THE ROLE OF SERUM AND URINARY CALCIUM LEVELS IN RENAL LITHIASIS

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Serum and urinary calcium levels play a major role in the aetio-pathogenesis of renal stone formation. In this study, serum and 24 hours' urinary calcium of 30 healthy controls and 60patients of renal lithiasis were studied. The mean age of controls and patients was 34.9 (range 3-60 years) and 37.5 years (range 3 to 70 years) respectively. The male to female ratio of controls and patients was 1.6:1 and 1.5:1 respectively. Chemical analysis of stones showed 42 (70.0%) were pure calcium oxalate, 2 (3.3%) pure uric acid, while the others were mixed stones. Calcium was found in 58 (96.7%) stones. The overall mean + SE of serum calcium among the controls and patients was 9.43 ± 0.14 and 9.46 ± 0.15 respectively, which is statistically not significant. Although the overall values of calcium of controls and patients was similar, its distribution of concentration showed that 1(3.3%) control and J1 (18.3%) patients were hypercalcemic, while 4 (13.3%) controls and 19(31.7%) patients were found hypercalciuric. Idiopathic hyper-calciuria was found in 3(10%) of controls as compared to 8(13.3%) patients. As hypercalciuria is the more common laboratory finding compared to hypercalcemia among these renal stone patients, so routine screening of 24 hours' urinary calcium is valuable in diagnosis and aetiology of calcium-containing renal stones.

INTRODUCTION

Calcium is the most abundant mineral in the body. It is about 1 kg in a 70 kg man. About 99% of the body calcium is present in bones as a complex salt known as hydroxy apatite, 3 Ca3(PCL)2, Ca(oH)2. The normal level of calcium in plasma is 8.4-10.5 mg/dL (2.1-2.6 mmol/L). It is present in plasma as ionized calcium (50-65%), bound to proteins mainly albumin (30- 45%), and as complexes with organic ions such as citrate etc. (5-10%)

Normal urinary calcium excretion is less than 300 and 250 mg per 24 hours in men and women respectively. The normal dietary intake of calcium is 500-1000 mg daily, the gut being the sole site of entry for calcium, which is regulated by the action of vitamin D. A high intake of refined carbohydrate, high protein intake and/or high salt intake increases the amount of calcium absorbed[2].

Adults maintain zero balance, with 99% of the body's total calcium content (1 kg) in the bone. The kidneys filter 10 g daily but only about 40-300 mg appears in the urine, i.e 98% is absorbed under the control of parathyroid hormone (PTH)[3]. Urinary calcium shows postprandial and diurnal variations. There is increased calcium and oxalate excretion in the summer months. This is due to increased vitamin D synthesis in the skin. Stone formers show a 50% rise in the recurrence rate of stones in the summer [4].

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This is the effect of the degree of saturation of urine. Hypercalciuria is important in stone formation because a rise in calcium concentration is as effective as oxalate in raising the activity product of calcium oxalate. According to international definition of hypercalciuria, urinary excretion of more than 300 mg calcium per day in men and more than 250 mg calcium per day in women or more than 4 mg calcium per Kg body weight per day regardless of sex or age was considered as hypercalciuria[3,6].

Hypercalciuria may occur due to increased intestinal absorption of calcium because of increased production of parathyroid hormones. Hypercalciuria is a frequent finding in patients with calcium oxalate nephrolithiasis and contribute to urine hyper-saturation with respect to calcium oxalate. Calcific stones are about 80% in England and 90% in North America[7].

Because hypercalciuria is important in stone formation, so the study was undertaken to access the aetio-pathogenesis of calcium in renal stone patients.

MATERIALS AND METHODS

Observations were made on 60 patients of urolithiasis from different hospitals of Lahore. Patients were selected before open renal surgery or Extracorporeal Shock Wave Lithotripsy (ESWL) and 30 healthy controls were selected from the students and staff of Sheikh Zayed Hospital with no history of stone or renal disease.

A day or two before the operation a random blood sample (10 ml) and 24 hours' urine sample were

collected. The collected samples were transferred to biochemistry laboratory for analysis. All the samples

were collected while the subjects were on normal daily activities and diet.

