Original Research Article

Correlation of plasma vitamin C levels with serum high sensitivity C – reactive protein and serum albumin levels in chronic kidney disease patients

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Abstract

Introduction: Subclinical inflammation is a common phenomenon in CKD patients. This inflammatory process is more seen in patients undergoing either continuous ambulatory peritoneal dialysis (CAPD) or maintenance hemodialysis (MHD). These inflammatory processes itself produce various inflammatory cytokines, which are responsible for, low levels of Hb, increased risk of atherosclerosis, and bone mineral diseases.

Aim of the study: To correlate plasma vitamin C levels with serum hsCRP and serum albumin levels in patients with chronic kidney disease stage.

Materials and methods: This single centered cross-sectional study was conducted in Medicine and Nephrology Departments at, VMMC and Safdarjung Hospital, New Delhi on 110 adult patients with CKD stage 5. 8 ml of venous blood was collected in a heparinized vial or syringe and the same amount was taken in the plain vial for serum separation for estimation of vitamin C, serum albumin levels, and serum hsCRP.

Results: The mean estimated GFR among the study population were cases (6.1±1.2 ml/kg/1.73m^2) and among controls were (126.42±11.59 ml/kg/1.73m^2) with a highly significant p-value. Among the control group, 11.5% were among insufficiency groups. Majority of the controls (88.5%) were having normal levels of plasma vitamin C levels. None of the control population was vitamin C deficient. A negative correlation was seen between plasma vitamin C and serum hsCRP in the cases group (-

0.634). There was a significant correlation observed between plasma vitamin C and serum hsCRP in the case group with a p-value of < 0.001. A negative correlation was seen between plasma vitamin C and serum PTH (-0.579), serum phosphate (-0.099) in the case group. A positive correlation was seen between plasma vitamin C and serum calcium (0.069) in the case group.

Conclusion: Plasma vitamin C level is positively associated with serum prealbumin levels and is negatively associated with the CRP level in both groups. Vitamin C deficiency may play an important role in the increased inflammatory status in dialysis patients. Further studies are needed to determine whether inflammatory status in dialysis patients can be improved by using vitamin C supplements.

Key words

Chronic kidney disease, Calcium sensing receptor in the parathyroid gland, Highly sensitive C-reactive protein, Parathyroid hormone.

Introduction

Chronic kidney disease (CKD) is a global threat to health in general and for developing countries in particular, because therapy is expensive and life-long [1]. Untreated CKD can result in end-stage renal disease and necessitate dialysis or kidney transplantation. Risk factors for CKD include cardiovascular disease, diabetes, hypertension, and obesity. Chronic kidney disease serves as a multiplier of risk in all populations [2, 3]. Risk factors such as obesity, diabetes, and hypertension are growing and superimposed on environmental and genetic influences that may serve to amplify existing incidence rates of disease over time [4]. It is seen that more than 10% of people, or more than 20 million, aged 20 years or older in the United States have CKD. CKD is more common among women than men. More than 35% of people aged 20 years or older with diabetes have CKD. More than 20% of people aged 20 years or older with hypertension have CKD [4]. In India's 1 billion populations, there are approximately 7.85 million CRF patients. Etiologically, diabetes (30-40%), hypertension (14-22%), chronic glomerular nephritis (16-20%), chronic interstitial disease (5.4-12.7%), hereditary/ familial disease (8.4%), obstruction including calculus (2.9%) constitutes the cause of CKD. Over 1 million people worldwide are alive on dialysis or with a functioning graft [8]. In India, approximately 90% of patients cannot afford the cost [5]. Increasing evidence, accrued in the past decades, indicates that the adverse outcomes of chronic kidney disease, such as kidney failure, cardiovascular disease, and premature death, can be prevented or delayed. Earlier stages of chronic kidney disease can be detected through laboratory testing. Treatment of earlier stages of chronic kidney disease is effective in slowing the progression toward kidney failure. Initiation of treatment for cardiovascular risk factors at earlier stages of chronic kidney disease should be effective in reducing cardiovascular disease events both before and after the onset of kidney failure [6]. It is seen that CKD patients are associated with low levels of vitamin C. It is due to dietary restrictions, regular dialysis, and oxidative stress, associated with pre-existing subclinical inflammation seen in CKD patients. It is observed that vitamin C deficiency is a factor in CVD morbidity and mortality [7]. It has been further observed that supplementation of vitamin C can decrease the level of PTH in hemodialysis patients with secondary hyperparathyroidism [8]. A high inflammatory state in the terminal stages of CKD patients causes a decrease in the nutritional status of the patients which shows a decrease in levels of serum albumin, pre albumin, and other nutritional markers [9]. It is thus presumed that measuring vitamin C levels in CKD patients will be beneficial and correcting the deficiency is supposed to prevent atherosclerosis [12], and ameliorate hyperparathyroidism [10]. This study was undertaken to correlate vitamin C levels with markers of inflammation such as hsCRP and albumin in CKD [4] patients.

