Effect of Oral Supplementation of Vitamin E on Urinary Risk Factors in Patients with Hyperoxaluria

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Summary The effects of administration of vitamin E on hyperoxaluria, hypercalciuria, hyperuricosuria, and hypocitraturia and on the decreased levels of antioxidants such as ascorbic acid, vitamin E, glutathione (GSH), and superoxide dismutase (SOD) in kidney stone patients were observed. Following the surgical removal of kidney stones from the patients, vitamin E (200 mg/day) was administered from the 7th day onwards to twenty patients for up to 90 days. The normalization process of urinary risk factors and antioxidants was rapid in the vitamin E-administered patients when compared with that in the untreated group. The increased excretion of citrate following vitamin E administration was suggested to reduce the retention of calcium oxalate crystals and thereby lower the rate of recurrence of stones.

Key Words: vitamin E, urinary risk factors, antioxidants, hyperoxaluria, citrate

Kidney stone disease is multifarious in origin and the precise mechanism by which kidney stones originate is still not well understood. The relationship between the concentrations of the precipitating substances in urine and the solubility of the salt to be formed [1], the role of nucleating substances [2], the role of crystal aggregation [3], and the part played by inhibitors of crystal formation and aggregation [4] have been a focus of several workers, and renal injury is considered as one of the prerequisites for calcium oxalate retention [5]. But the biochem-

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ical mechanism behind renal tubular injury and crystal retention is still not understood. Involvement of an enhanced lipid peroxidation reaction in stone formation was shown earlier [6]. Further depletion of antioxidants and altered activities of antioxidant enzymes were noted in the calculus kidneys and plasma of experimental urolithic rats and in the blood of kidney stone patients [7, 8]. Supplementation of vitamin E or methionine was found to normalize the antioxidant status and to prevent stone formation in experimental urolithiasis [9]. These studies suggest a role of antioxidants in the pathology of calcium oxalate deposition. However, the role of antioxidants in human stone disease and their relationship with the excretion of substances considered to be risk factors has not been well characterized. The present study was undertaken to determine whether or not treatment with vitamin E normalizes the excretion of such substances in kidney stone (hyperoxaluric) patients. As a result, we observed hypooxaluric and hypercitraturic effects of vitamin E on stone patients.

MATERIALS AND METHODS

Blood and urine samples were obtained from patients suffering from renal calculi and admitted to the Urology Department of Government Royapettah Hospital and Sri Ramachandra Medical College and Research Institute Hospital (Deemed University), Chennai. All chemicals were of analytical grade.

Totally 40 patients (30 males and 10 females in the age group of 15–55 years with a mean age of 42 years) with same age and sex as controls were included in this study. Willingness was obtained from each patient. The patients were grouped as follows: Group I, normal subjects ($n=30$, 21 males and 9 females); Group II, stone patients ($n=40$); Group III, 7th day after surgical removal of renal calculi ($n=40$). This was further divided into two groups. Group IV was untreated and the Group V was orally administered vitamin E (dl- α -tocopheryl acetate Evion, E-Merck, India) at a dosage of 200 mg/day ($n=20$) for a time period of 90 days. Group IVa, measurements at 30 days; Group Va, at 30 days; Group IVb, at 60 days; Group Vb, at 60 days; Group IVc, at 90 days; and Group Vc, at 90 days.

The blood sample was collected in tubes containing heparin as an anticoagulant. The heparinized samples were centrifuged at $3,500 \times g$ for 10–15 min, the plasma was separated, and the level of plasma lipid peroxides was measured by the thiobarbituric acid reaction method of Yagi *et al.* [10] and expressed as nmol in terms of malondialdehyde/ml of plasma.

