the RBCs' deformability was found in the VEM group compared with the PM group after 8 weeks (Fig. 1C and D). These results demonstrated that HD with VEMs alleviated the abnormalities of RBC morphology and deformability in patients with ESRD undergoing HD.

VEM restores the imbalance of redox metabolism in RBCs of patients with ESRD undergoing HD. To investigate the reason for the abnormal morphology and function of the RBCs of patients with ESRD undergoing HD, the ROS and antioxidant capacity indexes of the RBCs were detected. The levels of ROS, MetHb and MDA in RBCs were markedly increased in patients with ESRD undergoing HD compared with the healthy volunteers. However, a notable decline in the ROS, MetHb and MDA values in RBCs were found in the VEM group compared with the PM group after 8 weeks (Fig. 2A-C).

In addition, the activities of FRAP, CAT and SOD in RBCs were markedly decreased in patients with ESRD undergoing HD compared with the healthy volunteers (Fig. 2D-F). Moreover, after 8 weeks of VEM treatment, the activities of FRAP, CAT and SOD in the RBCs were markedly improved compared with the PM treatment group (Fig. 2D-F). These results indicated that HD with VEMs alleviated the imbalance of redox metabolites of RBCs in patients with ESRD undergoing HD.

VEM participates in the regulation of redox metabolism in RBCs of patients with ESRD undergoing HD by transferring

