Original Communication

Plasma Vitamin C Concentrations in Patients on Routine Hemodialysis and its Relationship to Patients' Morbidity and Mortality

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Abstract: *Background:* Some studies have hypothesized the protective role of vitamin C against cardiovascular disorders (CVD) in patients with end-stage renal disease (ESRD). This study was designed to assess plasma vitamin C concentration and its relationship to hemodialysis (HD) patients' morbidity and mortality. *Methods:* Plasma vitamin C concentrations were assessed in HD patients using spectrophotometry and subjects were prospectively followed for up eighteen months for all-cause mortality. Any association between vitamin C concentration and patients' demographic data, co-morbidities, or the cause of ESRD were investigated using the Chi-square test. *Results:* Ninety-one patients with a mean age of 56.7 ± 15.7 years were included in this study. The most frequent cause of ESRD was simultaneous hypertension and diabetes in 30 % of patients, followed by hypertension in 25.6 %, and diabetes in 11.1 %, respectively. About 34 % of patients had CVD as the most prevalent co-morbidity. Forty-nine patients (53.8 %) had low levels of vitamin C concentration. There was a significant relationship between vitamin C insufficiency and presence of any co-morbidity in HD patients ($p < 0.05$). There was a significant difference in vitamin C concentrations between patients without co-morbidities and those with cardiovascular ones ($F[2,88]=3.447$, $p = 0.036$). Twenty-two (24.2 %) patients died over a median duration of 227 days. There was a significant difference in time to death of patients with and without low levels of vitamin C concentration ($p = 0.04$). *Conclusions:* The results showed lower plasma vitamin C levels in HD patients who suffered any co-morbidity and sooner time to death in these patients.

Key words: Cardiovascular disease, clinical pharmacy, hemodialysis, plasma vitamin C concentration

Introduction

In patients suffering from end-stage renal disease (ESRD), cardiovascular diseases (CVD) including coronary artery disease and hypertension, are the leading causes of morbidity and mortality [1,2]. Increased oxidative stress is one of the hypothesized causes of cardiovascular diseases in hemodialysis (HD) patients [3]. The vulnerability to oxidative stress in these patients is mediated by lower antioxidant defenses. Previous studies have reported several deficiencies in hydrophilic antioxidant molecules in the plasma of HD patients who had lower plasma concentrations of vitamin C [4]. Plasma vitamin C levels are typically reduced by about 30–50 % in HD patients due to loss during hemodialysis and also decreased intake [5]. Hemodialysis patients may restrict their consumption of fresh fruits and vegetables to avoid hyperkalemia that may lead to low plasma vitamin C levels [5]. Secondary hyperparathyroidism is another proposed cause of low vitamin C levels in HD patients [6]. Moreover, supplementation of vitamin C has resulted in a decrease of biochemical markers of oxidative damage in HD patients [7,8]. Recently it was reported that a low plasma level of vitamin C in hemodialysis patients is a risk factor for cardiovascular morbidity and mortality [9]. Vitamin C is speculated to play a protective role against human CVD. Overall, there has been some encouraging but not overwhelming support for vitamin C as a protector against CVD [10]. However, there are some safety concerns regarding administration of vitamin C such as potential secondary oxalosis [11], especially in HD patients with increased serum oxalate levels [12,13]. Additionally, oral low- to moderate-dose vitamin C supplementation is shown to increase lipid peroxidation in hemodialysis patients [14].

Given the potential beneficial outcomes associated with the administration of vitamin C in HD patients but because of the existing uncertainties pertaining to the role of vitamin C and its possible adverse reactions in these patients [11–15], this study was designed to evaluate vitamin C concentrations and its relationship to HD patients' demographic data, co-morbidities, and their all-cause mortality in eighteen months of follow-up.