The ascorbic acid was estimated by the method of Omaye *et al.* [11] and expressed as mg%. The plasma vitamin E was estimated by the method of Barker and Frank [12] and expressed as mg%. After centrifugation the plasma and the buffy coat were removed. The red blood cells were washed three times with 0.9% normal saline by centrifugation for 10–15 min at $3,000 \times g$ after each wash. Cell lysis was carried out by adding a one-third volume of ice-cold redistilled water and mixing well. The lysate was allowed to stand for 1 h at 4°C, after which it was used

for the determination of glutathione (GSH) by the method of Moron *et al.* [13] and expressed as μg of GSH/mg of Hb. Superoxide dismutase (SOD) activity in the hemolysate was measured by the auto-oxidation of pyrogallol as described by Marklund and Marklund [14]. One unit of the latter enzyme activity corresponds to the amount of the enzyme that inhibits the reaction by 50% and was expressed as U/ml hemolysate. The glutathione peroxidase (GPX) activity in the hemolysate was estimated by the method of Rotruck *et al.* [15] and expressed as μg of GSH/mg Hb. The hemoglobin in the hemolysate was measured according to Drabkin and Austin [16]. Twenty-four hour urine was collected with toluene as the preservative, and analysis of oxalate was estimated by the method of Hodgkinson and Williams [17]. Citrate was estimated by the method of Rajagopal [18], and was expressed as mg/day. All the estimations were carried out within 24 h after sampling.

Calcium, phosphate, uric acid, and creatinine in urine as well as in blood were estimated by standard enzymatic methods in a Ciba Corning Express Plus automated clinical chemistry analyzer.

Stone analysis was carried out by a wet chemical method. Statistical analysis was carried out by Student's *t*-test. The values were expressed as mean $\pm$ SD. Correlation coefficient *r* was calculated for the parameters analyzed in plasma and hemolysate.

RESULTS

Stone analysis data revealed the presence of calcium, oxalate, and phosphorus as major constituents in most of the stones except three uric acid stones.

The urinary excretion of risk factors such as oxalate, uric acid, calcium, phosphorus, creatinine, and the inhibitor citrate is presented in Table 1. The oxalate excretion in the normal subjects was 24 mg/day, but it was doubled in the stone patients ($p < 0.001$). Excretion of citrate was drastically decreased to 22% of the control in the stone patients ($p < 0.001$). Both calcium and uric acid excretion were also significantly elevated in the stone patients when compared with control levels ($p < 0.001$). There were no significant differences in the parameters studied before vitamin E administration between groups IV and V. The patients at 7 days after the removal of stones showed no significant variation in the excretion of oxalate when compared with that before surgery. However, administration of vitamin E reduced the oxalate excretion by 34% by 60 days and normalized it by 90 days when compared with that before surgery, whereas the untreated group showed only a 27% reduction of oxalate by 90 days.

The administration of vitamin E elevated the excretion of citrate and rapidly brought to 31, 53, and 61% of the normal level on 30, 60, 90 days, respectively, whereas untreated patients showed 27, 32, and 40% of normal level on the corresponding days. The excretion of calcium was decreased after vitamin E treatment by 17% on day 60 and normalized by 90 days when compared with that in the

Table 1. Excretion of urinary risk factors and inhibitors in normal and hyperoxaluric and vitamin E-treated and -untreated patients.

Group	No.	Oxalate (mg/day)	Citrate (mg/day)	Calcium (mg/day)	Phosphorus (mg/day)	Uric acid (mg/day)	Creatinine (mg/day)
I	30	24.4 ± 5.5	676 ± 110	174 ± 40	540 ± 96	282 ± 81	820 ± 300
II	40	48.1 ± 12.5 ^a	151 ± 84 ^a	311 ± 50 ^a	455 ± 98 ^a	474 ± 149 ^a	889 ± 240
III	40	43.8 ± 8.7	173 ± 79	254 ± 65 ^b	468 ± 144	499 ± 145	970 ± 261
IVa	20	39.7 ± 10.2	188 ± 57	232 ± 40	370 ± 101	463 ± 135	864 ± 220
Va	20	35.9 ± 3.4	210 ± 35	227 ± 45	436 ± 204	450 ± 146	796 ± 185
IVb	20	37.9 ± 11.2	219 ± 48	218 ± 49	391 ± 107	456 ± 131	836 ± 190
Vb	20	31.9 ± 3.0 ^d	361 ± 52 ^d	180 ± 40 ^d	487 ± 100 ^d	391 ± 115 ^f	760 ± 148 ^f
IVc	20	34.8 ± 9.9	271 ± 50	207 ± 33	412 ± 112	411 ± 111	818 ± 120
Vc	20	26.9 ± 10.2 ^e	411 ± 53 ^e	177 ± 30 ^e	511 ± 102 ^e	311 ± 98 ^{e,g}	751 ± 130 ^g

The values are expressed as the mean ± SD. Statistically significant difference at *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. ^aSignificantly different from group I, ^bsignificantly different from group II, ^csignificantly different from group IVb, ^dsignificantly different from group IVc, ^esignificantly different from group IVa, ^fsignificantly different from group II, ^gsignificantly different from group II.

