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Biochemistry

CHEMICAL COMPOSITION OF URINARY TRACT CALCULI ASSESSED BY A BASIC METHOD

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ABSTRACT

Background: Urolithiasis is the presence or formation of stones at any anatomic location in the genitourinary system. It is a common condition, which affects both genders but has a predilection for males. Both environmental and genetic factors contribute to calculi formation. This retrospective study sought to determine the chemical composition of the inorganic minerals, which constitutes the uroliths at the University Hospital of the West Indies for the 4-year period January 2009 to December 2012.

Method: The study was conducted on 288 urinary tract stones received at the Chemistry laboratory. Qualitative chemical analysis was performed for calcium, magnesium, phosphate, oxalate, uric acid, cystine and bicarbonate by Wootton's method.

Results: The incidence of males and females was in the ratio of 1.4:1. Calcium was present in 96.2% of the stones, followed by phosphate 67.7%, oxalate 56.3%, magnesium 28.2% and uric acid 17.3%. Mixed uric acid was present in 16.3% and urinary stones containing bicarbonate accounted for 11.8%. There was one cystine stone.

Conclusion: A relatively high proportion of the urinary tract stones consisted of both pure and mixed calcium phosphate, followed by calcium oxalate and uric acid. More detailed information on both the composition and structure of the urinary tract stones may be garnered using more advanced methodology.

KEYWORDS : urolithiasis, stones, calcium, phosphate, oxalate, uric acid

Introduction

Urolith formation is quite heterogeneous and current investigations have alluded that this is due to the interplay between genetic, dietary, environmental and hormonal factors [1,2]. Urolithiasis is a fairly common disorder across the globe with lifetime prevalence between 5-10%. [3,4,5,6]. The incidence has gradually increased in the past decade and the common clinical presentation of the disease, "renal colic", currently accounts for up to 0.1% of all hospital admissions internationally [7]. Urolithiasis has a predilection for males, with a male to female ratio of approximately 3:1[1,3]. The disease usually affects the working age group, between the ages of 15-60 years, with younger aged patients usually having inborn errors of metabolism or protein deficient diet, therefore, a more specific chemical composition of the stones [8].

The majority of stones are composed primarily of calcium oxalate, calcium phosphate and magnesium ammonium phosphate (struvite), while a small number are comprised of uric acid and cystine [9]. About 70% to 80% of all stones are primarily calcium oxalate or calcium phosphate crystals [7,10]. The chemical composition of urinary tract stones differs based on site of formation, which is usually secondary to the underlying aetiology. As living standards progressed, the chemical composition of the stones in tropical countries mimicked those found in more affluent western societies; with a shift from being composed of ammonium acid urate crystals to calcium oxalate or calcium phosphate [6]. These calcium-based mixed stones are formed in the upper urinary tract and associated with acidification of urine due to a myriad of underlying causes which include obesity and insulin resistance [1]. Despite the well-known associations of urolithiasis with diet, temperature and sex, the genetic component and modes of stone formation are still poorly understood, even when it comes to the formation of the most banal variety, calcium oxalate/phosphate stones [11]. Evaluation of the chemical constituents has elucidated a possible pathway for the development of calcium oxalate stones via interstitial apatite crystal formation [12]. Despite this not being dictum, it illustrates the application of stone analysis in not just defining the constituents, but determining how these inorganic minerals may be utilized in eventually unraveling the mysteries of urolithiasis.

There is a notable paucity in data regarding the types and composition of urinary tract stones recovered from patients within the Caribbean region. This study, therefore, sought to determine the chemical constituents, particularly that of inorganic minerals of urinary tract stones collected at The University Hospital of the West Indies over a 4-year period.

Materials and Methods

This retrospective descriptive study was conducted in the Chemical Pathology Laboratory at the University of the West Indies. The study was performed in conformance with the Declaration of Helsinki and received approval from the University of the West Indies/University Hospital of the West Indies/Faculty of Medical Sciences Ethics Committee. The period under study was January 2009 to December 2012. There were no exclusion criteria and all patients irrespective of gender or age, diagnosed with urinary tract stones were included in the study. Qualitative chemical analysis of the stones for calcium, magnesium, phosphate, oxalate, uric acid, cystine and bicarbonate was performed. Data regarding the site of origin of the stones in the urinary tract was unavailable of the data. The samples were washed with deionized water, dried in air, stone powder obtained by pulverization in an agate mortar then divided into three portions.

