Clinical research

Profiling oxidative stress markers and cardiovascular complications in chronic kidney disease patients supplemented with vitamin E

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Abstract

Introduction: Cardiovascular diseases are common complications in chronic kidney disease (CKD). Oxidative stress associated with renal and metabolic dysfunctions is one of the cardiovascular complications (CVC) in haemodialysis patients. The aim of the present study is to analyse the oxidative stress markers in CDK patients supplemented with antioxidants and vitamin E, with monitoring of CVC.

Material and methods: This was a cross-sectional study conducted on 99 subjects. CKD patients received oral supplementation of vitamin E (300 mg/day) for 2 years. Oxidative stress markers, nitric oxide (NO); myeloperoxidase (MPO); oxidized low-density lipoprotein (LDLox); malondialdehyde (MDA) and glutathione were measured before and after the vitamin treatment.

Results: NO (62.62 ±2.80 µmol/l), LDLox (10.55 ±4.62 µmol/l), MDA (6.11 ±2.83 µmol/l) and MPO (53.35 ±3.82 UI/ml) were overconcentrated, while glutathione (62.09 ±4.15 UI/ml) was less concentrated in CKD patients with cardiovascular complications, compared to those without cardiovascular complications (67.08 ±1.90 µmol/l, 31.18 ±5.25 µmol/l, 16 ±6.47 µmol/l, 57.00 ±7.24 UI/ml, 43.09 ±3.33 UI/ml, respectively). After 2 years of vitamin E treatment, the overall cardiovascular complications were not significantly decreased.

Conclusions: These results showed that oral complementation with vitamin E did not affect the occurrence of cardiovascular complications associated with CKD. These findings may pave the way for future innovative strategies for antioxidant supplementation in CKD patients.

Key words: nephropathy, oxidative stress, cardiovascular complication, vitamin E.

Introduction

Chronic kidney disease (CKD) is a major public health issue, and the number of cases is continuously increasing worldwide [1]. Population aging and the increase of cardiovascular and metabolic pathologies such as diabetes strongly affect the prevalence of CKD [2]. Cardiovascular complications (CVC) are common in CKD patients and depend on the progression stage of the disease. For instance, moderate CKD is associated with 20% of CVC [3]. This latter is the leading cause of death in CKD patients, where the mortality rate is 10 to 30 times higher than in the general population [4]. Arrhythmia and cardiac arrest are the main cause of death (37%) in CKD patients, followed by myocardial infarction, atherosclerosis-related cardiovascular accidents, and heart failure [5]. CVC in CKD patients are multifactorial diseases, induced mainly by traditional risk factors such as age, sex, hypertension, diabetes, insulin resistance, sedentary and tobacco, and non-traditional risk factors such as anaemia, phospho-calcium imbalance, impairment of the renin-angiotensin-aldosterone system (RAAS), inflammation and oxidative stress [6, 7].

Oxidative stress is defined as a set of lesions induced by reactive oxygen species (ROS) resulting from an imbalance between the production of free radicals, oxidants and defence mechanisms, and antioxidants within the same organism [8]. Haemodialysis may further damage this antioxidant system, primarily through the loss of hydrophilic substances such as vitamin C (Vit C), trace elements and enzyme-regulating compounds [9].

The organism neutralizes oxidants by enzymatic and non-enzymatic defence systems, by which it reduces the formation of free radicals and eliminates them after accumulation, as well as tissue and cellular repair. Vitamin E (Vit E) is an important antioxidant molecule that is implicated in elimination of free radicals, stability and permeability of plasma membrane, mainly through protection from the harmful effect of endogenous lipid-degrading enzymes [10]. Interestingly, Vit E has anti-inflammatory and anti-coagulant activities by reducing neutrophil chemotaxis and inhibiting platelet aggregation, respectively [11, 12]. The main sources of Vit E are seed oils such as wheat germ oil (150 mg/ml), almond oil (95 mg/ml) and olive oil (15 mg/ml). Vitamin E is a lipophilic vitamin. Haemodialysis membranes remove only hydrophilic substances, so that α-tocopherol cannot be cleared by haemodialysis, but the undernutrition of CKD patients causes the blood level of vitamin E to be low [13]. Although Vit E therapy has been widely studied in CKD patients, there has been no consensus on its benefit in managing cardiovascular complications in haemodialyzed patients [14]. The present study aimed to measure oxidative stress markers in CKD patients supplemented with Vit E while monitoring CVC.

Material and methods

This was a descriptive longitudinal and prospective study, conducted during 2 years on 99 CKD patients at the haemodialysis stage for more than 6 months. As inclusion criteria, all patients over 18 years of age and suffering from chronic renal failure of various aetiologies and at haemodialysis stage, were included. In addition, patients were required to be clinically stable for 3 months before the onset of the study, and did not receive treatment with injectable iron, an important generator of oxygenated free radicals.

