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Biochemical Study of Serum Factors in Male Patients of Nephrolithiasis

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Abstract: *Nephrolithiasis a multi-factorial disorder resulting from the combined influence of environmental, biochemical and genetic factors. Maximum stones were in mixed form, Calcium oxalate and phosphate stones are more common in men; peak age of incidence in our study was in the fourth decade of life. Nephrolithiasis was slightly prevalent in non-Veg dietary habits and with average daily water intake was low (1-1.2Lit) as compared to controls (1.5-2Lit) In this study we find that stones were slightly prevalent in Hindus (53.33%) over muslims (46.67%). We find that Nephrolithiasis cases were higher in urban area (60% cases) in all age groups. 43.33% (n=13) cases had positive family history of nephrolithiasis. 76.67% cases (n=23) were diagnosed at first time while 23.33% cases (n=7) presented as recurent one. In 16.67% cases (n=5) also given the history of spontaneous passage of stones in their urine. The serum biochemical parameters were considerably higher, calcium (10.43 ± 0.66), phosphorus (4.01 ± 0.69) and uric acid (5.95 ± 1.64) in cases as compared to controls and significant.*

Key words*: Nephrolithiasis, serum biomarkers, hypercalcemia.*

I. Introduction

Predominance of Urinary tract stones (urolithiasis) incidence has shifted from lower urinary tract to upper urinary tract (Nephrolithiasis) with the passage of time. WHO kept this disease under "**Refractory diseases**" whose etiology is still questionable and medical management is incomplete. Kidney stone disease is mainly a multi-factorial disorder resulting from the combined influence of environmental, biochemical and genetic risk factors. It occurs both in men and women but the risk is generally high in men. Approximately 80% of kidney stones occur in men. Men commonly experience their first episode between ages 30–40 years, while for women the age at first presentation is somewhat later.

Life time recurrence rate is 80% [1] and 20% develops mild renal insufficiency [2]. Metabolic evaluation of renal calculus disease has shown one of the identifiable and treatable diseases in western countries and selective medical therapy reduces the recurrence up to 95% [3]. Environmental, genetic and dietary factors implicated in the pathogenesis of nephrolithiasis with wide variability [4]. Out of these urinary abnormalities are claimed by most.

Human urine contains stone forming metabolic substances (promoters and inhibitors) and has potential to induce spontaneous mineralization by their delicate balance. Tamm–Horsfall Protein (THP) has been reported to behave both as promoter and inhibitor depending on urinary pH, ionic strength and chemical milieu. When the urine is supersaturated with insoluble materials, because excretion rates are excessive and / or because water conservation is extreme, crystals form and may grow and aggregate to form a stone.

As there are limited data regarding the recurrence and metabolic evaluation in Indian context we studied the biochemical risk factors in male patient of nephrolithiasis in North Indian patients (Lucknow).

II. Material And Methods

This observational case control study comprised of cases that are coming to Era's Lucknow Medical College & Hospital Lucknow during the period from January 2011 to 31st December 2011. 30 cases and 30 controls were randomly selected with all due ethical and legal formalities. The subjects were relatively matched as much as possible. Only the male patients above 18 year were included in this study. The patient having other associated disease which could influence the stone risk factors and those with congenital and other anatomical defects were excluded from the study.

Detailed history regarding age, place of residence (rural or urban), dietary habits, any addiction, passage of stones spontaneously in urine, family history of stones, history of fresh case or recurrence and any treatment taken in the past were recorded.

Venous blood (antecubital vein) collected with all aseptic precautions after overnight fast.. Samples were centrifuged after being kept for 30 minutes for separation of serum. Then poured in cuvette and subjected for biochemical analysis, which was done using kit method on Transasia ERBA XL 300 Auto analyser following the technical bulletin of manufacturer. Test sample analyzed for total calcium (Arsenazo Method), uric acid (Uricase Method), creatinine (Jaffe's Method) and phosphorus by (UV molybdate method).

Stone were analyzed by Wet Chemical Analysis method. After collection of stones and washing with distal water and dry in a incubator. Larger stones which consist of several layers so it is cut in half and cut surface examined. Very small stones are powdered. Size, appearance and hardness of stones were noted.

