Low Total Vitamin C Plasma Level Is a Risk Factor for Cardiovascular Morbidity and Mortality in Hemodialysis Patients

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Hemodialysis patients are prone to deficiency of vitamin C, which constitutes the most abundant nonenzymatic antioxidant in blood. Because antioxidants are involved in the pathogenesis of atherosclerosis, the authors examined the association of total vitamin C plasma level with cardiovascular outcomes in such patients. One hundred thirty-eight consecutive maintenance hemodialysis patients (median age 61 yr, 90 males) were enrolled in a single-center study. At baseline, routine laboratory parameters were recorded, and predialysis total vitamin C plasma levels were measured by high-pressure liquid chromatography. Patients were prospectively followed-up for the occurrence of a primary composite endpoint consisting of fatal and nonfatal major adverse cardiovascular events (MACE) and for all-cause and cardiovascular mortality. MACE occurred in 35 patients (25%) over a period of median 30 mo, and 42 patients (30%) died [29 cardiovascular deaths (21% of total)]. Using Cox proportional hazards modeling, adjusted hazard ratios for the occurrence of MACE were 3.90 (95% confidence interval [CI]: 1.42 to 10.67; $P = 0.008$) and 3.03 (95% CI: 1.03 to 8.92; $P = 0.044$) for patients in the lower (<32 μmol/L) and middle (32 to 60 μmol/L) tertile of total vitamin C levels, compared with patients in the upper tertile (>60 μmol/L). Hazard ratios for cardiovascular death were 3.79 (95% CI: 1.23 to 11.66; $P = 0.020$) and 2.89 (95% CI: 0.89 to 9.37; $P = 0.076$). Total vitamin C levels were not independently associated with all-cause mortality. This study concludes that low total vitamin C plasma levels predict adverse cardiovascular outcomes among maintenance hemodialysis patients. Future studies should address the potential protective effect of an adequate vitamin C supplementation.

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Nutritional status plays a substantial role in the pathogenesis of atherosclerosis. In particular, deficiencies of antioxidant vitamins are suggested to exert deleterious effects on the vascular system and are associated with the development and progression of cardiovascular disease (1). It has been hypothesized that antioxidants may retard the growth of vascular plaques by opposing lipid peroxidation or the oxidative modification of low-density lipoproteins (LDL) (2), but the exact role of antioxidants in the prevention of vascular events like plaque rupture, vasoconstriction, or thrombosis remains to be elucidated. In this context, three prospective, large-scale, European studies confirmed a strong inverse relation between vitamin C plasma levels and all-cause or cardiovascular mortality in patients without kidney disease (3–5). The World Health Organization's MONICA study reported a similar observation (6), and the risk for coronary artery disease was higher in elderly Asian Indian subjects with vitamin C plasma levels in the lowest quartile as compared with those in the highest quartile (7). Consequently, approximate plasma cutoff levels have been proposed, above which the risk for apparent cardiovascular events should decrease (vitamin C: 40 to 50 μmol/L) (8).

Studies on the use of antioxidant supplements, however, showed inconsistent results. Observational cohort studies found some protection of antioxidant vitamin combinations against cardiovascular events (9), particularly in the elderly (10,11). Others, however, found no effect (12,13). The Heart Protection Study (14) reported no difference between a combination supplement of vitamin C, vitamin E, and β-carotene, or placebo on all-cause or all-vascular mortality, or on all major vascular events. As of June 2003, the US Preventive Services Task Force (USPSTF) concluded that there is not sufficient evidence to recommend or argue against vitamin A, C, or E use in the general population (15). It is of note that this statement is based on studies that measured intake of vitamins from supplements only. Data on patients with known or potential nutritional deficiencies including patients with chronic illnesses were not incorporated into this analysis. In particular, cohorts with ESRD were excluded from these studies (15,16).