The patients excluded from the study were those diagnosed with heart diseases before the diagnosis of CKD, such as severe valve disease, constrictive pericarditis, systolic dysfunction with an ejection fraction (EF) of less than 50%, patients on dialysis for less than 6 months and those on peritoneal dialysis. All patients with heart failure were excluded from the study if their ejection fraction was less than 50%.

All CKD patients received oral supplementation of 300 mg/day of α-tocopherol for 2 years. Blood samples were collected in tubes containing dipotassium ethylenediamine tetraacetic acid (EDTA K2) from all patients before the onset of Vit E treatment and after 2 years of treatment, for measurement of oxidative stress markers. Blood samples were centrifuged for 10 min at 4500 rpm (3900 g), then aliquoted and stored at –20°C until further analysis.

Measurement of oxidative stress markers

The glutathione assay was carried out using the colorimetric method based on the reducing properties of thiol groups (SH) in the presence of free thiol groups of the analysed samples. The disulphide bonds (S-S) present in the DTNB reagent (5,5′-dithiobenzoic acid; Pierce Biotechnology PO Box 117, 3747 N. Meridian Road Rockford, USA) are reduced and generate colorimetric changes measured by a spectrophotometer at 412 nm. The reduced glutathione concentration is calculated using the molar extinction coefficientI $\varepsilon = 13.6 \times 10^3$ mol³-L.cm from the following formula: DO = ε.C.I. The normal values of glutathione in the control group were 89.4 ±2.6 IU/ml.

Myeloperoxidase (MPO) measurement was conducted by the colorimetric enzymatic method. Briefly, plasma samples collected from control individuals and CKD patients were diluted in 50 mM phosphate buffer, pH 6.0. The mixture went through several cycles of freezing and thawing. Then, the suspensions were centrifuged at 4500 rpm for 15 min. Each supernatant was mixed

with 50 mM phosphate buffer, pH 6.0 containing o-dianisidine (0.167 mg/ml) and H_2O_2 (0.0005%). The enzymatic activity was evaluated by measuring the absorbance at 460 nm. The normal values of controls were 38.45 ±1.93 IU/ml.

In the present study, the nitric oxide (NO) secreted by inducible NO-synthase (iNOS) was measured, for its known pro-oxidative role, by the quantification of two physiological metabolites: nitrites (NO_2^-) and nitrates (NO_3^-). Briefly, the NO was measured in plasma following deproteinization using a solution of zinc sulphate (ZnSO4). To measure the total nitrites, each sample was incubated in the presence of 200 mg of cadmium and agitated for 10 min at room temperature. A volume of 400 ml of the previous solution was added to 1500 ml of sulfanilic acid. After incubation in the dark for 10 min, 1600 ml of N-naphthalene diamine was added to the mixture and incubated for 10 min in the dark. The colour intensity was measured at 550 nm. The normal values of the controls were 52.19 ±2.1 µmol/l.

To monitor the apolipoprotein oxidation, the oxidized low-density lipoprotein (LDLox) was measured by enzyme-linked immunosorbent assay (ELISA) (abx253899, Abbexa Ltd, 181 Cambridge Science Park, Cambridge CB4 0GJ, United Kingdom), following the manufacturer's instructions.

The malondialdehyde (MDA) was measured in plasma, aiming to monitor the lipid peroxidation, by high performance liquid chromatography (HPLC) using Chromolith RP-18 Endcapped HPLC Columns (Merck Worldwide Headquarters, 400 Summit Drive Burlington, Massachusetts, 1803USA).

General metabolic parameter measurements

Further general metabolic parameters were measured in the CKD patients, including total cholesterol, high-density lipoprotein (HDL), triglycerides, high-sensitivity C-reactive protein (hs-CRP) and phospho-calcium balance, to monitor the metabolic profile of the studied population.