According to the method as reported by Wootton [13], cold N-nitric acid was added to one portion of the powder. Effervescence indicated the presence of carbonate. The mixture was then boiled, cooled and filtered. The filtrate was then divided into three portions and used to detect (1) calcium with ammonium oxalate, magnesium with potassium phosphate and ammonia, (2) phosphate with ammonium molybdate and (3) oxalate with calcium chloride. A second portion of the powder was boiled with N-potassium hydroxide and Folin's uric acid reagent, then sodium cyanide was added to the filtrate to detect uric acid. (3) Cystine was detected with sodium nitroprusside and sodium cyanide. Ammonia was not tested for as it was not included in Wootton's methods [13]. Carbonates were determined by the effervescence test by exposing the stone powder to concentrated hydrochloric acid. Any compound which was less than 10% of the weight of the stone was disregarded. Data processing, analysis and comparison was carried out using SPSS 11.

Results

A total of 379 (225 males, 154 females) stones were received. Most were fragmented. Insufficient sample was obtained for 91 (59 males, 32 females) leaving 288 (166 males, 122 females) for complete analysis (Table 1).

Calcium the main constituent was present in 96.1% of the stones followed by phosphate 67.7%, oxalate 56.3%, magnesium 28.1% and uric acid 17.7%. Mixed calcium phosphate accounted for 42.4% of the stones while there were 25.3% pure calcium phosphate stones. Mixed calcium oxalate accounted for 43.4% of the stones while there were 12.8% pure calcium oxalate stones. Mixed uric acid was present in 16.3% and urinary stones containing bicarbonate accounted for 11.8%.

Table 1: Composition of all urinary tract calculi assessed over a four year duration

	Total 288	Male 166 (57.6%)	Female 122 (42.4%)
CaA-	204 (70.8%)	119 (58.3%)	85 (41.7%)
CaMgA-	73 (25.3%)	39 (53.4%)	34 (46.6%)
MgA-	8 (2.8%)	7 (87.5%)	1 (12.5%)
UA	3 (1.0%)	1 (33.3%)	2(66.7%)

Table 2 shows the 204 stones that contained calcium as the only cation and mixtures of the various anions. The males to female ratio was 1.4:1. Calcium phosphate (35.8%) was the main constituent in most stones from both males and females, followed by those with calcium, oxalate and phosphate (22.1%) and then calcium oxalate (18.1%).

There were 73 stones that contained both calcium and magnesium with other anions. The most common compositions were calcium, magnesium, oxalate and phosphate (27.4%), followed by calcium magnesium oxalate (24.7%) and then calcium magnesium phosphate (17.8%). One stone contained cystine (Table 3). There were 8 (2.8%) stones with magnesium as the sole cation, 7 of which were from males. Pure uric acid was present in only 3 (1.0%) stones (Table 1).

Table 2: Prevalence of urinary tract calculi comprised of calcium and mixtures of anions

	Total (%)	Male (%)	Female(%)
$CaPO_4$	73 (35.8)	45 (61.6)	28 (38.4)
$CaOxPO_4$	45 (22.1)	26 (57.8)	19 (42.2)
CaOx	37 (18.1)	21 (56.8)	16 (43.2)
CaOxUA	11 (5.4)	8 (72.7)	3 (27.3)
$CaOxPO_4UA$	9 (4.4)	6 (66.7)	3 (33.3)
$CaPO_4UA$	7 (3.4)	4 (57.1)	3 (42.9)
$CaOxPO_4HCO_3$	7 (3.4)	2 (28.6)	5 (71.4)
$Ca\ PO_4HCO_3$	4 (2.0)	(25.0)	3 (75.0)
$CaHCO_3OxUA$	3 (1.5)	1 (33.3)	2 (66.7)
$Ca\ PO_4HCO_3UA$	2 (1.0)	2 (1.0)	0
$CaHCO_3Ox$	2 (1.0)	1 (0.5)	1 (0.5)
CaUA	1 (0.5)	1 (0.5)	0
$CaHCO_3$	1 (0.5)	1 (0.5)	0
$CaOxPO_4HCO_3UA$	(0.5)	0	1 (0.5)
$CaHCO_3UA$	1 (0.5)	0	1 (0.5)
Total	**204 (100)**	**119 (58.3)**	**85 (41.7)**

Table 3: Urinary tract calculi with calcium, magnesium and a combination of different anions

