

CHEMICAL ANALYSIS OF URINARY CALCULI (KIDNEY STONES) OBTAINED FROM PATIENTS ADMITTED AT JOS UNIVERSITY TEACHING HOSPITAL, JOS-NIGERIA

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ABSTRACT

The chemical make-up of urinary calculi of six (6) patients admitted at the Jos University Teaching Hospital (JUTH), Jos – Nigeria, have been studied using both qualitative and quantitative procedures. The samples were found to contain calcium, magnesium, ammonia, oxalates, potassium, sodium, carbonates, phosphates and uric acid. The analytical results indicate that most of the samples contain the following range of values for the various parameters determined; Ca (3.08 – 4.44) mg/100g, Mg (0.18 – 0.38)mg/100g, Na (0.11-0.34mg /100g), K(0.10-0.35mg /100g), NH₃ (0.04 -0.76) mg /100g, PO₄³⁻ (15.20 -29.69) mg/100mg, CO₃²⁻ (200 -900) mg /100mg, C₂O₄²⁻ (200 -900) mg/100g and uric acid (0.96 -2.88) mg/100g. However, two of the samples presented abnormally high values for some parameters, which have been identified as infection – based (struvite) calculi. It is also evident from the results that most of the stones are mixed (binary and ternary), a manifestation of more than one underlying metabolic disorder in the patients possessing the samples. From the results of the investigation and other presentations by the patients, it can be inferred that the most common metabolic abnormalities in the population within the study area emanated from infection and nutrition.

INTRODUCTION

Urinary calculi are universal diseases, which endanger human health with an increasing incidence since the earliest recorded times. For instance, a bladder stone was found in an Egyptian skeleton more than 7000 years old (Goad, 2003). However, it was not until much later, at the end of the 18th century that the first reports were published on the chemical composition of urinary calculi (Kesner, 2000). Since then, many discoveries about urinary stones in human beings and other domestic animals have been in place. Urinary calculi can form anywhere within the urinary tract, the kidney, ureter or in the bladder and ranges in sizes and shapes (Chidambaranathan, 2004).

Both intrinsic and extrinsic factors affect the susceptibility of a population to develop urinary calculi and this varies around the world (Goad, 2003; Roozbeh, 2001). Early studies by McIntosh and Salter (1942), Winer and Mattice (1943), Domanski (1939), Kirby et al (1957), Varley ([1967), Brown et al[1939], Kleeberg[1997], Hodgkinson[1971] and many other has led to the discovery of some important components of the urinary calculi amongst which include, calcium oxalate, various calcium phosphate, uric acid, cystine, cholesterol, proteins, magnesium ammonium phosphate, bile salts and some stone forming drugs (triamterene, indinavir, opherdine, etc). The discovery of these components has attracted much attention and investigation on the formation and medical technology was in principle, an established routine. However, while the formation

mechanism of a particular stone types in an area is understood, it may varies in other parts of the environment with such influences as infection, genetic, dietary preference and metabolic imbalance identified [Rogers, 1998].This has left many questions about the general formation mechanism of urinary calculi and their promoting and inhibiting factors unanswered (Mattice, 1943).

Consequently, it is in realization of the potential causes of these stones in this part of the environment that this work was done in order to study the chemical composition of the urinary calculi obtained from these patients which may serves as clues to the kinds of metabolic factors responsible for the formation of these stones in the study area and to offer useful suggestions that can lessen the risk of occurrences in future.

EXPERIMENTAL

Materials and Methods

The samples were collected from the Urology Department of the Jos University Teaching Hospital, JUTH, Jos-Nigeria and labeled appropriately. The chemicals used were of A.R grade except otherwise specified.

The samples were soaked in warm (44°C) distilled water for 10minutes in a 50ml beaker to remove any blood or attached tissue. They were removed, rinsed with cold water and oven-dried on filter papers for 45minutes. Each sample was allowed to cool before

being grounded into powder and stored in sterilized stirrer bottles, ready for use.

