

# Antioxidant vitamins status in children and young adults undergoing dialysis: A single center study

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## ABSTRACT

Vitamin E and C are well-known antioxidant vitamins. Oxidative stress is common in chronic kidney diseases. We evaluated 43 dialysis subjects prospectively in a cross-sectional survey. Serum vitamin E concentration was checked in all subjects; 37 cases underwent blood sampling for measurement of serum vitamin C. The enrolled subjects consisted of 12 (27.9%) peritoneal dialysis (PD) and 25 (58.1%) hemodialysis (HD) patients. Six (13.9%) patients were switched from PD to HD or vice versa. Serum concentration of vitamin E was normal, low and high in 9 (20.9%), 31 (72%) and 3 (7.1%) patients, respectively. There were no significant differences regarding age, gender, modality and duration of dialysis, and characteristics of dialysis sessions, mean serum blood urea nitrogen, and albumin levels between vitamin E deficient cases with those with normal serum vitamin E concentration ( $P > 0.05$  for all). The serum vitamin C levels were low in 5 (13.5%) and normal in 32 (86.5%) patients. Vitamin C deficiency was more prevalent in HD versus continuous ambulatory peritoneal dialysis patients ( $P = 0.128$ ). Mean serum vitamin C concentration was higher in patients who were supplemented by vitamin C compared with those who didn't receive the vitamin supplement ( $P = 0.043$ ). Vitamin E deficiency was a prevalent finding and supplementary vitamin C 30–60 mg/day was sufficient to prevent deficiency. Regular assessments of serum vitamin E level may be needed in dialysis centers.

**Key words:** Continuous ambulatory peritoneal dialysis, hemodialysis, Vitamin C, Vitamin E

## Introduction

Free radicals are present in human cells as normal consequences of energy metabolism. Documented studies have shown oxidative damage to DNA, proteins and lipids by free radicals.<sup>[1]</sup> Vitamin E and C are two well-known antioxidant vitamins. Vitamin E has the ability to scavenge lipid radicals and terminate oxidative chain reactions.<sup>[2]</sup> Ascorbate represents one of the most prominent plasma as well as intracellular antioxidants

with lipid peroxidation inhibitory effect by reducing endothelial dysfunction.<sup>[3]</sup> Ascorbic acid maintains copper and iron in a reduced state, and can act both as an antioxidant and a pro-oxidant.<sup>[4]</sup> Different *in vivo* and *in vitro* studies have performed to show anti-atherogenic effects of these antioxidants.<sup>[2]</sup>

Tissue hypoxia with oxidative stress is common in chronic kidney diseases (CKD). Hemodialysis (HD) however ameliorates hypoxia and lowers antioxidant vitamins levels.<sup>[5]</sup> Oxidative stresses during HD is attributable to peripheral blood mononuclear cells interaction with bio-incompatible dialysis devices, which results in increased synthesis and release of pro-inflammatory cytokines and imbalance between pro and antioxidant activities. These conditions may lead to complications, including cardiovascular disease, atherosclerosis, anemia and malnutrition.

New evidence suggests that dialysis patients have an impaired mitochondrial respiratory system, which may be the consequence or the cause of enhanced oxidative stress.<sup>[2]</sup> The aim of this study is defining antioxidant vitamins (Vitamin E and C) status in dialysis patients.

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## Materials and Methods

Forty-three dialysis patients, including 18 girls (41.9%) and 25 (58.1%) boys aged 19–300 ( $166 \pm 76$ ) months, who consented to participate in the study were enrolled. They included 12 (27.9%) patients who were placed on continuous ambulatory peritoneal dialysis (CAPD) and 25 (58.1%) HD subjects. Six (13.9%) patients during treatment were switched from peritoneal dialysis (PD) to HD or vice versa because of some complications related to dialysis modality (such as intractable peritonitis in PD cases, which needed catheter removal).

Serum vitamin E concentration was checked in all cases, and blood samples for measurement of serum vitamin C were obtained in 37 of 43 subjects. Blood samples for measuring serum vitamin E and C and routine monthly laboratory tests (serum blood urea nitrogen [BUN], creatinine, serum electrolytes, and complete blood count and serum albumin levels) were obtained at the time of the study. The serum BUN and hemoglobin (Hb) levels at the time of the study and serum levels in 1 and 2 months before the study, which were available in patient files, were used to calculate mean serum BUN and Hb levels. As serum albumin levels routinely were checked every 3 months in our subjects, the result of the last serum albumin levels (recent 3 months) were used in the study.