Premature atherosclerosis constitutes a constant threat to chronic kidney disease patients, and oxidative stress is considered a potentially important source of morbidity and mortality in this population (17). Vitamin C represents the primary antioxidant defense in blood (18), and nonsupplemented maintenance hemodialysis patients are at high risk for vitamin C

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Table 1. Baseline demographic data and clinical characteristics of 138 maintenance hemodialysis patients stratified by lower and middle versus upper tertiles of total vitamin C plasma level

	Total Vitamin C <60 μmol/L (lower and middle tertiles) $n = 92$	Total Vitamin C ≥60 μmol/L (upper tertile) $n = 46$	*P* Value
	n (%)/Median (IQR[a])	n (%)/Median (IQR[a])	
Age, yr	60 (49 to 73)	63 (48 to 69)	0.7
Male	61 (66%)	29 (63%)	0.7
Body mass index, kg/m^2	24 (21.2 to 26.4)	24 (21 to 26.7)	0.8
Diabetes mellitus	21 (23%)	12 (26%)	0.7
Smoking, %	19 (21%)	11 (24%)	0.7
Noncompliance to prescribed multivitamin supplement	14 (15%)	6 (13%)	0.7
History of cardiovascular events (myocardial infarction, aortocoronary bypass grafting, stroke)	22 (24%)	10 (22%)	0.8
Mean blood pressure, mmHg	96 (87 to 104)	94 (84 to 103)	0.3
Duration of hemodialysis, mo	22 (11 to 38)	20 (11 to 38)	0.9
Mode of dialysis			0.9
Hemodialysis	23 (25%)	11 (24%)	
Hemodiafiltration	69 (75%)	35 (76%)	
Adequacy of dialysis, Kt/V[b]	1.39 (1.26 to 1.58)	1.42 (1.25 to 1.69)	0.7
Hemoglobin, g/L	122 (113 to 130)	121 (110 to 128)	0.3
Dose of rHuEPO,[c] IU[d]/kg per wk	199 (98 to 331)	151 (83 to 264)	0.1
Dose of iron sucrose, mg/kg per wk	0.8 (0.5 to 1.3)	0.7 (0.4 to 1.1)	0.3
Iron, μmol/L	8.6 (6.1 to 11.1)	9.7 (7.0 to 11.3)	0.3
Transferrin saturation, %	19 (14 to 24)	20 (15 to 26)	0.4
Ferritin, μg/L	291 (150 to 431)	360 (123 to 590)	0.3
C-reactive protein, mg/L	9.8 (5.5 to 16.6)	8.2 (5.5 to 13.5)	0.5
Malonyldialdehyde, μmol/L	1.3 (1.0 to 1.6)	1.3 (1.1 to 1.7)	0.3
Total cholesterol, mmol/L	4.4 (3.9 to 5.0)	4.6 (3.7 to 5.2)	0.5
HDL cholesterol	1.1 (0.9 to 1.5)	1.2 (1.0 to 1.5)	0.3
LDL cholesterol	2.5 (2.0 to 2.9)	2.3 (1.8 to 2.8)	0.4
Albumin, g/L	37.6 (35.8 to 39.2)	37.8 (35.9 to 40.0)	0.4
Parathyroid hormone, ng/L	126 (47 to 243)	182 (90 to 247)	0.1
Calcium, mmol/L	2.3 (2.1 to 2.4)	2.3 (2.2 to 2.4)	0.5
Phosphorous, mmol/L	1.7 (1.4 to 2.2)	1.8 (1.5 to 2.0)	0.5
Calcium × phosphorous, mmol/L	3.9 (2.9 to 4.9)	4.3 (3.4 to 4.9)	0.3

[a]IQR denotes interquartile range.
[b]Kt/V indicates the dialyzer clearance of urea multiplied by the duration of the dialysis treatment divided by the volume of distribution of urea in the body.
[c]rHuEPO recombinant Human erythropoietin.
[d]International units.

deficiency (19). A significant proportion of multivitamin-supplemented patients still exhibits plasma levels that are well below the reference range of healthy, nonsmoking, middle-aged European residents (20). Low vitamin C plasma levels are likely to contribute to the reported imbalance toward a more pro-oxidant state in ESRD patients. However, it is unknown whether vitamin C deficiency is associated with cardiovascular outcomes in maintenance hemodialysis patients. We therefore performed a prospective cohort study investigating the association between total vitamin C plasma level and major fatal and nonfatal cardiovascular events in this patient population.

