Spectroscopic Studies of Urinary Calculi in the Solid State

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Summary: Two hundred renal calculi were analysed by pellet technique. Chemical composition of the outer shell and core of urinary calculi was determined separately wherever possible, otherwise the whole stone was powdered and analysed. The most frequently occurring components in both pure and mixed stones were calcium oxalate (whewellite/weddellite) (73.5%), uric acid (anhydrous/hydrated) (31%), apatite/whitlockite (20%), struvite (18%) and ammonium acid urate (7.5%). Calcium oxalate (37%), mixture of calcium oxalate and apatite/whitlockite (19%), and uric acid (19.4%) served as nuclei of urinary stones. Whitlockite did not occur in its pure state but its presence was detected in mixed form with calcium oxalate, struvite and apatite. Statistical combinations in relation to chemical composition of urinary calculi were computed and compared with the results of other workers. Calcium oxalate was the commonest component of pure as well as mixed stones. Only one stone was composed of cystine. Stones from children and factors such as sex, age, and environment have been discussed.

Introduction

The importance of a knowledge of the chemical composition of renal stones cannot be neglected. The precise knowledge of the composition of urinary tract calculi is clinically important to determine the possible cause of stone formation and is absolutely necessary for planning medical management by dietary and other means to avoid recurrence. For example, in urinary tract infection, the urine becomes alkaline resulting in the precipitation of carbonate-apatite and magnesium ammonium phosphate [1,2]. Where the disease process does not involve infection, such as in hyperparathyroidism, stone formation occurs at acid pH due to the precipitation of calcium oxalate and hydroxy apatite. In hyperuricaemia, as in gout, stone which is more frequently formed, is uric acid. In case of genetic abnormalities, stones such as cystine and xanthine are formed.

While the classical wet chemical analysis is confronted with a number of difficulties in establishing the composition of stones, physical methods have been found to be the most helpful in this effort. The infrared spectrophotometric method, for the first time, was used by Beischer [3] to determine the chemical composition of renal calculi. This method is speedy, accurate, specific and convenient. It is quite adequate for small stones. Especially it is very useful in the analysis of mixed stones. Thermal methods of analysis (TGA, DTA) [4] and electron microscopy [5] were also carried out in addition to IR spectroscopy. The results of three methods are comparable. Here we are reporting the data taken by IR spectroscopy in the solid state (KBr disc technique).

Results and Discussion

The infrared spectra of the calculi were compared with those of pure compounds either commercially available or prepared in the laboratory according to the procedures [2] as well as reported

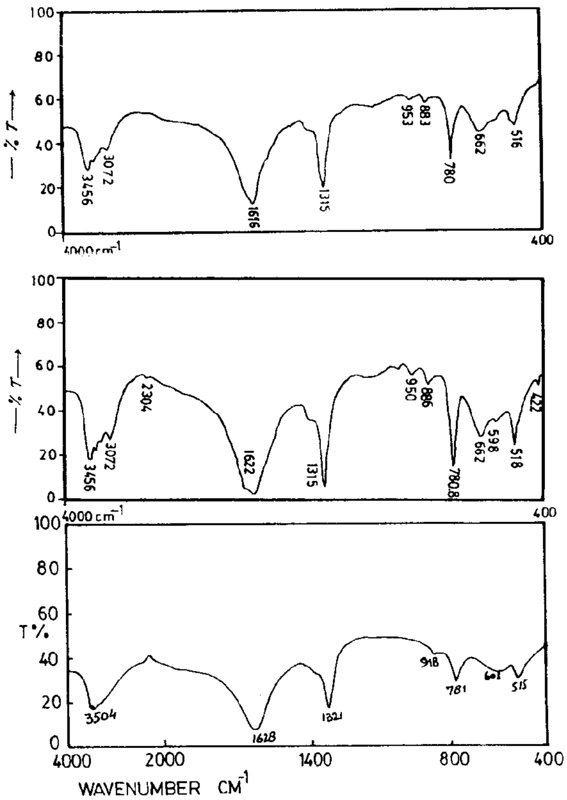

Fig.1: Infrared spectra, A; calcium oxalate monohydrate (pure) as KBr disc., B; calcium oxalate monohydrate (calculus) as KBr disc. and C; calcium oxalate dihydrate (calculus) as KBr disc.

by Haux [2], Beischer [4], Weissman *et al* [6], Chihara *et al.* [7] and Hesse [8]. Comparison was made for the purpose of identification of frequencies, where pronounced absorptions occurred. Identification of only those compounds is reported here, which were analysed during the present study.

