Analysis of Urinary Calculi by Chemical Methods

Pages with reference to book, From 147 To 153

S.A. Anwar Naqvi, S. Adibul Hasan Rizvi (Department of Urology, Dow Medical College and Civil Hospital, Karachi.)
S. Shahjehan (Department of Biochemistry, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi.)

Abstract

Two hundred twelve samples from 90 stones obtained from one hundred consecutive children with bladder stone disease (BSD) were analysed by qualitative and quantitative chemical methods. Majority (72%) of the stones were spherical in shape while 51% had mammilated surface. Children in older age group had heavier stones ($P < 0.05$). The calculi were predominantly of mixed type (88.9%). Calcium oxalate was the commonest (97.7%) compound detected by qualitative chemical method. The central portion of calculi showed a significantly higher ($P < 0.02$) occurrance of ammonium urate compared to the surface. Uric acid was present in surface layer in significantly higher ($P < 0.05$) number in patients with uninfected urine. On quantitative analysis calcium and oxalate occurred in significantly higher ($P < 0.01$) percentage in surface layers, while urate occurred more frequently ($P < 0.05$) in the central portion of calculi.
On comparing the two chemical methods of stone analysis, a good agreement was seen in the detection of calcium, oxalate and urate but phosphate was missed in 39% samples by the qualitative method and in 10% samples by quantitative method (JPMA 34 :147, 1984).

Introduction

The examination of the composition of individual urinary stone plays an important role in the diagnosis and treatment of various types of urolithiasis. The cummulative results of stone analysis of a particular type of patient, together with the data on distribution of different types of stone disease, is likely to enhance our understanding of the epidemiology and etiology of the disease (Sutor et al., 1974). Furthermore, it seems inescapable that the composition of the stone's nucleus or central portion must bear an even more fundamental relationship to its causation.
The ideal method of stone analysis should be sensitive, accurate, rapid and inexpensive. It should be semiquantitative and powerful enough to deal with small stone nuclei and intimate structural details (Prien and Prien, 1973). In practice none of the methods of examination of calculi (chemical or physical) can fulfill all the criteria. Nonetheless a combination of chemical and physical methods seems to offer the best solution to the problem of stone analysis in a clinical setting.
Scheele published the first analysis of calculi in 1776 (Larsson et al., 1981). Winer and Mattice (1942) and Winer (1959) emphasised the importance of analyzing stones in determining the etiology of its formation and devised simple spot tests to give qualitative chemical composition of calculi. Hodgkinson et al. (1969) published the quantitative analysis of 200 calcium containingstones, relating the stone composition to clinical history and composition of urine. To the clinician, analysis of calculi provides considerable help in the planning of any meaningful regime towards prevention of stone recurrenceS (Drach, 1978).
Composition ot calculi from many developing countries, where childhood BSD is endemic, shows considerable variation (Lonsdale et al., 1968; Sutor, 1981). Few studies have reported the composition of stones from Pakistan (Shahjehan and Rabman, 1971; RiZvi, 1975) which necessitated a study of analysis of stones, correlating their composition to clinical variables and other etiological factors.

Material and Methods

Ninety vesical calculi from one hundred children admitted to Urology Ward of Civil Hospital, Karachi between July 1979 and April, 1981 were included in the study. History and clinical examination of BSD children was recorded on a proforma. Urinalysis (Varley, 1969) and urine culture (Cruickshank et al., 1973) were done in all the patients.
The stones after washing with water to rid them of blood and cellular debris were dried in an oven. Their physical features were then described noting down their shape, size, colour and consistency. The weight of the stones was recorded. The calculi were fractured with a sharp needle. The central layer, containing the nucleus, as well as other distinguishable layers were identified by the help of a hand lens and samples from the different layers was taken by scrapping with a sharp knife. The samples were powdered and stored in labelled stoppered glass bottles. Qualitative analysis were carried on 80 surfaces, 59 middle layers and 73 centre samples while 10 stones, too small to have distinguishable layers, were pulverised and treated as a whole. Spot tests for calcium, magnesium, ammonium, phosphate, itrate, oxalate, carbonate, cystine and iron were done by the method described by Winer and Mattice (1942).
Quantitative analysis was performed on 76 surfaces, 50 middle layers and 63 centres of 90 calculi. Calcium was estimated by the method of Bauer et al. (1962), phosphorus by the method of Gomori, 1942 (cited by Varley, 1969) and oxalate by the method deséribed by Wooten (1 964).

