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EFFECT OF PYRIDOXINE (VITAMIN-B_6) SUPPLEMENTATION ON CALCIURIA AND OXALURIA LEVELS OF SOME NORMAL HEALTHY PERSONS AND URINARY STONE PATIENTS

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ABSTRACT

Effect of pyridoxine (Vitamin-B_6) supplementation on calciuria and oxaluria levels of 20 normal healthy persons and 17 urinary stone patients has been studied. Mean 24 hr urinary calcium and oxalate levels of controls (healthy persons) and stone patients were estimated in pre-supplementation period and at every 20 days interval during supplementation. Stone patients were divided into two groups viz., mild hyperoxaluriacs and moderate hyperoxaluriacs, based on their pre-supplementation (base line) oxaluria levels. 60 days of pyridoxine supplementation, at the rate of 10 mg/day, resulted in a significant decrease ($p<0.01$ for mild hyperoxaluriacs and $p<0.001$ for moderate hyperoxaluriacs) in mean 24 hr urinary oxalate levels of urinary stone patients. The corresponding decrement in mean oxaluria level of controls was, however, only mild. The decrease of mean calciuria level of controls as well as stone patients, upon pyridoxine supplementation, were also found to be only mild and not significant. Utility of pyridoxine therapy in oxalate urolithiasis has been discussed in the light of results.

KEY WORDS

Calciuria, Oxaluria, Pyridoxine supplementation, Urinary stones, Urolithiasis.

INTRODUCTION

Urinary stone formation is related to a number of risk factors. Hypercalciuria and hyperoxaluria are the two most important risk factors (1). These factors contribute to urolithiasis by precipitating calcium oxalate in the urinary tract. This, being highly insoluble, forms the most stubborn constituent of urinary stones. Calcium is an essential macronutrient metal. It has a number of vital functions to be performed in the body. Maintenance of calcium homeostasis is essential for the body. Oxalic acid, on the other hand, is a non-nutrient factor. It even impairs calcium absorption by forming insoluble calcium oxalate. The chief source of oxalic acid in the body is through endogenous synthesis. Some dietary contribution is also made. It does not have any useful role to play and is excreted out through urine. As such, the strategy in calcium oxalate urolithiasis prevention should be to tackle hyperoxaluria rather than hypercalciuria. Hyperoxaluria is, in fact, a more potent risk factor than hypercalciuria in urolithiasis (2).

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Hyperoxaluria arises due to an increased production of oxalates in the body. Oxalates can arise from two sources, from glyoxylate metabolism and ascorbate degradation. In humans, it is presumed to be due to increased glyoxylate formation and metabolism. Defective carboligase or D-glycerate dehydrogenase increases the glyoxylate pool size leading to excessive formation of oxalate (3). It is an autosomal recessive trait with a familial incidence. Attempt to control oxaluria by regulating the enzymes of oxalate biosynthesis has recently been receiving attention. Role of vitamins in endogenous oxalate synthesis is also being probed. Deficiency of certain vitamins such as vitamin B_1 and B_6 have also been attributed to increased oxalate biosynthesis as well as its enhanced absorption by the intestine (4, 5, 6). However, quantitative correlations of these deficiencies, as well as their supplementation, to urinary oxalate levels are not yet clearly known. As such, presently we have tried to correlate pyridoxine (vitamin-B_6) supplementation to calciuria and oxaluria levels of some normal healthy persons and urinary stone patients.

MATERIALS AND METHODS

Selection of Subjects for Studies

20 normal healthy persons (15 male and 5 female) and 17 urinary stone patients (14 male and 3 female) were selected for study. All subjects were in the age group

of 30-50 years. The healthy persons selected were those with no recent report of ill health of any kind and were engaged in different occupations. The stone patients were selected at random from among those attending the local clinics and were confirmed for suffering from urolithiasis as diagnosed by ultrasonography or x-ray. The patients, however, had no familial incidence of urolithiasis in the recent past. The stone patients were also not under any medication during experiment. All subjects (healthy persons and stone patients) were in the dietary habit of non-vegetarian food. An overview of their dietary recall suggest less chances of vitamin-B_6 deficiency. All of them belonged to middle to low income group.

Collection of Urine Samples

A 24 hour urine output was collected in sterilised plastic containers from each subject. 1% thymol was added as a preservative in each sample. The samples were analysed for calcium and oxalate content by adopting standard methods. Half of the portions of the urine samples was treated with 5ml conc.HCl; and this portion was used for calcium estimation. The samples were used out in minimum possible time after collection. Collection of urine sample, followed by analysis, was continued for 10 consecutive days for each subject. Mean 24 hr urinary calcium and oxalate contents of each subject were calculated out.