Qualitative Analysis

The samples were first tested for the presence of inorganic and organic components by igniting 0.5g each of the powders on a Pyrex glass slide. Little charring indicates organic matters while inorganic components were evidenced by presence of deposit after heating (Hodgkinson, 1971). Simple slide test methods by Kleeberg (1997) was employed to test for the presence of the constituents while the methods of McIntosh and Salter [1942] were used for their confirmation. Acid-insoluble components were separated by boiling 1.0g of the powder with 3:1ml HCl/deionised water to a clear solution with little residue at the bottom of the test. Ammonium ion, calcium oxalate, non-oxalate calcium and magnesium were confirmed from these solutions. The well known murexide test [Mcintosh and Salter, 1942] was satisfactory for uric acid and xanthine.

Digested samples using 10ml of concentrated HNO₃ were prepared and runned between 4000-500cm⁻¹ on an infrared spectrometer and the fingerprint spectra obtained compared to a number of library spectra of kidney stone components and the most similar spectrum characterized for each component in a sample.

Quantitative Analysis

Calcium, sodium, potassium and magnesium were determined using SOLAAR Atomic Absorption Spectrometer. A one-acid digestion method using 10ml of concentrated trioxonitrate (V) acid was used after which the clear solution was made up to 100ml. 5ml of the diluted solution was taken and further diluted to 50ml. It was then aspirated into the flame AAS at the various wavelengths of the metals [Ca(422.7nm), Na(589.0nm), K(766.5nm) and Mg(285.2nm)] and the conc. displayed in mg/l.

Colorimetric methods using vanado-molybdophosphoric acid and Nesler's reagent were used to determine phosphate and ammonia respectively. The concentrations were obtained from the absorbances and calibration graphs through extrapolation. Titrimetric methods using standard 0.01N KMnO₄ and 0.05N HCl were respectively used for the determination of oxalate and carbonates. For uric acid, a colour reagent was prepared by dissolving 14.0g of molybdenum free sodium tungstate dehydrate in 50ml of deionised water in a 1000ml flask which served as a reflux reactor. 11.0ml of 85% orthophosphoric acid was added and refluxed and until dissolution. The volume was made up to 1000ml with deionised water and stored in a refrigerator for use. 5mM/L of uric acid standard was prepared using 60mg of lithium carbonate, 84.055mg uric acid and 1ml of a 50:50 acetic acid/deionised

water [14]. Dilute stock standard (1 in 10) was also prepared and a blank before taken the absorbances at 710nm and the concentration of uric acid determined.

RESULTS AND DISCUSSION

The results of the qualitative analysis give an estimate of the concentration of the constituents in the samples based on the quantity of precipitate obtained and intensity of colour and absorption. The results are presented in Table 1. The quantitative results are shown in Table 2. The infrared absorption spectra of the samples were compared with those of the pure components and the absorptions characterized as follows; (3650-3100)cm⁻¹ stretching vibrations for OH-(alc.), N-H (Prim. & sec.) amine, NH₄⁺ group, Hydrate H₂O, sp²CH Ar/olefins.spCH; 2900 cm⁻¹ stretching vibrations for Sp³CH alkanes; 2850 cm⁻¹ stretching vibrations for C - H, (C = O)-H aldehyde; (2370-2330) cm⁻¹ stretching vibrations for P-H (hydrogen bonded phosphate); (2200-2000) cm⁻¹ stretching vibrations for N = C=O (isocyanates) C = C=O; 1650 cm⁻¹ stretching vibrations for Metallic salts of oxalates (-M-O-CO-CO-O-M-), C = C, C = N, Amides due to C=O stretch, R-S-CO-S-R.; (1400-1350) cm⁻¹ stretching vibrations for S = O, C=O (carboxylic acids), sulphonic acids (SO₂) derivatives and (1100-1000) cm⁻¹ stretching vibrations for PO₄³⁻, P-O-C (aliphatic). Samples of the spectra are shown in Figures 1 and 2.