Materials and Methods

Study Design, Study Population, and Outcome Measures

The study was designed as a prospective analysis including all consecutive maintenance hemodialysis patients at a tertiary care university hospital in January 2002. The study complied with the Declaration of Helsinki, and was approved by the local ethics committee.

Table 2. Cox proportional hazard models assessing the association between total vitamin C plasma levels (in tertiles) and occurrence of major adverse cardiovascular events (MACE, cardiovascular death, and major nonfatal cardiovascular events [myocardial infarction, aortocoronary bypass grafting, stroke, limb amputation because of peripheral vascular disease]), cardiovascular mortality, and all-cause mortality among 138 maintenance hemodialysis patients

	Hazard Ratio	95% Confidence Interval	*P* Value
Unadjusted model			
MACE			
Total vitamin C <32 μmol/L	3.81	1.41 to 10.33	0.009
Total vitamin C 32 to 60 μmol/L	2.82	1.00 to 7.90	0.049
Total vitamin C >60 μmol/L	1.0	—	—
Cardiovascular mortality			
Total vitamin C < 32 μmol/L	3.81	1.25 to 11.56	0.018
Total vitamin C 32 to 60 μmol/L	2.88	0.92 to 9.04	0.070
Total vitamin C > 60 μmol/L	1.0	—	—
All-cause mortality			
Total vitamin C < 32 μmol/L	1.97	0.91 to 4.29	0.086
Total vitamin C 32 to 60 μmol/L	1.49	0.65 to 3.30	0.35
Total vitamin C > 60 μmol/L	1.0	—	—
Adjusted model			
MACE			
Total vitamin C <32 μmol/L	3.90	1.42 to 10.67	0.008
Total vitamin C 32 to 60 μmol/L	3.03	1.03 to 8.92	0.044
Total vitamin C >60 μmol/L	1.0	—	—
Cardiovascular mortality			
Total vitamin C < 32 μmol/L	3.79	1.23 to 11.66	0.020
Total vitamin C 32 to 60 μmol/L	2.89	0.89 to 9.37	0.076
Total vitamin C > 60 μmol/L	1.0	—	—
All-cause mortality			
Total vitamin C < 32 μmol/L	1.84	0.84 to 4.05	0.13
Total vitamin C 32 to 60 μmol/L	1.51	0.67 to 3.49	0.33
Total vitamin C > 60 μmol/L	1.0	—	—

MACE indicates major adverse cardiovascular events comprising cardiovascular mortality and nonfatal cardiovascular events.

Written informed consent was obtained from each participant. Cardiovascular death and major nonfatal cardiovascular events (myocardial infarction, aortocoronary bypass grafting, stroke, limb amputation because of peripheral vascular disease served as the primary endpoint (major adverse cardiovascular events [MACE]). Cardiovascular mortality and all-cause mortality were secondary objectives. Patients were advised not to take any vitamin supplements on the day of the baseline visit (January 14 and 15, 2002). Baseline visit included the recording of each participant's medical history, the individual dry weight (the prescribed, postdialysis weight) and height for the calculation of body mass index (dry weight in kilograms divided by the squared height in meters), and blood sampling through dialysis access before commencement of dialysis. During 30 mo of follow-up, individual major nonfatal cardiovascular events and date and cause of death were prospectively registered.

Laboratory Evaluation

Standard methods were used for the estimation of routine parameters. We applied a validated method for the measurement of total vitamin C, as described (21). Blood was collected in chilled tubes containing lithium-heparin as an anticoagulant. Subsequently, the blood was transported in an ice bath to the laboratory and was centrifuged at 4°C. Total vitamin C was analyzed with commercial high-pressure liquid chromatography (HPLC) kits from Immundiagnostik (Bensheim, Germany). Because vitamin C is unstable in plasma, 200-μl samples were immediately stabilized by adding 200 μl of an acidic precipitating agent supplied with the kit. After high-speed centrifugation, samples were stored at −80°C until HPLC analysis within 3 wk. Interassay coefficients of variation amounted to 13.8% and 8.6% at 23.5 and 76.9 μmol/L, respectively.