Groups IVa, IVb, and IVc were untreated, and parameters were measured at 30, 60, and 90 days, respectively. Groups Va, Vb, and Vc were treated, and parameters were measured at 30, 60, and 90 days, respectively.

vitamin E-untreated group who still showed a 20% higher level even after 90 days when compared with the normal value. The excretion profiles of phosphorus and uric acid were also significantly brought to near normal values following vitamin E treatment for 90 days.

Table 2 presents the serum level of calcium, phosphorus, uric acid, and creatinine in normal and stone patients. A significant rise in calcium and uric acid was noted in the stone patients. The stone patients receiving vitamin E treatment for 60 days showed a significant decrease in their uric acid level; and normalization occurred by 90 days when comparison was made with the level in vitamin E-untreated patients, suggesting an improvement in the normalization on oral feeding of vitamin E.

The plasma lipid peroxide level in the normal subjects was 2.5 nmol/ml, and this level was increased by 265% in stone formers (Table 3). The levels of plasma ascorbic acid, blood GSH, and vitamin E were significantly decreased in stone formers ($p < 0.001$).

SOD in the hemolysate was significantly decreased by 50% in the stone formers as compared with that of the control. GPX as well as GSH were decreased by 26 and 35%, respectively, in the stone formers.

Following the surgical removal of stones from the patients, the lipid peroxide level was reduced by 28% on the 60th day, whereas patients treated with vitamin E for 60 days showed a 47% reduction and near normalization by 90 days. Untreated patients still showed 171% of the control by 90 days.

Vitamin E administration to the surgically treated patients significantly increased plasma vitamin E levels by 250% (2.1) on the 60th day from the initial

Table 2. Level of serum calcium, phosphorus, uric acid, and creatinine in normal and hyperoxaluric patients.

Group	No.	Calcium (mg/dl)	Phosphorus (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
I	30	9.7±0.8	3.4±0.5	4.5±0.8	0.8±0.2
II	40	11.0±1.3 ^{a***}	3.8±1.3	5.8±1.2 ^{a***}	1.0±0.2 ^{a***}
III	40	11.1±1.1	4.1±1.1	5.8±1.1	0.9±0.2
IVa	20	10.4±1.0	3.7±1.1	5.6±1.1	1.0±0.2
Va	20	9.8±0.7	3.7±0.9	5.0±1.1	0.8±0.2
IVb	20	10.1±1.0	3.6±0.9	5.4±1.2	0.9±0.2
Vb	20	9.7±0.5 ^{f***}	3.6±1.0	4.6±1.0 ^{a*,f***}	0.8±0.2 ^{f***}
IVc	20	9.9±1.0	3.6±0.9	5.2±1.0	0.9±0.2
Vc	20	9.6±0.8 ^{g***}	3.4±0.8	4.5±0.9 ^{e*,g***}	0.8±0.2 ^{g***}

The values are expressed as the mean ± SD. Statistically significant difference at *** $p < 0.001$, * $p < 0.05$. ^aSignificantly different from group I, ^dsignificantly different from group IVb, ^esignificantly different from group IVc, ^fsignificantly different from group II, ^gsignificantly different from group II.

Groups IVa, IVb, and IVc were untreated, and parameters were measured at 30, 60, and 90 days respectively. Groups Va, Vb, and Vc were treated, and parameters were measured at 30, 60, and 90 days, respectively.

Table 3. Lipid peroxides and antioxidant levels.