The preliminary investigation revealed that all the stones contain both organic (characteristic flames and smells) and inorganic (presence of deposits after burning) constituents. The powders were found to be generally insoluble in water while the colours, shapes and homogeneities differ from each other. The high concentration of calcium carbonate and oxalate are perhaps responsible for the insolubility of the powders in water whereas the different colours and shapes result from the pathogenesis, location and the time spent in the body before extraction. The inhibiting roles of potassium and magnesium [Roozbeh, 2001; Calero, 2003] are evidenced by their low concentrations and absence of non-oxalate calcium.

Considering the concentration of the constituents in the samples as shown in Table 2, it can be observed that there is a statistical difference in the concentration of all the constituents between the samples. However, this is not to conclude that the patients' cases are entirely different from one another. For uric acid, the concentration is in direct proportionality with calcium for each of the patients. One possible explanation to this is the hypothesis that calcium stone crystals are more likely to grow in the presence of uric acid crystals such that high urine

uric acid excretion potentiates the formation of calcium containing stones [Calero, 2003].

Higher sodium concentration plays an important role in calcium excretion, which promote its supersaturation [Calero, 2003]. This effect is clearly seen in the direct relationship of both cations in the samples. The results for ammonia agreed favorably with the estimated quantities (quantity of ppt. and intensity of colour) obtained from qualitative analysis. The high concentration of ammonia in four patients, 'MUS 001', 'SUS 013', 'SUS 021', and 'US 002' coupled with their phosphate and

magnesium concentrations suggest that they are struvite (infected) calculi. Perhaps the most interesting part of this finding is the extreme difference between the concentration of oxalate/carbonate and that of other constituents. Pure calcium carbonate stones hardly occur in human urine [Kesner et al, 2000], but the carbonate concretions are formed as complex salts with apatite. The high concentration is an indication that most of the stones are carbonate apatite. May be a further study of the mineral content of the soil in the environment where the patients come from may give a clearer explanation to this.

Table 1. Estimated quantity of constituents present in six urinary calculi samples

Sample	Magnesium	Calcium	Phosphate	Ammonia	Uric acid	Cystine	Carbonate	Xanthine	Oxalate
MUS 001	+++	++	++	++	+	+	++	+	+++
SUS 009	++	++	+++	++	++	+	+++	-	++
SUS 013	++	+++	++	+++	+	+	+++	-	+
SUS 016	+	+++	++	+	++	+	+++	+	++
SUS 021	++	+++	++	+++	++	+	+++	-	+++
US 002	++	++	+++	++	+	+	++	-	++

KEY: +++ = strong; ++ = mild; + = less; - = non detectable.

Table 2: Concentration of constituents in urinary calculi samples

Sample	Na	Mg	Ca	K	PO ₃ ²⁻	NH ₃	U.A	CO ₃ ²⁻	Ox
MUS 001	2.11±0.00	0.32±0.01	3.08±0.01	ND	29.60±0.00	2.800±0.03	1.20±0.01	750.0±0.02	2000±0.02
SUS 009	1.21±0.00	0.32±0.00	4.13±0.00	0.13±0.00	424.0±0.02	0.08±.00	2.88±0.00	300.0±0.01	450.0±0.02
SUS 013	0.18±0.01	0.25±0.01	4.01±0.00	0.35±0.01	15.20±0.00	0.76±.00	1.92±0.01	450.0±0.02	200.0±0.02
SUS 016	3.41±0.01	0.18±0.01	4.44±0.00	0.12±0.00	20.00±0.01	0.04±0.00	2.64±0.00	600.0±0.00	400.0±0.01
SUS 021	1.34±0.01	0.40±0.00	4.05±0.02	0.10±0.00	28.00±0.01	1.22±0.01	2.64±0.00	900.0±0.01	900.0±0.01
US 002	0.33±0.01	0.38±0.00	3.37±0.00	ND	280.0±0.02	0.40±0.00	0.96±0.00	340±0.10	780±0.30

