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Research Paper

Supplementation of Vitamin E on Urinary Risk Factors And Calcium Oxalate Monohydrate Binding Proteins in Urolithiasis

Medical Science

KEYWORDS : Urolithiasis,Calcium Oxalate Monohydrate,Vitamin E .

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ABSTRACT

The efficacy of supplementation of vitamin E in preventing stone formation was assessed in stone formers. The excretion of total COM binding proteins was elevated in stone formers when compared with that of healthy individuals and normalized in vitamin E treated patients. Apart from these factors, the macromolecules present in the urine are capable of altering the crystallization rate. Hence, the Calcium oxalate monohydrate binding proteins were isolated from these patients yielded three protein peaks and they were designated as fractions I, II and III according to their order of elution. Among the three COM binding protein fractions, the proportion of F I and F III was increased and F II was reduced in stone formers. Identical distribution pattern of COM binding proteins with those of healthy individuals were observed for vitamin E treated patients after 9 months.

Introduction:

Nephrolithiasis is a common multifactorial disorder that initiates with the formation of micro crystals in the urine and terminates with the formation of mature renal calculi. Free radicals have been implicated to be one of the causes for the pathological biomineralization process within the urinary tract namely urolithiasis.

Preliminary studies (1,2) in our laboratory haveshown that vitamin E supplementation to hyperoxaluric patients brings down the urinary lithogenic risk factors. The effectiveness of vitamin E therapy should be judged not only by the risk factors but also by the status of urinary lithogenic proteins as these proteins direct the act of stone formation by mimicking the process of biomineralization.

Patients and methods

This study was approved by the institutional ethical committee. The patients were also given a clear picture of the benefical effct of vitamin E (400 mg)

Normal subjects and Kidney stone patients (KSP) without any complications were included in the present study. Patients admitted in the Urology ward of Stanley Medical College & hospital and confirmed for the presence of stones by x- rays, KUB scan and intravenous ureterogram (IVU) and had undergone surgery for the removal stones.

EXPERIMENTAL DESIGN

Group I - Normal healthy individuals (control)

Group II a - Stone patients

Group IIb - Stone Patients supplemented with vitamin E for 3 months

Group IIc - Stone Patients supplemented with vitamin E for 6 months

Group IId - Stone Patients supplemented with vitamin E for 9 months

METHODS

24 hrs urine samples were collected from KSP before supplementation of vitamin E and at three months interval for the duration of nine months after supplementation.VitaminE supplementation was given along with their regular treatment regime.

Routine urinary parameters like oxalate, calcium,uric acid and total protein were assayed. COM binding proteins were isolated (3).COM crystals were freshly prepared before use by mixing 1.5M $CaCl_2$ and 0.3M potassium oxalate in the ratio of 1:5 (adjusted to pH 6.5 using Tris – HCl buffer) with constant shaking at room temperature. After30 minutes of stabilization of the system by agitation, protein (3 mg protein / mg crystal) was added and made upto twice the volume prior to the addition of protein using 0.3 M potassium chlorid and allowed to interact with the proteins with constant shaking of the solution for 1 hour. The solution was centrifuged at 4000 g for 10 minutes and precipitated calcium oxalate was washed with water thrice for removal of the extraneously bound protein. 25mM Ethylene diamine tetra acetic acid(EDTA) was used for extraction of the bound protein. EDTA extract was separated by centrifugation at 4°C at 10000 g for 10 minutes and dialyzed against water at 4°C overnight with two changes of water.Then isolated COM binding proteins were subjected to DEAE cellulose column chromatography.

About 1.2 mg of protein was loaded onto a DEAE cellulose column (10 x 1 cm) pre – equilibrated with 0.05M Tris – HCl buffer. Elution was carried out first with 1 in buffer. Twenty `two` ml fractions were collected in each step of elution and the elution of the protein was monitored in a UVIKON 930spectrophotometer at 220 nm.

Three major proteins were eluted in each buffer, and the protein fractions were designated as fraction I (buffer eluant), fraction II (eluant of 0.05 M NaCl in buffer) and fraction III (0.3 M NaCl in buffer).

Statistical evaluation

Data are presented as mean ± S.D. Statistical analysis was carried out using ANOVA SPSS for windows, Release 9.05.

Results

Summarises urinary constituents of control, stone patients and vitamin E treated stone patients.The 24 hr urinary excretion of oxalate was 2.22 fold higher in the stone formers group when compared with that of the control [Table 1]. However, this increase in oxalate excretion was brought to near normal during treatment with vitamin E. It decreased by 33 %, 49 % and 52 % when compared to stone formers [$p<0.001$] progressively during 3rd, 6th, 9th month of treatment with vitamin E. Citrate levels were found to be significantly lowered in stone formers when compared to control subjects ($p<0.001$). Supplementation of vitamin E to these patients leads to a gradual increase in the excretion of citrate. The urinary excretion of calcium was observed to be approximately 1.5 fold increased in the stone forming patients.

