ANALYSIS OF URINARY CALCULI BY PHYSICAL METHODS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

by

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LIST OF ABBREVIATIONS

Al	aluminium
AP	activity product
C	carbon
Ca	calcium
CaOx	calcium oxalate
CaP	calcium phosphate
Cl	chlorine
Cl	chlorine
COD	calcium oxalate dihydrate
COM	calcium oxalate monohydrate
COT	calcium oxalate trihydrate
Cu	copper
CYS	cystine
EDX	Energy Dispersive X-ray
EDAX	Elemental distribution analysis X-ray
ESWL	Extracorporeal shock wave lithotripsy
F	fluorine
FTIR	Fourier Transform Infrared
FT-IRS	Fourier Transform Infrared Spectroscopy
GAG	glycosaminoglycan
HUSM	Hospital Universiti Sains Malaysia

ICDD	International Centre for Diffraction Data
IQR	Interquartile range
JCPDS	Joint Committee on Powder Diffraction Standards
K	kalium
KBr	potassium bromide
Ksp	thermodynamic solubility product
LUTC	lower urinary tract calculi
Mg	Magnesium
N	nitrogen
Na	sodium
O	oxygen
P	phosphorus
PCNL	percutaneous nephrolithotripsy
PCVL	percutaneous vesicolithotripsy
S	sulfur
SEM	scanning electron microscopy
SP	solubility product
SPSS	Statistic Package of Social Science
STR	struvite
THP	Tamm-Horsfall glycoprotein
UA	uric acid
URS	urethroscopy
UTI	urinary tract infection

UUTC	upper urinary tract calculi
Vit-D	vitamin D
XRD	X-ray Diffraction
XRF	X-ray Fluorescence

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ABSTRAK

Pengenalan

Dalam kajian batu karang sama ada secara praktikal dan klinikal, analisis batu karang secara jitu dan sistematik adalah amat penting. Pengetahuan yang tepat berkenaan komposisi sesuatu batu karang adalah prasyarat terhadap sebarang rawatan perubatan untuk pesakit batu karang. Penyakit batu karang sangat kerap didapati di Negeri Kelantan, namun kajian analisa terhadap batu karang tidak dilakukan secara rutin di HUSM.

Pengetahuan berkenaan komposisi batu karang memberikan maklumat penting berkenaan punca utama terhasilnya penyakit batu karang, termasuk penyakit metabolik, kehadiran jangkitan, kemungkinan terdapatnya artifak, di samping kesan metabolism ubat-ubatan yang diambil pesakit. Analisis batu karang berdasarkan kualiti yang tinggi adalah keperluan penting dalam pengurusan pesakit, di mana ia membolehkan punca penyakit dikenalpasti dan membolehkan strategi rawatan yang berkesan boleh diimplantasi bagi mengelakkan berlaku penyakit batu karang yang berulang.

Setiap teknik analisis mempunyai kelebihan masing-masing bergantung kepada keadaan. Dimana satu teknik sahaja kemungkinan tidak mencukupi untuk tujuan semua analisis, oleh itu kombinasi teknik-teknik berkenaan adalah digunakan dan memberikan keputusan yang lebih tepat dan jitu. Oleh yang demikian, kami telah mengkaji satu ratus lima puluh lima sampel batu karang di HUSM dengan menggunakan teknik-teknik fiziko-kimia

seperti X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Scanning electron microscopy (SEM) dan X-ray fluorescence (XRF), di mana ia memberikan keputusan yang terbaik dalam mengenalpasti komposisi batu karang.

Tujuan

Tujuan kajian ini adalah untuk mengenalpasti pelbagai komposisi batu karang di Hospital Universiti Sains Malaysia dengan meggunakan kaedah-kaedah fizikal moden. Kami melihat keputusan yang diperolehi berkenaan komposisi, keberkesanan kaedah-kaedah tersebut dan kepentingan ujian analisis untuk mengenalpasti komposisi batu karang dalam keadaan rutin. Ia seterusnya dapat membantu meningkatkan rawatan dan pengurusan pesakit.

Tatacara

Ini merupakan kajian secara 'cross-sectional' yang melibatkan satu ratus lima puluh lima batu karang telah dikumpulkan daripada para pesakit batu karang di HUSM, bermula daripada bulan Januari 2003 sehingga Disember 2009 untuk tujuan analisa secara fizikal. Data demografi asas seperti nama, nombor identifikasi dan nombor pendaftaran hospital, umur, jantina dan kumpulan etnik juga dicatatkan. Lokasi batu karang yang dikeluarkan dan kaedah rawatan juga diambil. Batu karang yang mempunyai berat lebih daripada 500mg telah dipilih dan disediakan untuk tujuan kalian. Spesimen tersebut dicuci dengan air, dikeringkan, ditumbuk hingga halus dan dibahagikan kepada empat sampel dan

disediakan untuk analisis menggunakan Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction Crystallography (XRD), Scanning Electron Microscopy (SEM) dan X-ray Fluorescence (XRF). Semua analisa dijalankan di Makmal Sains, Sekolah Kejuruteraan Bahan dan Sumber Mineral, Kampus Kejuruteraan USM Transkrian Pulau Pinang.

Keputusan

Berdasarkan kajian ini, sebanayak 155 sampel telah diperolehi berdasarkan kriteria yang ditetapkan. Umur median (IQR)) bagi populasi kajian ini ialah 60.0 (21) tahun, dengan julat umur dari 6 tahun hingga 86 tahun. Kebanyakan pesakit berumur di antara 50 hingga 70 tahun. Daripada 155 pesakit, 124 adalah terdiri daripada kalangan lelaki (80%), 31 adalah terdiri daripada kalangan wanita (20%). Nisbah lelaki wanita ialah 4:1. Kebanyakan pesakit terdiri daripada kalangan kaum Melayu iaitu seramai 151 orang (97.4%) dan bukan Melayu seramai 4 orang pesakit (2.6%).

