The composition and quantitative analysis of urinary calculi in patients with renal calculi

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ABSTRACT
To get complete information about the chemical composition and physicochemical principles underlying the formation of stone there is a need for more precise information. At present there is no single analytical procedure that provides an exact quantitative analysis of urinary calculi. In this present procedure it is assumed that only calcium oxalate monohydrate, hydroxyapatite, or magnesium ammonium phosphate hexahydrate are present in stone, therefore the percent chemical composition of stone is calculated on the basis of molecular weight of an element and related molecular formula of the stone. By analysis of 100 stones the chemical composition observed with Mean ± SD value for calcium is 25.68 ± 5.38, for Calcium oxalate hydrate crystal is 32.84 ± 17.28 which contains oxalic acid 20.35 ± 10.70, for Apatite crystal is found to be 41.70 ± 16.56 which contains inorganic phosphate 8.09 ± 3.08. Total uric acid found in these stones is 27.12 ± 11.42. Major component are calcium oxalate 32.8% (combined monohydrate and dihydrate), Phosphate 41.7%, Magnesium ammonium phosphate hexahydrate 3.3% and uric acid 27.1%. No pure stone was obtained. Calcium oxalate was present in nearly all stones, and the majority of renal calcium stones contains mixtures of calcium oxalate and calcium phosphates in addition some contains uric acid or magnesium ammonium phosphate. The mixture of calcium oxalate / uric acid / magnesium ammonium phosphate hexa hydrate has never been observed.

Keywords: Calcium oxalate, hydroxyapatite, uric acid, urinary calculi.

INTRODUCTION
Better understanding of the physico-chemical principles underlying the formation of stone there is a need for more precise information, particularly with regard to the common calcium containing stones. Qualitative chemical tests provide only a rough indication of the relative amounts of the different constituents in a mixed stone and the results may be misleading. A number of physical methods have been used for the analysis of calculi including optical crystallography, x ray diffraction, infrared spectroscopy, x ray spectroscopy and thermogravimetry, but these techniques require elaborate apparatus, are generally only semi quantitative and do not detect minor constituents of mixed calculi.1 Chemical analysis therefore remains the most convenient procedure for routine use. The method is relatively rapid will detect minor components of mixed calculi and can readily be made quantitative.2

At present there is no single analytical procedure that will provide an exact quantitative analysis of urinary calculi. In the present procedure it is assumed that only calcium oxalate monohydrate, hydroxyapatite, or magnesium ammonium phosphate hexahydrate are present. Although the monohydrate is the commonest form of calcium oxalate in urinary calculi.3 Some stones contain appreciable quantities of the dihydrate and in such cases the present assumptions will result in an under estimation of the amount of calcium oxalate present. A further possible source of error is that calcium and phosphate may be present as brushite (CaHPO₄·2H₂O), Whitlockite (αCa₃(PO₄)₂) Octacalcium phosphate (Ca₈H₂(PO₄)₆·5H₂O), while magnesium may be present as newberyite (Mg H PO₄·3H₂O). However, these minerals are usually present only as minor components so that errors from these sources are probably small.2

The nomenclature used in this study to identify stone composition was designed to identify the actual chemical make up as well as the specific crystalline components of each stone. The chemistry of a stone is a reflection of what chemical groups or moieties are available in the urine at the time of formation and growth of the stone.

MATERIALS AND METHODS
Urinary calculi of 100 patients of kidney stone were collected and analysed quantitatively statistical analysis was done through SPSS 12 software. Student’s t test was applied wherever necessary.

The stone was powdered in a pestle and mortar. The main elements and radicles of interest are calcium, magnesium, inorganic phosphate, uric acid and oxalate.
From knowledge of these constituents it is possible to calculate the composition of a stone in terms of calcium oxalate, calcium phosphate, and magnesium ammonium phosphate.

The procedure was followed as per Hodgkinson A. The procedure was as follows:

Dissolve 20 mg of powdered stone in 2 ml of 50 % (V/V) HCl, with warming, and dilute to 10 ml with water in a volumetric flask (Solution A). This solution is used for all the subsequent determinations. The stone solution stored at 2-8°C when not in use.

RA-50 Chemistry analyser was used for the estimation of Calcium by Trinder’s method, inorganic phosphate by Fiske and subbaraw method, uric acid by Caraway’s method, magnesium by Neil and Neely method can be determined by standard procedures.

The oxalate radicle can also be determined by a simple permanganate titration.

Estimation of oxalate

Procedure: Mix 1 ml of the dissolved stone (Solution A) with 2 ml of 2 N H₂SO₄. Add one drop of an aqueous solution of 5.0% (W/V) MnSO₄, heat to between 70 and 80°C, and titrate rapidly with standard 0.01 N KMnO₄ till persistent pink colour end point.

A ‘control’ sample of powdered calcium oxalate (20 mg) when treated in a same manner should give a recovery of 99 to 100 % after allowing for titration blanks (1 ml of 0.01 N KMnO₄ is equivalent to 0.45 mg of oxalic acid). Accuracy of experiment was confirmed by recovery experiment.

Calculations

1) One ml of 0.01 N KMnO₄ is equivalent to 0.45 mg of oxalic acid.