On the basis of mean urinary oxalate level, the urinary stone patients were divided into two groups. Those suffering from mild hyperoxaluria (oxalate < 110 mg/ 24 hr) were grouped together. The other group consisted of stone patients suffering from relatively high oxaluria levels (oxalate > 110 mg/24 hr). Thus, all the subjects were divided into three groups as detailed below:

Group-I: This group comprised of all the 20 normal healthy persons.

Group-II: This group comprised of 9 urinary stone patients (7 males and 2 females) who were exhibiting mild hyperoxaluria (oxalate < 110 mg/24 hr).

Group-III: This group consisted of 8 urinary stone patients (7 males and 1 female) who were showing relatively higher (moderate) urinary oxalate levels (oxalate > 110 mg/24 hr).

Subjects in all the three groups were supplemented with pyridoxine hydrochloride (vitamin-B_6) at the rate of 10 mg per day in the form of tablets (Benadon tablets), orally. The supplementation was continued for 60 consecutive days. All subjects were asked to continue their usual dietary pattern during this period. Urine samples (24 hr) of each subject were collected at the interval of every 20 days after the start of vitamin-B_6 supplementation. The samples were analysed for calcium and oxalate contents.

Mean 24 hr urinary calcium and oxalate levels of each group at 20 days intervals were calculated out. Next, mean change of urinary calcium as well as oxalate levels in 60 days of supplementation of vitamin B_6 were calculated out. Corresponding mean percentage changes were also calculated out. t- test was applied to find the level of significance between initial and final urinary calcium/oxalate levels.

Estimation of Calcium in Urine

Calcium in the urine samples was estimated by Trinder's method. The method is based on the principle that calcium present in the sample is precipitated with napthylhydroxamic acid (calcium reagent). The precipitate is dissolved in EDTA reagent and calcium from this solution is complexed with ferric nitrate (colour reagent) to give a coloured complex that is measured colorimetrically (7).

Estimation of Oxalate in urine

The method is based on the principle that oxalate present in the sample is precipitated as calcium oxalate by calcium chloride solution. The precipitate is dissolved in 1.0*N*-H_2SO_4 and titrated against standard potassium permanganate solution to first permanent pink colouration (8).

RESULTS AND DISCUSSION

Hyperoxaluria is one of the most pertinent risk factors in urinary calculogenesis. It precipitates out urinary calcium to deposit as a stone over a suitable nidus (matrix) in the urinary tract. Oxalate stones are of most stubborn type. Urinary oxalate arises from endogenous synthesis as well as dietary source. A majority of the oxalate (85%) is, in fact, from endogenous synthesis only. Increased oxalic acid synthesis is related to metabolic disorders. Deficiency of certain vitamins such as vitamin B_6 have also been attributed to increased oxalate synthesis. Attempts have also been made to delineate molecular mechanism of hyperoxaluria in pyridoxine deficiency (5). Oxalate absorption by the intestine was found to be enhanced in pyridoxine deficiency due to induction of a biphasic carrier mediated phenomenon for oxalate uptake in intestinal brush border membrane (5). Pyridoxine deficiency also led to increased endogenous biosynthesis of oxalate by several folds due to significant increase in activities of liver and kidney peroxisomal enzymes, glycolic acid oxidase and glycolic acid dehydrogenase as well as cytosolic enzyme, lactate dehydrogenase, which are mainly responsible for conversion of oxalate precursor viz., glycolate into oxalate (5). Effect of pyridoxine on the production of enzymes like carboligase and D-glycerate dehydrogenase (defect of which increases

the glyoxylate pool size) is not yet clear. Requirement of pyridoxine is quite low (2 mg/day) and is generally met from sources like milk, liver, meat, egg-yolk, fish, whole grain cereals, legumes and vegetables. Isoniazid, an anti-tuberculosis drug is a recognized antagonist of pyridoxine. Requirement of pyridoxine is also related to dietary protein intake, as it is involved as coenzyme in many metabolic reactions of amino acid metabolism. Since pyridoxine deficiency leads to increased synthesis and absorption of oxalate, pyridoxine therapy might control hyperoxaluria in general.

Data concerning effect of pyridoxine supplementation on urinary calcium and oxalate levels are recorded in Table 1 and Table 2, respectively.

Urinary calcium excretion depends on dietary calcium level as well as its absorption from intestine. Calcium absorption depends on reaction of intestinal contents. Increased alkalinity of the intestinal contents causes its precipitation as phosphate, which is lost in the faeces. Calcium absorption increases with increased acidity of intestinal contents. Urinary calcium excretion is hormonally controlled by parathromone and calcitonin. On an average calcium diet (800 mg/day) the urinary excretion of calcium lies between 100-300 mg/day (9). On a very average to low calcium diet, it might, however, fall between 50-150 mg/day (9). Since most of the subjects, studied presently, belong to middle to low income group, a pre-supplementation (base line) calciuria level of 181.7±23.8 mg/day (stone patients of gr-II) and 192.3 ± 32.7 mg/day (stone patients of gr-III) might be considered as mild hypercalciuria cases.