Total cholesterol

Total cholesterol was measured using the Siemens Dimension system and Reagent Dimension Flex DF27 (Siemens, UK). The detection limit was 50 mg/dl and desirable, upper limit, and high expected values were < 200 mg/dl (5.2 mmol/l), 200–239 mg/dl (5.2–6.2 mmol/l) and ≥ 240 mg/dl (6.2 mmol/l), respectively. HDL was determined by the enzymatic colorimetric method (CHOD/PAP), using a COBAS INTEGRA 400 plus analyser (Roche Diagnostics, Nederland) with a detection limit of 0.39 mg/dl. Results were considered normal at values > 0.35 g/l for males and > 0.45 g/l for females. Triglyceride was measured using the Siemens Dimension system and Reagent Dimension Flex DF 69A (Siemens, UK), according to the manufacturer's instructions. The detection limit was 15 mg/dl and expected normal values were < 150 mg/dl. hs-CRP was measured according to the particle-enhanced immunonephelometric method using BN II/BN ProSpec Systems Reagent. The detection limit was 0.175 mg/l and the expected normal value was ≤ 3 mg/l. Calcium was measured according to a modification of the calcium o-cresolphthalein-complexone (OCPC) reaction method, using the Siemens Dimension system and Reagent Dimension Flex DF 23A (Siemens, UK), according to the manufacturer's instructions. The detection limit was 5 mg/dl (1.25 mmol/l) and expected normal values were 8.5–10.1 mg/dl (2.12–2.52 mmol/l). Phosphorus measurement was conducted according to a modification of the classical phosphomolybdate method using the Siemens Dimension system and Reagent Dimension Flex DF 61A (Siemens, UK). The detection limit was 0.5 mg/dl and expected normal values were 2.6–4.7 mg/dl (0.84–1.52 mmol/l). The Vit E levels were measured, in the experimental and control groups, in micromoles per liter of blood (µmol/l) in a blood sample taken after 12 to 14 h of fasting. The samples were collected using an EDTA tube, and HPLC-DAD was employed for the measurement, with a reference range of 8.60–19.24 mg/l.

Cardiovascular complication monitoring

Patients were clinically monitored to detect cardiovascular complications such as amputation of a limb due to arteritis, death after cardiovascular complication, stroke, myocardial infarction, and aneurysm rupture, before and after treatment, every 3 months for 2 years. Lower limb arteritis refers to a condition affecting the arteries of the legs, typically caused by deposition of cholesterol in the arteries. This deposition can lead to disrupted blood circulation and reduced oxygen supply to the muscles, resulting in symptoms such as leg pain, cramping, and difficulty walking. Lower limb arteritis is commonly referred to as lower limb peripheral arterial disease. During the monitoring period for CKD patients, visits occurred every 3 months with clinical, biological, and radiological surveillance. Clinically, we monitored for cardiac complications, while biologically, we monitored inflammatory markers and oxidative stress, and radiologically, we performed ECGs and echocardiograms. For healthy patients, monitoring occurred every 6 months with clinical, biological, and radiological surveillance to detect cardiac complications. The haemodialysis patients were evaluated for cardiovascular complications before the

start of vitamin E treatment. During the 2 years of oral vitamin E supplementation, the patients were re-examined every 3 months to monitor the progression of cardiovascular complications, primarily cerebrovascular accidents (strokes), myocardial infarction (heart attacks), and cardiac arrest.

Statistical analysis

Statistical analysis was performed using the SPSS 22 (IBM, Armonk, NY) and Microsoft Excel 365 software. The comparison of two averages was analysed using Student's *t* test. The comparison of more than two means of the continuous variables was made by the ANOVA test for the parametric tests and by Welch and Brown-Forsythe tests for the nonparametric tests. Tukey and Hartman tests were used for multiple comparisons. The chi square (χ^2) test was used for comparison of the qualitative variables. Pearson's test was used for linear correlations. Test results were considered significant at *p*-values < 0.05.

Table I. Characteristics of the CKD patients included in the study. Values are presented as mean ± standard deviation (SD)

Parameters	CKD patients (*n* = 99) Mean values ± SD
Age [years]	45.3 ±11.56
Sex-ratio (male/female)	1.3
BMI	19.5 ±1.34
Hb [g/dl]	10.4 ±1.7
Cholesterol [g/l]	1.54 ±0.05
HDL [g/l]	0.32 ±0.03
Triglyceride [g/l]	2.04 ±0.05
hs-CRP [mg/l]	7.56 ±0.56
Ca [mg/l]	87.6 ±1.2
P [mg/l]	46.7 ±4.4
NO [µmol/l]	80.7 ±2.15
MPO [UI/ml]	90.76 ±2.34
LDLox [µmol/l]	41.5 ±0.98
Glutathione [UI/ml]	32.45 ±0.9
MDA [µmol/l]	9.87 ±0.23
Vit E[mg/l]	3.75 ±0.34

BMI – body mass index, Hb – haemoglobin, HDL – high-density lipoprotein, hsCRP – high-sensitivity C-reactive protein, Ca – calcium, P – phosphorus, NO – nitric oxide, MPO – myeloperoxidase, LDLox – oxidized low-density lipoprotein, MDA – malondialdehyde, Vit E – vitamin E.

Results

The study was conducted on 99 CKD patients at the chronic haemodialysis stage (Table I).

Investigating the origin of nephropathy in the studied population, hypertensive (32%) and diabetic (26%) nephropathies were the most important (Figure 1).