Average of duplicate determination. ND = Not detectable

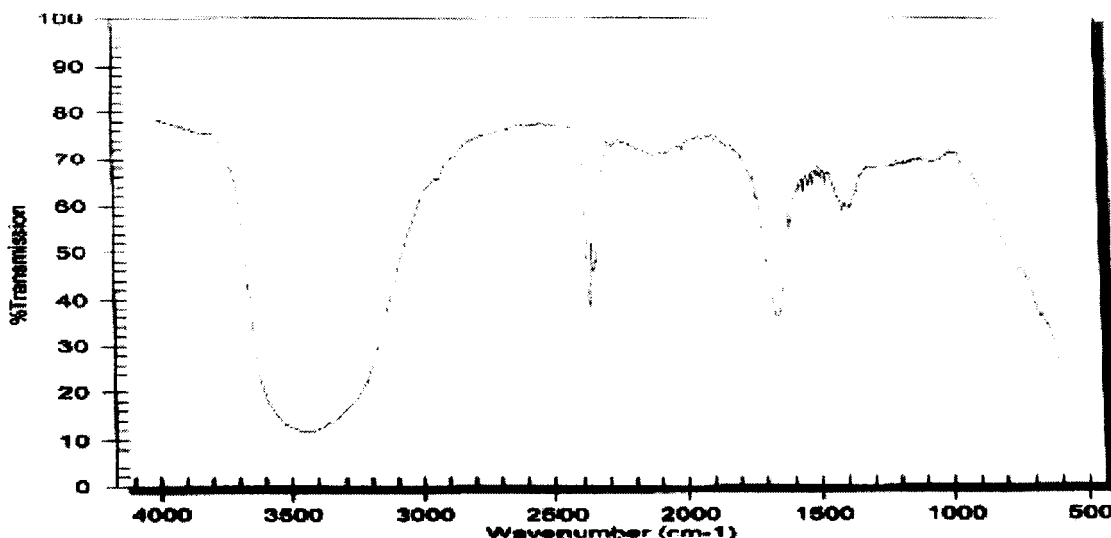


Figure 1: IR spectrum of a urinary calculus 'MUS 001'

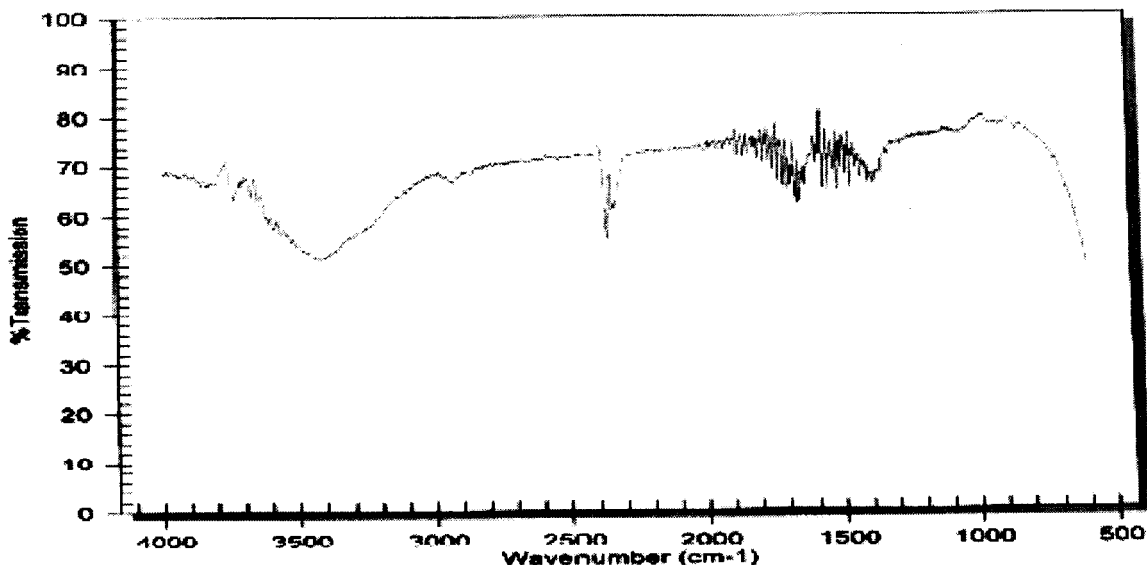
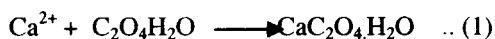