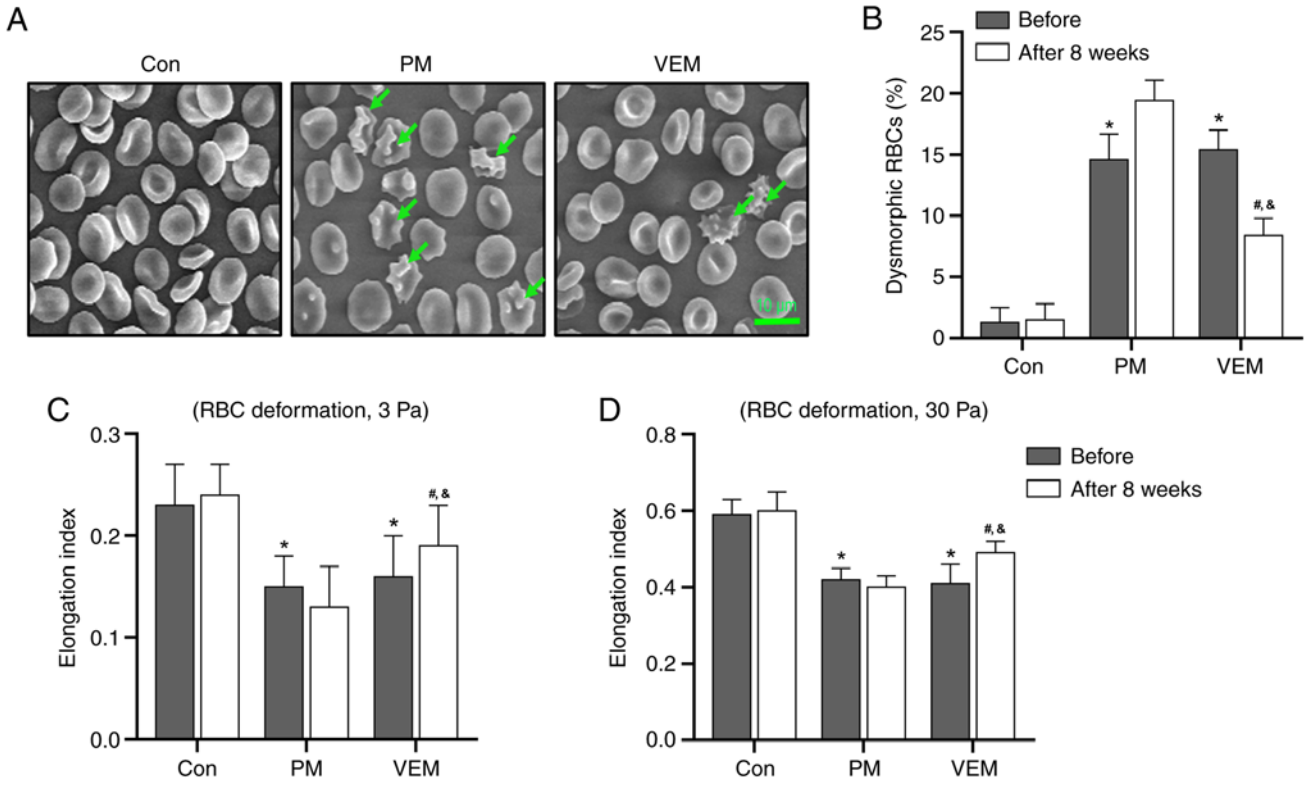

Figure 1. RBC morphology and deformability in patients with ESRD undergoing HD and healthy volunteers. (A) Representative RBC images were obtained using a scanning electron microscope in healthy donors and in patients with ESRD undergoing HD after 8 weeks (green arrows indicate dysmorphic RBCs; scale bar, 10 μm). (B) Percentages of dysmorphic RBCs. Erythrocyte deformability in healthy donors and patients with ESRD undergoing HD as reflected by the elongation index were tested under shear stress of (C) 3 Pa and (D) 30 Pa. All data are expressed as the mean ± SD. *P<0.05, PM or VEM group vs. control group before examination; $^{\#}$P<0.05, VEM group vs. control group 8 weeks after examination; $^{\&}$P<0.05, VEM group vs. PM group 8 weeks after examination. PM, polysulfone dialyzer membranes; VEM, vitamin E-coated dialyzer membrane; RBC, red blood cell; ESRD, end-stage renal disease.