The mechanism of the reaction between oxalate and permanganate react slowly with oxalic acid but after a small amount of manganous salt has been formed the reaction occurs almost instantaneously in hot solution. The manganous ions appear to catalyse the reaction between permanganate and oxalic acid and the addition of a drop of MnSO₄ solution therefore ensures a sharp endpoint from the start of the titration. Manganese Sulphate serves a double purpose since it also prevents any reaction between permanganate and hydrochloric acid.

2) The percentage composition of a stone in terms of calcium oxalate, magnesium ammonium phosphate, uric acid, hydroxyapatite can be calculated from the following equations:

1. Calcium oxalate monohydrate = Oxalic acid (grams of anhydrous acid per 100 gm of calculus) × 1.62

2. Magnesium ammonium phosphate hexahydrate = Magnesium (grams per 100 gm of calculus) × 10.2

3. Hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂) = Magnesium ammonium phosphate × 31 / 247.3

4. A = The total calcium contained in calcium oxalate monohydrate

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A = \text{Calcium Oxalate} \times \frac{40}{146.12}
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B = The total calcium contained in Hydroxyapatite

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B = \text{Hydroxyapatite} \times \frac{400}{1004}
\]

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\therefore \text{Total calcium contained in calculus} = A + B
\]

5. Uric acid = Uric acid × 100 / 20

RESULTS

The Hydroxyapatite calcium oxalate and uric acid stones are commonly observed stones in this area revealed by Table-1.

Using the present procedures it was possible to account for 90.0% ± 5.0% of the total weight of most stones, the remainder was accounted for mainly by water and protein.
Human urinary stones generally contain more than one type of crystals and there is a tendency for certain crystals to occur together. Calcium oxalate and calcium phosphate are the two most common components of urinary stones. Fig. 1. shows calcium is the most common major component of the stones. The total calcium found by analysis of 100 stones is 25.68 ± 5.38. Calcium oxalate hydrate is the most common crystal present is 32.84 ± 17.28, which contains oxalic acid 20.35 ± 10.70. Apatite is the second most common crystal found 41.70 ± 16.56 contains inorganic phosphate 8.09 ± 3.08. CaOX / apatite is the most common combination.

In case of uric acid stones it is present in combination with CaOX and CaP. Total uric acid found in these stones is 27.12 ± 11.42.

In case of magnesium ammonium phosphate (MAPH) is present in combination of CaOX and CaP. MAPH is 3.33 ± 6.66 magnesium present is 0.33 ± 0.65. All stones contains a organic matrix. The percentage present as organic matrix in stones varied from 2.0-30.0%.

A statistical analysis performed on 100 urinary calculi showed the following distribution, with regard to the major component are calcium oxalate 32.8% (combined monohydrate and dihydrate), Phosphate 41.7%, Magnesium ammonium phosphate hexahydrate 3.3% and uric acid 27.1%.

**DISCUSSION**

Urinary calculi are formed as a result of a biological maladjustment of urine. The stone formation is attributed to supersaturation of urine and retention of solid particles. Its composition varies from being homogeneous (from the nucleus to the periphery) to heterogeneous. This latter state results from successive and different aggregations and crystal growths due to various diets.9

For diagnosis, understanding of etiology and treatment of lithiasis, it will be necessary to characterize and analyze the ions and molecules participating in the formation of urinary calculi. The purpose of this study was to quantify hydrate forms of calcium oxalate (COM and COD) in calcium oxalate stones, calculi containing uric acid or magnesium ammonium phosphate hexahydrate (MAPH).

Our results showed the presence of calcium oxalate in all stones. Although calcium oxalate because of its high solubility is the most common component of renal calculi, its presence in 99.8% of the stones analyzed. The majority of renal calcium stones contains mixtures of calcium oxalate and calcium phosphates, and it is evident that the precipitation of calcium oxalate is fundamental for the formation of these stones. A pure calcium phosphate stone was unusual.

Among the urinary calculi studied, several ones consisted of pure calcium oxalate hydrate and uric acid were observed, where as some consisted of calcium oxalate hydrate was associated with free water, organic matter and variable amounts of calcium phosphate. In case of uric acid stones, uric acid can be presented in mixtures with calcium oxalate hydrate, free water and organic matter. In case of magnesium ammonium phosphate hexahydrate (MAPH), obtained mixture of calcium oxalate hydrate, organic matter and calcium phosphate.

The mixture calcium oxalate hydrate / uric acid / MAPH has never been observed. Calcium phosphates are often present in oxalate stones. Calcium phosphate was present in more than 80.0% of calcium oxalate calculi. All stones contains a second principal component, referred as organic matrix (albumins, proteins, non amino sugars, nitrogen and water being major constituents). The percentage by mass of the organic matrix (as a percentage of the original mass) varied from 2.0-20.0% but with a mean of 2.5%.

Calcium carbonate and cystine was not component of any stone analyzed. Absence of cystine was confirmed by chromatography.

Using the present procedures it was possible to account for 90.0% ± 5.0% of the total weight of most stones; the remainder was accounted for mainly by water and protein.10

We have used the quantitative procedure for the analysis of 100 urinary calculi and it has been found to be reliable and suitable for routine use. Moreover it requires only apparatus, which is currently available in most modern hospital laboratories.
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REFERENCES