Subjects and methods

During a prospective study, fasting plasma vitamin C concentrations were assessed in HD patients of Imam-Khomeini Hospital affiliated with the Tehran University of Medical Sciences. Ninety-one adult patients on hemodialysis for at least six weeks were included. The study was in compliance with the Declaration of Helsinki and all subjects provided informed consent; the study protocol was approved by a local Ethics Committee. All hemodialysis patients were dialyzed thrice weekly for four hours using conventional polysulfone dialyzer and bicarbonate buffer. The pre-dialysis blood samples were collected in heparinized tubes and were centrifuged immediately. The samples were transferred into four different vials and then stabilized with 60 g/L metaphosphoric acid (1:1 volume) before storage at –70 °C until analysis. All samples were analyzed within three months [16]. In this study, total serum vitamin C measurements were performed with a 2,4-dinitrophenylhydrazine (DNPH) reagent procedure [17]. To prepare this solution, 0.04 g thiourea was mixed with 0.005 g $CuSO_4.5H_2O$ and 0.3 g 2,4-dinitrophenylhydrazine and the mixture was made up to a volume of 10 mL with 9 N H_2SO_4. A volume of 400 µL samples was mixed with 80 µL DNPH solution and incubated for 3 hours at 37 °C. After 3 hours, 600 µL ice-cold 65 % H_2SO_4 was added, mixed well and the solutions were allowed to stand at room temperature for an additional 30 minutes. Absorbance was determined with a UV-visible spectrophotometer at 520 nm against a blank of distilled water [17]. For standard curve construction, the working vitamin C standard solutions (5–25 cm/mL) were used as samples and the absorption was determined. Then the standard curve was calculated between the absorptions and concentrations using linear regression analysis. All measurements were performed in triplicate [17] .The spectrophotometric method for vitamin C analysis was adapted from the well-known McCormick method [17]. This method was used and validated by some other reports [18–20] and its validity was comparable to the high-performance liquid chromatography (HPLC) method [21]. In this study a low level of vitamin C was defined as plasma vitamin C concentrations of less than 30 micromole/L (µM) [9]. Serum albumin concentrations in enrolled patients were measured using the Bromocresol Green test to evaluate patients' nutritional status, as the nutritional status may reflect a diet extremely deficient or even absent in protein but adequate in carbohydrate-derived calories [22], which is seen in malnourished Iranian hemodialysis patients.

The serum level of intact parathyroid hormone (iPTH) was quantified by the enzyme-linked immunosorbent assay (ELISA) method and its relationship with the concentration of vitamin C was evaluated with Pearson's correlation test. Any associations between

vitamin C concentration and patients' demographic data, co-morbidities, or the cause of ESRD were investigated using the Chi-square test. Cardiovascular co-morbidity was defined as the history of any previous cardiac events such as myocardial infarction or any positive findings on a patient's electrocardiogram or angiogram. Pearson's correlation test was used to assess any relationship between vitamin C concentration and time since dialysis program initiation. Survival according to vitamin C level was analyzed by Kaplan-Meier and compared by log-rank, Breslow, and Tarone-Ware tests. P values less than 0.05 were considered significant.

Results

From a total of 97 patients, six patients failed to fill the consent form. Ninety-one patients were included in this study, with the mean ± standard deviation age of 56.7 ± 15.7 (range 23–86) years; of these patients, 61 (67.4 %) were male. The length that patients were on hemodialysis was between 6 weeks to 22 years, with a median of 3 years. The most frequent cause of ESRD was simultaneous hypertension and diabetes in 27 (30 %) patients, followed by hypertension in 23 (25.6 %), diabetes in 10 (11.1 %), polycystic kidney disease in 9 (9.7 %), and glomerulonephritis in 6 (6.6 %) patients, respectively. Sixteen (17.6 %) patients were classified as ESRD with unknown origin. Table I depicts patients' co-morbidities, with CVD as the most prevalent at 34.4 %. The mean concentration of vitamin C in the included patients was 42.70 ± 39.63 (mean ±SD) μM. The results revealed low vitamin C levels in 49 (53.8 %) of patients. There was a significant negative relationship between plasma vitamin C level and the presence of any co-morbidity ($p < 0.05$). In comparing plasma vitamin C concentrations between patients without any underlying diseases, patients with cardiovascular co-morbidities, and those with co-morbidities other than cardiovascular ones, there was a significant difference in vitamin C concentrations between patients without co-morbidities and those with cardiovascular ones ($F[2,88]=3.447$, $p = 0.036$). The concentration of vitamin C was categorized into four levels: elevated, normal, deficient, and severely deficient. The patients' demographic data was also shown for each group in Table II.

Table I: Co-morbidities of patients enrolled in the study.