Statistical Analyses

We present continuous data as the median and the interquartile range (IQR) (25th to the 75th percentile) and discrete data as counts and percentages. χ^2 tests and Mann-Whitney *U* tests were applied for univariate analyses. Mortality and event-free survival according to the baseline levels of total vitamin C were analyzed by the Kaplan-Meier method and compared by the log-rank test. Multivariate Cox proportional hazards models were used to assess the association between total vitamin C levels and event-free survival, adjusting for potential confounding factors. Variables that were imbalanced between patients with high and low total vitamin C levels (upper tertile *versus* the

remaining patients) indicated by $P < 0.2$, as well as established risk factors for poor cardiovascular outcome, were considered as potential confounders and included in the multivariate model. We calculated hazard ratios with 95% confidence intervals (CI) for the analysis of outcome. We tested for interactions between baseline variables, and the model fit was assessed using Cox and Snell residuals and scaled Schoenfeld residuals. Calculations were performed with Stata, release 8 (Stata, College Station, TX) and SPSS for Windows (version 10.0, SPSS Inc, Chicago, IL).

Results

Patients

We enrolled 138 patients in the study and we excluded none of these individuals from the analysis. All baseline and follow-up data were complete. Patients' characteristics have been described previously (21). Median age was 61 yr (IQR, 68 to 71) and 90 patients (65%) were male. Baseline total vitamin C level was 45 μmol/L (IQR, 24 to 75). Oral multivitamin supplementation containing 100 mg vitamin C, 0.16 mg folate, 0.03 mg biotin, 8 mg thiamine, 8 mg riboflavine, 10 mg pyridoxine, 50 mg nicotinamide, 10.9 mg calcium pantothenate (Dreisavit; GRY-Pharma, Kirchzarten, Germany) was routinely prescribed to all patients independently of the study; 112 (81%) subjects were compliant with this regimen as assessed by personal interviews in approximately 3-mo intervals. Two (1.5%) patients took a second pill of the supplement over a period of 8 and 26 mo, respectively. Four (2.9%) patients occasionally received intravenous multivitamin supplements containing 113 mg vitamin C, 3.1 mg thiamine, 4.9 mg riboflavine, 40 mg nicotinamide, 4.9 mg pyridoxine, 60 μg biotin, 0.4 mg folic acid, 5 μg cyanocobalamin (Soluvit-Neu; Fresenius Kabi AB, Stockholm, Sweden) in addition to their oral multivitamin. Five (3.6%) patients did not take the supplement during 4 to 26 mo. Fifteen (11%) patients did not take any multivitamin supplements. The median total vitamin C plasma level of compliant patients (46 μmol/L, mean 60, IQR 25 to 75, $n = 118$) did not differ from that of the noncompliant individuals (40 μmol/L, mean 51, IQR 18 to 76, $n = 20$, including the five partially compliant subjects; $P = 0.59$). Table 1 presents demographic, clinical, and laboratory data of patients in the two lower tertiles of total vitamin C (<32 μmol/L and 32 to 60 μmol/L) in comparison to patients in the upper tertile (>60 μmol/L). None of the factors listed was significantly associated with total vitamin C levels in univariate or multivariate linear regression analyses (vitamin C levels logarithmically transformed).

Association of Low Total Vitamin C Plasma Level with MACE

MACE occurred in 35 of 138 patients (25%) during the median follow-up period of 30 mo (range, 5 to 30 mo). Twelve nonfatal MACE were observed (two myocardial infarctions, two aortocoronary bypass graftings, three cerebrovascular accidents, and five limb amputations), and six of these patients died from a secondary cardiovascular event during follow-up. No patient was lost to follow-up.

