

Research Article

Antioxidants deficiency: a sensitive indicator of cardiometabolic risk in chronic renal failure?

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Received: 25 March 2013

Accepted: 9 April 2013

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ABSTRACT

Background: Antioxidant depletion occurring in chronic renal failure patients is an important cause of associated morbidity & mortality, which in turn imposes a great socioeconomic burden of health care. Early diagnosis & targeted management of this preventable deficiency may have a positive impact on the management of co morbidities associated with chronic renal failure.

Aims & Objectives: To evaluate the status of antioxidants as an early indicator of cardiometabolic risk in chronic renal failure patients.

Settings & Design: This was a randomised case Control study including 10 controls of either sex with normal renal function between age group 20-60 years and 15 patients of chronic renal failure on dialysis between the age group of 16 - 60 years.

Methods: 12 hour fasting venous blood samples were collected from all the participants and were assayed for various antioxidants.

Statistical analysis: Results were analyzed by unpaired t test, p value was determined & Correlation coefficient was calculated amongst various parameters.

Results: In the present study, significantly low levels of vitamin C (Cases: 0.367 ± 0.13 mg/dl controls: 1.324 ± 0.61 mg/dl; $p < 0.01$) & vitamin E (cases: 0.235 ± 0.102 mg/dl, controls (0.854 ± 0.28 mg/dl; $p < 0.01$) were observed in chronic renal failure patients as compared to controls.

Conclusion: Diminished levels of Vitamin C & E in our study may be an indicator of increased oxidative stress which can be a responsible factor for increased incidence of cardiovascular complications. Supplementing these patients with recommended dosage of these vitamins may provide an essential tool to reduce the burden of suffering.

Keywords: Chronic renal failure (CRF), Antioxidants, Vitamin C, Vitamin E

INTRODUCTION

Chronic kidney disease (CKD) is a gradual and progressive loss of the ability of the kidneys to function normally over a period of months or years.¹

Vitamins have a variety of biochemical functions. Some vitamins play a crucial role in mineral metabolism

(vitamin D), some act as antioxidants (vitamins C and E) while some form cofactors of enzymes in various metabolic activities of the body (vitamins of the B - complex).²⁻⁶

The vitamin C acts as an antioxidant and helps to fight against the oxidative stress. It is also required in the synthesis of collagen, carnitine and neurotransmitters,

synthesis and catabolism of amino acid tyrosine and the metabolism of microsomes. Researchers in the Linus Pauling Institute at Oregon State University discovered that vitamin C can react with and neutralize the toxic byproducts of human fat metabolism.⁷ Vitamin C is also known to help in the prevention of cancer, heart disease and inflammatory conditions like arthritis. Dr Linus Pauling, often referred to as the "Father of Vitamin C" proposed that vitamin C aids anti-cancer activity within the body. A team of Johns Hopkins scientists have shown that vitamin C and potentially other antioxidants - can inhibit the growth of some tumors in mice.⁷ Researchers have also found that vitamin C supplements can boost the immune system and help in the prevention and treatment of the common cold.

Vitamin E is a fat soluble vitamin which acts as an antioxidant. Vitamin E is a collective term for a set of 8 related α -, β -, γ -, and δ -tocopherols and the corresponding four tocotrienols of which α -tocopherol has the highest biological activity.⁸ The major sources of vitamin E in the diet include vegetable oils (sunflower, safflower, olive, cottonseed oils), green leafy vegetables, nuts such as almonds and hazel nuts, cereals, meats, egg yolks, wheat germ, and whole wheat products.