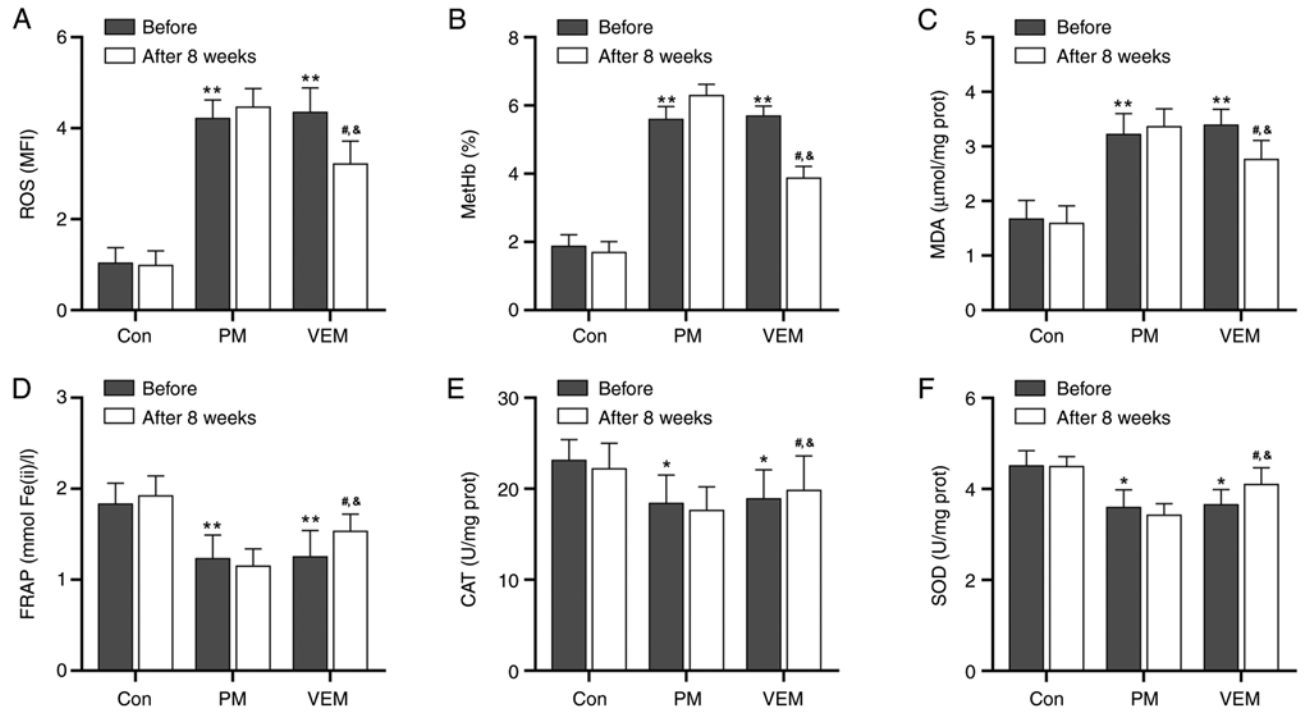

Figure 2. RBC redox metabolism parameters in patients with ESRD undergoing HD and healthy volunteers. The oxidative stress parameters of (A) ROS, (B) MetHb and (C) MDA in RBCs of healthy donors and patients with ESRD undergoing HD. The antioxidant parameters of (D) FRAP, (E) CAT and (F) SOD in RBCs of healthy donors and patients with ESRD undergoing HD. All data are expressed as the mean ± SD. *P<0.05, **P<0.01, PM or VEM group vs. control group before examination; $^{\#}$P<0.05, VEM group vs. control group 8 weeks after examination; $^{\&}$P<0.05, VEM group vs. PM group 8 weeks after examination. PM, polysulfone dialyzer membrane; VEM, vitamin E-coated dialyzer membrane; RBC, red blood cell; SOD, superoxide dismutase; FRAP, ferric-reducing ability of plasma; CAT, catalase; ROS, reactive oxygen species; MDA, malondialdehyde; MetHb, methemoglobin; ESRD, end-stage renal disease.

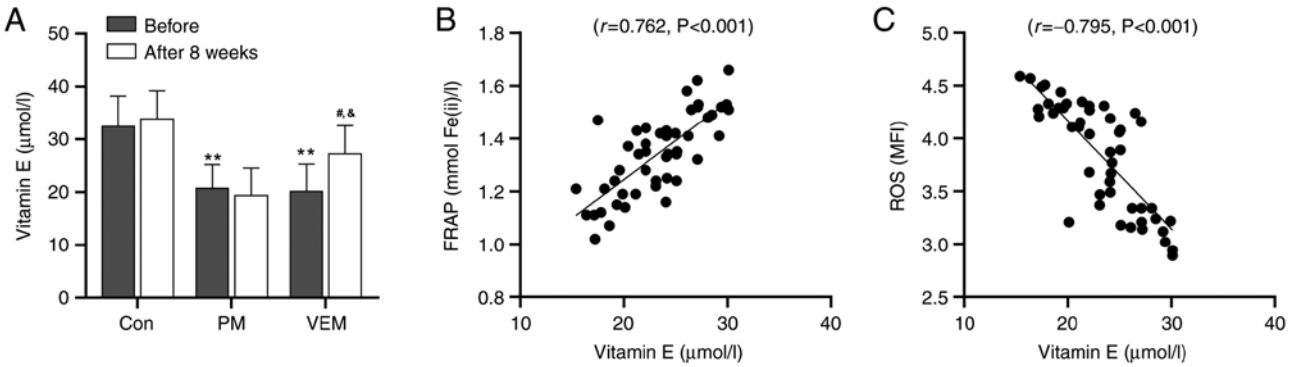

Figure 3. Red blood cell serum vitamin E concentration in patients with ESRD undergoing HD and healthy volunteers. (A) Concentration of serum vitamin E in healthy donors and patients with ESRD undergoing HD. All data are expressed as the mean ± SD. **P<0.01, PM or VEM group vs. control group before examination; $^{\#}$P<0.05, VEM group vs. control group 8 weeks after examination; $^{\&}$P<0.05, VEM group vs. PM group 8 weeks after examination. (B) Pearson correlation analysis between the concentration of serum vitamin E and the FRAP level in patients with ESRD undergoing HD (r=0.762, P<0.001). (C) Pearson correlation analysis between the concentration of serum vitamin E and the ROS level in patients with ESRD undergoing HD (r=0.795, P<0.001). FRAP, ferric-reducing ability of plasma; ROS, reactive oxygen species; ESRD, end-stage renal disease; PM, polysulfone dialyzer membrane; VEM, vitamin E-coated dialyzer membrane.