From a total of 200 stones, 106 (53%) were pure and 94 (47%) were mixed. The frequencies of occurrence of renal stones in terms of chemical composition are shown in Tables 1-2. Of 106 pure stones, 53 (26.5%) were composed of calcium oxalate dihydrate, 26 (13.0%) were anhydrous uric acid, 15 (7.5%) were magnesium ammonium phosphate hexahydrate, 5 (2.5%) were ammonium acid urate and 1 stone (0.5%) comprised of pure cystine.

Of 94 mixed stones, calcium oxalate (whewellite/weddellite) was detected in 88 (44.0%) cases. Out of 200 stones, 166 were with nucleus and 34 were without central portion, hence they were

Table 1: Frequency of occurrence of pure renal calculi
Total number of stones analysed = 200
Number of pure stones = 106 (53%)
Number of mixed stones = 94 (47%)

Chemical compostion	No. of stones	%	Upper urinary tract (renal)	Lower urinary tract (bladder)
Whewellite	53	26.5	42	11
Weddellite	06	3.0	06	-
Uric acid anhydrous	26	13.0	05	21
Struvite	15	7.5	02	13
Cystine	01	0.5	01	-
Urate	05	2.5	05	-

Whewellite = Calcium oxalate monohydrate, $CaC_2O_4.H_2O$
Weddellite = Calcium oxalate dihydrate, $CaC_2O_4.2H_2O$
Struvite = Magnesium ammonium phosphate hexahydrate, $MgNH_4PO_4.6H_2O$
Urate = Ammonium hydrogen urate, $C_5H_3N_4O_3NH_4$
Cystine = $[-SCH_2CHNH_2COOH]_2$

Table 2: Frequency of occurrence of mixed renal calculi (outer shell + inner core)

Chemical composition	No. of stones	%	Upper urinary tract (renal)	Lower urinary tract (bladder)
Calcium oxalate + uric acid	24	12.0	07	17
Calcium oxalate + whitlockite	25	12.5	11	14
Calcium oxalate + sttruvite	11	5.5	03	08
Calcium oxalate + Apatite	07	3.5	04	03
Calcium oxalate + Urate	04	2.0	02	02
Uric acid (anhydrous + hydrated)	02	1.0	-	02
Calcium oxalate + Struvite + Whitlockite	05	2.5	02	03
Ca-oxalate + Uric acid + Urate	03	1.5	02	01
Ca-oxalate + Uric acid + Struvite	01	0.5	-	01
Ca-oxalate + Whitlockite + Apatit	01	0.5	01	-
Weddellite + Struvite +	03	1.5	02	01
Weddellite + Whitlockite +	01	0.5	01	-
Struvite + Apatite +	01	0.5	-	01
Uric acid + Urate	03	1.5	01	02
Ca-oxalate + Uric acid hydrated	02	1.0	-	02
Ca-oxalate + Uric acid (anhydrous + hydrated)	01	1.0	-	01

Ca = Calcium
Urate = Ammonium hydrogen urate
Whitlockite = Tricalcium phosphate
Weddellite = Calcium oxalate dihydrate

taken as whole for analysis. The nuclei of only 52 stones (26.0%) were composed of calcium oxalate. It was also found in the nuclei mixed with other components: tricalcium phosphate/apatite in 26 cases (13.0%), uric acid in 24 cases (12.0%), am-

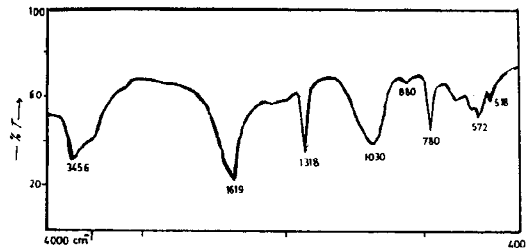

Fig.2: Infrared spectrum of calcium oxalate monohydrate and apatite (mixed calculus) as KBr disc.