Composition	Total	Male	Female
$CaMgOxPO_4$	20	10	10
CaMgOx	18	12	6
$CaMgPO_4$	13	8	5
$CaMgPO_4HCO_3$	6	2	4
CaMgOxUA	4	3	1
$CaMgPO_4UA$	3	2	1
$CaMgPO_4HCO_3UA$	3	0	3
$CaMgHCO_3$	1	0	1
$CaMgOxPO_4UA$	1	1	0
$CaMgOxPO_4HCO_3$	1	0	1
$CaMgOxHCO_3UA$	1	0	1
$CaMgOxHCO_3$	1	0	1
$CaMgOxHCO_3UACyst$	1	1	0
Total	**73**	**39**	**34**

Discussion

Urolithiasis is one of the most common urological disorders [14]. Urinary tract stones are most often formed in the kidney, ureter and to a lesser extent in the bladder. The proper management of patients with renal stone disease involves the analysis of urinary calculi and various methods have been employed including chemical, thermo-analytical, infrared spectrophotometry, solid state nuclear magnetic resonance spectroscopy and X-ray diffraction crystallography [15-17]. All these methods have been reported to be reasonably accurate in the determination of the composition and structure of the urinary stones. The rapid and inexpensive method that was used in our laboratory to determine the constituents of renal calculi is a simple qualitative chemical analysis [13].

In this study, males were more likely to present with urinary tract stones, with a ratio of 1.4:1. In general a male gender preponderance for stone formation is well established and this is a constant trend seen in most populaces. For the most part comparative studies done in South East Asia, Balkan region and the Middle East have reported higher male to female ratios such as 3.3:1 in Pakistan [18], 2.1:1 in Kurdistan [19], 1.8:1 in Macedonia [20], and 1.5:1 in India [21]. The ratio of 1.4:1 in our study population is constant and directly mirrors the value reported by Choo Kang [22] in a retrospective study in this institution, the University Hospital of the West Indies. However, some outliers to the norm exists and in a Thai population a slight female preponderance of 0.96:1 has been reported [23], which begs to question the ethopathogeny of stones in the varied populations. The male preponderance for lithogenicity may be due to a number of factors including the effects of androgens while the genitourinary anatomical structure where the urethra is smaller in diameter makes it more likely to be obstructed by calculi. Other aetiologic considerations include reduced activity of macromolecular and micromolecular inhibitors to calcium stone formation [24] and lower citrate concentrations in urine compared with their female counterparts [25].

The study revealed that most of the urinary stones consisted of calcium phosphate, calcium oxalate and uric acid. The presence of calcium, the main cation, in 96.1%, was marginally higher than the 93.9% reported by Choo-Kang [22]. Magnesium was detected in 28.1% reflecting an increase of greater than three-fold when compared to 8.3% [22]. No clinical information was available, however, this may indicate increased lithiasis as a result of urinary tract infections. Phosphate was present in 67.7% of the urinary stones followed by oxalate in 55.7%. The major combination was that of calcium phosphate, followed by a mixture of calcium phosphate and oxalate, then calcium oxalate.

A multifactorial etiology may be responsible for the presence of mixed stones found in this study. Calcium being the most common cation present in the stones suggests hypercalciuria due to hypercalcaemia likely secondary to conditions such as primary hyperparathyroidism, sarcoidosis, vitamin D intoxication, phaeochromocytoma, thiazide diuretic therapy, primary hyperthyroidism and a high protein diet. The synthesis of calcium phosphate is usually associated with hypercalciuria due to primary or secondary hyperparathyroidism, renal tubular acidosis and hypocitruria [26]. A treatment for calcium oxalate stones utilizes potassium citrate which promotes the formation of calcium phosphate recurring stones by increasing urinary pH and the retention of apatite [27], a possible cause for the increased prevalence of calcium phosphate stones in this study as opposed to the higher prevalence of calcium oxalate stones in a previous study by Choo Kang [22].

Mixed calcium phosphate stones accounted for 42.4% of cases which is higher than the 36.5% reported by Choo-Kang [22] and 11.6% reported by Ullah et al in a Pakistani referral centre. [18]. Approximately one-quarter of the urinary stones (25.3%) were pure calcium phosphate while 12.8% were pure calcium oxalate. The latter result is marginally lower than the 24.4% pure calcium oxalate stone reported previously by Choo Kang [22] and greater than twice higher than the 9.2% found by Aiken and Bennett [28], both retrospectives studies conducted at this institution. With respect to pure calcium phosphate, Choo-Kang [22] reported a lower figure of 17.7% and Aiken and Bennett [28] reported 6.1%, showing evidence of increasing numbers. Mixed calcium oxalate stones accounted for 43.4% of cases observed. This figure was lower than reported values of 48.8% recorded by Aiken and Bennett [28] but higher than 39.3% documented by Choo Kang [22] and 34.8% by Ullah et al [18].