### Clinical characteristics

The duration from onset of dialysis was 1–128 ( $44.3 \pm 32.1$ ) months. Vitamin C supplement was recommended routinely before or immediately after placing on dialysis, but unfortunately about half of cases stopped the vitamin consumption or used it irregularly. Eighteen patients were supplemented by vitamin C 30–60 (51.2) mg/day, whereas 11 and 14 subjects stopped consumption or were not sure about regular consumption, respectively. No vitamin E supplementation was recommended routinely.

The types of dialysis machines were Fresenius 4008 (Germany), AK95 and AK96 (Swiss) and the types of dialyzer were low flux (R3-R5 and low flux polysulfone membranes). The blood flow rates were regulated on 100–300 cc/min. The duration of dialysis session routinely was three 4-hour sessions per week, but a group of patients with severe volume overload symptoms were placed on four dialysis sessions per week and in some patients 3 h of dialysis in each session were recommended instead of a standard (4 h in each session) dialysis duration.

In CAPD group, the number of dialysis cycles per day was 4–6 ( $4.82 \pm 0.52$ ), with dwelling time of 2–4 ( $3.58 \pm 0.71$ ) h and dwelling solution volume of

27–77 ( $39.65 \pm 16.14$ ) cc/kg. The study was funded by a research grant from Mashhad University of Medical Sciences. Written consents were obtained from patients or their parents and the study was approved by local Ethic Committee.

### Laboratory methods

Serum vitamin E and C levels were measured by HPLC.<sup>[6,7]</sup> For vitamin C values, 0.6–2 mg/dl was defined as normal, serum levels <0.6 mg/dl and >2 mg/dl were defined as low and high levels, respectively.

For vitamin E, the reference values of 3–9 µg/ml, 6–10 µg/ml and 5–18 µg/ml were defined normal values for children, teenagers and adult age groups, respectively. Values lower than 3 µg/ml, 6 µg/ml and 5 µg/ml in children, teenagers and adults were defined as vitamin E deficiency, respectively.

Blood samples in HD patients were obtained before dialysis sessions and during routine monthly sampling.

### Statistical analysis

Descriptive statistics included mean values  $\pm$  standard deviation (SD) for continuous and percentages for categorical data. Chi-square and independent *t*-tests were used for data analysis and  $P < 0.05$  was considered as significant statistical differences. Univariate analysis was performed using a model with vitamin E and C as the dependent variables, and age, gender, modality, and duration of dialysis and characteristics of dialysis sessions (number of cycles/day, dwelling time and volume in CAPD patients and hours of dialysis per week in HD cases), mean serum BUN and albumin levels (as important nutritional indexes) as independent variables.

## Results

Serum concentration of vitamin E was normal in 9 (20.9%), low in 31 (72%) and high in 3 (7.1%) patients. Serum levels ranged 0.6–20 ( $3.5 \pm 4.1$ ) µg/ml. Eight CAPD (25.8%), 21 HD (67.7%) and 2 (6.5%) patients who received both modalities had vitamin E deficiency. Serum vitamin E concentration was reported high in 1 HD and 2 cases that received both modalities [Table 1].

Comparing different independent variables in vitamin E deficient subjects with those who had normal serum vitamin E levels didn't show any significant statistical differences ( $P > 0.05$  for all) [Table 1].

Serum vitamin C levels were checked in 37 subjects and ranged 0.45–1.45 ( $0.92 \pm 0.27$ ) mg/dl. The serum

levels were low in 5 (13.5%) and normal in 32 (86.5%) patients. All cases with vitamin C deficiency were HD patients [Table 2]. Vitamin C deficiency was more prevalent in HD versus CAPD patients ( $P = 0.128$ ).

Mean serum vitamin C concentration in patients who were supplemented by vitamin C and those who didn't

receive vitamin C supplements were  $1.04 \pm 0.25$  and  $0.84 \pm 0.23$  mg/dl, respectively ( $P = 0.043$ ) [Table 2].