Materials and methods

This single centered cross-sectional study was conducted in Medicine and Nephrology Departments at, VMMC and Safdarjung Hospital, New Delhi in the year 2014-2015 on 110 adult patients with CKD stage 5. 8 ml of venous blood was collected in a heparinized vial or syringe and the same amount was taken in the plain vial for serum separation for estimation of vitamin C, serum albumin levels, and serum hsCRP.

Inclusion criteria: Adult male and female patients with CKD stage 4-5 were included. Age and sex-matched subjects without chronic kidney disease were included.

Exclusion criteria: Malignancy, Acute Hepatitis, Current usage of steroids, or immune-suppressants. Positive human immunodeficiency virus (HIV) serology, Chronic smoking, Chronic alcoholism, Acute infections or acute inflammatory state within one month, Tuberculosis, Malabsorption syndromes were excluded from the study.

Methods of estimation

Plasma Vitamin C: This was a micro method for the determination of ascorbic acid in plasma using orthophosphoric acid and ferric ions.

Principle: The method was based on the principle that there was a reduction of ferric ions by ascorbic acid followed by the formation of complex ferrous ion products and alpha, alpha1 dipyridyl, which was measured calorimetrically. This method can be used to determine as low as 0.1micro gram of ascorbic acid in as low as 0.01 ml of human plasma.

Reagents used : Trichloroacetic acid 5% aqueous solution, orthophosphoric acid 85%, ferric chloride 3%, alpha,alpha1 dipyrimidyl 1%.

Serum albumin estimation was done using the Beacon kit (lysozyme albumin).

Determination of albumin in serum or plasma was based on the binding behavior of albumin with dye 33' 55'tetrabromoM cresol sulphonapthaline (BCG) in the acidic medium at Ph 4.2. The blue-green colored complex was formed the absorption of which was proportional to albumin concentration in the sample and it's measured at 600 nm (600-650 nm or with red filter).

Reaction: Albumin +BCG= Albumin-BCG Complex. The kit was standardizing to perform accurately up to albumin concentration of 10 gm/dl. Samples above this concentration were diluted with saline and results were multiplied by the dilution factor. The expected normal value using this procedure: 3.4-5.5gm/dl.

Serum hsCRP estimation: This test was based on a two-site sandwich enzyme immunoassay principle. The tested specimen was placed into the microwells coated by specific murine monoclonal to human CRP-antibodies. Antigen from the specimen was captured by the antibodies onto the microwell surface. Unbound material was removed by the washing procedure. Second antibodies – murine monoclonal to human CRP, labeled with a peroxidase enzyme, are then added into microwells. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface was detected and quantified by the addition of chromogen substrate mixture, stop solution, and photometry at 450 nm. The optical density in the microwell was directly related to the quantity of measured analyte in the specimen. This kit was intended to use with serum or plasma. Grossly hemolytic, lipemic, or turbid samples were avoided. Specimens were stored at 2^0-8^0C before testing.

Statistical analysis

The descriptive statistics for the quantitative variables under stage 5 CKD patients such as vitamin C, hsCRP, albumin were presented by mean and standard distribution. The correlation between vitamin C with hsCRP and albumin was calculated by Pearson correlation coefficient. Non-parametric Spearman rank correlation in case the data do not follow the normal distribution. Overall under categories such as diabetic and non-diabetic patients, $p < 0.05$ was

taken as a level of statistical significance. The entire data were analyzed using standard statistical software.

Results

35.45% of the case group was in the age group 28-37 years and 33.64% were in the age group 38-47 years. 61.54% of the control group was in the age group 28-37 years (**Table – 1**).