Results

The physical examination of the stones showed that 72% stones were oval or spherical in shape (Table 1).

Table I
Relationship of Shape and Weight of Stones to Age Group.

	Age group in years	
	0–6	6–12
Shape of Stone		
Oval/spherical (65)	48	17
Irregular (25)	14	11
Weight of stone		
0–6 Gm (66)	52	14
More than 6 Gm (24)	10	14*

*$P < 0.05$ when weight of stones and age groups are compared.

Mammilated surface was seen in 46 (51.2%) stones. Seventy three percent stones weighed between 0-6 grams.

On relating the weight of stones to age groups a significantly higher number ($P < 0.05$) of older patients had stones weighing more than 6Gm. (Table 1). No significant Eelationship could be seen between the weight of stones and their composition or duration of symptoms in these patients.

Calcium oxalate was the commonest (97.7%) compound detected in the calculi analysed, followed by ammonium urate (6 1.2%) while magnesium ammonium phosphate was seen in 8.9% (Table II).

Table II

Occurrence of Common Constituents in Different Layers of the Calculi.

Stone layers	Calcium oxalate	Ammonium urate	Calcium phosphate	Uric acid	Magnesium ammonium	Uniden-tified
Surface (80)	78	26	26	20	6	1
Middle (59)	57	32	22	14	2	1
Centre (73)	59	46*	20	12	4	1
Whole stone (90)	88	55	35	24	8	1

*$P < 0.02$ when surface and centre are compared.

The centre showed a significantly higher ($P < 0.02$) occurrence of ammonium urate compared to surface (figure 1). Majority (88.9%) of calculi were of mixed type (Table III).

Table III

Occurrence of Type of Calculi according to Radicals and Their Combinations.

Group	Oxalate-urate	Oxalate-phosphate	Oxalate-phosphate-urate	Urate-phosphate	Oxalate	Urate	Phosphate	Unidentified
Surface (80)	32	15.	11	1	20*	0	1	0
Middle layer (59)	30	14	7	1	6	1	0	0
Centre (73)	40	13	7	3	4	6*	0	0
Whole stone (90)	36	32	11	1	9	0	0	1

*$P < 0.02$ when surface and centre are compared.

When classified on the basis of radicals and theft combitiations, oxalate-urate was seen in 40% stones. It was found that surface layer had significantly higher ($P < 0.02$) occurrence of pure oxalate compared to the central layer of calculi. Pure urate was seen in 9.2% samples in centre, and compared to the surface layer the difference was significant ($P < 0.02$) (Table III).

The constituents showed no significant relationship with age, sex or urinary pH of the patients. On comparing the surface composition of calculi in infected and non-infected groups of children, it was seen that uric acid was present in significantly higher ($P < 0.05$) number of patients with uninfected urine. When the symptoms of BSD children was correlated with surface composition of calculi, it was seen that frequency of micturition was always associated with urate as the predominant constituent on stone surface.

The analysis of surface layer and central portion of calculi by quantitative chemical method showed that the percentage of calcium and oxalate was significantly higher ($P < 0.01$) in the surface compared to centre (Table IV).

Table IV

Comparison of Surface and Central Layers by Quantitative Analysis (Mean ± SE).

Group	Calcium %	Oxalate %	Phosphate %	Urate %
Surface (76)	18.4** ± 0.66 (70)	36.4** ± 1.52 (70)	5.6 ±1.24 (54)	12.3 ± 1.25 (44)
Centre (63)	15.2 ± 0.82 (63)	29.7 ± 1.74 (58)	6.5 ± 1.25 (60)	15.2* ± 1.04 (56)

*P <0.05
**P <0.01

Moreover, the percentage of urate was significantly higher (P11< 0.05) in the centre compared to surface of calculi. On correlating the percentage of phosphate to urinary pH a significantly higher ($P < 0.05$) percentage of phosphate was seen in the groupwith urinary pH greater than 6.5 (Table V).

Table V

Percentage of Calcium, Oxalate, Phosphate and Urates in Stone surfaces and its relationship with Urinary pH (Mean ± SE).

Urine pH	Calcium%	Oxalate %	Phosphate %	Urate %
>6.5 (26)	18.7 ± 0.98 (26)	35.1 ± 2.49 (26)	8.2* ±2.64 (25)	12.8 ± 1.09 (17)
>6.5 (50)	19.0 ± 0.81 (49)	39.7 ± 1.71 (48)	2.9 ±0.59 (35)	11.53 ± 1.82 (26)

*$P < 0.05$

Comparison of qualitative and quantitative chemical methods showed agreement in the detection of calcium in 96.5%, of oxalate in 93.9% and of urate in 95.1% samples (Table VI).