Kaplan-Meier survival analysis revealed a significant association between total vitamin C levels (in tertiles) and cardiovascular outcome (Figure 1): Lower total vitamin C levels were associated with a higher rate of MACE ($P = 0.0059$). Being aware of several potential confounding factors, we then applied a multivariate model to adjust for baseline imbalances (age, sex, body mass index, smoking, C-reactive protein levels, mean BP, diabetes, and cholesterol level): Adjusted hazard ratios for the occurrence of MACE were 3.90 (95% CI: 1.42 to 10.67; $P = 0.008$) and 3.03 (95% CI: 1.03 to 8.92; $P = 0.044$) for patients in the lower (< 32 μmol/L) and middle (32 to 60 μmol/L) tertile of total vitamin C levels, compared with patients in the upper vitamin C tertile (>60 μmol/L), respectively (Table 2).

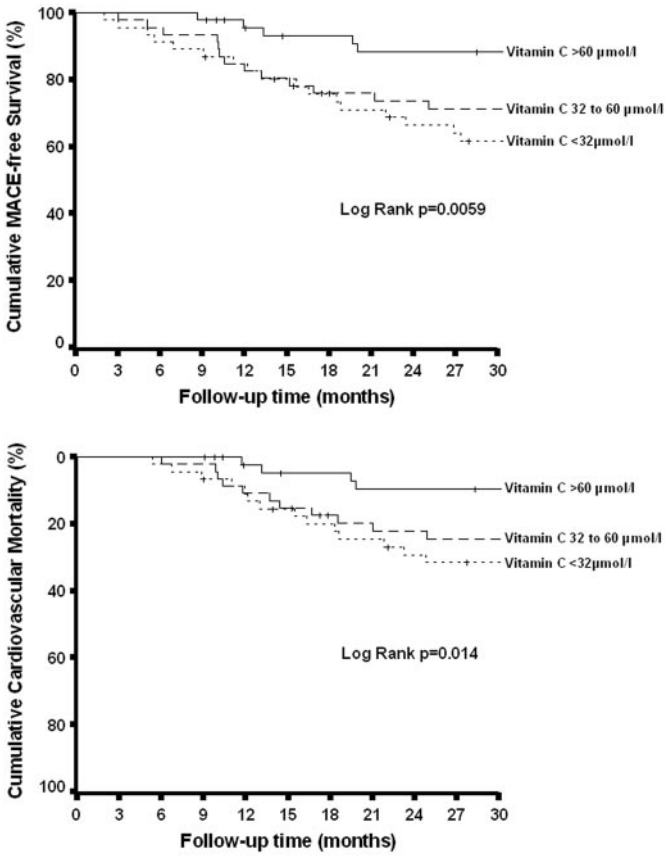

Figure 1. Cumulative major adverse cardiovascular events (MACE)-free survival and cumulative cardiovascular mortality of 138 maintenance hemodialysis patients stratified by tertiles of total vitamin C plasma level (<32, 32 to 60, >60 μmol/L).

Patients with a history of cardiovascular events (myocardial infarction, aortocoronary bypass grafting, stroke) showed a trend toward higher cardiovascular event rates during follow-up ($P = 0.058$). However, additional adjusting for this variable only marginally changed the effect sizes (adjusted hazard ratios for total vitamin C plasma levels in tertiles: 3.66 and 2.93 as compared with the upper tertile) without improvement of the model fit and without a significant interaction between total vitamin C levels, history of cardiovascular events, and cardiovascular outcome (log likelihood ratio test $P > 0.2$). We have therefore not included this variable into the final model. Although duration of hemodialysis was not significantly associated with vitamin C (Table 1), we included

duration of hemodialysis (quartiles) into the multivariate model and tested for a possible interaction between duration of hemodialysis, total vitamin C, and outcome: No significant effect modification, however, was observed and no statistical interaction between the three variables was found (log likelihood ratio test, $P > 0.05$). Therefore, duration of hemodialysis was likewise not included into the final model.