Significant differences were found in serum iron, TIBC, serum ferritin levels of the cases and controls with a highly significant p-value. No significant differences were found in the transferrin saturation % among both the groups with a p-value of 0.624 (**Table – 2**).

Significant differences were found in serum PTH, serum calcium, serum phosphate levels of the cases and controls with a highly significant p-value (**Table – 3**).

Table - 1: Distribution of age among the study population.

Age group (Years)	Case		Control		Total Count
	Number of cases	Percentage	Number of cases	Percentage	
18-27	13	11.82%	15	19.23%	28
28-37	39	35.45%	48	61.54%	87
38-47	37	33.64%	8	10.26%	45
48-57	14	12.73%	6	7.69%	20
58-67	4	3.64%	1	1.28%	5
68-77	3	2.73%	0	0.00%	3
Grand Total	110		78		188

Table - 2: Distribution of iron profile parameters among cases and controls.

	Serum iron (mcg/dL)	TIBC (mcg/dL)	Serum Ferritin (mcg/L)	Transferrin saturation (%)
Cases	64.1±37.2	427.6±132.4	100.2±70.6	29.7±16.6
Controls	140.2±21.5	283.8±46.0	172.6±46.5	30.6±7.4
p value	<0.01	<0.01	<0.01	0.624

Table - 3: Distribution of PTH, serum calcium, and serum phosphorus.

	PTH (pg/ml)	Serum calcium (mg/dl)	Serum phosphate (mg/dl)
Cases	515.10±251.12	7.6±0.6	4.8±0.5
Control	31.6±9.3	10.1±0.7	3.3±0.4
p value	<0.01	<0.01	<0.01

Table - 4: Distribution of vitamin C levels among cases and controls.

Vitamin C	Cases		Control	
	Number	Percentage	Number	Percentage
Deficiency	41	37.27%	0	0
Insufficiency	46	41.82%	9	11.5%
Normal	23	20.91%	69	88.5%

Among the control group 11.5% were among insufficiency groups. Majority of the controls (88.5%) were having normal levels of plasma vitamin C levels. None of the control population was vitamin C deficient. Among the case group, 37.27% were among the vitamin C deficiency group, 41.82% were among vitamin C

insufficiency group and 20.91% of the control population was normal (**Table – 4**).

In the cases, group 78 patients (70.9%) were diabetic and 32 patients(29.1%) were non diabetic. Among the diabetic group, 16.67% were having normal vitamin C values. 47.44% among the diabetic group were vitamin C insufficient and 35.90% were vitamin C deficient. No significant correlation was observed between diabetic patients and plasma vitamin C levels with a p-value of 0.107 (**Table – 5**).

Table - 5: Comparison of vitamin C levels according to diabetic status.

	Non-diabetic		**Diabetic**		**Total**	**P-value**
Normal	10	31.25%	13	16.67%	23	P=0.107
Insufficiency	9	28.13%	37	47.44%	46	
Deficiency	13	40.63%	28	35.90%	41	
Total	32		78		110	

Table - 6: Correlation of plasma vitamin c with serum hsCRP cases.

	Correlation of vitamin C(Pearson's Coefficient)	**P value**
hs-CRP	-0.634	<0.001

Table – 7: Correlation of plasma vitamin C with serum albumin cases.

	Correlation of vitamin C (Pearson's Coefficient)	**P value**
Serum albumin	0.191	0.046

Table - 8: Correlation of plasma vitamin with **markers of mineral bone disease** cases.

Markers of mineral bone disease	**Correlation of vitamin C (Pearson's coefficient)**	**P value**
Serum pth	-0.579	<0.001
Serum calcium	0.069	0.476
Serum phosphate	-0.099	0.305

Table - 9: Correlation of plasma vitamin with iron profile cases.

Iron profile	**Correlation of vitamin C (Pearson's coefficient)**	**P value**
Serum iron	0.235	0.014
Serum ferritin	0.224	0.019
TIBC	-0.284	0.003
Transferrin saturation	0.286	0.002

Negative correlation was seen between plasma vitamin C and serum hsCRP in the cases group (-0.634). There was a significant correlation observed between plasma vitamin C and serum hsCRP in the case group with a p-value of < 0.001 (**Table – 6**).