Table VI

Comparision of Qualitative and Quantitative Method of Analysis of Calculi.

Group	Agreement	Missed on qualitative analysis	Missed on quantitative analysis
Calcium (200)	193	7	0
Oxalate (198)	186	11	1
Phosphate (115)	58	45	12
Urate (143)	136	1	6

Phosphate was missed in 39.1% samples by qualitative method and could not be detected in 10.4% samples by quantitative analysis.

Discussion

Majority of the stones were oval or spherical in shape. A significantly greater number of older children had: stones weighing more than 6 grams. The weight of the calculi showed no correlation to the duration of symptoms. On relating the symptomatology with the type of calculi it was found that urate was always associated with frequency of micturition while oxalate was associated with frequency in 69.2%. Intermittent retention was associated with urate as surface constituent in 34.8% cases while its association in case of phosphate was seen in 12.5% cases. No comparative study on these aspects was available in literature.

In the occurrence of calcium oxalate, the present series closely resembles reported incidence in bladder stones from Thailand (90%), Turkey (100%), Afghanistan (100%), India (93.3%), Pakistan (97.8%) and han (84%) with overwhelming majority of calculi having mixed composition (Lonsdale et al., 1968; Balakrishna Rao et al., 1970; Gharib, 1970; Shahjehan and Rehman, 1971; Aurora, 1977). However calcium oxalate was less commonly reported in childhood bladder stones from Egypt (58%). Again anunonium urate was more frequently reported from Turkey (88%) and Thailand (95%) (Lonsdale et al., 1968) as compared to 61.2% in the present study. In the occurrence of ammonium urate the present study resembles studies from Egypt and India (Loutfi et al., 1974; Balakrishna Rao et al., 1970). The occurrence of uric acid was similar to bladder stones from Turkey, Afghanistan, India and Thailand, while Egyptian series showed a very high occurrence (54.8%) of uric acid as compared to 28.8% in the present series. Sinillar to present study the occurrence of calcium phosphate in bladder stones from Turkey, Afghanistan, india, was 20%-40%, whereas the occurrence was 51.6% from Egypt and only 14% from Thailand, (Balakrishna Rao et al., 1970; Lonsdale et al., 1968; Loutfi et al., 1974). In occurrence of magnesium ammonium phosphate, only bladder stones from Thailand and India resembled the present series while a very low occurrence was reported from Turkey and Afghanistan and a high occurrence from Egypt (Sutor, 1980). The present series also resembled the Norwich collection of nineteenth century from England in the occurrence of calcium oxalate, ammonium urates and magnesium ammonium phosphate (Lonsdale, 1968). Figure 2 gives a comparative analysis of results from some of the countries where endemic vesical calculi in children have been clinically important.

The significantly high occurrence of ammonium urate in the centre of calculi in the present study (figure 1) corrobrates the finding of many workers from developing countries (Prien and Frondel, 1947; Gershoff et al.; 1963; Sutor et al., 1974; Balakrishna Rao et al., 1970). The higher occurrence of urates in the centres of calculi has been postulated to be the result of acidaemia and increased ammonia excretion by some workers (Brockis et al., 1981; Teotia et al., 1981). Evidence for greater occurrence of ammonium urate in the centre of bladder stone in our BSD children warrants greater attention to the role of urate as the primary event in the formation of bladder stones in addition to the current practice of considering calcium oxalate crystallization as the primary process.

The preponderance of mixed calcuui in the reporting mainly on analysis of childhood renal calculi have shown increased occurrence of magnesium ammonium phosphate with increasei urinary pH due to ureasplitting proteus infection (Ghazali, 1975; Churchill et al., 1980; Fishbein, 1981; Bruce and Griffith, 1981). However in case of bladder stone disease most workers could not correlate the stone compositions to the type of infection (Eckstein, 1961; Gharib, 1970; present series agrees well with most series from the developing countries, except with the series from Sudan where pure uric acid calculi are seen in 57% of cases (Ibrahim et al., 1977; Ibrahim, 1979). These mixed stones do not appear to be due to infection alone as infection appears to contribute in increased occurrence of apatite and struvite and again infection has been reported in about one third of cases only (Eckstein, 1961; Valyasevi et al., 1967). Thus a more plausible explanation of mixed calculi would appear to be on the basis of epitaxy, where primary process may be in the crystallization of oxalate or urate from supersaturated polyionic solution like the urine (Lonsdale, 1968; Sutor and Wooley, 1972; Mayers, 1977; Mandel and Mandel, 1981).