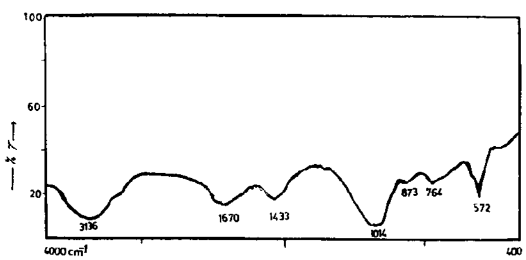

Fig. 3: Infrared spectrum of magnesium ammonium phosphate hexanhydrate (struvite) and apatite (mixed calculus) as KBr disc.

monium hydrogen urate in 2 cases (1.0%) and struvite in 6 cases (3.0%). The nuclei of 29 stones (14.5%) were of uric acid anhydrous alone. Pure ammonium hydrogen urate was present in the nuclei of 5 stones (2.5%). Struvite in pure form was detected in the nuclei of 18 stones (9.0%). The nucleus of one stone (0.5%) was composed of uric acid dihydrate only. Calcium oxalate dihydrate alone was present in the nuclei of 2 stones (1.0%). The central portion of one stone was composed of pure cystine.

Calcium oxalate, the most frequently occurring constituent of pure as well as mixed urinary calculi, was the result of this series, which was also confirmed by other workers [9,10]. Analysis of our statistical data regarding the chemical composition of renal calculi indicated that out of 200 stones, 171 samples were with nuclei, while 29 were without them. The without nuclei were taken and analysed as such.

Pure calculi

Statistical analysis of 200 samples yielded the data: 106 (53%) pure stones, 94 (47%) impure stones, 53 (26.5%) calcium oxalate monohydrate, 6 (3%) calcium oxalate dihydrate, 26 (13%) uric acid anhydrous, 15 (8.5%) magnesium ammonium phosphate hexahydrate, 5 (2.5%) ammonium hydrogen urate and 1 (0.5%) cystine.

Mixed calculi

Of 200 calculi examined, calcium oxalate (mono/dihydrate) was found in 147 (73.5%), i.e. in 59 pure and in 88 mixed stones. The frequency of subsequently deposition of calcium oxalate in both pure and mixed forms in the outer portions of calculi was also quite high (57%). Calcium oxalate occurred as single constituent in the outer shells (25.5%) or mixed with other constituents (31.5%). Uric acid-calcium oxalate combination (13%) was also observed in which the frequency of occurrence as anhydrous uric acid was the highest. Association of calcium oxalate and apatite was also found (5.5%) in the outer shells of the stones. Calcium oxalate-ammonium acid urate combination was found only in the nuclei of stones. Calcium oxalate was present in the nuclei, alone (21%) or frequently mixed with uric acid (15%), tricalcium phosphate (15.5%) and less frequently with apatite (8.5%) and ammonium acid urate (2.5%). Struvite-calcium oxalate combination was present (9.5%) and in such cases stone formation might have started due to urinary infection. It was further found that 38 calcium oxalate calculi (19%) had developed around nuclei consisting of calcium oxalate alone.