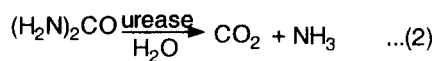
Figure 2: IR spectrum of a urinary calculus 'SUS 016'

Oxalate, a metabolic product, which is eliminated mainly in the urine [Batanjac, 2000], is a common metabolic disorder amongst the population under study. This must have resulted from eating a variety of foods rich in oxalic acids such as spinach, waterleaf, bitter leaf, sorrel, pumpkin, lettuce, garden egg, cocoyam leaf, rhubarbs and tomatoes [Roosbeh, 2001; Calero, 2003; Ladeji et al. 2004] which are commonly consumed in the area of study. Most of these foods contain digestible cellulose, which aids fermentation processes in caecum, leading to the generation of these oxalates, which can easily complex with excess calcium in the body to form calcium oxalate. The generated oxalates can be injurious to renal epithelial cells which may result in their death and breaking apart into vesicles which can act as nucleators of the calcium oxalate and promote its crystallization from the solution since it contains these solutes in high concentrations [Calero, 2003]. Renal tubular diseases can as well impair the kidney from proper excretion of the generated oxalate and this is easily complexed with any available calcium.



More worrisome is infection which is associated with over 75% of the stones analyzed. Most diseases of the kidney usually starts as apatite under a chemical driving force (supersaturation), when the concentration of urinary solutes (Ca^{2+} , $\text{C}_2\text{O}_4^{2-}$, PO_4^{3-} , CO_3^{2-} , uric acid, etc) become too high [Calero, 2003].

Proteus species and other urease-secreting bacteria (*klebsiella* and *pseudomonas species*) are key actors infection stones formation [Roosbeh, 2001; Calero, 2003]. Secreted urease can split urea (always present at high concentration in urine) as follows:



The ammonia hydrolyses, increasing the pH, which facilitates the precipitation of ammonium and magnesium phosphate. These can combine to form struvite (infection) stone [Calero, 2003].

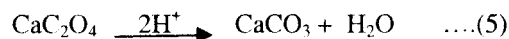


Another mechanism through which the calcium carbonate accumulates in the body is through drinking hard water which is a characteristic of most ground water in the study area [Egga, 2002]. The calcium and magnesium hydrogen carbonates can break down as follows:

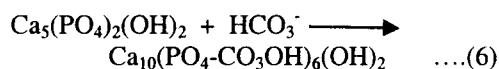


Where M = Mg and Ca

The high concentration of oxalate in the patients can as well contribute to the accumulation of the carbonate in the body when the urine becomes acidic due to high uric acid.



Most of the carbonates accumulate in the kidney region, combining with the apatite with time under favorable conditions of high concentration, supersaturation and temperature.



CONCLUSION

The results demonstrated that stones form in most cases in this environment are as a result of a combined effect of several factors ranging from metabolic abnormalities emanating from infection in the kidney or bladder and nutrition (continues eating of common food items which are rich in oxalic acids such as spinach, green vegetables, nuts, rhubarbs, cocoa etc which are very common in the study area. As a whole, hygiene is a factor that must be considered serious in order to curtail the rate at which infection (struvite) stones occur in this environment and these vegetables should be properly washed before consumption to reduce the concentration of insoluble antinutritional nutrients like oxalate.

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