24 hours' urine was collected in disposable plastic jars. 10 ml HCL was added as preservative and to dissolve any crystals of calcium salts that might be present. The acidified samples were analyzed immediately or stored at -18°C for 1-2 days before analysis.

Serum and urinary calcium was determined with automatic analyzer (Technicon 2010) by using o-cresophthalein complexone method[8]. Urine was diluted 1:4 ratios with deionized water prior to assay. Renal stones were analyzed for chemical composition using E. Merck's urinary calculi kit based on Maurer and Gotz[9].

RESULTS

Sixty patients of urolithiasis and 30 normal healthy controls were included in this study. The mean age of the patients was 37.5 years (range 3-70 years) and of controls 34.9 years (range 3-62 years). Their male and female ratios were 1.5:1 and 1.6:1 respectively. The highest incidence of stone disease was in the age groups of 26-55 years (61.6%).

Among this group, the peak incidence was between 26-35 years (23.3%) followed by 36-45 years (20.0%) and 46-55 years (18.3%). The incidence in lower and upper age groups was comparably low (Table-1).

AGE AND SEX DISTRIBUTION OF PATIENT AND CONTROL SUBJECTS

Age group (years)	Controls			Patients		
	Male	Female	Total	Male	Female	Total
Upto 15	1	-	1	3	3	6
16 - 25	5	3	8	6	2	8
26 - 35	7	1	8	10	4	14
36 - 45	3	3	6	5	7	12
46 - 55	2	1	3	9	3	12
56 - 65	-	4	4	3	3	6
Over 65	-	-	-	1	1	2
Total	18	12	30	37	23	60
M:F ratio	1.6:1			1.5:1		

Most of the patients (83.7%) belonged to urban area. Ninety-three percent of patients belonged to low and middle socio-economic status.

TYPE OF CALCULUS

Calcium oxalate pure stones were the commonest of all the stones analyzed, i.e. in 42 patients (70%), while calcium oxalate mixed with other compounds were found in 15 patients (25.1%). Pure uric acid stones were found in 2 (3.3%) of the stones analyzed while in mixed types, uric acid component was found in 11.7%. One patient (1.7 %) had a pure calcium phosphate stone. (Table-2).

TABLE-2: TYPE OF CALCULUS FORMATION (n = 60)

Type of Calculus	Number	%
Calcium Oxalate Calcium Oxalate and	42	70.0
Magnesium Ammonium Phosphate	04	6.7
Uric Acid(mostly) and Calcium Oxalate	04	6.7
Calcium Oxalate (mostly) and Uric Acid	03	5.0
Calcium Phosphate and Calcium Oxalate	03	5.0
Uric Acid	02	3.3
Calcium Oxalate and Magnesium Phosphate	01	1.7
Calcium Phosphate	01	1.7
Total	60	100

CHEMICAL COMPOSITION OF RENAL STONES

Chemical composition of stones showed that calcium was present in 58 (96.7%) stones analyzed, while oxalate was in 57 (95%) stones, followed by phosphate (9, 15%), uric acid (9, 15.0%), magnesium (4, 6.7%) and ammonium (4, 6.7%) (Table-3).

TABLE-3: CHEMICAL COMPOSITION OF RENAL STONE (n=60)

RADICAL	NUMBER OF STONES	%
Calcium	58	96.7
Oxalate	57	95.0
Phosphate	09	15.0
Uric Acid	09	15.0
Magnesium	04	6.7
Ammonium	04	6.7

In controls and patients, the overall mean ± SE urine volume was 1650 ± 75 and 1440 ± 59 ml respectively, which is statistically significant ($p < 0.005$). The overall urine specific gravity of controls and patients was 1.015 ± 0.0011 and 1.019 ± 0.0092 respectively, which is statistically highly significant ($P < 0.005$). The 24 hours' urine pH of controls and patients was 5.81 ± 0.1 and 5.6 ± 0.1 respectively, which is statistically not significant. The overall mean ± SE of pH, specific gravity and volume is shown in table-4.

The overall mean ± SE of serum calcium in controls and stone formers was 9.43 ± 0.14 (range 7.4-11.1 mg/dl) and 9.46 ± 0.15 (range 6.0-11.8 mg/dl) respectively, while the mean serum calcium levels in stone formers was slightly greater than controls, but statistically it was not significant. The distributions of serum calcium levels among the subjects show that

hypercalcemia was found in 3.3% controls and 18.3% patients; while 2 (6.7%) controls and 6 (10.0%) patients were hypocalcemic (Tables- 5,6).