The phosphates in the urinary calculi are present as tricalcium phosphate, struvite, apatite, and calcium hydrogen phosphate dihydrate (brushite). The latter is not very common and was not found in this series of stones. Tricalcium phosphate $Ca_3(PO_4)_2$ was not observed in its pure state but its presence was detected in 35 cases frequently mixed with other components in the nuclei as well as in the outer shells of stones. It was found that it mixed with both forms of calcium oxalate in the nuclei (15.5%) and also in the peripheral portion (11.5%). Its association with struvite was observed in 8 cases (4%) and with apatite in 5 cases (2.5%). One stone was a mixture of weddellite and tricalcium phosphate/apatite in the centre as well as in the outer shell. Out of 200 stones, 38 contained struvite, 15 (7.5%) in the pure state, while 23 (11.5%) in the mixed state. These stones were found to be associated mostly with calcium oxalate (10%) but less frequently with whitlockite or

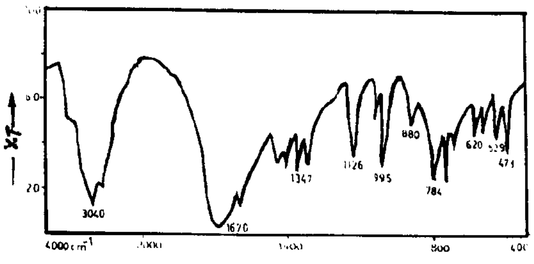

Fig.4: Infrared spectrum of uric acid anhydrous calculus.

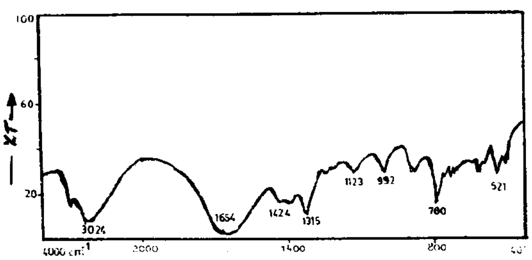

Fig. 5: Infrared spectrum of calcium oxalate and ammonium hydrogen urate (mixed calculus) as KBr disc.

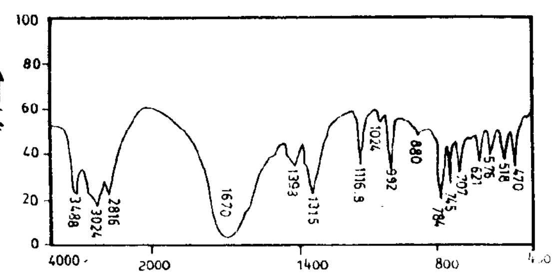

Fig. 6: Infrared spectrum of calcium oxalate and uric acid (mixed calculus) as KBr disc.

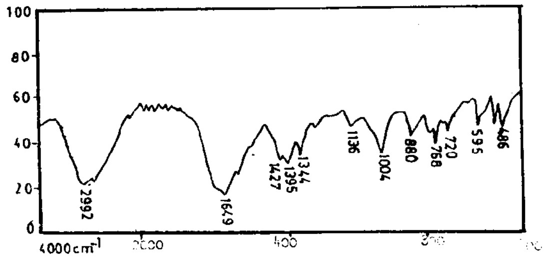

Fig. 7: Infrared spectrum of ammonium hydrogen urate calculus.

apatite (5.5%). Whitlockite was observed in the outer shells of such calculi. Association of struvite with carbonate-apatite was observed in 3 cases. The only two apatites which form the components of renal stones are carbonate-apatite $Ca_{10}(PO_4)_{6-x}(OH)_{2-y}(CO_3)_{x-y}$ and hydroxy-apatite $Ca_{10}(PO_4)(OH)_2$. Both apatites were observed in 9 cases (4.5%) mixed with either calcium oxalate (6%), tricalcium phosphate (4%) or struvite (3%).

Out of 200 stones, 62 (31%) contained uric acid in pure as well as mixed form 26 (13%) stones were composed of pure uric acid and 36 (18%) were mixed with calcium oxalate, urate, struvite or uric acid hydrate. Calcium oxalate monohydrate formed the nuclei of 28 (14%) uric acid calculi. The only urate found during the present study was ammonium acid urate. It was detected as one of the components of 14 (7%) calculi. Urate was observed in the nuclei of 4 (2%) stones in pure form and mixed with calcium oxalate monohydrate in 5 (2.5%) stones. Ammonium acid urate can be formed in urines with a high concentration of NH_4^+ secreted by the renal tubule and in alkaline urine through the bacterial production of ammonium hydroxide [11]. With ordinary methods, it is difficult to differentiate between uric acid and its sodium or ammonium salt, various hydrates of calcium oxalate and uric acid, and different kinds of phosphates but infrared spectroscopy shows the difference clearly [7].