In 1922, Herbert M. Evans and Katherine S. Bishop discovered vitamin E in green leafy vegetables. They performed fertility experiments on the rats in which they fed the rats with a purified diet consisting of casein 18%, corn starch 54%, lard 15%, butter fat 9%, and salts 4%, adequate vitamin A (as cod liver oil), vitamin B (as yeast), vitamin C (as orange juice) but found that the rats could not reproduce. The fertility was restored by the feeding of lettuce leaves. Therefore they concluded that 'natural foods, as opposed to purified diets contained a substance not needed for normal growth, but essential for reproduction'.⁹ In 1924, B. Sure¹⁰ called the substance 'Vitamin E' since vitamins A, B, C and D were already known. Since the activity of the vitamin was first identified as a dietary fertility factor in rats, it was named as 'tocopherol' coming from the Greek words *toco* meaning child, *phero* meaning to bring forth and the ending *ol* to indicate the alcoholic properties of the molecule. Henry. A. Mattill has been attributed the discovery of the antioxidant property of vitamin E.¹¹

The human body has developed an antioxidant defense system to counter the free radical activity which can cause extensive damage to the body tissues. These antioxidants involve a number of enzymes like superoxide dismutase, catalase and glutathione peroxidase. Sometimes the endogenous antioxidants are not sufficient to act against the free radical damage. Therefore the body requires certain exogenous antioxidants provided by the diet. These include micronutrients like vitamin C, vitamin E, vitamin A, selenium, zinc, flavonoids and coenzyme Q10. Vitamin C and vitamin E are known to be the most abundant of the micronutrient antioxidants in the body.

Vitamin C, being a water soluble vitamin, exerts its antioxidant activity in the aqueous compartments of the body. It is capable of scavenging a variety of reactive oxygen species like hydroxyl, alkoxy, peroxy as well as reactive nitrogen species such as nitrogen dioxide and nitroxide. In addition, it can also regenerate other antioxidants such as α -tocopheroxyl, urate and β -carotene radical cation from their radical species. Thus vitamin C can be said to act as a co - antioxidant along with vitamin E.

The primary role of vitamin E is believed to be that of an antioxidant. The α - tocopherol form is known to be the most potent antioxidant that prevents the peroxidation of the polyunsaturated fatty acids in the cell membrane and protects the membrane against oxidative damage. In the process it gets converted to α - tocopheryl radical which is recycled back to α - tocopherol by ascorbic acid.

Thus vitamin E and vitamin C prevent oxidative damage by countering the free radical activity and provide protection against cardiovascular complications, cataract and diseases like Alzheimer's disease, Parkinson's disease, rheumatoid arthritis and even cancer. The Cancer Institute recommends at least 5 servings of fruits and vegetables per day for efficient protection against the free radical damage.^{2,6}

METHODS

10 controls between age group 20-60 years and 15 CRF patients between age group of 16-60 years were included in the study. CRF patients having serum creatinine level of more than 3 mg/dl and not taking any were included in this study. Patients who were positive for HIV, HBsAg, HCV and patients on vitamin supplementations were excluded from the study.

The Vitamin C levels were estimated by colorimetric 2, 6- Dichlorophenol Indophenol (DCPIP)¹² method & Vitamin E levels were estimated by Baker and Frank method.¹³ The BUN & serum creatinine values were obtained from Medicine Laboratory for controls and from patient files in department of Nephrology for patient. Regarding clinical investigation, creatinine level >3 mg/dl was considered as CRF patient. Data analysis was performed by unpaired t-test and correlations between variables were studied by using Pearson's correlation coefficient test. The level of significance was set at $p < .001$.

RESULTS

Table 1 shows a significantly ($p < 0.0005$) elevated blood urea nitrogen (BUN) levels in the CRF patients (27.6 ± 9.49 mg/dl) as compared to the healthy controls (10.9 ± 2.5 mg/dl). We also observed significantly ($p < 0.0005$) increased levels of serum creatinine in the chronic renal failure patients (6.88 ± 1.98 mg/dl) as compared to the healthy individuals (0.91 ± 0.26 mg/dl).

Table 1: Serum creatinine and blood urea nitrogen in CRF patients.