vitamin E. The effect of VEM treatment on the serum concentration of vitamin E and its association with the change of redox metabolism was further evaluated in patients with ESRD undergoing HD. Patients with ESRD undergoing HD were characterized by a markedly lower concentration of serum vitamin E compared with the healthy volunteers (Fig. 3A). In addition, the level of serum vitamin E was markedly improved in the VEM group compared with the PM group after 8 weeks. Additionally, Pearson's correlation analysis suggested a notable positive correlation between the concentration of serum vitamin E and the FRAP level in patients with ESRD undergoing HD (r=0.762, P<0.001) (Fig. 3B). Conversely, a notable negative correlation between the concentration of serum vitamin E and the ROS level was found in patients with ESRD undergoing HD (r=-0.795, P<0.001) (Fig. 3C). These data revealed that HD with VEMs restored the imbalance of redox metabolism of RBCs in patients with ESRD undergoing HD by transferring vitamin E.

VEM attenuates the oxidative phosphorylation of Band 3 in RBCs of patients with ESRD undergoing HD. To further clarify the potential molecular mechanism of VEM treatment in alleviating the abnormal morphology and deformability of RBCs in patients with ESRD undergoing HD, the degree of oxidative phosphorylation damage of the membrane skeleton protein Band 3 in RBCs was detected. Densitometric analyses revealed markedly higher levels of clustered and tyrosine-phosphorylated (p)-Band 3 in patients with ESRD undergoing HD compared with the healthy volunteers. In addition, after 8 weeks of VEM treatment, the levels of clustered and p-Band 3 were markedly decreased compared with the PM group (Fig. 4A-C). Subsequent microscopic analysis of Band 3 verified the presence and differences of Band 3 aggregates in RBCs of patients with ESRD undergoing HD (Fig. 4D).

Discussion

RBCs are vulnerable to oxidative damage and abnormal deformability under OS induced by a variety of physiological and pathological conditions; this is considered a potential triggering factor of various cardiovascular and cerebrovascular diseases. It is now accepted that the RBCs of patients with ESRD undergoing HD are susceptible to OS and chronic inflammation due to the primary diseases and HD process. Therefore, effective therapy to restore the imbalances of redox metabolism in RBCs of patients with ESRD undergoing HD is desirable for improving the curability of the condition and the survival rate. The present study has shown that HD with VEM may alleviate the abnormalities of RBC morphology and deformability in patients with ESRD undergoing HD by restoring the equilibrium of redox metabolism.

Accumulating evidence has suggested that patients with ESRD are subject to enhanced OS, which triggers oxidative damage to nucleic acids, lipids and proteins (24,25). Furthermore, patients with ESRD undergoing HD usually present a higher level of OS due to the accumulation of oxidative products and the loss of antioxidants during HD procedures (3,26). Consistent with previous studies, the present study also found markedly higher OS levels (ROS, MetHb and MDA) and lower antioxidant capacity (FRAP, CAT and SOD) in patients with ESRD undergoing HD compared with healthy volunteers. It was evidenced that patients undergoing HD suffer from serum vitamin E deficiency, as vitamin E is the most important lipophilic antioxidant in cell membranes, and this is considered to be the potential reason for the decrease of antioxidant capacity in patients undergoing HD (13). Based on this, a therapeutic schedule of vitamin E supplementation may be beneficial to these patients. Maccarrone *et al* (12) showed that vitamin E intake could alleviate the membrane lipid peroxidation of blood cells in patients undergoing HD. In addition, *in vivo* and *in vitro* studies have reported that VEM administration can alleviate vitamin E deficiency in patients undergoing HD, improve the antioxidant capacity and reduce the extent of OS injury (16,27-29). Similarly, the present study detected markedly increased levels of antioxidant capacity and decreased OS in patients with ESRD undergoing HD under VEM treatment compared with the PM treatment group. In addition, VEM treatment led to a marked improvement in the values of serum vitamin E in patients with ESRD undergoing HD, and this was notably correlated with the antioxidant capacity of RBCs. These results indicated that VEM treatment