Children's calculi

Of the 200 stones, 17 were from children (8.5%). Three stones were analysed as a whole while rest of the stones were treated by taking nucleus and outer shell separately. Calcium oxalate was the most frequently (50%) occurring component of the children's calculi. Pure calcium oxalate (whewellite/weddellite) stones were found with (12.5%) occurrence. Other major constituents were ammonium acid urate (23.53%) and uric acid anhydrous (17.65%). Calcium oxalate in the nuclei of such calculi were detected in 4 cases (23.53%). Calcium oxalate was also found mixed with tricalcium phosphate/apatite (17.65%) and uric acid (17.65%) in the nuclei as well as outer shell. One calculus was a mixture of struvite and calcium oxalate in the outer shell while its nucleus was composed of pure

Table 3: Frequency of occurrence of various constituents of renal stones in the outer shell and the nucleus

Composition of stones	Stones with nucleus		Stones without nucleus
	No. of stones with outer shell	No. of stones with nucleus	No. of stones
Ca-oxalate monohydrate	50 (25.0%)	42 (21.0%)	11 (5.5%)
Uric acid anhydrous	28 (14.0%)	25 (12.5%)	1 (0.5%)
Struvite	21 (10.5%)	14 (14.0%)	1 (0.5%)
Ammonium acid urate	8 (4.0%)	4 2.0%)	2 (1.0%)
Ca-oxalate dihydrate	1 (0.5%)	1 (0.5%)	5 (2.5%)
Cystine	-	-	1 (0.5%)
Ca-oxalate + uric acid	24 (12.0%)	28 (14.0%)	-
Ca-oxalate + Whitlockite	15 (7.5%)	17 (8.5%)	-
Ca-oxalate + Struvite + Whit.	11 (5.5%)	16 (8.0)	-
Ca-oxalate + Apatite + Whit.	7 (3.5%)	11 (5.5%)	3 (1.5%)
Ca-oxalate + Urate	-	5 (2.5%)	-
Weddellite + Whitlockite	1 (0.5%)	3 (0.5%)	-
Weddellite + struvite	3 (1.5%)	3 (1.5%)	-
Struvite + Apatite + Whit.	-	-	3 (1.5%)
Ca.Ox + Uric acid hydrate	2 (1.0%)	2 (1.0%)	-
Uric acid (anhydrous + hydrated)	-	-	2 (1.0%)

Total number of stones = 200
Number of stones with nucleus = 171 (85.5%)
Number of stones without nucleus = 29 (14.5%)
Ca-Ox = Calcium oxalate, Whitlockite/white = Tricalcium phosphate
Weddellite = Calcium oxalate dihydrate
Struvite = Magnesium ammonium phosphate hexahydrate
Urate = Ammonium hydrogen urate

Table 4: Location of stones and sex incidences

Location	Sex		Total
	Male	Femal	
Renal	66	25	91
Ureteric	05	03	08
Bladder	89	12	101
Total	160	40	200

Table 5: Age and site occurrence

Age group	Upper tract	Lower tract	Total	%
0 - 10	12	05	17	8.5
11 - 20	10	04	14	7.0
21 - 30	20	15	35	17.5
31 - 40	16	14	30	15.0
41 - 50	10	16	26	13.0
51 - 60	16	19	35	17.5
61 - 70	09	17	26	13.0
71 - 80	05	07	12	6.0
81 - 90	01	04	05	2.5

calcium oxalate. These observations agree with the results of Sutor and Wooley [12] and Sutor et al [13] who stated that approximately 89% of bladder stones in children from underdeveloped areas contained ammonium acid urate or calcium oxalate or both. Our results are also similar to those of Chutikorn et al [14] who surveyed the problem in Thailand and found that most of children's stones were formed of ammonium acid urate or calcium oxalate.