	Serum creatinine		Blood urea nitrogen	
	Control	CRF	Control	CRF
No. of cases	10	15	10	15
Range (mg/dl)	0.5-1.3	4.3-10.6	8-15	17-53
Mean (mg/dl)	0.91	6.88	10.9	27.6
S.D.	± 0.26	±1.98	±2.5	±9.49
S.E.	±0.08	±0.51	±0.79	±2.45
t-test	-	9.42	-	5.4
p	-	<0.0005	-	<0.0005

Table 2: Vitamin C and Vitamin E levels in CRF patients.

	Vitamin C		Vitamin E	
	Control	CRF	Control	CRF
No. of cases	10	15	10	15
Range(mg/dl)	0.53-2.23	0.2-0.588	0.523-1.493	0.127-0.485
Mean(mg/dl)	1.324	0.367	0.854	0.235
S.D.	± 0.61	±0.13	±0.28	±0.102
S.E.	±0.19	±0.034	±0.09	±0.026
t-test	-	6.02	-	7.94
p	-	<0.001	-	<0.001

Table 2 shows significantly ($p < 0.001$) low levels of vitamin C (0.367 ± 0.13 mg/dl) in the patients of CRF as compared to the healthy controls with normal renal function (1.324 ± 0.61 mg/dl). We observed significantly

($p < 0.001$) low level of vitamin E in the chronic renal failure patients (0.235 ± 0.102 mg/dl) as compared to the healthy individuals with normal renal function (0.854 ± 0.28 mg/dl).

Table 3: Correlation of Vitamin E & Vitamin C with serum creatinine.

	Vitamin C		Vitamin E	
	Control	Patients	Control	Patients
Correlation between vitamins and serum creatinine	- 0.1289	- 0.13	- 0.385	- 0.519
Correlation coefficient probability	Not significant	Not significant	Not significant	< 0.05

In the present study, we also found a significant ($p < 0.05$) negative correlation between vitamin E levels and serum creatinine in the chronic renal failure patients ($r = -0.519$) (Table 3). In contrast, no such correlation was observed in the healthy controls. Further, we also did not observe any correlation between vitamin C levels and serum creatinine in both the controls as well as the patients.

DISCUSSION

Chronic renal failure has been associated with an increased prevalence of malnutrition. Although gastrointestinal abnormalities may be responsible for this condition, more than often a reduced oral intake has been implicated as an important contributory factor. The dietary restrictions prescribed to the patients are believed to be responsible for the deficient levels of certain nutrients observed in these patients. Deficiencies of micronutrients like trace elements and vitamins have been observed in chronic renal failure. The factors

thought to be responsible are imposed dietary restrictions, uremic toxins, the drug – nutrient interactions and in case of ESRD (End Stage Renal Disease), the procedure of dialysis. A deficient level of water soluble vitamins has been observed in uremic patients, especially those on dialysis.¹⁴ The levels of lipid soluble vitamins have been found to be variable in the patients of chronic renal failure.

The levels of vitamins C and vitamin E have been widely studied in chronic renal failure, primarily due to their significance as micronutrient antioxidants in the body. The α – tocopherol form of vitamin E is believed to prevent oxidative damage to polyunsaturated fatty acids in the cell membranes from the reactive oxygen species and in the process gets converted to tocopheryl radical. The ascorbic acid then helps to recover the native form of α – tocopherol from its reduced form.

Sullivan et al (1972) found deficient plasma levels of vitamin C in the patients on maintenance hemodialysis. They proposed that an addition of the vitamin in the dialysate concentrate could prevent the loss of the ascorbic acid.¹⁵ Reports indicate that unsupplemented dialysis patients show a decreased plasma ascorbate level as compared to the healthy controls, mostly due to loss in the dialysate. In case of patients who are not on dialysis, the reduced values are attributed to the urinary losses.¹⁶

VA DeBari et al (1984) also observed that the levels of ascorbate were significantly reduced in the hemodialysis patients.^{15,17} Marion Morena et al reported that patients who were subjected to hemodiafiltration session with highly permeable membranes showed a decreased vitamin C level.¹⁸ Robert Deicher, Walter H. Hörl et al (2005) reported low plasma vitamin C levels in the patients on hemodialysis and observed that the reduced vitamin levels were responsible for the prevalence of cardiovascular disease among the patients.