Group	No.	Plasma			Hemolysate		
		Lipid peroxide level (nmol/ml)	Vitamin E (mg/dl)	Ascorbic acid (mg/dl)	SOD (U/mg Hb)	GSH (μ g/mg Hb)	GPX (μ g of GSH utilized/min/mg Hb)
I	30	2.5 $\pm$ 0.6	1.8 $\pm$ 0.4	0.5 $\pm$ 0.1	0.05 $\pm$ 0.003	3.4 $\pm$ 1.1	3.8 $\pm$ 1.3
II	40	6.6 $\pm$ 2.1 ^{a***}	0.8 $\pm$ 0.2 ^{a***}	0.3 $\pm$ 0.1 ^{a***}	0.025 $\pm$ 0.002 ^{a***}	2.5 $\pm$ 0.8 ^{a***}	2.5 $\pm$ 1.1 ^{a***}
III	40	6.7 $\pm$ 2.2	0.8 $\pm$ 0.2	0.3 $\pm$ 0.1	0.025 $\pm$ 0.004	2.5 $\pm$ 1.0	2.7 $\pm$ 1.1
IVa	20	5.7 $\pm$ 1.7	0.9 $\pm$ 0.2	0.33 $\pm$ 0.1	0.027 $\pm$ 0.005	2.7 $\pm$ 0.9	2.8 $\pm$ 0.9
Va	20	4.3 $\pm$ 1.5 ^{c**}	1.8 $\pm$ 0.4 ^{c***}	0.39 $\pm$ 0.1	0.034 $\pm$ 0.007 ^{c*}	2.9 $\pm$ 0.8	3.1 $\pm$ 1.2
IVb	20	4.8 $\pm$ 1.2	1.0 $\pm$ 0.2	0.34 $\pm$ 0.1	0.030 $\pm$ 0.005	2.8 $\pm$ 0.7	2.8 $\pm$ 1.0
Vb	20	3.5 $\pm$ 1.6 ^{d***,f***}	2.1 $\pm$ 0.4 ^{d***,f***}	0.45 $\pm$ 0.1 ^{d***,f***}	0.043 $\pm$ 0.005 ^{d***,f***}	3.3 $\pm$ 1.0 ^{f**}	3.2 $\pm$ 1.2 ^{f*}
IVc	20	4.3 $\pm$ 1.4	1.1 $\pm$ 0.2	0.41 $\pm$ 0.1	0.035 $\pm$ 0.005	3.1 $\pm$ 0.9	2.9 $\pm$ 0.8
Vc	20	3.1 $\pm$ 1.2 ^{e***,g***}	2.0 $\pm$ 0.3 ^{e***,g***}	0.51 $\pm$ 0.1 ^{e***,g***}	0.049 $\pm$ 0.006 ^{e***,g***}	3.3 $\pm$ 0.96 ^{g**}	3.653 $\pm$ 1.2 ^{e***,g***}

The values are expressed as the mean $\pm$ SD. Statistically significant difference at *** p < 0.001, ** p < 0.01, * p < 0.05. ^aSignificantly different from group I, ^csignificantly different from group II, ^dsignificantly different from group IVb, ^esignificantly different from group IVc, ^fsignificantly different from group II, ^gsignificantly different from group II.

Groups IVa, IVb, and IVc were untreated, and parameters were measured at 30, 60, and 90 days, respectively. Groups Va, Vb, and Vc were treated, and parameters were measured at 30, 60, and 90 days, respectively.

Fig. 1

Fig. 1. Correlation between parameters measured in normals and hyperoxaluric patients. (a) between vitamin E and GSH ($r=0.59$, $p<0.001$); (b) between GSH and SOD ($r=0.51$, $p<0.001$); (c) between GSH and GPX ($r=0.24$); (d) between vitamin E and lipid peroxide level ($r=-0.69$, $p<0.001$); (e) between GSH and lipid peroxide level ($r=-0.53$, $p<0.001$).

level (0.8) to nearly normal, and this normalization was rapid when compared with that of the untreated group (1.0). Further administration of vitamin E did not increase the value significantly. Similarly, vitamin E treatment rapidly brought the level of ascorbic acid near to 90% of normal level by the 60th day, and complete normalization occurred by 90 days, when compared with the values for the vitamin E-untreated group. The GSH level was completely restored by the 60th day of administration of vitamin E, whereas the untreated group showed only 84% of the normal value. Both SOD and GPX activities were restored to 85% of normal on treatment with vitamin E for 60 days and complete restoration was found by 90 days, whereas the untreated group showed only 60–75% normalization.

A positive correlation between vitamin E and GSH ($r=0.59$) (Fig. 1a), between GSH and SOD ($r=0.51$) (Fig. 1b), and between GSH and GPX ($r=0.24$) (Fig. 1c), and a negative correlation between vitamin E and lipid peroxide ($r=-0.69$) (Fig. 1d) and between GSH and lipid peroxide ($r=-0.53$) (Fig. 1e) were obtained.