Positive correlation was seen between plasma vitamin C and serum albumin in the cases group (0.046). A significant correlation was observed between plasma vitamin C and serum albumin among the case group with a p-value of 0.046 (**Table – 7**).

Negative correlation was seen between plasma vitamin C and serum PTH (-0.579), serum phosphate (-0.099) in the case group. A positive correlation was seen between plasma vitamin C and serum calcium (0.069) in the case group. Significant correlation was observed between

plasma vitamin C and serum PTH, p-value < 0.001. No significant correlation was observed between plasma vitamin C and serum calcium (p = 0.476), serum phosphate (p = 0.305) among the case group (**Table – 8**).

Positive correlation was seen between plasma vitamin C and serum iron (0.235), serum ferritin (0.224), transferrin saturation (0.286) in the case group. Negative correlation was seen between plasma vitamin C and TIBC (-0.284) in the case group. Significant correlation was observed between plasma vitamin C and serum iron (p = 0.014), serum ferritin (p = 0.019), TIBC (p = 0.003), transferrin saturation (p = 0.002) among the case group (**Table – 9**).

Discussion

Diabetes was present in 78 patients (70.91%). As well as reinforcing the trend that diabetes is the most common cause of CKD in our population, this also highlights the fact that the global diabetic pandemic, more so in India, will in the long term lead to a rapid rise in the number of patients with CKD. The WHO estimate of the diabetes burden in India is 35 million by 2025 and studies by Diabetes India show a prevalence of 4.3% of diabetes in the general population [11]. Vitamin C has a low molecular weight and high water solubility, hence it could be easily cleared from plasma during dialysis [12]. As vitamin C is prominently consumed because of oxidative stress and inflammation in patients on dialysis, there are low vitamin C levels in these patients. It is well established that plasma vitamin C level is generally low in patients on dialysis compared with the general population and it has been seen that patients on dialysis with low plasma vitamin C levels are associated with increased risk of cardiovascular morbidity and mortality [13]. It is proposed that the interaction between BiPTH and vitamin C observed is explained by the action of vitamin C on post-receptor events in the superfamily of seven membrane-spanning receptors which includes the PTH receptor in the bone and calcium-sensing receptor in the parathyroid gland (CaSR) [14]. Binding of PTH to its receptor initiates signaling through the alpha subunit of the stimulatory G-protein resulting in elevated cAMP and activation of cAMP-dependent protein kinases Vitamin C enhances the cAMP response to PTH in preosteoblast-like cells and other organ systems [15]. C-reactive protein (CRP) is the prototype acute phase protein primarily synthesized in the liver and its release is stimulated by interleukin 6 (IL-6) and other pro-inflammatory cytokines [16]. Composed of five 23-kDa subunits, C-reactive protein (CRP) is a liver-derived pentraxin that plays a key role in the innate immune response. CRP has a long plasma half-life and is now understood to be a mediator as well as a marker of atherothrombotic disease setting. hsCRP not only may be a marker of low-grade chronic systemic inflammation but also may be directly involved in atherosclerosis and hsCRP can also amplify the anti-inflammatory response through complement activation, tissue damage, and activation of endothelial cells [17]. Among the case group, 37.27% were among the vitamin C deficiency group, 41.82% were among the vitamin C insufficiency group and 20.91% of the case population was having normal plasma vitamin C levels. The mean plasma vitamin C levels among the case group were 0.290±0.25, and among the control group were 0.911±0.7 when the mean values of plasma vitamin C were compared, there was a significant difference between the case group and the control group (p < 0.001) [18]. A study conducted by Khankin EV et al. shows the prevalence of low levels of vitamin C. hence our study also proves the low prevalence of plasma vitamin C among CKD patients on MHD. Among the cases group majority of the females (45.8%) were in the insufficiency group, 39.6% were in the vitamin C deficiency group. Among the males (38.70%) were vitamin C insufficient, 35.5% were vitamin c deficient [19]. In the cases group, 78 patients (70.9%) were diabetic and 32 patients (29.1%) were non-diabetic. Among the diabetic group, 16.67% were having normal vitamin C values. 47.44% among the diabetic group were vitamin C insufficient and 35.90% were vitamin C deficient. No significant