TABLE-4: OVERALL MEAN ± SE OF 24 HOURS URINARY pH, VOLUME AND SPECIFIC GRAVITY

GROUPS	pH VOLUME (ML) SPECIFIC GRAVITY
Controls	5.80 ± 0.10 1651 ± 75 1.015 ± 0.0011
Patients	5.65 ± 0.10 1444 ± 59 1.019 ± 0.00092
p value	N.S < 0.05 < 0.005

TABLE-5: THE OVERALL MEAN ± SE OF SERUM CALCIUM OF SUBJECTS

GROUPS	SERUM CALCIUM (mg/dl)
Controls	9.43 ± 0.14
Patients	9.46 ± 0.15
p Value	N.S.

TABLE-6: THE DISTRIBUTIONS OF DIFFERENT CONCENTRATIONS OF SERUM CALCIUM LEVELS AMONG THE SUBJECTS

	SERUM CALCIUM LEVELS (mg/dl)			
GROUPS	< 8.0	8.1-9.0	9.1-10.5	> 10.6
Controls	2 (6.7%)	8 (26.7%)	19 (63.3%)	1 (3.3%)
Patients	6 (10.0%)	12 (20.0%)	31 (51.6%)	11 (18.3%)

The overall mean + SE of urinary calcium excretion in controls and patients was 210 + 13.76 and 266 ± 23.26 mg/24 hours respectively, which is statistically significant ($p < 0.05$). In our study, 4 (13.3%) controls and 19 (31.7%) patients were hypercalciuric (Table-7,8).

Although only 1(3.3%) control and 11(18.3%) patients were hypercalcemic, but hypercalciuric among the controls and patients were 4(13.3%) and 19(31.7%) respectively, which shows that 3(10.0%) controls and 8(13.3%) patients were having idiopathic hypercalciuria.

TABLE-7: THE OVERALL MEAN ± SE OF 24 HOURS URINARY EXCRETION OF CALCIUM AMONG THE SUBJECTS

GROUPS	URINARY CALCIUM (mg/DAY)	P VALUE
Controls M F	210.4 ± 13.76 226 ± 4.64 186 ± 4.64	
Patients M F	266.6 ± 23.26 299.2 ± 5.66 214.3 ± 4.46	< 0.05 <0.05 N.S.

TABLE-8: THE DISTRIBUTIONS OF IFFERENT CONCENTRATIONS OF URINARY CALCIUM EXCRETION AMONG THE SUBJECTS

GROUPS	**CALCIUM (MG/DAY)** **< 250 251-300 > 300**
Controls (n=30) M (n=18) F (n=12)	 14 02 02 10 02
Patients (n=60) M (n=37) F (n=23)	 15 09 13 10 02 --

DISCUSSION

The results show that hypercalcemia and hypercalciuria are important risk factors, but in our study hypercalciuria is the more predominant risk factor.

Actually the amount of calcium excreted in urine is not directly correlated with calcium intake, and the restriction of dietary calcium has little effect on hypercalciuria[10,11]. This is probably because a feedback mechanism involving PTH and calcitriol suppresses the absorption of calcium from the intestine.

The urinary calcium level is correlated with the intake of animal protein rather than calcium intake[12]. When the protein intake is increased, the amount of calcium excreted in urine increases irrespective of content of calcium in diet[13]. As for the mechanism underlying the increase in urinary calcium, an increase in glomerular filtration rate was proposed, but it seems more likely that the ingestion of protein increases the production of fixed acid to incline the body fluids towards acidosis, in which state the amount of calcium reabsorbed in the renal tubules is reduced[14]

Hypercalciuria is defined as urinary calcium excretion of 300 mg/day in men and 250 mg/day in women or more than 4 mg calcium per Kg body weight per day regardless of age or sex[5]. In the last decade or so the practical usefulness of the 24 hours' urinary calcium determination has come to be questioned [1516]. Increased urinary calcium excretion is a risk factor for nucleation and precipitation of calcium oxalate and/or calcium phosphate from the urine.