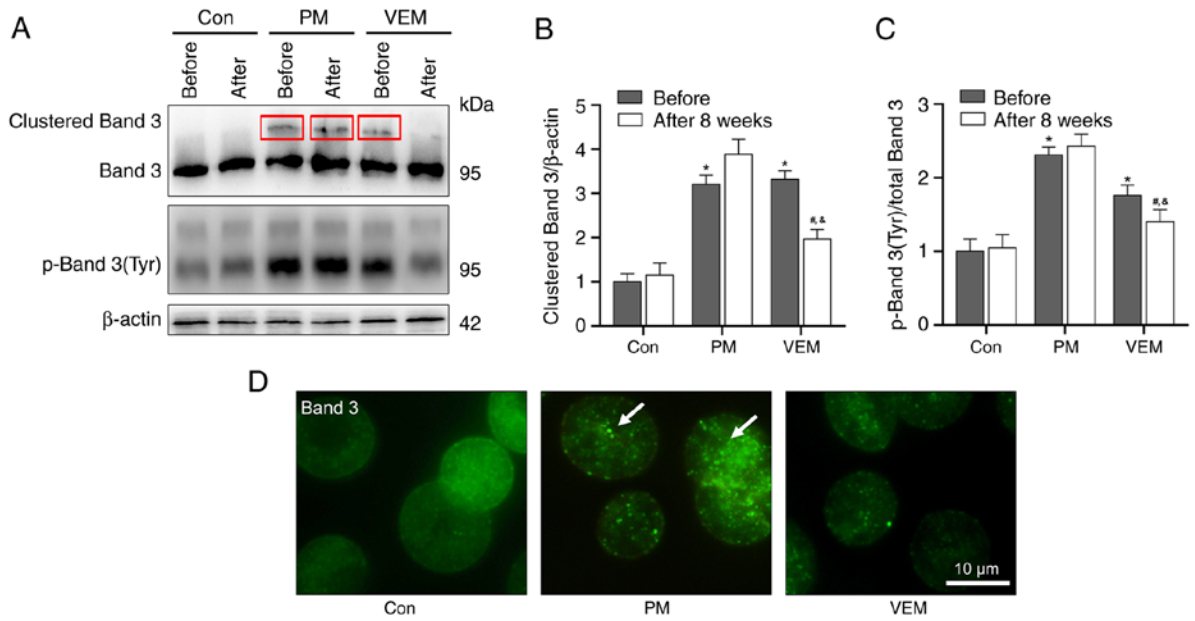

Figure 4. Oxidative phosphorylation of Band 3 in RBCs of patients with ESRD undergoing HD and healthy volunteers. (A) Expression levels of Band 3 and tyrosine-p-Band 3 (Tyr) in healthy donors and patients with ESRD undergoing HD (red boxes indicate highly expressed clustered Band 3). (B) Densitometric analyses of clustered Band 3. (C) Ratio of p-Band 3(Tyr)/total Band 3 is shown. (D) Representative fluorescence micrographs for Band 3-labeled RBCs in healthy donors and patients with ESRD undergoing HD after 8 weeks (white arrows indicate the crosslink of Band 3 in RBCs; scale bar, 10 μm). All data are expressed as the mean ± SD. *P<0.05, PM or VEM group vs. control group before examination; $^{\#}$P<0.05, VEM group vs. control group 8 weeks after examination; $^{\&}$P<0.05, VEM group vs. PM group 8 weeks after examination. ESRD, end-stage renal disease; PM, polysulfone dialyzer membrane; VEM, vitamin E-coated dialyzer membrane; RBC, red blood cell.

alleviated the imbalance of redox metabolism of RBCs by transferring vitamin E in patients with ESRD undergoing HD.