In PD group, 7 cases were supplemented by vitamin C regularly, four had stopped the vitamin consumption and 1 patient was not sure about regular consumption. Vitamin C and E deficiencies were reported

**Table 1: Mean±SD serum vitamin E concentration in different groups of patients**

Patients' group	n (%)	Mean±SD of variable	P value
Age (month)			
Normal serum vitamin E levels	9 (20.9)	137.33±84.7 (month)	0.206
Low serum vitamin E levels	31 (72)	175.2±75.7 (month)	
High serum vitamin E levels	3 (7.1)	168±101.8 (month)	
Male gender			
Normal serum vitamin E levels	5 (20)	-	0.897
Low serum vitamin E levels	18 (72)	-	
High serum vitamin E levels	2 (8)	-	
Serum concentration of vitamin E	25	1.78±0.42 (µg/ml)	
Female gender			
Normal serum vitamin E levels	4 (22.2)	-	-
Low serum vitamin E levels	13 (72.2)	-	
High serum vitamin E levels	1 (5.6)	-	
Serum concentration of vitamin E	18	1.76±0.43 (µg/ml)	
Hemodialysis patients			
Normal serum vitamin E levels	3 (12)	-	0.144
Low serum vitamin E levels	21 (84)	-	
High serum vitamin E levels	1 (4)	-	
Serum concentration of vitamin E	25	1.87±0.33 (µg/ml)	
CAPD patients			
Normal serum vitamin E levels	4 (33.3)	-	-
Low serum vitamin E levels	8 (66.7)	-	
High serum vitamin E levels	0	-	
Serum concentration of vitamin E	12	1.66±0.46 (µg/ml)	
Duration on dialysis (month)			
Normal serum vitamin E levels	9 (20.9)	42.2±28.7 (month)	0.927
Low serum vitamin E levels	31 (72)	43.3±32.5 (month)	
High serum vitamin E levels	3 (7.1)	96±0 (month)	
Dialysis cycle per day in CAPD patients			
Normal serum vitamin E levels	4 (33.3)	4.66±0.51	0.473
Low serum vitamin E levels	8 (66.7)	4.9±0.06	
High serum vitamin E levels	0	-	
Dwelling time in CAPD patients (h)			
Normal serum vitamin E levels	4 (33.3)	3.33±0.81 (h)	0.415
Low serum vitamin E levels	8 (66.7)	3.66±0.7 (h)	
High serum vitamin E levels	0	-	
Dwelling volume in CAPD patients (cc/kg)			
Normal serum vitamin E levels	4 (33.3)	41.5±19.2	0.893
Low serum vitamin E levels	8 (66.7)	40.18±16.5	
High serum vitamin E levels	0	30±0	
Dialysis hours per week in HD patients			
Normal serum vitamin E levels	3 (12)	11±2 (h/week)	0.748
Low serum vitamin E levels	21 (84)	10.43±3 (h/week)	
High serum vitamin E levels	1 (4)	9±0 (h/week)	
Serum BUN levels (mg/dl)	25		
Normal serum vitamin E levels	9 (20.9)	53.2±9.3 (mg/dl)	0.643
Low serum vitamin E levels	31 (72)	58.1±22.6 (mg/dl)	
High serum vitamin E levels	3 (7.1)	84±28	
Serum albumin levels (g/dl)			
Normal serum vitamin E levels	9 (20.9)	3.5±0.41 (g/dl)	0.483
Low serum vitamin E levels	31 (72)	3.38±0.38 (g/dl)	
High serum vitamin E levels	3 (7.1)	2.8±0.2	

SD: Standard deviation, CAPD: Continuous ambulatory peritoneal dialysis, HD: Hemodialysis, BUN: Blood urea nitrogen