Similarly, we assessed the potential impact of compliance to vitamin C supplementation by including this variable into the fully adjusted model and testing for interaction; again, no significant change of the model fit was observed (log likelihood ratio test, $P > 0.05$) and the adjusted hazard ratios of the middle and lower tertiles of vitamin C with respect to MACE (2.84, 95% CI: 0.98 to 8.25; 4.33, 95% CI: 1.57 to 11.92), cardiovascular death (2.83, 95% CI: 0.88 to 9.12; 4.01, 95% CI: 1.32 to 12.70), and all-cause mortality (1.44, 95% CI: 0.62 to 3.32; 1.64, 95% CI: 0.73 to 3.69) also remained consistent.

Association of Low Total Vitamin C Plasma Level with Cardiovascular and All-Cause Mortality

Fatal cardiovascular events occurred in 29 of 138 (21%) patients (26 cardiac deaths, 3 cerebrovascular accidents) and 42 of 138 patients (30%) died of any cause. Kaplan-Meier survival analysis revealed a higher cardiovascular mortality ($P = 0.014$) with lower total vitamin C plasma levels (Figure 1). Similarly, patients with lower total vitamin C showed a trend toward higher all-cause mortality ($P = 0.080$) compared with patients in the upper tertile. Adjusted hazard ratios for cardiovascular death (Table 2) were 3.79 (95% CI: 1.23 to 11.66; $P = 0.020$) and 2.89 (95% CI: 0.89 to 9.37; $P = 0.076$). Total vitamin C levels were not independently associated with all-cause mortality in the multivariate model (Table 2).

Discussion

Main Study Outcome

We provide the first evidence to our knowledge that low total vitamin C plasma concentrations are associated with an increased risk for fatal and major nonfatal cardiovascular events in maintenance hemodialysis patients. A total vitamin C plasma level of <32 μmol/L conferred a 3.9-fold higher risk for a fatal or major nonfatal cardiovascular event in comparison to a total vitamin C plasma level >60 μmol/L. Intermediate total vitamin C plasma levels (32 to 60 μmol/L) resulted in a threefold higher risk. We observed similar trends for cardiovascular mortality.

Comparison with Available Evidence

Numerous studies have dwelled on the relation of antioxidants with cardiovascular outcome, but only a few have been focused on population cohorts with a notably higher risk for cardiovascular events, *i.e.*, ESRD patients or the elderly. An early, community-based, 20-yr follow-up British study of elderly subjects reported a significantly higher risk of stroke with a lower vitamin C intake or plasma level (22), but it failed to document any association of vitamin C status with coronary risk. Validity of this study is hampered by the uniformly low levels of vitamin C, the range of which might have been too narrow to detect any association with coronary risk. Correlation of plasma vitamin C with intake was only modest, and the performance of the spectrophotometric method for the measuring of vitamin C is inferior to current HPLC methods. Importantly, smoking, serum cholesterol, or body mass index had no impact on coronary outcome, very much like our findings and observations derived from the Framingham cohort (23,24). Another large-scale study of an elderly population cohort (25) reported a continuous decrease of the risk for all-cause and cardiovascular disease mortality with increasing vitamin C plasma concentrations. Of note, plasma α-tocopherol, β-carotene, and retinol had no impact on outcomes, and inclusion of the three antioxidants in the model did not cause any effect modification, nor did extensive adjustment change the effects on outcomes.

Interventional antioxidant trials of patients at the highest risk for development of premature atherosclerosis are rare. The multicenter, randomized, placebo-controlled Secondary Prevention with Antioxidants of Cardiovascular disease in End-stage renal disease (SPACE) study (26) included ESRD patients with apparent cardiovascular disease and found a significant reduction of the primary composite cardiovascular disease endpoint, and a decreased rate of myocardial infarction with 800 IU vitamin E per day. The study outcome, however, may have been affected by baseline imbalances: A higher rate of noncardiac mortality was observed in the treatment group, and data were *a priori* not controlled for the intake of other antioxidant vitamins. In particular, 100 to 500 mg vitamin C per day was prescribed to approximately 40% of study participants. Vitamin C may interact with vitamin E to keep the latter in a reduced state (27), but *post hoc* analysis of the trial data revealed no such interaction (28). Another randomized, placebo-controlled study on ESRD patients reported a reduction of a composite cardiovascular endpoint with oral acetylcysteine (600 mg twice daily) over a median follow-up of 14.5 mo (29). This trial and the SPACE study, however, did not provide any assessment of oxidative stress with regard to vitamin C. The randomized, placebo-controlled Harvard Intravascular Ultrasonography (IVUS) trial (30) found a twice-daily vitamin C (500 mg) plus E (400 IU) supplement to retard the early progression of cardiac transplant–associated coronary atherosclerosis, as assessed from direct measurements of intimal thickness. These findings suggest that oxidant-mediated processes support the obliteration of coronary arteries in subjects who are continuously exposed to increased oxidative stress. Antioxidant vitamin supplements might thus oppose the premature progression of vascular disease among ESRD patients, although no association between vitamin plasma concentrations and the progression of coronary atherosclerosis was noted in this study. Our study yielded a strong and independent association of plasma total vitamin C with combined fatal and major nonfatal adverse cardiovascular events. Similar trends were observed for all-cause and cardiovascular mortality.