III. Result And Discussion

Acute renal colic is probably the most excruciatingly painful event a person can endure. Striking without warning, the pain is often described as being worse than childbirth, broken bones, gunshot wounds, burns, or surgery. A variety of investigative programs have been suggested to elucidate etiopathogenesis of renal calculi. Amongst these although the more important and uninanimously agreed protocol is "URINALYSIS" and "STONE ANALYSIS" (if and when available) for prevention as well as control of recurrence but serum biochemical parameters also suggested.

The serum and urinary profile shows large fluctuation depending upon genetic and familial disposition, living style, occupation, nutritional factors, and idiosyncrasies, geographical/climatic variations, aggravated by pollution. This has been the reason for difference in incidence and prevalence of urolithiasis in different population belonging to different pockets in India.

Peak incidence of nephrolithiasis cases occurs in 4^{th} decade of life (46.67%), followed by 5^{th} decade (40%) and lowest in 6^{th} decade and onwards (3.33%) overall mean age (39± 6.7) [table 1]. Although the disease affects all age groups from less than 1 year to more than 70 years. Julka et al. in their study observed similar finding with mean age 38 ± 10.2 years. [4] Kumar et al. also have the similar findings with mean age of patients being 39 years and similar observations were in the studies of various authors.[5,6] Baker et al. reported that peak age for the development of calcium oxalate stones was in between 50–60 years [7]. In men, the incidence of kidney stones declines markedly after 60 years of age, suggesting that the pathophysiology of nephrolithiasis is different in the elderly [8, 9].

Our study Shows that stones were slightly prevalent in Hindus (53.33%) over muslims (46.67%) [Table 1]. Our finding similar to the population based study in Manipur where Hindus were affected more than Muslims; this could be due to the characteristic dietary habits of Hindus of that area [10]. Although Pendse et al. showed that Muslims were affected more than Hindus. Being a smaller sample size and lack of population based study this could be the fortuitous finding [11].

The renal stones are slightly more prevalent in urban areas 60% cases(n=18) and lesser in rural areas 40% cases (n=12) [Table 1]. Nephrolithiasis cases were higher side in urban area in all age groups. Similar findings are reported elsewhere [12].

The most dreadful aspect of the disease is its recurrence. In our study 23% (n=7) patients showed recurrence within 5 years supporting the findings of Rajabala who had reported a recurrence rate of 20.9% and Singh et al. in their two series found it to be 15.8% and 16.8% respectively [13, 14].

Average daily water intake were somewhat lesser in patients (5.6 glasses) as compare to (6.67 glasses) in controls. One glass of water is approx 250ml. Majority of patient having the habit of daily water intake between 5 to 6 glasses whereas controls have 7 to 8 glasses [Table 2]. Supersaturation of the urinary environment with stone -forming constituents is prerequisite for calculus formation. A low fluid intake (<1200 ml/day) predisposes to stone formation [15].

Stones were more prevalent in non-vegetarian (56.67%) than vegetarian (43.33%). Nephrolithiasis in non-vegetarian dietary habits had higher incidences from 4^{th} decade onwards [Table 3]. Increased incidence of renal calculi is associated with a more 'affluent' diet (increased animal protein, refined sugar and salt) [16, 17] Animal protein increases urinary calcium, oxalate and uric acid along with causing more acidic urine, contributing to calcium oxalate over saturation and precipitation. Awasthi, et al. reported that non vegetarians have higher risk of stone formation (67.95 % of total male patients were non-vegetarian while 19.23% were vegetarian) [18].

The cases included in this study were symptomatic at the time of presentaion. In 43.33% (n=13) cases patients gave positive family history of nephrolithiasis. 76.67% cases (n=23) were new cases and diagnosed first time while 23.33% cases (n=7) presented as recurent one. In 16.67% cases (n=5) history of spontaneous passage of stones in their urine were present. [Table 4] Familial risk of nephrolithiasis has been reported in about 16-37% of patients. People with family history of kidney stones are at higher risk for the development of stones. [19, 20, 21]. This implies that the influence of extrinsic factors superimpose on intrinsic causes [22]. It may be suggested due to living in the same environment along with having the similar dietary habits, lifestyles and activities.