Factors

Sex

The correlation between recurrent urinary infection and chemical composition of stones has been investigated in the earlier work of Westbury and Omenogor [15]. 40 (20%) stones out of 200 were related to females and 160 (80%) to males. In case of females, 28 (70%) were kidney stones and 12 (30%) were blader stones. While males showed 69 (71.13%) kidney stones and 91 (88.35%) bladder stones. Present study showed that the typical male stone was mainly composed of calcium oxalate. Major constituent of females was also calcium oxalate (whewellite/weddellite) (97.5%), which was present in both pure state in 22 cases (55%) and mixed form with tricalcium phosphate/apatite (17.5%), with struvite (5%), with ammonium acid urate (12.5%) and with uric acid (7.5%). One stone was composed of pure uric acid anhydrous.

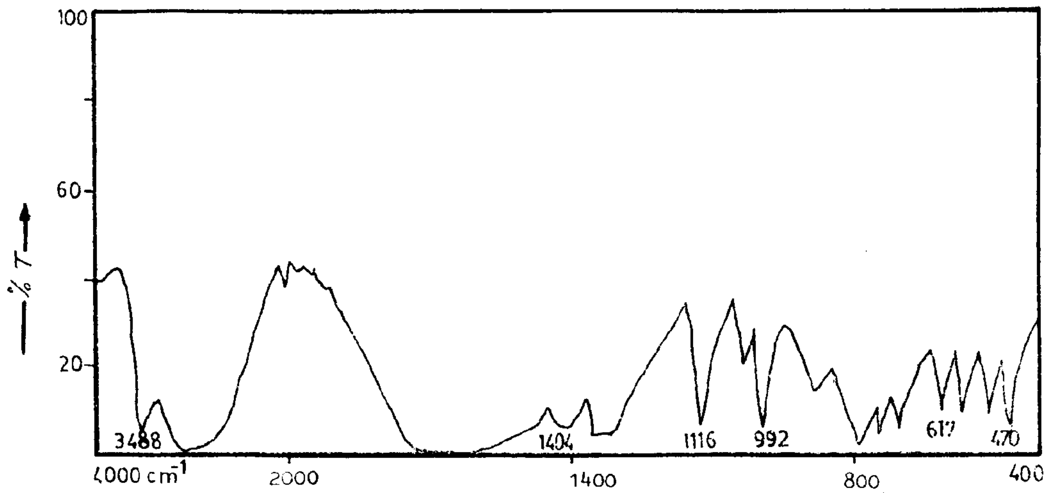

Fig. 8: Infrared spectrum of uric acid hydrated and calcium oxalate (mixed calculus) as KBr disc.