Generalizability of Findings

Our study was not designed to target vitamin C as a causative agent for the progression of cardiovascular disease in

general or in renal failure patients, but it suggests that plasma total vitamin C constitutes a promising tool for future trials on the efficacy of antioxidants on cardiovascular outcomes. Plasma vitamin C levels of chronic renal failure and dialysis patients are lower when compared with healthy subjects (17,19), and associations with elevated amounts of various (per) oxidation products or deficiencies in antioxidants have been reported frequently (31–33). Evidence, however, for a causal link between a low vitamin C status and increments of oxidative stress parameters is limited. Eiselt *et al.* (34) found no change of erythrocyte glutathione and superoxide dismutase content or whole-blood glutathione peroxidase after 4 wk of intradialytic vitamin C supplementation to 20 maintenance hemodialysis patient (504 mg during each session, equal to a net amount of approximately 200 mg), although vitamin C plasma levels indicated vitamin C deficiency at baseline. The patients, however, dialyzed on a vitamin E–modified cellulose membrane, which *per se* resulted in a reduction of lipid peroxidation and higher plasma levels of reduced vitamin C as compared with baseline. Notably, vitamin C administered during dialysis with nonmodified cellulose membranes prevented increments of lipid peroxidation (34). A recent randomized, placebo-controlled trial on maintenance hemodialysis patients (35) reported a lower spontaneous and stimulated intracellular production of reactive oxygen species in concert with a reduction of oxidatively modified lymphocyte DNA after 8 wk of intravenous vitamin C supplementation (300 mg after each dialysis session). Although similar findings have been reported in a sample of chronic heart failure patients after long-term vitamin C therapy (36), validity of the aforementioned study results (35) is questionable because data were not analyzed by between group comparisons.

Vitamin C may not only scavenge excess free reactive oxygen and nitrogen species, but also may affect the bioavailability of nitric oxide either by increasing its synthesis or release from endogenous nitrosothiols or by protection from premature deactivation in conjunction with superoxide (37,38). In mice, vitamin C increased vascular levels of tetrahydrobiopterin, an essential cofactor of nitric oxide synthases, restored endothelial nitric oxide synthase activity, improved endothelial dysfunction, and reduced atherosclerotic lesions (39). Plasma levels of S-nitrosothiol were found to be increased in vitamin C–deficient maintenance hemodialysis patients (37), and it was hypothesized that the release of nitric oxide from nitrosothiols may be impaired in vitamin C deficiency (37,40). Importantly, high plasma levels of S-nitrosothiols independently predicted the occurrence of MACE (cardiac death, nonfatal myocardial infarction, and unstable angina requiring coronary revascularization) in a single-center cohort of 250 maintenance hemodialysis patients after a follow-up of 1 yr (40). Vitamin C plasma levels, however, were not reported, and no relationship was found between plasma levels of S-nitrosothiol and vitamin C in a small-scale, cross-sectional survey (37). A recent study on predialysis and maintenance hemodialysis patients found parenteral vitamin C to increase serum antioxidant activity and, simultaneously, to improve the endothelial, nitric oxide-dependent dilation of forearm resistance vessels (38). No effect of vitamin C was observed in healthy subjects, suggesting that vitamin C might ameliorate vascular endothelial dysfunction only in patients with known deficiencies in antioxidants. It is of note that the reported improvement of endothelial function was not associated with uniform changes of biochemical measures of oxidative stress: Whole-blood and plasma glutathione levels remained unaffected by vitamin C but lipid hydroperoxides increased, similar to observations in chronic heart failure patients (36). It is therefore conceivable that vitamin C conveys nonantioxidant-mediated effects on the endothelial cell as well.