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Vitamin E treatment to these patients normalized the excretion of calcium. Urinary protein excretion for normal subjects was found to be 58.5 ± 8.7 mg, while in stone formers, it was found to be 85.9 ± 11.6 mg, which is nearly 1.5 fold higher. On treatment with vitamin E, it gradually decreased and by nine months, the protein excretion was non-significant from the control. Uric acid excretion was elevated by 47% in stone formers when compared to non-stone formers. There was a gradual decrease in the excretion of uric acid by supplementation of vitamin E

Control subjects excreted nearly 0.875 mg of COM binding proteins / 24 hrs. It accounted for only 1.5 % of the total protein excretion. Stone formers exhibited 1.46 fold-increased excretion of total protein, while the increase was nearly 2.4 fold for COM binding protein alone. The COM binding protein excretion was normalized by 6 months of vitamin E treatment (Table 2)

Table 1:Urinary constituents of control, stone patients and vitamin E treated stone patients

Particulars	Oxalate	Total protein	Calcium	Citrate	Phosphorus	Uric acid
Control(n= 50) (GP I)	23.9±5.2 ^{b***}	58.5±8.7 ^{b***}	189.4±20.7 ^{b***}	428.1±63.7 ^{b***}	343.0± 28.2 ^{b***}	268.0± 31.5 ^{b***}
KSP (n = 42) (GP IIa)	53.2±8.4 ^{a***}	85.9±11.6 ^{a***}	302.4±40.5 ^{a***}	161.4±24.4 ^{a***}	503.4 ± 40.3 ^{a***}	394.0 ± 33.6 ^{a***}
VitaminE Treated						
3rd Month (n= 30) (GP IIb)	35.7±3.8 ^{a***b***}	66.4 ± 7.9 ^{a***b***}	236.6±19.6 ^{a***b***}	216.5 ± 23.9 ^{a***b***}	422.2±38.9 ^{a***b***}	344.2±26.1 ^{a***b***}
6th Month (n = 26) (GP IIc)	27.1 ± 4.6 ^{a**b***}	63.8 ± 8.7 ^{a***b***}	208.3 ± 23.2 ^{a***b***}	375.1 ± 43.3 ^{a***b***}	384.6 ± 50.9^{a***b***}	291.3 ± 26.9 ^{a***b***}
9th Month (n = 24) (GP IId)	25.2 ± 2.6 ^{aNSb***}	61.2 ± 8.1^{aNSb***}	198.4 ± 18.9 ^{aNSb***}	399.0 ± 32.1 ^{a**b***}	359.9 ± 50.1^{aNSb***}	279.7 ± 22.7 ^{a**b***}

Values are mean ± S.D., expressed as mg/ 24hr, Comparisons made with a= control Vs various groups, b= KSP Vs various-groups,* p< 0.001, ** p< 0.01, * p< 0.05, NS Non - Significant**

Table 2:Effect of vitamin E treatment on the urinary excretion of total COM binding protein

Particulars	Total Protein	Total COM	% Excretion
Control (GP I)	58.5± 4.7	0.875 ± 0.079	1.495
KSP (GP IIa)	85.9± 7.6	2.104 ± 0.288	2.444
Treated			
3rd month (GP IIb)	66.4± 5.9	1.535 ± 0.124	2.304
6th month (GP IIc)	63.8 ±5.7	0.956 ± 0.116	1.498
9th month (GP IId)	61.2 ± 5.1	0.915 ± 0.098	1.495

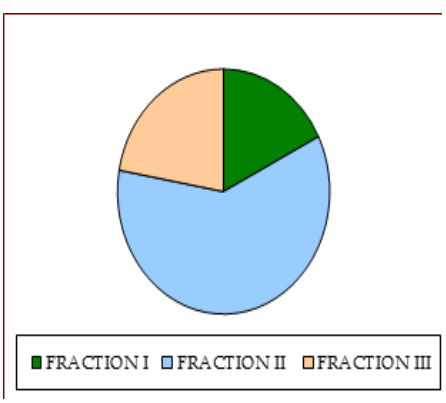
Values are mean ± S.D. for 21 patients. Values are expressed as mg of COM binding protein / 24 hrs.