However, Iain S. Henderson et al (1984) have reported a high level of vitamin C in the chronic renal failure patients. They have attributed this increase to the fact that none of the patients included in the study were on a potassium restricted diet, which is thought to be one of the reasons for the deficient intake of vitamin C in the CRF patients. Also, the patients were supplemented daily with 100 mg of ascorbic acid to replace any loss that may occur in dialysis.¹⁹ Hidetsugu Nakayama et al (2001) did not observe any significant difference in the plasma ascorbate levels in the CRF patients and the healthy controls.²⁰ These studies indicate that not all uremic patients exhibit a deficient vitamin C level.

The reduced values in our study can be attributed to the process of dialysis. The patients included in the study were receiving dialysis thrice a week. They were on dialysis for the past two years on an average. The hydrophilic nature of vitamin C may cause its increased loss in the dialysate. None of the patients included in the

present study were on any vitamin supplementation. The patients of CRF, especially those on dialysis, are recommended certain dietary restrictions particularly pertaining to potassium rich diets since the dialysis procedure cannot effectively eliminate potassium which may lead to hyperkalemia. Most of the fruits and vegetables rich in potassium are also vitamin C rich. Therefore, a reduced intake of these foods could lead to a low vitamin C intake in the patients. Another possible reason for the ascorbate deficiency could be the decreased levels of reduced glutathione. It has been suggested that the recycling of the ascorbate from dehydroascorbate, which is the oxidized form of ascorbic acid, is largely dependent on reduced glutathione.¹⁸ Current recommendations for maintenance hemodialysis (MHD) patients advise supplementation with ascorbic acid 75-90 mg daily to replace the losses of this water-soluble vitamin that occur during dialysis.²¹

The levels of vitamin E in the chronic renal failure patients have been reported to be low, normal or high. R B Bhogade et al (2008) observed significant decreased levels of vitamin E in the chronic hemodialysis patients before as well as after the process of dialysis. They proposed that vitamin E supplementations could increase the levels of vitamin E as well as vitamin C.²² Ioannis Karamouzis et al (2008) and Mehri Kadkhodaei et al (2008) observed lower values of plasma vitamin E in CRF patients as compared to controls.^{23,24} Francesco Galli et al also observed decreased vitamin E levels in patients of chronic renal failure patients on hemodialysis.²⁵

In contrast, some studies have indicated normal or higher values of vitamin E in the chronic renal failure patients. Iain S. Henderson et al (1984) have reported increased values of vitamin E in the patients and have suggested that the elevated levels may be due to relatively good diets.¹⁹ Stein G et al (1983) and Drukker. A et al (1988) observed higher than recommended serum vitamin E levels in the CRF patients.^{26,27} while Himmelfarb J et al (2003) and Fellah H et al (2006) did not observe any significant changes in the serum vitamin E levels as compared to the control group.^{28,29}

The diminished levels in our study may be indicative of an increased oxidative stress. Chronic renal failure patients are known to be under severe oxidative stress due to increased free radical activity and it is said to be more prevalent in the patients on dialysis therapy. The dialyzer membrane may have a low biocompatibility which can lead to neutrophil activation causing formation of reactive oxygen species. The lack of ultrapure dialysis water may also be one of the causative agents for the increased free radical activity.

Vitamin E is the most potent antioxidant vitamin in the body which prevents lipid peroxidation. Due to an increased level of free radicals in the chronic renal failure, there is an overconsumption of the vitamin E in

countering the oxidative damage. The resultant tocopheryl radical formed is converted back to the reduced α - tocopherol by vitamin C. Therefore, the increased oxidative stress has a two – fold effect: the more and more conversion of vitamin E to vitamin E radical and the subsequent reconversion of oxidized vitamin E to the reduced form by vitamin C. thus a deficiency of both the vitamins is observed. It has been reported that use of vitamin E coated dialyzer's can help in the prevention of the oxidative stress.