difference was observed between diabetic and nondiabetic CKD patients concerning plasma vitamin C levels (p= 0.107).We estimated the mean eGFR among the cases. Mean eGFR among the diabetic population (6.012±1.16) and non-diabetic (6.253±1.35) when eGFR compared between diabetics and non-diabetics significant difference in the value was observed. (p 0.01) [20]. When vitamin C concentration and eGFR relationships were compared in the two groups, a significant correlation was not seen. This was in contrary to the study conducted by Kher V, et al. [21] showed that plasma vitamin C concentration had a positive linear relationship with eGFR in both diabetic and nondiabetic patients (P = 0.006 and P = 0.004, respectively). The mean hsCRP levels among the case group were (33.06±9.18mg/L) and among controls were (9.86±7.77 mg/L). Significant differences were found in serum hsCRP levels of the cases and controls with a highly significant p-value. A negative correlation is seen between plasma vitamin C and serum hsCRP in the cases group (-0.634). There was a significant correlation observed between plasma vitamin C and serum hsCRP in the case group with a p-value of < 0.001. The mean serum albumin levels among the case group were (2.67±1.04 gm/dl) and among controls were (3.58±0.34 gm/dl). Significant differences were found when serum albumin levels of the cases and controls were compared (p < 0.001). A positive correlation was seen between plasma vitamin C and serum albumin in the cases group (0.046) (p-value 0.04) [21]. A study conducted by Levine M, et al. which were conducted among dialysis patients, plasma vitamin C level was positively associated with albumin (Spearman r = 0.161, P = 0.007), and inversely associated with hsCRP (Spearman r = -0.201, P = 0.001), this shows the similarity between our study in the correlation of plasma vitamin C with serum hsCRP and serum albumin in CKD patients [22]. These data indicate that plasma Vitamin C is positively associated with higher Hb levels and estimating the Vitamin C status could play a major role in helping PD patients to utilize iron for erythropoiesis and achieve a better Hb response during anemia management. A positive correlation was seen between plasma vitamin C and serum albumin (0.191), BMI (0.210), triceps skinfold thickness (0.613), handgrip strength (0.651) in the case group. A significant correlation was observed between plasma vitamin C and serum albumin (p = 0.046), BMI (p = 0.002), triceps skinfold thickness (p < 0.001), and handgrip strength (p < 0.001) among the case group [23]. Study conducted by May JM, et al. assessed the Parameters of Nutritional Status in Moroccan Hemodialysis patients. They have used triceps skinfold thickness, BMI, for evaluation of the nutritional status of hemodialysis patients and proven to be a good indicator for assessing the nutritional status of CKD patients [24]. A study conducted by McCauley et al concluded that HGS represents a marker of the body lean muscle mass that is not confounded by inflammation or the hydration status of CKD patients. It predicts all-cause and cardiovascular mortality in PD patients independent of other confounding covariates, including CRP and serum albumin. HGS may be used in conjunction with serum albumin as a regular nutrition monitoring tool in PD patients [25]. We found that estimating plasma vitamin C levels could reflect on the iron profile status of CKD patients. Since vitamin C can affect the level of absorption of iron from the gastrointestinal tract, mobilization of iron from kupffer cells, reticuloendothelial cells. Hence the change in plasma vitamin C can affect the iron profile of these patients [26, 27].

Conclusion

In our study, we estimated the plasma vitamin C levels in chronic kidney disease stage 5 patients and a healthy control population. We found that there was a significant fall in the levels of plasma vitamin C in chronic kidney disease stage 5 patients who were on hemodialysis when compared with healthy control subjects. Thereby proving the prevalence of low levels of plasma vitamin C in chronic kidney disease stage 5 patients. We also found a significant positive correlation between plasma vitamin C levels and

serum albumin and a significant negative correlation between plasma vitamin C and serum hsCRP. Since hsCRP and albumin are a part of acute phase reactants, we show that by estimating the levels of plasma vitamin C, can reflect the inflammatory status prevailing in CKD stage 5 patients.

We also showed the significant positive correlation of plasma vitamin C and markers of nutritional status such as serum albumin, BMI, triceps skinfold thickness, and handgrip strength. There by proving the fact that low levels of plasma vitamin C denote poor nutritional status in CKD stage 5 patients.

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