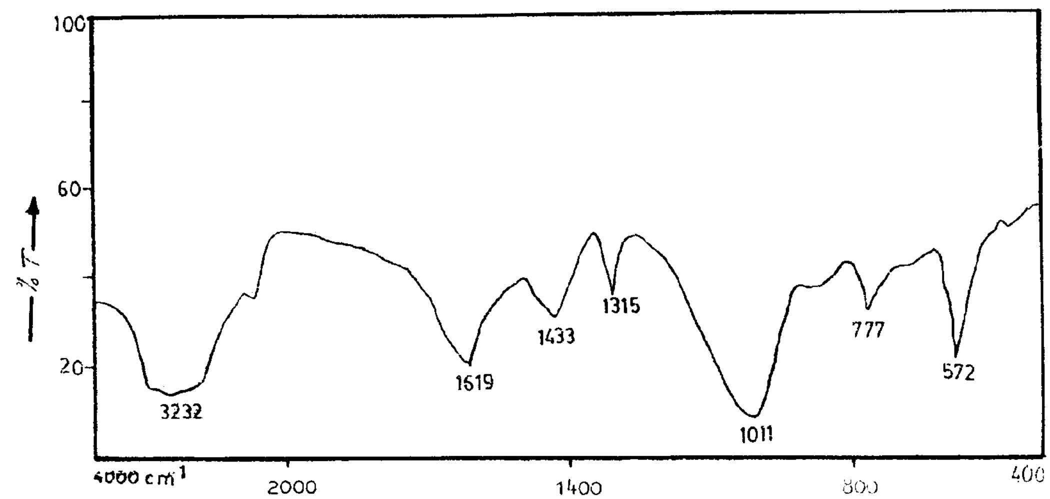

Fig. 9: Infrared spectrum of calcium oxalate and struvite (mixed calculus) as KBr disc.

Age

Children's stones contained less phosphates, and more calcium oxalate and ammonium acid urate. The stones of adults were composed of mainly pure calcium oxalate and its mixture with apatite. While stones of old people were comprised of uric acid, struvite and their mixtures with calcium oxalate.

Environment

Stones of people living in the city were mainly composed of either pure calcium oxalate or in the mixed form with apatite or uric acid. While stones of people residing in villages contained mixed stones of calcium oxalate with apatite, struvite and ammonium acid urate. Greater proportion of phosphate in the stones in both upper and lower urinary