The chemical composition of recovered stones, Calcium was inherent component in 93.33% (n=28) cases. Oxalate was the next common component with its presence in 76.67% (n=23)cases. Next common components with decreasing orders of frequency were phosphate, uric acid and carbonate with 53.33% (n=16), 16.67% (n=5) and 13.33% (n=4) respectively [Table 5].

The most of the Indian authors closely resemble with our result in that the percentage of calcium and oxalate stones were the highest. Kaur, et al. observed that stones of mixed chemical composition were the commonest (90.4%). Calcium was the main constituent (90.4%) in renal stones followed by oxalate (81.9%), uric acid (47.8%) and phosphates (43.6%). The least encountered was carbonate (11.7%) [23].

The constituents of recovered stones analysed qualitatively. The kidney stones are mainly found in mixed forms 93.33% cases (n=28) and scarcely in pure forms only in 6.67% cases (n=2). Calcium and oxalate were the most common components in mixed form of stones 76.67%. In mixed forms most prevalent composition of stone were the mixture of $Ca+Ox+UA+PO_4$ in 30% cases (n=9). Next common composition were Ca+Ox (23.33%), $Ca+Ox+PO_4$ (13.33%), $Ca+CO_3$ (10%), Ca+Ox+UA (6.67%), $Ca+PO_4$ (6.67%) and $Ca+Ox+UA+PO_4+CO_3$ (3.33%) in decreasing order of frequency [Table 6].

Jehangir, from Lahore demonstrated that 80% of renal stones were of mixed type [24]. Singh et al. from Delhi showed urate in 38.2% of cases [25]. Some of the reasons for this high incidence of mixed stones in local population might be as follows: Non-vegetarian diets (animal protein lowers citrate excretion and increases calcium and uric acid excretion). Insufficient dietary intake of fruits and potassium rich vegetables, which can affect urine chemistry considerably. Diet with high oxalate content, high carbohydrate intake (especially rice), which provides acidic medium to urine favoring calcium oxalate stone formation.

Serum biochemical parameters in patient blood shows mean calcium is 10.43 ± 0.66, phosphorus 4.01±0.69, uric Acid 5.95±1.64 and mean creatinine is 0.94±0.21. Mean serum calcium is highest in 3rd decade (10.83±0.91) and lowest in 6th decade. Mean serum PO_4 was observed highest in 6th decade (4.5±0.0) and lowest in 5th decade (3.9±0.73). Mean serum Uric acid was observed highest in 3rd decade (7.8±2.6) and lowest in 6th decade (5.4±0.0). Mean serum creatinine was observed highest in 4th decade (0.96±0.21) and lowest in 6th decade (0.69±0.0) [Table 7].

In our patients with nephrolithiasis, though the levels of serum calcium, uric acid, phosphorus and creatinine are within normal limits, but their levels are on higher side in patients than controls. Although blood analysis is not as important as urinalysis but various workers have reported its usefulness for detection of some specific condition eg. Hypercalcaemia may indicate the involvement of parathyroid and hyperuricemia usually indicates error in enzymes of purine metabolism or gouty nephrolithiasis. Tanksale et al. also shown the similar findings calcium 10.2± 0.5 mg/dl, phosphorus 3.3± 0.6 mg/dl, uric acid 6.1± 0.3 mg/dl [26]. Gyawali and Fuad hussain et al. found more or less similar observations [27, 28].

In our study, though none of the patients showed distinct tendency towards hypercalcemia (above 10.5 mg/dl) with 83.33% cases showing hypercalciuria with calcium level of >200mg/day. Tanksale et al. found hypercalciuria in 78% with calcium level of 10.2 ± 0.5 mg/dl. After comparing the results of various studies we infer that disturbance in calcium metabolism and even marginal hypercalcemia is responsible for hypercalciuria [26].

In our study we found that uric acid level was 5.95 ± 1.64 mg/dl, which was within normal limit but towards higher side. Two patients had pure uric acid stones with serum uric acid level was 8.5 mg/dl and 6.1 mg/dl. The creatinine levels are within normal limit.