**Table 2: Characteristics of patients with low versus those with normal serum vitamin C levels**

Patients' group	n (%)	Mean±SD of variable	P value
Age (month)			
Normal serum vitamin C levels	32 (86.5)	163.7±80	0.687
Low serum vitamin C levels	5 (13.5)	179.2±67	
Male gender			
Serum vitamin C concentration	22 (59.5)	0.92±0.26 (mg/dl)	0.881
Female gender			
Serum vitamin C concentration	15 (39.5)	0.93±0.3 (mg/dl)	
Hemodialysis patients			
Normal serum vitamin C levels	18 (78.2)	-	0.448
Low serum vitamin C levels	5 (21.8)	-	
Serum vitamin C concentration	23	0.87±0.28 (mg/dl)	
CAPD patients			
Normal serum vitamin C levels	9 (100)	-	
Low serum vitamin C levels	0 (0)	-	
Serum vitamin C concentration	9	0.96±0.24 (mg/dl)	
Duration were placed on dialysis			
Normal serum vitamin C levels	32 (86.5)	45±33.5 (month)	0.868
Low serum vitamin C levels	5 (13.5)	41.6±26.8 (month)	
Hour of dialysis per week in HD patients			
Normal serum vitamin C levels	18 (78.2)	10.6±3 (h/week)	0.588
Low serum vitamin C levels	5 (21.8)	9.8±2 (h/week)	
Serum BUN levels (mg/dl)			
Normal serum vitamin C levels	32 (86.5)	53±16 (mg/dl)	0.34
Low serum vitamin C levels	5 (21.8)	64.5±19 (mg/dl)	
Patients supplemented by vitamin C $\Omega$			
Normal serum vitamin C levels	18 (100)	-	0.043
Low serum vitamin C levels	0 (0)	-	
Mean±SD serum vitamin C concentration	18	1.04±0.25 (mg/dl)	
Patients stopped consumption of vitamin C supplements			
Normal serum vitamin C levels	9 (81.8)	-	
Low serum vitamin C levels	2 (19.2)	-	
Mean±SD serum vitamin C concentration	11	0.84±0.23 (mg/dl)	

CAPD: Continuous ambulatory peritoneal dialysis, HD: Hemodialysis, BUN: Blood urea nitrogen

in 2 and 13 girls, respectively and 3 and 18 boys had low serum vitamin C and E concentration, respectively. Frequency of vitamin C and E deficiencies was as common in boys as in girls [Tables 1 and 2]. Combined vitamin E and C deficiencies were detected in 3 of 37 (8.3%) patients. All cases with combined vitamin E and C deficiencies were HD patients! Figures 1 and 2 present the ranges of serum vitamin E and C concentration changes according to dialysis modalities. Mean serum Hb levels (in recent 2 months) in patients with vitamin C deficiency ( $9.6 \pm 2.96$  mg/dl) were not significantly lower than those with normal serum vitamin C levels ( $10.62 \pm 2.27$  mg/dl) ( $P = 0.55$ ).

Patients were followed for a 4-year period. During the follow-up, 11 patients (25.6%) died because of different complications of CKD. Of 31 patients with low serum vitamin E levels 8 cases died during follow-up, whereas in cases with normal serum vitamin E (9 patients) 3 died during follow-up ( $P = 0.175$ ). Ten of 37 cases that underwent serum vitamin C measurement died during follow-up; all of them had normal serum vitamin C levels. Five subjects with low serum vitamin C levels were alive at final follow-up! ( $P = 0.143$ ).

## Discussion

Vitamin status depends on nutritional habits and lifestyle. Serum vitamin C concentrations depend on gender, age, smoking status, seasons, dietary intake and location of residence.<sup>[8]</sup> Ascorbic acid is involved in the formation and repair of collagen, development of bones and teeth, amino acid metabolism, synthesis of hormones, wound healing, and absorption and utilization of iron.<sup>[9]</sup> Adequate intake of vitamin E defined as per age ranged from 4 mg in infants younger than 6 months to 15 mg after the age of 14 years.<sup>[10]</sup> To avoid hyperkalemia, limiting the intake of potassium-rich foods especially in HD patients is recommended. As the major portion of dietary vitamin C is provided by potassium-rich foods, vitamin C deficiency has complicated the management of dialysis patients.<sup>[11]</sup> In addition, vitamin C metabolism results in oxalate production. To prevent oxalate accumulation in renal failure, vitamin C with a dose of 60–100 mg/day<sup>[11]</sup> or lower doses (50 mg)<sup>[12]</sup> is recommended that may not be optimal.<sup>[11]</sup>

The mean recommended dietary allowances (RDAs) for vitamin C is 65 and 75 mg/day for girls and boys, respectively.<sup>[13]</sup> Data from the US Department of Agriculture