DISCUSSION

Hyperoxaluria and hypercalciuria with hypocitraturia have been observed in kidney stone patients. Administration of vitamin E following surgical removal of stones speeded up normalization of oxalate and calcium when comparison was made with the untreated group. There was a significant elevation of citrate excretion following administration of vitamin E up to 90 days.

Several methods of treatment for prevention of stone formation or for dissolu-

tion of stones have been attempted for different types of stones, but complications also have been reported in these cases [19]. Lipid peroxidation, a degradative process of polyunsaturated fatty acids, has been shown to be involved in the pathogenesis of experimental urolithiasis [6, 7]. Further it has been recently shown that free radical-damaged cells produce a favorable environment for crystal development [20]. Administration of methionine [19], vitamin E [21], mannitol [22], GSH monoester [23] to hyperoxaluric rats was found to effectively prevent deposition of calcium oxalate crystals. The mechanism for this action is attributed to its action of prevention of cell injury, which is a prerequisite factor for the retention of calcium oxalate crystals. Further, peroxidized membranes can facilitate the entry of calcium into the cell from extracellular fluids. Conditions in which free radicals accumulate were shown to increase cellular and mitochondrial calcium in the kidney. Also, peroxidized microsomes were shown to increase the uptake of calcium [24].

In the present study, the significant increase in the plasma lipid peroxide level was found to be associated with a decrease in vitamin E, ascorbic acid, and blood GSH and hemolysate SOD and GPX in stone patients. When vitamin E was administered to the patients following the surgical removal of stones, the normalization process of above parameters was found to be rapid when compared with that in the untreated group. The restoration process achieved nearly 100% normalization after 90 days of vitamin E administration except in the case of citrate, suggesting that the treatment should be prolonged further for its restoration. This restoration trend suggests that there are disturbed antioxidant defense systems in the stone patients.

Few studies have shown the beneficial effect of vitamin E administration on hemodialysis patients [25], which would occur by lowering the lipid peroxide level, whose elevation was shown to be involved in a variety of diseases [26] including end-stage renal disease. Treatment with citrate has been advocated in patients with hypocitraturia because of its lowering effect on calcium oxalate supersaturation by binding calcium [27].

Administration of vitamin E to the stone-depleted patients for 60 days increased the excretion of citrate effectively faster than that in the untreated group, suggesting that prolonged administration of vitamin E could be beneficial for preventing the recurrence. The citrate level is extremely low in the hyperoxaluric patients when compared with the normal, and this level was raised significantly by vitamin E treatment. This increase in citrate could lower the recurrence rate considerably. The lower excretion of citrate in stone patients is attributed to intracellular acidosis with potassium depletion [28].

Treatment of stone patients with potassium citrate was shown to increase the citrate level from 223 to between 475 and 578 mg/24 h, after over two years of treatment [29]. The present study shows a rapid restoration of citrate from 150 to 410 mg/24 h within 90 days of vitamin E treatment.

The observed decreases in antioxidants, both scavengers and enzymes, resem-

bled those in the experimental urolithic condition [7] as well as those in hemodialysis patients [30] and rheumatoid arthritis patients [31]. This decreased antioxidant status in stone patients is suggested to enhance lipid peroxidation by oxalate itself and cause peroxide-mediated inhibition of the catalase enzyme [32]. There was a negative correlation between lipid peroxide and GSH and a positive one between GSH and vitamin E or GSH vs. SOD in the stone-forming conditions. Since GSH, ascorbic acid, and vitamin E are linked in the free radical detoxification and recycling process, supplementation of vitamin E restores the levels of GSH and ascorbic acid [33]. Cell injury associated with lipid peroxidation is suggested for the retention of oxalate in renal cells [6]. Feeding citrate to a calculogenic diet-fed rats was shown to prevent stone formation without altering the excretion of oxalate [34], or improving reduced antioxidant defense system, similar to the case for rats with kidney stones [34]. This study provides evidence that exogenous vitamin E administration results in restoration of antioxidants as well as the excretion of oxalate and calcium with increased excretion of citrate and thereby in protection against stone formation as well as oxidative stress. Thus, vitamin E administration reduces the cell injury.

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