Elution profile and % distribution of human urinary COM adsorbing proteins

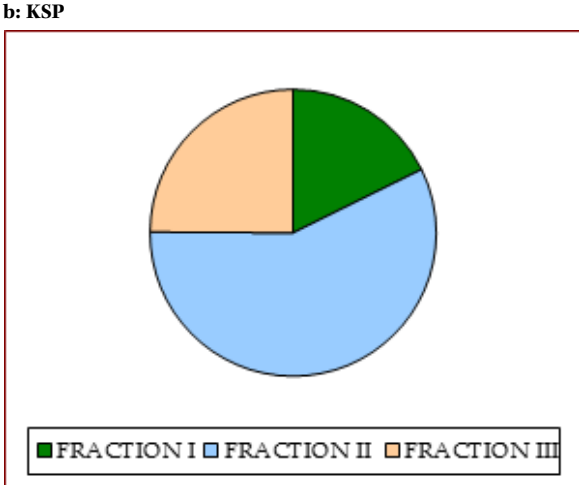
COM binding proteins isolated from human urine were subjected to DEAE cellulose column chromatography and eluted with a stepwise gradient of NaCl; three major protein peaks were obtained. The peak obtained in the Tris-HCl buffer [0.01M; pH 7.0] was designated as fraction I and the peaks obtained in 0.05M and 0.3M sodium chloride in buffer were designated as fraction II and III respectively. All the three fractions were present in all the groups. Among the various COM binding proteins, the proportion of FII was maximum in control and it was found to be 60.2 %, while in stone formers it was only 37.5 %, which accounts for only half of that excreted in non – stone formers. FI was increased by 43 % in KSP and FIII by 68 %(Fig 1)

Fig1: EFFECT OF VITAMIN E THERAPY ON THE ON THE % DISTRIBUTION OF COM BINDING PROTEINS

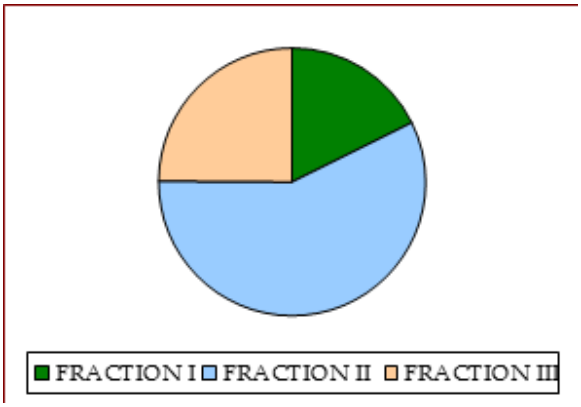
a:Control



b: KSP



KSP + VIT E 9 TH MONTH



Discussion

Hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria (4). Oxalate in urine can cause tubular damage by the production of free radicals leading to cell death (5). High concentrations of oxalate promote stone formation in two ways 1. By providing urinary conditions favorable to the formation of calcium oxalate crystals and 2. By inducing renal injury that generates cellular debris and promotes crystal nucleation and attachment.(6). Hypocitraturia is recognized as one of

the important risk factors for stone pathogenesis (7,8). Hypercalciuria remains the most prevalent risk factor both in male and female renal calcium stone formers (9).

COM adsorbing protein excretion is increased in kidney stone patients. Over -expression of proteins involved in lithogenesis has been already reported in humans as well as in urolithic rats (10).. Excretion of COM binding protein is associated with injurious hyperoxaluria, and is within normal limits in non-injurious hyperoxaluria (11).

Fraction I and II exhibit oxalate binding activity at pH 7.4, which is nearer to physiological urinary pH, suggesting that they can bind urinary oxalate leading to biological consequences that are involved in lithogenesis. A significant high oxalate binding activity is observed for hyperoxaluric fractions. The total urinary calcium binding protein activity is significantly greater in the active calcium oxalate stone formers when compared with either inactive calcium oxalate stone formers or non-stone forming controls.

Conclusion

In Vitamin E therapy as assessed by the routine urinary risk factors reveal that it is successful. Inorder for a molecule to take part in the lithogenic event, it should have the capacity to bind crystals. Hence, the Calcium oxalate monohydrate binding proteins were isolated from these patients and studied. . On fractionation, three COM binding proteins were obtained among which the one eluted in the buffer alone designated as fraction I was comparatively high in stone formers than non – stone formers. Stone formers fraction I exhibits higher promoting activity and forms COM aggregates which worsens the situation. Upon vitamin E supplementation, the excretion of COM binding protein was well within the non – stone formers range.

The adverse effects of oxalate / oxalate induced oxidative stress to the protein is reverted by Vitamin E.

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