Aiming to investigate the effect of oxidative stress markers on the cardiovascular complications in haemodialysis patients, it was found that NO, LDLox, MPO, MDA and glutathione were significantly associated with CVC ($p < 0.0001$) (Figure 2). Patients with CVC had higher average values of NO, LDLox, MPO and MDA, but lower glutathione values, compared with CKD patients without CVC (Figure 2). Importantly, these distinctions were evident at the initiation of treatment and continued to increase over time during the 2 years of treatment. There was a significant difference in the average levels of pro-oxidant stress markers (i.e., NO, oxidized LDL) between patients with cardiovascular complications and those without, with the averages being higher in the former group.

Pro-oxidizing markers (NO, MPO, LDLox and MDA) and an anti-oxidizing marker (glutathione) were measured in haemodialysis patients after 2 years of oral supplementation with Vit E (300 mg/day), to verify the possible protective effect of Vit E against oxidative stress (Figures 3 and 4).

In this cross-sectional study, 99 subjects with CKD patients were orally supplemented with 300 mg/day of vitamin E for 2 years.

The average MPO value was persistent before and after treatment (53.86 ±4.61 against 53.44

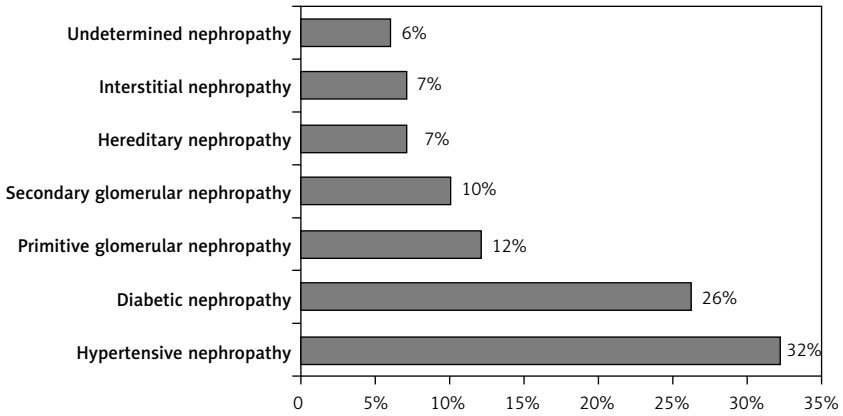

Figure 1. Nephropathy origin in chronical kidney disease (CKD) patients at haemodialysis stage