Type of co-morbidity	Frequency (percent)
Cardiovascular diseases	31 (34.4 %)
Hepatitis B	5 (5.5 %)
Hepatitis C	3 (3.3 %)
Smoker	3 (3.3 %)
HIV/AIDS	2 (2.2 %)
Cancer	1 (1.1 %)
None	46 (50.5 %)

HIV/AIDS: Human immunodeficiency virus/Acquired immune deficiency syndrome

Nineteen (20.4 %) patients had received vitamin C supplements with a mean daily dose of 167.1 ± 86.4 mg in their routine medications; however, there was no association between vitamin C consumption and the presence of a normal plasma vitamin C level ($\chi^2 = 0.76$, $p = 0.09$).

The results showed no correlation between plasma vitamin C concentration and length of dialysis program ($r=0.027$; $p = 0.80$), underlying disease leading to ESRD ($p = 0.07$), or sex ($p = 0.78$).

There was no relationship between the concentration of vitamin C and serum iPTH or albumin levels (p values = 0.26 and 0.88 respectively). Mean serum albumin and iPTH levels in included patients were 3.76 ± 0.4 g/dL and 660.51 ± 679.76 pg/mL, respectively.

In the evaluation of patients' outcome, 22 (24.2 %) patients died over a median of 227 days. The albumin level in this group of patients was 3.74 ± 0.37 g/dL (only two patients had albumin level of less than 3.5 g/dL). The median survival period for patients with low (12 patients) and normal (10 patients) plasma vitamin C levels was 85 and 264 days, respectively. Kaplan-Meier analysis revealed that low vitamin C level is a death predictor in an eighteen month follow-up period. By considering vitamin C plasma level of less than 30 μM as low, we found a significant correlation between vitamin C concentration and time to death. Time to death was significantly different between patients with low and normal levels of vitamin C ($p = 0.04$) (Figure 1).

Discussion

The mean value of plasma vitamin C concentration of our hemodialysis patients was 42.70 ± 39.63 μM, which is about half of the measured value of 81.93 ± 39.4 μM in healthy Iranian subjects (unpublished data, by Hajimahmoodi *et al.*). Using a cut-off point of plasma concentration of less than 30 μM to signify a low plasma vitamin C level [9], the results of our study showed that about 54 % of the patients on maintenance hemodialysis had low levels of vitamin C plas-

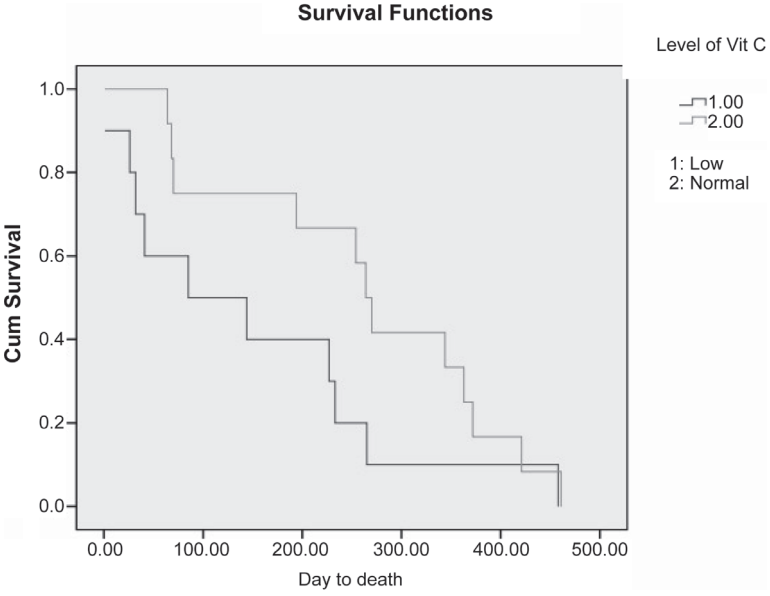

Figure 1: The survival curves of patients with low or normal concentration of vitamin C.

ma concentration. If we considered plasma vitamin C levels of less than 11.4 μM as vitamin C deficiency, as presented in the large epidemiologic study, the National Health and Nutrition Examination Survey (NHANES) [23], 14 patients (15.4 %) were vitamin C-deficient. This vitamin C deficiency or insufficiency could be explained by weekly vitamin C loss of several hundred milligrams during hemodialysis due to the vitamin's low molecular weight [24, 25]. Furthermore, increased vitamin oxidation catalyzed by iron, unusual oxidant production, and defective antioxidant mechanisms may result in a decrease in the reduced form of vitamin C in hemodialysis patients [26]. Decreased intake is another possible reason behind vitamin C deficiency, however, the results of albumin level measurements in our enrolled patients can be considered as evidence against malnutrition in the population included in the study. There is a high prevalence of hypovitaminosis C in acutely hospitalized patients, which may be associated with systemic inflammation [27]. However, we did not include critically ill patients in our study.