Feasibility of considering plasma vitamin C levels as a valid marker of oxidative stress has been scrutinized because of the inconsistent results of general population cohort trials. It has been argued that plasma vitamin C may not adequately represent tissue levels, nor may it reflect intake of the relevant protective substances contained within fruits and vegetables. Nonfasting plasma vitamin C, however, correlates considerably better with fruit and vegetable intake than carotenoids or tocopherols (41). Oral intake and serum vitamin C levels correlated strongly in peritoneal dialysis patients (42), but the loss during hemodialysis might attenuate this relation (31). Our finding of a lower than normal total vitamin C plasma level suggests a correlation with a lower intake of vitamin C, carotinoids, and fibers in maintenance hemodialysis patients (43). In addition, the pro-oxidant burden of ESRD favors increased vitamin C consumption. Because cardiovascular outcome was found to be less worse in patients with a plasma level >60 μmol/L, it might be prudent to raise individual total vitamin C plasma levels above this cutoff, which lies close to the reference range of healthy subjects (20). Continuous supplementation, however, is restricted by the threat of secondary oxalosis. Future studies should assess cardiovascular outcomes of ESRD patients in relation to a safe vitamin C dosing scheme.

Strengths and Limitations

Although we adjusted our final model for a number of established risk factors for poor cardiovascular outcome, our study does not prove that low total vitamin C plasma levels are involved in the pathogenesis of cardiovascular damage. In particular, we performed a prospective, observational, single-center study on a relatively small number of cases; hence, our results might have been affected by a possible selection bias. Differences of dialysis vintage might have conferred a potential survival bias, but the inclusion of this variable into our model did not cause any significant effect modification. We further applied a conventional multivariate Cox proportional hazards model that used a baseline measure to predict prospective events. It is conceivable that time-varying changes of the individual vitamin C status (nutrition, infection, noncompliance with the prescribed multivitamin, or other) would exert a significantly different exposure–outcome constellation. It may be argued, however, that our patients were at a steady state with regard to vitamin C. The multivitamin supplement containing 100 mg vitamin C was continuously consumed by $>85\%$ of our patients. This amount largely outweighs the mean estimated weekly loss of approximately 200 mg by the dialysis procedure itself (31). Compliance, however, was not strictly confirmed,

nor did we estimate the oral intake of vitamin C. We sampled plasma vitamin C at baseline after a 1-day cessation of the multivitamin to preclude perturbations by supplementary intake. The impact of the latter on baseline plasma concentrations should be negligible at steady state. We did not observe any significant differences between baseline and 24-h follow-up total vitamin C plasma concentrations after the ingestion of a single dose of the multivitamin supplement by 10 study subjects (data not shown). Furthermore, baseline and 24-h follow-up vitamin C plasma concentration did not differ after a single oral dose of 200 mg of vitamin C to nonsupplemented maintenance hemodialysis patients (44).

It is, finally, a strength of our study that we did not include percutaneous vascular interventions in our analysis in order to avoid ambiguity, which might derive from the relative necessity for intervention. We therefore rather underestimated the cardiovascular event rate in comparison to other studies (29).

Conclusion

From a prospective study, we found a 3.9-fold increased risk for fatal and nonfatal major adverse cardiovascular events among maintenance hemodialysis patients with total vitamin C plasma levels <32 μmol/L, and a 3.0-fold increased risk for patients with total vitamin C levels <60 μmol/L. We observed similar trends for a higher cardiovascular and all-cause mortality. Future studies should address the feasibility of targeting total vitamin C plasma levels >60 μmol/L.

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