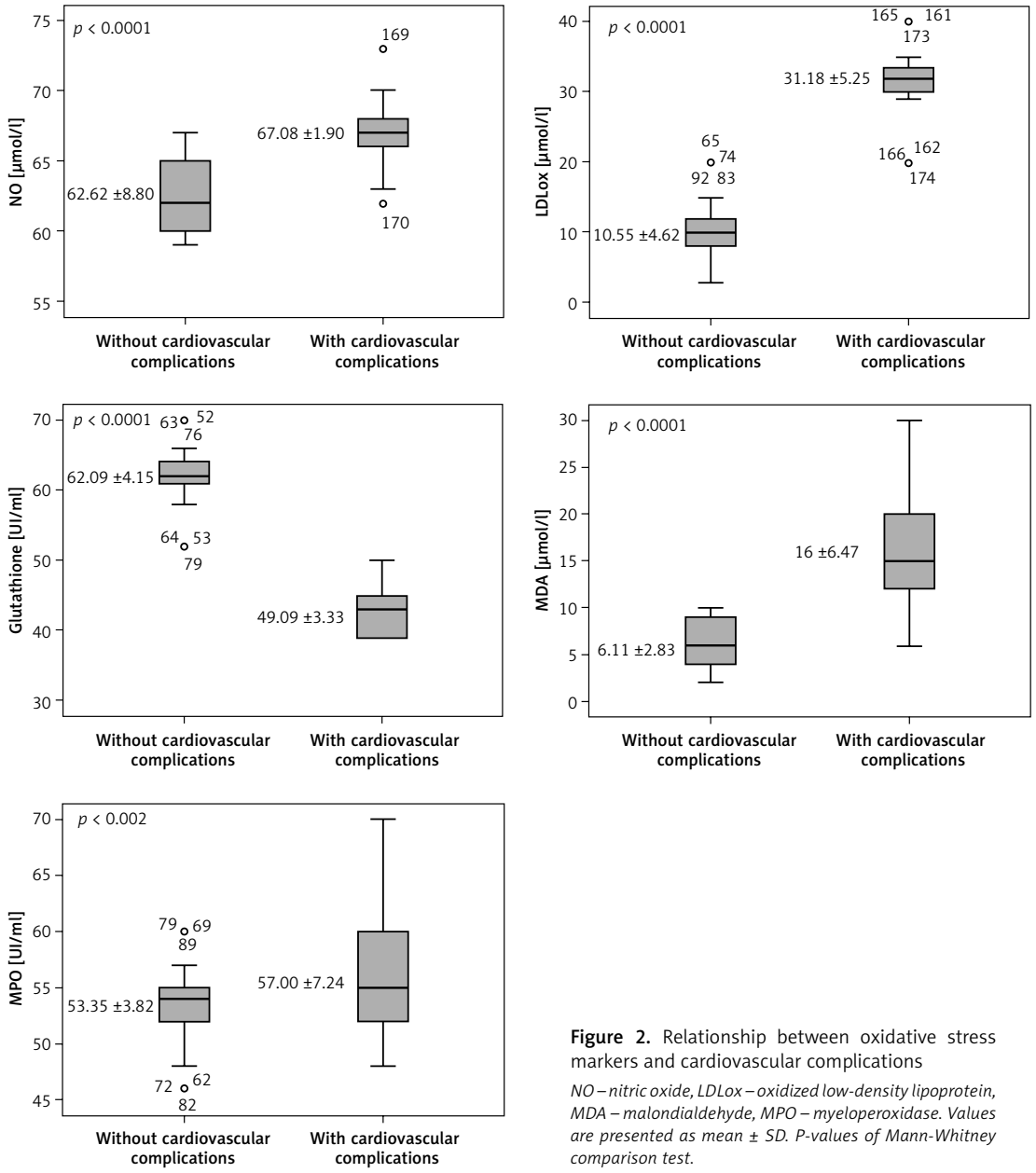

Figure 2. Relationship between oxidative stress markers and cardiovascular complications

NO – nitric oxide, LDLox – oxidized low-density lipoprotein, MDA – malondialdehyde, MPO – myeloperoxidase. Values are presented as mean ± SD. P-values of Mann-Whitney comparison test.

±7.38, $p > 0.05$). In contrast, glutathione increased significantly after oral supplementation with Vit E (71.44 ±9.99 versus 61.54 ±4.76, $p < 0.0001$). The mean values of NO, MDA and LDLox significantly decreased after 2 years of Vit E treatment (6.72 ±3.73 versus 3.7 ±2.42, $p < 0.0001$ and 10.96 ±5.01 versus 9.18 ±3.08, $p < 0.023$).

The haemodialysis patients were explored for cardiovascular complications before the onset of Vit E treatment. During 2 years of oral supplementation with Vit E, the patients were re-examined every 3 months for the development of CVC, mainly cerebrovascular accidents (CVA), myocardial infarction (IDM) and cardiac arrest (Table II).

The overall CVC were not significantly affected by the oral supplementation of Vit E for CKD patients (Table II). Rupture of the aneurysm occurs due to hypertensive peaks and weakened arteries [15] and the association of stroke and myocardial infarction was completely absent after Vit E treatment. The results were reported based on statistical analysis. The findings indicate that the outcomes were random, suggesting that vitamin E supplementation did not produce any significant change.

Discussion

In haemodialysis patients, several studies have revealed increased oxidative stress markers, mainly lipid peroxidation markers, such as thiobarbituric acid reactive substances (TBARS) and 4-hydroxynonenal (HNE) that react with thiobar-

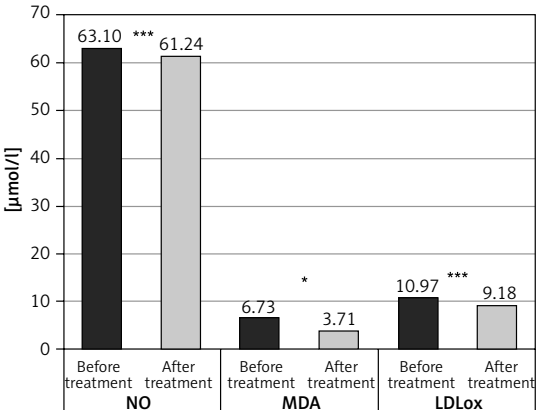

Figure 3. Evolution of oxidative stress markers' average values before and after 2 years of oral vitamin E supplementation. NO – nitric oxide, LDLox – oxidized low-density lipoprotein, MDA – malondialdehyde. Values are presented as mean ± SD. *P*-values of Mann-Whitney comparison test. *Significant difference at $p < 0.05$, ***significant difference at $p < 0.0001$