An increased serum creatinine level is the first indicator of an impaired renal function. People having an increased muscle mass may show slightly elevated creatinine levels. Creatinine levels also differ with age and sex with slightly higher values in the elderly and males as compared to the younger population and females. However, in case of chronic renal failure, a gradual and significant increase is observed over a period of time. In the initial stages, there may not be any marked increase in the serum creatinine levels. But as the GFR declines and reaches below 10 mL/min/1.73 m², their levels increase rapidly and lead to systemic manifestations.² Therefore, even mild elevations of serum creatinine persisting for a longer time are worthy of evaluation for possible decrease in kidney function.

A high value of BUN, most often, indicates a less than normal kidney function. BUN levels may also increase due to other factors like heart failure, dehydration or a diet rich in protein. Bleeding in the intestines and certain medications may also make the BUN higher than normal. However a marked increase in BUN along with an increased creatinine level in the serum may be indicative of renal failure. The patients included in our study showed significantly elevated levels of BUN as well as serum creatinine indicating a greatly impaired renal function.

In summary, our results showed a significant decrease in the levels of vitamin C as well as vitamin E in the chronic renal failure patients as opposed to the healthy individuals with normal renal function. Vitamin E and vitamin C being the most abundant micronutrient antioxidants in the body which provide protection against free radical damage, their decreased levels could be indicative of oxidative stress. An increased oxidative stress can lead to increased incidences of cardiovascular complications. Hence, it is important to supplement these patients with the recommended dosage of the vitamins.

CONCLUSION

Chronic renal failure is fast becoming a major public health threat worldwide. It is a serious condition which is associated with decrease quality of life and pre - matured mortality. The incidence of CRF has doubled in the last 15 years. Over 1 million people worldwide are alive on dialysis or with a functioning graft.³⁰ Based on the worldwide estimates, it is suggested that there are ~ 7.85

million CRF patients among the one billion population in India.³¹

Dietary restrictions are often suggested to the patients of chronic renal failure, particularly those pertaining to protein, sodium and potassium intake. The reduced oral intake could lead to a deficiency of the important nutrients required by the body like the vitamins. Our study indicated that there are significant deficient levels of vitamin C and vitamin E in the CRF patients. Besides the dietary restrictions, the process of dialysis is also known to impose additional strain on the levels of the vitamins.

Vitamins C and vitamin E are important antioxidants in the body that help in the prevention of lipid peroxidation especially related to LDL. A deficiency in their levels could be an important contributing factor towards the increased levels of oxidative stress observed in the patients. Oxidative stress has been implicated in the high prevalence of cardiovascular morbidity and mortality in case of CRF. Also a deficiency of vitamin C could interfere with the absorption of dietary iron and could be responsible for the symptoms of scurvy observed in the patients like bleeding gums. Therefore it is important to supplement the patients with these vitamins.

However an increased dosage of the vitamins can have side effects. A mega dosage of vitamin C is known to cause oxalosis characterized by deposition of calcium oxalate crystals in the joints, myocardial tissues, retina and also in renal tubules. The oxalosis is known to play an important role in the progression of the renal failure. An increased dosage of vitamin E can led to headaches, tiredness, double vision, and diarrhea. Hence it is important to evaluate the levels of the vitamins in the patients of chronic renal failure and to supplement them with a carefully designed dosage of the vitamins.

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DOI: 10.5455/2320-6012.ijrms20130510

Cite this article as: Rai PSK, Kamath S, Bakshi AA, Deokar SA, Rai AB. Antioxidants deficiency: a sensitive indicator of cardiometabolic risk in chronic renal failure? *Int J Res Med Sci* 2013;1:87-92.