On correlating the occurrence of common constituents of calculi with urinary tract infection, it was seen that a significantly higher number of uric acid calculi were seen in absence of infection. Calcium phosphate and magnesium phosphate was more often associated with infected urine although the difference was not significant. Many workers reporting mainly on analysis of childhood renal calculi have shown increased occurrence of magnesium ammonium phosphate with increased urinary pH due to ureasplitting proteus infection (Ghazali, 1975; Churchill et al., 1980; Fishbein, 1981; Bruce and Griffith, 1981). However in case of bladder stone disease most workers could not correlate the stone compositions to the type of infection (Eckstein, 1961; Gharib, 1970; Shahjehan and Rehman, 1971; Thalut et al., 1976; Noronaha et al., 1979).

No significant relationship could be elicited between the occurrence of common constituent and pH of the urine by qualitative chemical method. On correlating quantitative analysis of the surface layer it was found that children with alkaline urine (pH above 6.5) showed a significantly higher ($P < 0.05$) percentage of phosphate as surface constituents as compared to the group with urinary pH below 6.5. Few workers have tried to correlate surface layer constituent with pH of urine in bladder stone disease.

However Shahjehan and Rehman (1971) have shown that in surface layer phosphate and ammonium urate were more commonly present in alkaline urine, and magnesium ammonium phosphate occurred with higher urinary pH, while uric acid as a surface tonstituent was present only in acid urine.
A comparison of qualitative and quantitative method of stone analysis was made and there was good agreement in detection of calcium, oxalate and urates while phosphate was missed in ?9.1% of samples by qualitative method and in 10.4% of samples by quantitative method. Similarly Larsson et al. (1981) while comparing qualitative chemical and semiquantitative method for stone analysis reported disagreement in the detection of phosphate as well as ammonium. The comparison in. the present study indicates a need for further modification of both methods for detection and estimation of phosphate.

Acknowledgements

We wish to thank Mr. Saeedullah and Mr. Yawar for their help in the analysis of stone samples and Miss Rehana for bacterial examination of urine samples. To the members of the urology unit, we owe gratitude for all their help in the management of the patients and to Mr. Rafiullah for typing the manuscript.

References

1. Aurora, A.L. Vesical calculus disease of childhood, in idiopathic urinary bladder stone disease. Sponsored by the John E. Fogarty International Centre. Edited by Robert Van Reen. Washington, DHEW Publication, 1977, P. 215.
2. Balakrishna Rao, B.B., lrnirya, K.N., Upadhyay P.and Mishra, P.K. (1970) Juvenile bladder stones; analysis by x-ray diffraction technique. Indian J. Med. Res, 58:876.
3. Brockis, J.G., Bowyer, R.C., McCulloch, R.K., Taylor, TA., Wisniewski, Z.S., Kamardi, T. and Rizal, A. Physiopathology of endemic bladder stones in urinary calculus, international urinary stone conference. Edited by J. Gwynne Brockis and Birdwell Finlayson. Littleton, PSG Publishing, 1981, p. 225.
4. Bruce, R.R. and Griffith, V.P. Retrospective follow-up of patients with struvite calculi, in urolithiasis; clinical and basic research. Edited by Lynwood H. Smith et al. New York, Plenum Press, 1981, p. 191.
5. Caraway, W.T. (1963) cited by Varley, H. Practical clinical biochemistry. 4th ed. New York, Interscience, 1969, p. 205.
6. Churchill, D.N., Maloney, C.M., Nolan, R., Gault, M.H. and Winsor, G. (1980) Pediatric urolithiasis in the 1970s. Eckstein, H.B. (1961) Endemic urinary lithiasis in J. Urol., 123:237.
7. Turkish children a clinical study of 119 cases. Arch. Dis. Child., 36: 137.
8. Fishbein, W.N. Urease inhibitors in the treatment of' infection induced; some chemical, pharmacologic and clinical considerations, in urolithiasis; clinical and basic research. Edited by Lynwood H. Smith et al. New York, Plenum Press, 1981, p. 209.
9. Gershoff, S.M., Prien, E.L. and Chandrapanond, A. (1963) Urinary stone in Thailand. J. Urol., 90:285.
10. Gharib, R. (1970) Lithiasis in the urinary tract of children. Clin. Pediatr., 9:157.
11. Ghazali, S. (1975) Childhood urolithiasis in the United Kingdom and Eire. Br. J. Urol., 47:739.
12. Gomorri, G. (1942) cited by Varley, H. Practical clinical biochemistry. 4th ed. New York, Interscience, 1969, p.431.
13. Hodgkinson, A., Peacock, M. and Nochalson, M. (1969) Quantitative analysis of calcium containing urinary calculi. Invest. Urol., 6:549.
14. Ibrahim, A., Zein, M. and Beleil, 0. (1977) Uric acid lithiasis in the Sudan. J. Trop. Med. Hyg., 80:173.