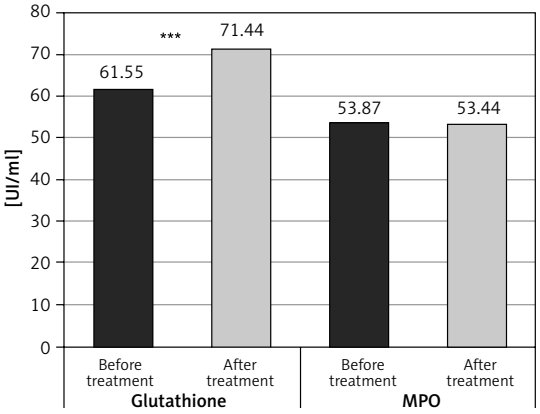

Figure 4. Evolution of oxidative stress markers' average values before and after 2 years of oral vitamin E supplementation. MPO – myeloperoxidase. ***Significant difference at $p < 0.0001$

bituric acid. The general inflammatory profile of haemodialysis patients is dominated by the abundant production of polynuclear neutrophils (PNN) and monocytes [16]. These cells increase the secretion of MPO and NADPH oxidases (NOX), leading to high reactive oxygen species (ROS) production, impairing vascular endothelial function and increasing lipid peroxidation, thus aggravating the CKD progression [17].

Isoprostanes, prostaglandin-like compounds formed by free radical lipid peroxidation of essential fatty acids independent of the enzyme cyclooxygenase (COX), are more specific markers of the peroxidation of polyunsaturated fatty acids in CKD [18]. Markers of DNA base alteration, such as 8-hydroxy 2′-deoxyguanosine (8-OHdG), were detected in haemodialysis subjects, in addition to protein peroxidation markers, such as advanced oxidation protein products (AOPP) [19]. During oxidative stress in CKD, the oxidized proteins react with the oxidation products of lipids or carbohydrates and create carbonyl derivatives, known as carbonyl stress [20]. CKD patients, especially those at the haemodialysis stage, present a chronic inflammatory condition maintained and worsened by oxidative stress, leading to malnutrition-inflammation-atherosclerosis (MIA) syndrome [21]. The MIA syndrome is one of the non-traditional cardiovascular risk factors in CKD since haemodialysis patients have high prevalence of protein-energy malnutrition and inflammation. Because these two conditions often occur concomitantly in haemodialysis patients, they have been commonly referred to as MIA to emphasize the important association with atherosclerotic cardiovascular disease. The three pathophysiological factors linked to these patients are dialysis-related nutrient loss, increased protein catabolism, and hypoalbuminemia [22].

For instance, haemodialysis patients were anaemic and had calcium and metabolic disorders, in accordance with literature findings [23, 24]. The investigation of the nephropathy origin in CKD patients revealed that hypertensive nephropathy was the most detected, followed by diabetic nephropathy. The Annual Report of the ERA-EDTA Registry, in 2017 revealed that hypertension is the second or third most common cause of renal replacement therapy (RRT), in relation to glomerulonephritis, in Europe [25].

The present study confirmed a significant relationship between CVC and all the measured oxidative stress markers in haemodialysis patients: MDA, LDLox, NO, MPO and glutathione. MDA was higher in patients with CVC. A study conducted on 76 patients at the haemodialysis stage showed

Table II. Evolution of cardiovascular complication before and after vitamin E treatment

Cardiovascular complication	Amputation of limb due to arteritis	Death after cardiovascular complication	Stroke	Myocardial infarction	Stroke and myocardial infarction	Aneurysm rupture	Total
Before vitamin E treatment	15.38%	30.76%	26.92%	19.23%	3.84%	3.84%	16.04%
After vitamin E treatment	17.64%	29.64%	23.52%	29.41%	0	0	11.03%
P-value	0.93	0.95	0,99	0.093	–	–	0.118

that MDA levels were higher in patients with CVC such as stroke, transient ischaemic attack (TIA), myocardial infarction (IDM), angina pectoris, sudden death and peripheral arterial disease, compared to those without CVC (2.8 ±0.6 µmol/l vs. 2.4 ±0.4 µmol/l, $p = 0.001$) [26]. This indicates a potential association between increased MDA levels and the presence of cardiovascular complications in these patients. Mechanistically, MDA is believed to play an active role in the development of CVC by binding to LDLox, which is then absorbed by resident macrophages, subsequently leading to the formation of foam cells and atheromatous plaques, ultimately contributing to cardiovascular diseases [27]. Therefore, the difference observed in MDA levels between patients with and without CVC underscores the potential role of oxidative stress in the pathogenesis of cardiovascular complications in haemodialysis patients.