Calcium oxalate is one of the major urinary stones found in this study and it may be caused by idiopathic hypercalciuria, hyperoxaluria, hypocitruria, hyperuricosuria and a number of conditions that facilitates increased calcium in the urine. Elevated oxalate in the urine may be as a result of increased intake of foods containing oxalate such as cocoa, spinach, nuts, pepper, tea, beet and rhubarb [29]. There are also a number of diseases such as chronic pancreatitis, bacterial overgrowth syndromes and biliary disease that causes increased absorption of oxalate [30]. There is also evidence that elevated oxalate in the blood and hyperoxaluria could be due to mutation in the gene which controls the transportation of oxalate, uric acid and chloride in the renal tubules [31].

Uric acid was present in 17.7% of the urinary stones. Only 3 stones were pure uric acid stones, constituting 1% of the total number of uroliths evaluated. Pure uric acid stones are quite infrequent and tend to be representative of systemic disorders of urate metabolism. Despite previous studies in similar cohorts from developing countries having higher volumes of pure uric acid stones; 5.3% reported by Choo-Kang [22]; 4.6% reported by Aiken and Bennett [28]; and 9.0% reported in an earlier study [32]; they never exceed the purported 10% prevalence seen in industrialized ones. The figure of 16.7% mixed uric acid stones is less than values of 68.0% seen in a Pakistani population [32], 32.8% [27] and 32.4% [22] seen in previous studies from this institution but more akin to 15.8% [33] reported out of Japan by Rayhan et al.

There are a number of risk factors which may contribute to the formation of uric acid stones. These include: hyperuricosuria, above average intake of vitamin D, gout, persistent acidity of urine with pH less than 5, urinary tract infections, low urine volume as seen in dehydration, and obstruction of the urinary tract [34]. The climate in Jamaica is tropical with fairly high temperatures which contributes to dehydration with reduced urinary output, and with a diet rich in purines and persistently low urine pH, there is a chance of uric acid stone formation [35].

Magnesium ammonium phosphate stones are often associated with urinary tract infection potentiated by urea-splitting bacteria such as *Pseudomonas, Klebsiella, Mycoplasma* and *Proteus*. These urease-producing bacteria infect the urine, hydrolyzing urea with the formation of ammonia and increased urinary pH, leading to calcium and magnesium precipitates and stone formation [31, 36]. In this study urinary calculi containing magnesium accounted for 28.1% of the cases which was higher than other reports such as 8.6% [22] and 20.0% [37]. It is unclear whether or not all the magnesium composite stones in our cohort was directly associated with urinary tract infections as the clinical information was not readily available at the time of data analysis.

In this study one stone contained cystine (0.3%) which is equivalent to values of 0.6% [22] and 0.8% [28] observed previously at our institution. Cystine stones are a rarity and even in patients known to have the genetic predisposition to formation, there appears to be sustained cystinuria without the formation of stones. [38]. Cystinuria is an autosomal recessive disease caused by mutation in the SLC7A9 and SLC3A1 genes. This causes defective transepithelial transport and inadequate reabsorption of dibasic amino acids such as cysteine, lysine, ornithine and arginine in the proximal convoluted tubules [39]. There is increased excretion of cystine in the urine and in the presence of acidic conditions cystine crystals develop. These crystals are hexagonal in shape, white or translucent in appearance and may turn green on exposure to air [40].

In summary, males are more likely to present with urolithiasis. Consistent with established dictum, a relatively high proportion of the urinary tract stones contain calcium as the sole cation with a combination of calcium and phosphate, or calcium and oxalate being more prevalent. Simple qualitative techniques such as the Wootton [13] or Hodgkinson [41] methods provide useful information about the constituents of uroliths and afford insight into the pathogenesis of these lesions. Armed with such information treatment protocols can be instituted in an attempt to decrease disease burden. The use of more advanced methods of chemical analysis such as solid state nuclear magnetic resonance spectroscopy or X-ray diffraction crystallography, does garner more information about the chemical composition of the urinary tract stones. In the future utilization of these techniques may assist in evaluation which will increase our understanding of their etiology and improve the management of patients with urolithiasis.

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