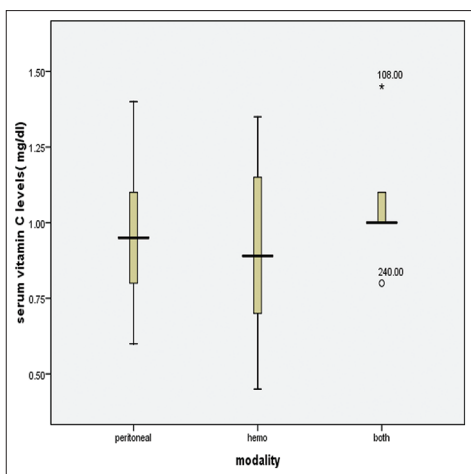


Figure 1: Serum vitamin C concentration changes according to dialysis modalities

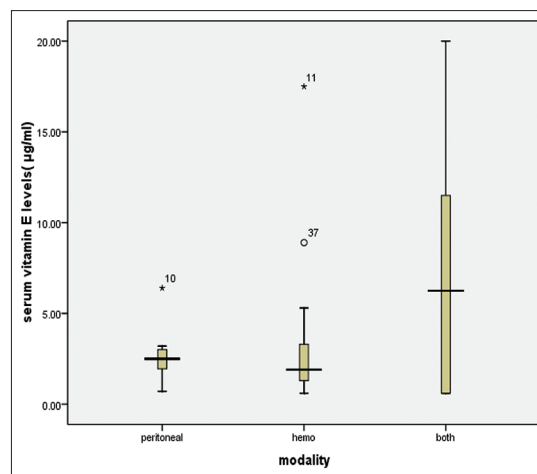


Figure 2: Serum vitamin E concentration changes according to dialysis modalities

indicate that daily intake of vitamin C in age group 13 to 18 years olds is <30 mg in 14% of males and 20% of females.<sup>[14]</sup> Recent reports suggest that a dietary intake between 60 and 100 mg/day is sufficient to maintain adequate plasma vitamin C levels in subjects with normal kidney function.<sup>[4]</sup> Studies by the Renal Research Institute revealed that 15% of HD patients have vitamin C plasma levels <10 µmol/L, which indicates severe vitamin C deficiency<sup>[15]</sup> and is associated with scurvy.<sup>[16]</sup>

Zhang *et al.* assessed a large group of dialysis patients and noted vitamin C deficiency in about 1/3 of their cases.<sup>[16]</sup> In contrast to our findings they found vitamin C deficiency as common in HD as PD cases. A national health and nutrition examination survey by Hampl *et al.*<sup>[17]</sup> reported vitamin C deficiency among 5–17% and depletion in 13–23% of respondents. Dialytic vitamin C clearance, restricted intake of vitamin C-rich foods and increased vitamin C catabolism due to inflammatory process accounted for vitamin C deficiency as a common finding in dialysis patients.<sup>[4]</sup>

Approximately 10–25% of dialysis patients have vitamin C deficiency.<sup>[18]</sup> Vitamin C depletion during HD is substantial with plasma levels typically decreasing by 50% or more after a single dialysis treatment; this represents a dialytic removal of 100–300 mg of vitamin C depending on plasma levels,<sup>[19,20]</sup> and Lower plasma levels of ascorbate in dialysis patients (who don't receive supplementary vitamin) compared to healthy controls have been reported mainly due to a loss into the dialysate or increased urinary losses, so vitamin C supplementation 60 mg/day are recommended for CKD patients.<sup>[3]</sup>

Very high levels of vitamin C can also occur in dialysis patients. The normal kidney clears vitamin C when the plasma level exceeds 60 millimole (mM).<sup>[21]</sup> The lack of

normal renal clearance mechanisms can result in very high plasma levels (>200 mM) in patients who take large amounts of vitamin C supplements.<sup>[22]</sup> Eighteen of our subjects were supplemented by 30–60 (51.2) mg/day vitamin C regularly. This range of the vitamin supplement resulted in normal serum concentration in all cases that were supplemented by vitamin C.