Although secondary hyperparathyroidism was hypothesized to have a relationship with low levels of vitamin C [6], the results of our study was not consistent with this finding. Nevertheless, there were 19 patients in our study who had received vitamin C supplementation that had not resulted in higher vitamin C plasma concentrations in these subjects. The possible reason may be an inadequate supplemented dose of vitamin C to correct vitamin C status. Systemic vitamin C supplementation has been advocated in maintenance hemodialysis patients, while no agreement exists on its proper dose. In a recent study, the majority of patients who received weekly supplemental vitamin C doses of 1500 mg showed serum vitamin C concentrations within reference limits. Their administered vitamin C dose was somewhat higher than that consumed by our patients [14].

Consistent with previous studies, we observed a strong relationship between the presence of co-morbidities, with the top ranked one being CVD, and the plasma level of vitamin C. Low vitamin C levels may be an independent factor in predicting adverse cardiovascular outcome in HD patients [8]. Moreover, in patients with low vitamin C concentration the episodes of cardiovascular events were higher compared to those with normal concentrations [28]. Viral infections were present in 11 % of our patients. Patients infected with hepatitis B or C were reported to have depleted hepatic vitamin C content and vitamin C deficiency, even in those with normal renal function [29, 30]. One of the other co-morbidities of our enrolled patients was being positive for human immunodeficiency virus (HIV). The HIV-infected population is known to be oxidatively stressed and scarce in antioxidant micronutrients, and this may explain the finding of a low vitamin C concentration in this group of patients [31]. Furthermore, it is speculated that by providing ESRD patients with nutritional supplements and antioxidant therapies such as vitamin C, overall inflammation may be decreased and HD outcomes improved [32]. This can provide potential treatment options to improve the high mortality and morbidity in ESRD patients [33]. Smoking also may play a significant role in plasma vitamin C reduction [34].

Table II: The comparison of patients' demographic data for each level of vitamin C concentration.

Concentration of Vitamin C	Number of patients (%)	Gender (Female %)	Age (mean±SD)	Time on dialysis (Years)	Cause of renal disease	Kt/V per session (mean±SD)	Co-morbidity
Elevated (> 80 uM)	10	21.4 %	53.38 ± 16.7	4	HTN; 3, DM; 1, HTN and DM; 2	2.29 ± 1.06	None; 9 HBV; 1
Normal (30–80 uM)	32	43.8 %	59.66 ± 15.51	3	HTN; 6, DM; 4, HTN & DM; 12	1.25 ± 0.72	None; 20 CVD; 10 HBV; 1 HCV; 1
Deficient (10–30 uM)	37	35.3 %	55.58 ± 15.8	3	HTN; 8, DM; 5, HTN & DM; 9	1.54 ± 0.89	None; 14, CVD; 14, Smoker; 2, HBV; 3, HCV; 1, HIV; 2, Cancer; 1
Severely deficient (< 10 uM)	12	0 %	57.9 ± 15.19	2	HTN; 6, HTN & DM; 3	0.87 ± 0.43	None; 3, HCV; 1, Smoker; 1, CVD; 7

Using a cut-off point of less than 30 μM as an indicator of low plasma vitamin C concentration in this study, we found a significant correlation between vitamin C concentration and time to death; however, if the range defined by NHANES was employed (less than 12 μM), no significant correlation can be seen. This could be attributed to the limited number of enrolled patients, or the fact that the ranges used for classification of vitamin C concentration as low or normal in studies are arbitrary and an exact normal range is not elucidated for this vitamin.

In conclusion, low vitamin C levels may be more common in hemodialysis patients with any co-morbidity, especially cardiovascular co-morbidities. Additionally, low plasma vitamin C concentrations in these patients were attributed to higher all-cause mortalities in an eighteen-month follow-up period. Further studies, including a more detailed analysis of patients' survival and possibly intervention with vitamin C administration in HD patients with cardiovascular co-morbidity, are needed to further elucidate the impact of vitamin C supplementation in this population.

Conflict of interest

This research has been supported by a grant from Nephrology Research Center, Tehran University of Medical Sciences.

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