A study of Table 1 suggests that the urinary calcium levels are not much effected by pyridoxine supplementation. The mean 24 hr urinary calcium levels of any of the groups (healthy as well as stone patients) have not changed appreciably even after 60 days of supplementation. The normocalciuriac persons (Group- I) have shown a meager decrease of 4.7±2.4 mg/24 hr only. Even the hypercalciuriacs (stone patients, Group-III) registered only a small decrement of 8.6±3.2 mg/24 hr. This comes to only 4.5%

Table 1. Effect of pyridoxine supplementation on urinary calcium levels

Group	Mean urinary calcium (mg/24 hr)				Net decrease in calcium level (Initial - Final)	Percentage decrease in calcium level	Level of significance (Initial vs. final calcium levels)
	Pre-supplementation (Initial)	Post supplementation					
		Period (Days)					
		20	40	60 (Final)			
Group-I	122.3±12.5	118.2±14.3	120.2±15.8	117.6±22.6	4.7±2.4	3.8±1.7	NS
Group-II	181.7±23.8	179.6±18.1	175.8±12.3	172.9±18.3	8.8±2.8	4.8±1.9	NS
Group-III	192.3±32.7	194.6±21.7	188.4±24.6	183.7±28.4	8.6±3.2	4.5±2.1	NS

Table 2. Effect of pyridoxine supplementation on urinary oxalate levels

Group	Mean urinary oxalate (mg/24 hr)				Net decrease in oxalate level (Initial - Final)	Percentage decrease in oxalate level	Level of significance (Initial vs. final oxalate levels)
	Pre-supplementation (Initial)	Post supplementation					
		Period (Days)					
		20	40	60 (Final)			
Group-I	46.4± 8.2	45.2± 9.3	44.6± 5.7	42.6± 9.3	3.8±1.7	8.2±1.9	NS
Group-II	93.6±12.3	90.2±13.4	84.3± 9.3	77.4± 6.9	16.2±3.2	17.3±2.4	P < 0.01
Group-III	138.5±16.1	133.4±13.5	118.2±12.3	82.7±14.5	55.8±6.4	40.8±4.6	P < 0.001

decrease over initial value. Thus it looks that vitamin-B6 does not have any direct role in urinary calcium output.

A study of Table 2, however, suggests that vitamin-B_6 has an effect on urinary oxalate levels. Reference intervals of urinary oxalate lie between 20-60 mg/day for men and 20-55 mg/day for women (9). Subjects of Group-I, who had normal urinary oxalate levels, showed a decrease of 3.8±1.7 mg/24 hr oxalate after 60 days of supplementation. The corresponding percentage increase has been 8.2±1.9. The stone patients, suffering from mild hyperoxaluria (Group-II), have shown a decrease of 16.2±3.2 mg/24 hr oxalate after 60 days of supplementation of vitamin-B_6 at the rate of 10 mg/day. This amounted to a 17.3±2.4% decrease. In case of stone patients, suffering from initially elevated oxaluria (Group-III),vitamin-B_6 supplementation has significantly lowered the urinary oxalate levels by 60 days. The decrease has been as high as 55.8±6.4 mg/24 hr. Compared to the mean initial value, the decrease has been by 40.8±4.6%. The rate of decrement of oxalate levels, however, been quite slow in the beginning in all the cases. By first 20 days of supplementation, none of the groups showed any significant change in urinary oxalate levels. Even after 40 days, some significant lowering was visible only with Group-III. It seems, a long term supplementation of vitamin-B_6 can only effect (decrease) urinary oxalate levels significantly. This might, perhaps, be due to a slow onset of decrease of endogenous oxalate synthesis caused by increased vitamin-B_6 load.

CONCLUSION

Our observations in the present work show that oral supplementation of pyridoxine (vitamin-B_6) for a long term to hyperoxaluriac urolithiasis patients can significantly lower the urinary oxalate levels. The urinary calcium levels, however, are not affected to any significant extent. Hypercalciuria, in fact, is not a direct index of urolithiasis risk. It is the urinary anions, that precipitate out calcium, are more pertinent in urinary calculogenesis. Thus oxalate level in urine assumes more importance in stone formation. A control on endogenous oxalate synthesis and related hyperoxaluria is desirable in the prevention of urinary stone formation. On the whole our present study indicates that the urolithiasis patients suffering from moderate/severe hyperoxaluria should be benefited by a long term pyridoxine therapy.

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