In contrast to our findings (no cases of vitamin C deficiency in PD patients), in a study by Lims *et al.*<sup>[23]</sup> although all of patients except one had total oral vitamin C intake above that of the US RDA (60 mg/day), 40% of CAPD patients had vitamin C deficiency. We found vitamin C deficiency in patients who either stopped the vitamin supplement consumption or their intakes were irregular. In contrast to our findings (no cases of vitamin C deficiency in PD patients), a wide range of plasma vitamin C levels, including both very low and high levels have been reported in dialysis patients.<sup>[11,24-27]</sup>

It's suggested that normal serum vitamin levels are not good markers of deficiency, particularly for retinol,<sup>[28]</sup> vitamin E,<sup>[29]</sup> and C.<sup>[30]</sup> Vitamin C concentration in leukocytes is more reliable than simple serum concentrations for assessing tissue concentrations.<sup>[31]</sup> Like our series, Zhang *et al.*<sup>[32]</sup> reported that vitamin C deficiency is more common in HD than in PD patients. Yang *et al.*<sup>[33]</sup> suggested that intravenous vitamin C plus a vitamin E-coated dialyzer is effective in palliating oxidative stresses in HD patients.

Vitamin E is a powerful lipophilic chain-breaking antioxidant that delays lipid peroxidation. New functions of this antioxidant have been shown in polymorphonuclear leukocytes.<sup>[34]</sup> One study in Tunisia showed that 5.4% of school children have vitamin E deficiency (serum levels <6.97 µmol/l).<sup>[35]</sup>

Gentile *et al.*<sup>[36]</sup> recommended avoiding fat-soluble vitamin supplements (A and E) in dialysis patients and believed that their dietary intakes should be limited. Stein *et al.*<sup>[37]</sup> suggested that there is no need for vitamin E supplementation in dialysis patients, while Galli *et al.*<sup>[38]</sup> found relative vitamin E deficiency (low vitamin E/triglyceride ratio) in dialysis patients. They believed that vitamin E supplementation may be an effective therapy to combat oxidative stress in HD patients. Despite elevated plasma levels of vitamin E in dialysis patients, intracellular and clinical signs of hypovitaminosis are frequently found.<sup>[39]</sup> Cristol *et al.*<sup>[40]</sup> found abnormally low red blood cell vitamin E concentrations in dialysis patients.

Majority of our CAPD (66.7%) and HD (84%) patients had low serum vitamin E concentration. Neither characteristics of dialysis modalities (number of cycles, dwelling time and volume) in CAPD cases and hours of dialysis per week in HD patients or gender had any impact on the frequency of vitamin E deficiency in our series [Table 1]. Nutritional indexes such as serum BUN and albumin levels that were affected by amounts of protein intake were similar in vitamin E deficient group and patients with normal serum vitamin E concentration. As serum  $\alpha$ -tocopherol is positively linked to the dietary intake, serum cholesterol and triglycerides,<sup>[8]</sup> it may be suggested that decreased fat intake is responsible for high incidence of vitamin E deficiency in our series. To determine the cause of vitamin E deficiency in our subjects, a study should be designed that focuses on the amounts of fat intake and the main dietary sources of vitamin E. Although it's reported that  $\alpha$ -tocopherol is higher in males, whereas serum vitamin C is higher in females.<sup>[8]</sup>

Plasma vitamin C is positively associated with higher Hb level.<sup>[41]</sup> In our cases, mean serum Hb levels in patients with vitamin C deficiency were not lower than those with normal serum vitamin C levels.

Overall we noted that although first investigation in dialysis patients commonly reported high serum vitamin E concentration, it seems that vitamin E deficiency is not uncommon in dialysis subjects. What are the reasons for this trend of vitamin E deficiency in dialysis patients? Whether changes in dietary habits play any role is not clear and needs more investigations.

Results of our study showed that although literature review commonly suggested high serum vitamin E concentration in dialysis patients, it seems that vitamin E deficiency may be a prevalent finding in some dialysis centers and because of well-known antioxidant effects of vitamin E, serial screening for vitamin E deficiency in

dialysis subjects should be considered especially in centers where vitamin E deficiency is a common finding. Overall, considering the cost-effectiveness of regular assessments of these vitamins, it needs to be determined whether measurement of vitamin E and C should be considered in patients on a regular dialysis? In addition, we found that supplementary vitamin C 30–60 mg/day is sufficient to prevent vitamin C deficiency in majority of patients and it also maintains the serum vitamin C concentration within normal ranges.

Higher prevalence of vitamin E and C deficiencies in HD compared to PD patients in our report is another positive point that adds to different advantages of PD modality. We didn't find any significant differences in mortality rates between cases with vitamin E or C deficiency and those with normal levels of these vitamins.

The limitations of our study were low patient numbers, lack of nutritional status assessments and its effects on serum concentrations of vitamin E and C that should be emphasized by further studies.

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