Severe OS injury may lead to lipid peroxidation and cross-linking of membrane skeleton proteins, resulting in an abnormal deformability and increased incidence rate of cardiovascular disease in patients with ESRD. Previously, it has been shown that patients with ESRD undergoing peritoneal HD presented with abnormal morphology and hemorheology of RBCs (30-32). The present study also found the occurrence of abnormal morphology and deformability of RBCs in patients with ESRD undergoing dialysis compared with healthy controls. Furthermore, VEM treatment alleviated the dysfunction of RBCs in terms of morphology and deformability in patients with ESRD undergoing HD compared with the PM treatment group. Therefore, the use of VEMs in HD may provide a means of reducing the risk of cardiovascular disease in patients with ESRD by repairing the abnormal rheological properties of RBCs.

Previous studies have confirmed that Band 3 has a vital role in maintaining the structural stability and mechanical properties of RBCs by linking phospholipid bilayer and spectral-based skeletal networks (23,33). Band 3 cluster caused by OS is usually accompanied by a decrease in the deformability of RBCs (19,34). Indeed, the present study showed notably increased levels of oxidative phosphorylation and clustering of Band 3 in patients with ESRD undergoing HD. In addition, VEM treatment could effectively reduce the oxidative phosphorylation and clustering of Band 3 in patients with ESRD undergoing HD compared with PM treatment group. This may potentially be a molecular mechanism for VEM treatment to alleviate the abnormal morphological and rheological properties of RBCs in patients with ESRD undergoing HD.

Although a number of studies have shown that adequate vitamin E supplementation can alleviate the oxidative damage of tissues and organs in a variety of physiological and pathological environments by improving antioxidant capacity (35,36), there is some evidence to show that an excessive vitamin E intake is also associated with side effects (37,38). A dose-response analysis showed that high-dosage vitamin E supplements may increase all-cause mortality risk (37). A meta-analysis study showed that high-dose vitamin E supplements may interact with prescription drugs, such as aspirin, warfarin, tamoxifen and cyclosporine A, and this was suggested as a possible underlying mechanism of the side effects of high-dose vitamin E supplements (38). In addition, it has been reported that an excessive vitamin E intake, when combined with salmon oil in the diet, lowers the activities of antioxidant enzymes in erythrocytes without affecting *in vivo* hemolysis (39). The present study, however, did not find that VEM treatment caused significant side effects in patients with ESRD.

The present study was associated with a couple of important limitations. First, there was a lack of information on the time- and dose-dependence of VEM treatment. Another limitation was that the numbers of patients and control individuals were relatively small.

In conclusion, the present study has presented evidence that patients with ESRD undergoing HD suffered from severe redox metabolic disorder, which led to the abnormal morphology and deformability of RBCs, thereby becoming a potential pathogenic factor for ESRD-associated cardiovascular disease. In patients with ESRD undergoing HD, VEM treatment effectively improved the antioxidant capacity, repaired the oxidative phosphorylation damage and resolved the problem of clustering of Band 3 in RBCs by delivering

vitamin E. This process was demonstrated to alleviate the abnormal morphological and mechanical properties of RBCs, and it is anticipated that this will lead to improvements in the nursing care and cure rates of patients with ESRD.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XL conceived and designed the experiments. YZ and WG performed the experiments. YZ and XL analyzed the data. XL wrote the paper. All authors read and approved the final version of the manuscript. YZ and XL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

This study protocol was reviewed and approved by the Ethics Committee of Yantai Hospital of Traditional Chinese Medicine (approval no. 2020-06). Patients and healthy volunteers were informed in detail and written consent was obtained before the examination.

Patient consent for publication

Written informed consent was obtained from the patients prior to publication at the time of admission.

Competing interests

The authors declare that they have no competing interests.

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