CKD patients with CVC had elevated LDLox levels compared to those without CVC. Likewise, a study conducted on 94 haemodialysis patients where CVC were recorded for 4 years revealed that patients who died from CVC had higher levels of LDLox compared to the other patients (17.4 ±2.8 vs. 13.9 ±1.5 µmol/l, $p = 0.019$) [28]. Lipid disorders, often observed at the early stages of chronic renal failure, play a major role in CVC development [28]. Among the various cardiovascular complications, atherosclerosis remains a predominant cause of morbidity and mortality in CKD [29]. However, oxidative stress plays a crucial role in the initiation of atherosclerosis, with LDLox serving as a pivotal factor [30]. The process begins with endothelial artery damage, triggering an inflammatory response and recruitment of macrophages that bind to apolipoproteins. Subsequently, these apolipoproteins undergo oxidation to form oxidized apolipoproteins, which then bind to macrophages, transforming them into foam cells and promoting the formation of atheromatous plaques [27].

In the present study, CVC were positively correlated with NO levels. Notably, NO exhibits contrasting effects on the cardiac pathogenesis depending on its origin [31]. The distinction between individuals with CVC and those without lies in the nuanced roles of NO within the cardiovascular system. In individuals with CVC, such as those with CKD or other cardiovascular disorders, there exists a dysregulated balance between endothelial nitric oxide synthase (eNOS)-derived NO and inducible nitric oxide synthase (iNOS)-derived NO. eNOS-derived NO, primarily produced by endothelial cells, typically exerts a cardioprotective effect by promoting vasodilation and reducing myocardial workload, thus attenuating left ventricular hypertrophy (LVH) and cardiomyopathy. Conversely, iNOS-derived NO, induced in response to inflammatory stimuli, contributes to adverse cardiac remodelling, including LVH and cardiac fibrosis [32]. The dysregulation of NO production and its opposing effects on cardiac pathogenesis delineate the difference between individuals with CVC and those without, highlighting the importance of NO balance in cardiovascular health and the potential therapeutic implications for managing cardiovascular complications.

MPO, a haem peroxidase, secreted mainly by neutrophils, is strongly implicated in the atherogenesis process and several cardiovascular diseases [33]. High levels of MPO are associated with oxidative stress, inflammation and higher mortality related to CVC [32]. In the present study, MPO plasma levels were higher in CKD patients with CVC, compared to those without CVC. Likewise, several studies have confirmed that low levels of MPO would have a protective effect against cardiovascular diseases [34]. This elevation in MPO levels promotes the progression of atherosclerosis by facilitating lipid oxidation, endothelial dysfunction, and leukocyte recruitment, ultimately leading to plaque formation and instability. Additionally, higher MPO levels are associated with poorer clinical outcomes and increased mortality rates in individuals with CVC, underscoring its prognostic significance in cardiovascular diseases. Conversely, lower levels of MPO may confer a protective effect against cardiovascular diseases by reducing oxidative stress and inflammation, suggesting a potential therapeutic target for mitigating cardiovascular complications.

Glutathione level was negatively correlated with CVC. A study conducted by Zuo *et al*. [35] found that glutathione level was lower in haemodialysis patients with CVC than those without CVC ($p < 0.01$). Glutathione exerts its protective effects on the cardiovascular system through its antioxidant properties, which involve scavenging ROS and maintaining cellular redox homeostasis. This antioxidant capacity is particularly important in endothelial cells, where glutathione helps prevent oxidative damage to lipids, proteins, and DNA, thus preserving endothelial function. Additionally, glutathione plays a critical role in supporting the activity of NO, a key vasodilator produced by endothelial cells, by preventing its inactivation by ROS [36]. Consequently, individuals with higher glutathione levels are expected to have enhanced protection against CVC, given the antioxidant and NO-preserving effects of glutathione. Therefore, strategies aimed at optimizing glutathione levels may hold promise in reducing the risk of cardiovascular complications, particularly in populations predisposed to oxidative stress and endothelial dysfunction.

Haemodialysis patients included in the present study were treated with an antioxidant treatment based on Vit E oral supplementation at a rate of

300 mg/day for 2 years, and oxidative stress markers were measured before and after treatment. It has been noted that haemodialysis patients exhibit lower levels of Vit E compared to individuals without renal issues. This discrepancy may stem from gastrointestinal disturbances commonly seen in uraemic patients, potentially leading to impaired absorption, and consequently resulting in diminished plasma concentrations of Vit E in this population [37].

The mean value of MDA clearly decreased after 2 years of treatment. Several literature findings confirm the decrease of MDA levels after treatment with Vit E. Using the same concentration of Vit E for 20 weeks, Uzum *et al.* [38] reported a significant decrease of MDA levels after treatment (2.2 ±0.76 vs. 2.77 ±0.87 µmol/l, $p = 0.018$). Likewise, the same oral supplementation of Vit E (300 mg/day) for 2 years resulted in a decreased concentration of MDA (2.73 ±0.4 vs. 3.78 ±0.3 µmol/l, $p < 0.001$) [38]. In contrast, another study reported a non-significant decrease of MDA after Vit E supplementation (400 UI/day) for 3 weeks (9.08 ±2.83 vs. 8.42 ±2.63 µmol/l, $p > 0.05$) [39]. This latter finding could be explained by the short duration of treatment, compared to the other studies. Other parameters could affect the difference in treatment responses such as study design, treatment with the anti-anaemia drug Venofer, patient's medical history, underlying pathologies, and associated risk factors. The significant decrease of MDA levels after Vit E supplementation in haemodialysis patients reported in the present study suggests a direct protective effect of Vit E against lipid peroxidation [40].