15. Larsson, L., Sorbo, B., Tiselius, H.G. and Ohman, 5, On the analysis of urinary calculi, in urinary calculus, international urinary stone conference. Edited by J. Gwynne Brockis and Biidwell Finlayson. Littleston, PSG Publishing, 1981, p. 383.
16. Lonsdale, K. (1968) Human stone; limited studies give some details of composition, rates of growth, distribution, and possible causes. Science, 159, 1199.
17. Lonadale, K., Sutor, D.J. and Wooley, S.E. (1968) Composition of urinary calculi by x-ray diffraction. Collected data from various localities. Parts II. Czechoslayakia, III. Turkey, N. Thailand, V. India, VI. Spain and Indonesia. Br. J. Urol., 40:402.
18. Loutfi, A.H., Van Reen, R. and Abdel-Hanild, G. (1974) Composition of bladder stones. J. Egypt. Med. Assoc., 57: 124.
19. Mandel, N.S. and Mandel, G.S. Epitaxis between stone-former crystals at the atomic level, in urolithiasis; clinical and basic research. Ediftd by Lynwood H. Smith et al. New York, Plenum Press, 1981, p.469.
20. Myers, N.A.A. (1957) Urolithiasis in childhood. Arch. Dis. Child., 32:48.
21. Noronaha, R.F.X., Gregory, J.G. and Duke, J.J. (1979) Urolithiasis in children. J. Urol., 121 :478. Prien, E.L. and Frondel, C. (1947) Studies in urolithiasis. the composition of urinary calculi. J. UroL, 57:949
22. Prien, E.L. and Prien, E.L., Jr.Optical crystallographic analysis of urinary stones: A simplified approach, in urinary calculi, recent advances in aetiology, stone structure and treatment. Edited by L. Cifuentes Delatte et al., Base!, S. Karget, 1973, P. 206.
23. Rizvi, S.A. (1975) Calculous disease; a survey of 400 patients. JPMA., 25:268. Shahjehan, S. and Rehman, M.A. (1971) Studies on the etiology of urolithiasis in Karachi. Am. J. Clin. Nutr., 24:32.
24. Sutor, DJ. Stones; epidemiology and composition, in urology; tutorials in postgraduate medicine. Edited by G.D. Chishoim. London, Heinemann, 1980, p. 95.
25. Sutor, D.J. and Wooley, S.E. (1972) Composition of urinary calculi by x-ray diffraction collected data from various localities. Part XII-XIV. Br. J. Urol., 44:287.
26. Sutor, D.J., Wooley, S.E. and Illungworth, J.J. (1974) A geographical and historical survey of the composition of urinary stone. Br. J. Urol., 46:393.
27. Teotia, M., Teotia, S.P. and Slngh, D.P. Effect of diet on urinary constituents, uric acid solubility and inhibitors of crystallization in children with endemic bladder stone disease, in urinary calculus; international urinary stone conference. Edited by J. Gwynne Brockis and Birdwell Finlayson. Littleton, PSG Publishing, 1981, p. 251.
28. Thalut, K., Rizal, A., Brockis, J.G., Bowyer, R.C., Taylor, T.A. and Wisniewski, Z.W. (1976) The endemic bladder stones of Jndonesia; epidemiology and clinical features. Br. J. Urot, 48:617.
29. Valyasevi, A., Haistead, S.B. and Dhanamitta, S. (1967) Studies of bladder stone disease in Thailand. VI. Urinary studies in children, 2-10 years old, resident in a hypoand hyperendemic area. Am. J. Clin. Nutr., 20:1362.
30. Winer, J.H. and Mattice, M.R. (1942) Routine analysis of urinary calculi- a rapid simple methods using spot tests. J. Lab. Clin. Med., 28:898.
31. Wootton, I.D.P. Analysis of calculi; qualitative analysis and quantitative analysis, in micro-analysis in medical biochemistry, by Earl J. King. 4th ed. London, Churchill, 1964, p. 207.