The serum biochemical parameters in cases and controls, mean age of nephrolithiasis cases in our study was 39.03±6.74, mean serum calcium was found higher in cases (10.43 ± 0.66) than controls (9.29 ± 0.88) and found significant. The mean serum phosphorus was higher in cases (4.01 ± 0.69) than controls (3.43 ± 0.72), the mean serum uric acid was also higher in cases (5.95 ± 1.64) than controls (4.55 ± 0.77) with significant p value [Table 9].

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Table 1: Age wise distribution of nephrolithiasis between two major religion Hindu/Muslim and Rural/Urban. **(Fig. 1-3)**

Age-Group	Cases (%)	Hindu	Muslim	Rural	Urban
20-30	3 (10%)	2	1	1	2
31-40	14 (46.67%))	7	7	7	7
41-50	12 (40%)	6	6	4	8
>51	1 (3.33%)	1	0	0	1
Total	30	16(53.33%)	14(46.67%)	12(40%)	18(60%)

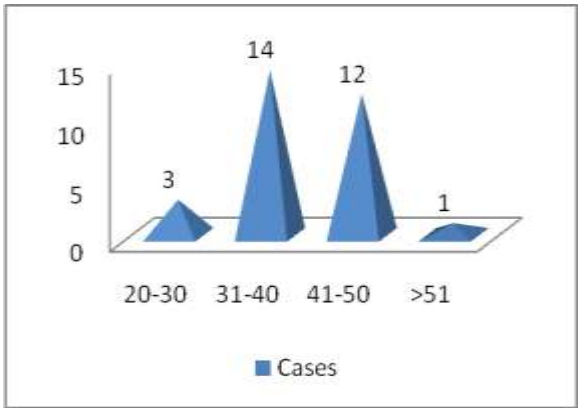

Fig.1

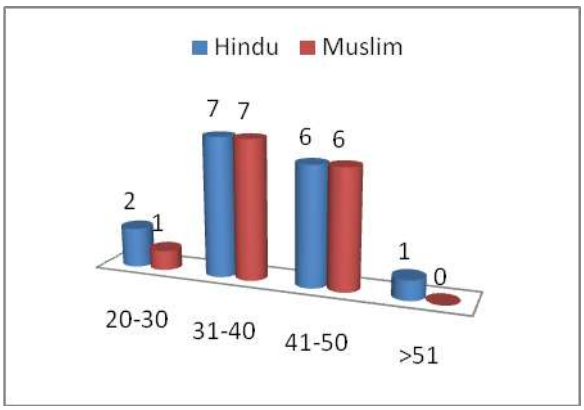

Fig. 2

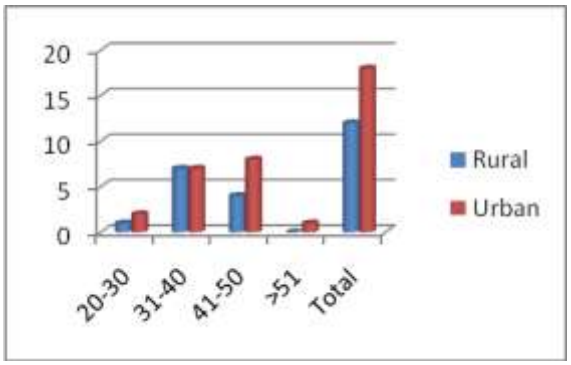

Fig. 3

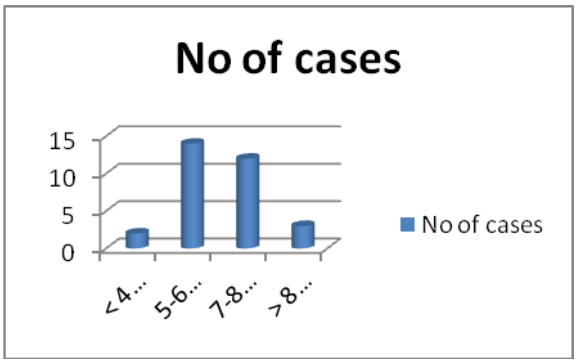

Fig.4

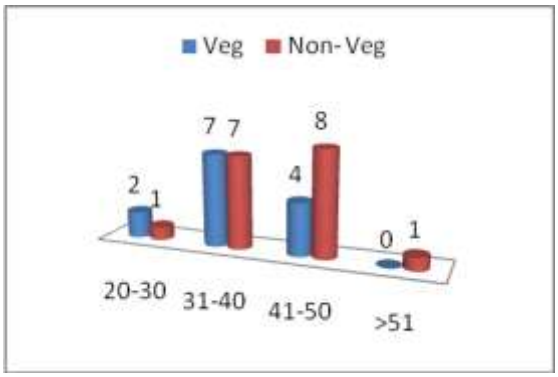

Fig. 5

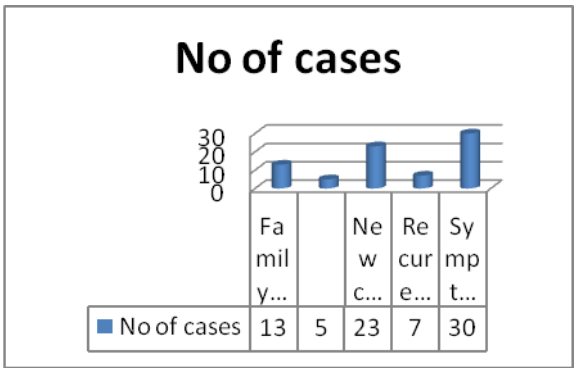

Fig. 6

Table 2: Average daily water intake. (Fig. 4)

Water intake	Cases	Controls
4 Glasses & <	2	2
5-6 Glasses	22	12
7-8 Glasses	5	14
> 8 Glasses	1	2