Hypercalciuria has been observed in patients of renal lithiasis since 50 years ago[18]. There are groups of patients with renal lithiasis who have exhibited hypercalciuria without hypercalcemia, although these patients did not have a history of excessive consumption of calcium, vitamin D intoxication,

sarcoidosis, hyperthyroidism, Cushing syndrome, malignant tumor, rapid bone loss or renal tubular acidosis. For these patients the term idiopathic hypercalciuria was used[19].

In our study the overall mean ± SE of urinary calcium excretion of controls and patients was 210 ± 13.13.76 and 266.6 ± 23.26 mg/24 hours. The difference was statistically significant ($p < 0.05$).

According to international definition of hypercalciuria, in our study 4 (13.3%) controls and 19 (31.7%) stone formers had hypercalciuria. In our study the daily excretion of calcium was higher than other studies done in Pakistan. The comparisons are shown in Table-9.

TABLE-9: 24 HOURS EXCRETIONS OF CALCIUM IN VARIOUS STUDIES DONE IN PAKISTAN

STUDIES DONE IN PAKISTAN	STONE FORMERS	FIRST EPISODE	RECURRENCE	CONTROLS
Shahjahan & Rehman 1971 in Karachi	157.9±24.2	-	-	148.7 + 13.0
Khanum 1981 in Karachi	151 ± 4.86	-	-	136.91 ±7.2
Wyne 1982 in Lahore	-	168.4 ±84.7	194.4 ± 80	136.2 ±47.2
Hussain 1985 in Lahore	-	188 ± 63	208 ± 30.4	108.8 ±30.4
Khan 1993 in Dera Gazi Khan	101.7±64.3	-	-	76.39±30.8
Present study	266.6±23.2	-	-	210 ± 13.76

All these studies show that the urinary calcium in patients is higher as compared to controls. The presence of higher levels of hypercalciuria in our study compared to other studies done in Pakistan is a positive finding, which shows that either the calcium intake among our subjects is higher due to better economic conditions, or that we used better determination methods.

In the west, hypercalciuria among the renal lithiasis patients is about 50%[19]. Rose and Harrison[20] demonstrated that hypercalciuria was found in 60% of the patients. More recent studies from the west have reported an incidence of 30-40% hypercalciuria in renal lithiasis patients.

In our study, hypercalcemia was found in 1(3.3%) control and 11(18.3%) patients, while hypercalciuria was found in 4 (13.3%) controls and 19 (31.7%) patients. This shows that 24 hours urinary calcium is of more value than serum levels in the diagnosis of calcium containing urinary stones. Further in early hyperparathyroidism (if calcium intake is adequate), mild hypercalcemia and hypercalciuria are common findings.

In the present study, the urinary calcium was significantly increased in renal lithiasis cases, which shows that hypercalciuria is a risk factor and knowledge of the source(s) of excess urinary calcium may provides insight into pathogenesis and optimal therapy.

' Excessive urine calcium may result from increased bone resorption or defective renal tubular reabsorption[21].

Quantitative estimation of Hypercalciuria may distinguish patients with primary hyperparathyroidism (relatively less excretion) from those with idiopathic hypercalciuria (relatively more excretion). Idiopathic hypercalciuria may be the result of a primary defect of the enterocytes (with increased absorption), or a primary over-production of 1, 25(OH)2 D3; there is evidence for both.

It is necessary to reduce urine calcium among people having hypercalciuria either due to increase bone resorption or idiopathic hypercalciuria, so that the risk of stone disease could be prevented. This may be achieved by giving phosphate buffers in the diet, which complex with intestinal calcium to form insoluble and nonabsorbable calcium phosphates.

In our study the daily excretion of calcium by both male and female stone formers was higher than normal men & women, but higher excretion of calcium among the female stone formers as compared to normal females was statistically not significant. The higher excretion of calcium among the stone formers of both sexes indicated that hypercalciuria is clearly a function of their disease, though in females larger and more sensitive studies may be needed.

It is also concluded that higher excretion of calcium among male subjects as compared to female subjects, regardless of presence or absence of stone disease, shows that gender is indeed instrumental in lowering the occurrence of stone disease in women; if their excretion of calcium is increased to male levels, then their susceptibility to stone disease increased accordingly.

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