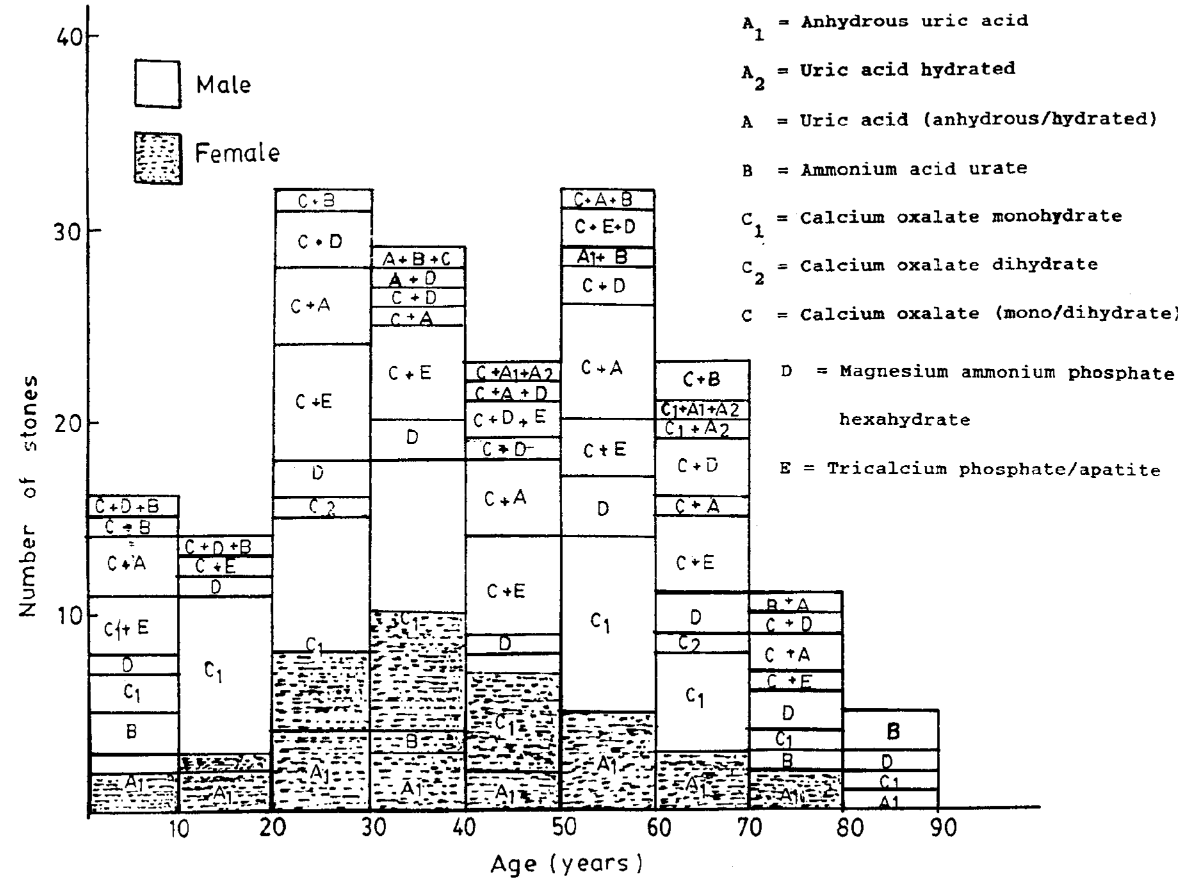

Fig. 10: Histogram of urinary stones frequency with the age and sex of the patients.

tracts of rural people as compared to those of urban may be attributed to alkaline pH of the urine caused by the nutritional variations. A significant increase in urinary pH is confined to patients preferably eating vegetables and fruits [16]. These facts suggest that diet could be one of the causative factors in this region.

Experimental

One hundred and forty stones were provided by the urology unit of the Christian Hospital Taxila and sixty stones by the Urology unit of Pakistan Institute of Medical Sciences (PIMS) Islamabad. These 200 urinary calculi were collected over a period of three years (April 1986- June 1989) as the patients presented themselves for operation or they produced the stones for analysis when the stones passed out naturally through urine.

Pretreatment

Firstly each stone was documented, its shape and colour noted. In the case of very large stones, an apparently representative portion was selected for analysis. Each calculus was freed from extraneous matter (blood, tissue etc.), dried and weighed. The calculi were cut into sections and each section was pulverised with pestle and mortar. If the specimen consisted of many discrete calculi, only one was selected at random for analysis. The core and outer shell of stones were separated and analysed, where it was possible. Otherwise the whole stone was ground to powder and subjected to IR study.

Preparation of reagent

Potassium bromide of spectroscopic grade was pulverised into fine particles in the agate mortar and equalised the grain size by the furnished filter paper. This powder was heated and dried at about 120°C for twenty four hours to expel the absorbed moisture. The dried salt was stored in a stoppered bottle and kept in a desiccator over silica gel. It was redried for two hours before making pellet.

Preparation of KBr pellet

The technique used for the analysis of urinary stones was the same as the used by Weissman et al. [6] and Chihara et al. [7]. 1-2 mg of ground sample was taken and thoroughly mixed with 250- 300 mg of predried KBr by grinding in the mortar. The powder was transferred to tablet frame and the pellet was pressed at a pressure of 8 tons in the evacuated die for approximately 5-10 minutes into a thin transparent pellet of about 1 mm thickness. The pellet was mounted in the holder and inserted into the sample path. IR spectra were taken by a double beam infrared spectrophotometer, Model IR-460, covering the frequency range between 4000- 400 cm^{-1}.

Conclusion

Out of 200 stones, 99 were upper urinary tract (renal) and 101 were lower urinary tract (bladder) calculi. 48 renal stones and 11 bladder stones contained pure calcium oxalate, while 36 renal and 52 bladder stones had calcium oxalate mixed with other components. Uric acid occurred in 5 renal and 21 bladder stones. 9 renal and 25 bladder stones contained uric acid mixed with calcium oxalate or urate or apatite. The occurrence of phosphates either mixed with calcium oxalate or in the pure state is higher in bladder (45) than renal stones (28). Ammonium acid urate was found only in renal stones. But urate mixed with calcium oxalate or uric acid was also observed in renal (4 cases) and bladder stones (5 cases). The tendency is for the nucleus and interior to contain calcium oxalate, uric acid, phosphates and urate, while for the outer shell to contain more calcium oxalate and uric acid. In our series, (23.53%) of childhood stones had urate composition which suggests that children from Pakistan with Urolithiasis probably suffer from nutritional disorders. Most stones were of mixed composition (47%) which points to a multiplicity of environmental factors.

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