Average	**5.6 Glasses**	**6.67 Glasses**

Table 3: Showing major dietary habits of the cases. (Fig. 5)

Age-Group	Vegetarian	Non- Vegetarian
20-30	2	1
31-40	7	7
41-50	4	8
>51	0	1
Total(%)	**13(43.33%)**	**17(56.67%)**

Table 4: Showing some features revealed by history through questionnare. (Fig. 6)

S. No.	Feature	No of cases (%)
1	Family History of stone	13(43.33%)
2	History of passage of stone	**5(16.67%)**
3	New cases	23(76.67%)
4	Recurrence	**7(23.33%)**
5	Symptomatic	30(100%)

Table 5: Showing the frequency of renal stone according to their chemical composition (Qualitative). **(Fig. 7)**

Chemical constituents	No of renal Stones	Percentage
Calcium	28	93.33%
Oxalate	**23**	76.67%
Phosphate	16	53.33%
Uric Acid	**5**	16.67%
Carbonate	4	13.33%

Table 6: Distribution of chemical composition of renal stones. **(Fig. 8)**

Chemical constituents	No of renal Stones	Percentage
Pure		
Oxalate	**0**	0.00%
Uric Acid	2	6.67%
Mixed		
Ca+Ox	7	23.33%
Ca+Ox+UA	**2**	6.67%
Ca+Ox+UA+PO_4	9	30.00%
Ca+Ox+UA+PO_4+CO_3	1	3.33%
Ca+Ox+PO_4	4	13.33%
Ca+PO_4	2	6.67%
Ca+CO_3	3	10.00%

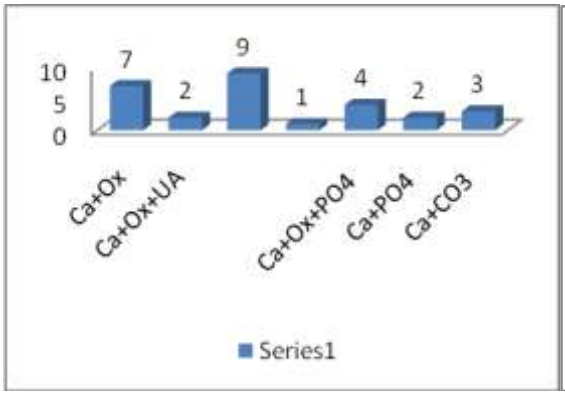

Fig. 7

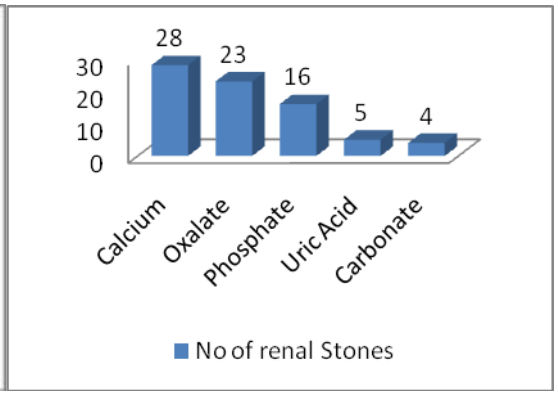

Fig. 8

Table 7: Shows age group wise mean biochemical parameter in serum of patients. (**(Fig. 9)**)