LDLox levels significantly decreased after Vit E supplementation, which is concomitant with most literature findings that support the beneficial effect of Vit E on LDLox levels whether taken orally [41] or on dialysis membranes [42]. The most likely explanation is that Vit E increases resistance to LDL oxidation, which explains the decline in LDLox levels [41]. Furthermore, Vit E inhibits the production of proinflammatory cytokines by the endothelial and immune cells, suppresses the expression of adhesion molecules on endothelial cells and ligands on monocytes, and reduces their adhesive interactions, which is an important event in the initiation of fatty streak formation and atherogenesis [43]. NO values decreased after oral supplementation with Vit E for 2 years, which is similar to literature findings [32].

The MPO levels were similar before and after Vit E supplementation. The literature findings about MPO as a marker are limited. A recent study revealed that a daily consumption of 450 mg of pomegranate peel extract (PPE) combined with 400 IU of Vit E for 8 weeks was more effective to reduce MPO levels than Vit E alone [44]. Even though Vit E is a good antioxidant, combining it with other antioxidants seems important for better management of oxidative stress.

Glutathione, a powerful antioxidant and oxidative stress marker, significantly increased in haemodialysis patients after 2 years of oral supplementation with Vit E. A significant increase in glutathione concentration was reported after Vit E supplementation at a rate of 600 mg/day for 14 weeks (26.2 ±3.26 vs. 20 ±2.89 UI/g, $p < 0.001$) [45]. Similar findings were reported in infants supplemented with 15 mg/day of Vit E for 15 days. However, in another study and after 3 months of supplementation with 800 IU/day of Vit E, the glutathione level did not increase significantly [46]. This discrepancy is mainly due to the fact that study results are affected by many factors, such as dose and duration of the treatment and route of administration.

It seems that the antioxidant treatment using oral Vit E did not protect haemodialysis patients from CVC. Numerous prospective randomized clinical trials have been performed over the past 15 years to assess the effect of Vit E on protecting against CVC, with contradictory results. Another study, with a large population size (n = 14,641), reported no significant difference in CVC and death rates in patients receiving 400 mg/day of Vit E combined with 500 mg/day of Vit C for 10 years [47]. Similar findings were reported for a larger population (20,536 patients) with high risk of CVC, where a combination of 600 mg Vit E, 250 mg of Vit C, and 20 mg of β-carotene did not affect the CV and death rate [48].

Patients with peripheral arterial disease (PAD) experience decreased arterial perfusion in the lower extremities, commonly referred to as "poor circulation". In most cases of PAD, atherosclerotic plaques narrow the arterial lumen, restricting blood flow to the distal extremities. Reduced blood flow can cause thigh or calf pain during walking due to temporary ischemia of the leg muscles during exertion [49]. This walking pain, referred to as intermittent claudication, causes patients to limp. Many patients with PAD either have no symptoms or exhibit atypical complaints that do not strictly conform to the definition of claudication. Some patients may develop limb-threatening blood flow compromise, necessitating emergent surgery. Various treatments are proposed for this condition. Notably, the VOYAGER-PAD study demonstrated the efficacy of adding rivaroxaban 2.5 mg twice daily to the standard treatment with aspirin (ASA) for secondary prevention of both cardiovascular and peripheral events [50]. This combination did not significantly increase the risk of bleeding in patients who underwent lower extremity revascularization. However, for optimal post-revascularization management of the dis-

ease, a multidisciplinary approach is essential to control risk factors such as dyslipidaemia, smoking, and physical inactivity.

In conclusion, cardiovascular disease is the most common cause of morbidity and mortality in haemodialysis patients. Traditional and non-traditional risk factors, including oxidative stress, worsen this process. The present study confirmed that pro-oxidative stress markers are higher in haemodialysis patients. Aiming to modulate oxidative stress status, Vit E supplementation increased glutathione and decreased MDA as a process of oxidative stress control. Vit E therapy led to a nonsignificant reduction in cardiovascular complications. A more recent approach involves the use of Vit E in dialysis membranes; however, this technique remains at the development level and is highly expensive. The combination of other antioxidants with Vit E supplementation remains the best strategy for better management of oxidative stress in CKD patients.

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Ethical approval

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Conflict of interest

The authors declare no conflict of interest.

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