S. No.	Age Group (yrs)	No. of Cases	Calcium (mg/dl)	Phosphorus (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
1	20-30	3	10.83 ± 0.91	4.03 ± .32	7.8 ± 2.6	0.95 ± 0.39
2	31-40	14	10.67 ± 0.53	4.1 ± 0.74	5.7 ± 0.88	0.96 ± 0.21
3	41-50	12	10.14 ± 0.57	3.9 ± 0.73	5.9 ± 1.98	0.93 ± 0.17
4	➢ 51	1	9.2 ± 0	4.5 ± 0.0	5.4 ± 0.0	0.69 ± 0.0
5	**overall mean**		**10.43 ± 0.66**	**4.01 ± 0.69**	**5.95 ± 1.64**	**0.94 ± 0.21**

Table 8: Shows age group wise mean biochemical parameter in serum of controls. **(Fig. 10)**

S. No.	Age Group (yrs)	No. of Control	Calcium (mg/dl)	phosphorus (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
1	20-30	9	9.6 ± 0.8	3.9 ± 0.57	4.5 ± 0.54	0.95 ± 0.01
2	31-40	10	8.9 ± 0.7	3.4 ± 0.23	4.9 ± 0.78	0.9 ± 0.02
3	41-50	9	9.3 ± 0.95	3.3 ± 0.52	4.2 ± 0.57	1.05 ± 0.03
4	➢ 51	2	9.6 ± 0.01	2.4 ± 0.3	4.8 ± 0.08	0.98 ± 0.1
5	**Overall mean**		**9.29±0.88**	**3.43±0.72**	**4.55±0.77**	**0.97±0.16**

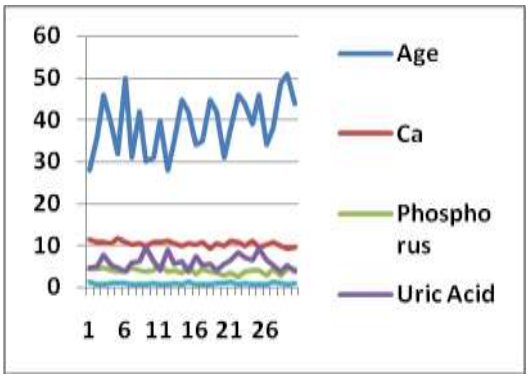

Fig. 9

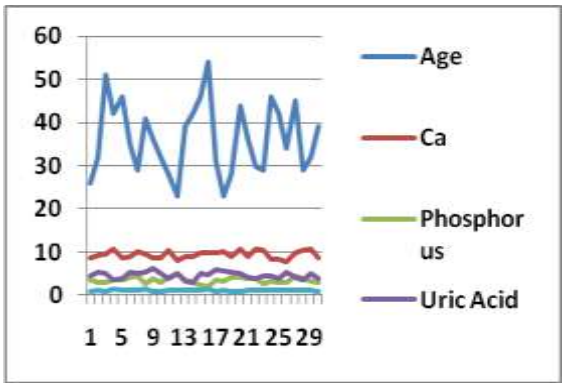

Fig. 10

Table 9: Value for serum chemistry in tests of Cases and Control (mean±stdev).

Features	**Age (years)**	**Calcium (mg/dl)**	**Phosphorus (mg/dl)**	**uric acid (mg/dl)**	**Creatinine (mg/dl)**
Cases (n=30)	39.03 ± 6.74	10.43 ± 0.66	4.01 ± 0.69	5.95 ± 1.64	0.94 ± 0.21
Controls (n=30)	38.23 ± 7.03	9.29 ± 0.88	3.43 ± 0.72	4.55 ± 0.77	0.97 ± 0.16
P value	0.655	**0**	**0.002**	**0**	0.59