Sebanyak 76 pesakit (47.8%) terdiri daripada batu karang saluran bawah, 75 pesakit (47.2%) terdiri daripada batu karang saluran atas dan 8 pesakit (5.0%) mempunyai kedua-dua batu karang di saluran atas dan bawah.

Seramai 145 (91.2%) telah menjalani pembedahan minimal endourology, 12 (7.5%) menjalani pembedahan terbuka dan 2 (1.3%) pesakit telah menjalani kedu-dua prosedur.

Berhubung dengan faktor lokasi batu karang, terdapat hubungkait yang signifikan berhubung faktor lokasi batu karang dengan umur dan jantina pesakit (p-value < 0.001).

Namun, tiada hubungkait yang signifikan antara lokasi batu karang dengasn kaum dan pembedahan.

Keputusan kajian menggunakan teknik FTIR menunjukkan bahawa komponen-komponen mineralogy batu karang yang dikumpul daripada pesakit HUSM kebanyakannya terdiri daripada komposisi asid urik iaitu sebanyak 59 (38.1%). Sebanyak 42 pesakit (27.1%) mengandungi batu karang jenis struvite, di ikuti oleh calcium oxalate monohydrate sebanyak 32 pesakit (20.6%), dan calcium oxalate dehydrate 17 pesakit (11.0%). Apatite terdiri sebanyak 5 pesakit (3.2%).

Menggunakan teknik XRD, majoritinya ialah campuran batu karang jenis calcium oxalates (COM/COD) iaitu sebanyak enam puluh empat pesakit (41.3%), diikuti oleh calcium oxalate dihydrate sebanyak dua puluh enam pesakit (16.8%). Sebanyak dua puluh enam pesakit (16.8%) ialah calcium oxalate monohydrate (10.3%). Jumlah keseluruhan batu karang jenis calcium oxalate ialah sebanyak 106 (68.4%). Sebanyak lima belas jenis asid urik (9.7%) dan enam (3.9%) terdiri daripada batu karang jenis struvite dan dua (1.3%) adalah batu karang jenis campuran calcium oxalate monohydrate dan asid urik.

Keputusan daripada ujian batu karang menggunakan'scanning electron microscope' mencadangkan kristal calcium oxalate monohydrate (whewellite) dan calcium oxalate dihydrate (weddellite) mempunyai sistem monoclinic dan leper. Batu karang uric acid menunjukkan sistem monoclinic dan bentuk prisma, manakala sturvite mempunyai sistem monoclinic.

Berhubung persetujuan antara FTIR dan XRD teknik yang digunakan, hanya empat komposisi dapat dikesan oleh kedua-dua teknik. Persetujuan antara kedua-dua teknik adalah menggunakan kaedah statistik Kappa. Berdasarkan keputusan komposisi batu karang yang didapati, persetujuan antara keduanya adalah sederhana ($\kappa = 0.42$, p-value < 0.001)

Kesimpulan

Kesimpulannya, kombinasi sekurang-kurangnya dua teknik analisis fizikal adalah berfaedah dan member keputusan yang tepat yang diperlukan dalam analisis batu karang pelbagai komponenen. Dengan hanya meggunakan satu teknik sahaja adalah tidak tepat kerana setiap mesin dan teknik mempunyai faedah dan kekurangan masing-masing. Kami dapati teknik XRD dan FTIR spektroskopi adalah dua teknik yang patut diberi pertimbangan untuk analisis komposisi batu karang secara tepat dan berkesan. Namun begitu, untuk tujuan klinikal yang menitikberatkan berkenaan kos dan penyelenggaraan, kami dapati FTIR spektroskopi merupakan alat yang bagus untuk tujuan analisis komposisi batu karang kerana mudah untuk didapati di HUSM. Kosnya lebih murah dan mempunyai koleksi data yang besar, di mana ia membantu dalam analisis batu karang.

ABSTRACT

Introduction

In urolithiasis or kidney stone disease, research and in clinical practice the highly specific and systematic analysis of urinary calculi is of great importance. The exact knowledge of the type of the stone is a precondition of any medical therapy for urinary calculus. Urolithiasis is a very frequent finding in the Kelantan, but stone analysis is not routinely performed in HUSM.

Knowledge of the composition of calculi yields fundamental information concerning the pathogenesis of the disease, including metabolic abnormalities, presence of infection, possible artifacts, and even drug metabolism. Accurate high-quality urinary calculi analysis is an essential requirement for this approach to patient management, allowing possible causes to be defined, logical treatment strategies to be implemented and prevention of stone recurrence.

Each method has merit in specific situations. Whereas a single method may not be adequate for all analyses, a combination of methods used in a complementary fashion may be relied on for accurate results. We therefore set out to analyze one hundred and fifty five samples of urinary calculi in HUSM using the modern physicochemical methods, X-ray diffraction, Fourier Transform Infrared Spectroscopy, Scanning electron microscopy and

X-ray fluorescence which yield the best results in the differentiation of the constituents of urinary calculi.

Objectives

The objectives of this study are to determine the composition of urinary calculi in HUSM and correlate the urinary calculi composition using various techniques under physical methods. Apart from that, we would also like to determine the association of demographic features and location of the urinary stone formers. Last but not least, is to determine the agreement between these four methods. We study the results, the reliability of these methods and the important analytical tests for urinary calculi on identical stone material under routine conditions. Through accurate high-quality urinary calculi analysis, allowing logical treatment strategies to be implemented.

Methodology

This is an observational cross-sectional analytical study involves a total of one hundred and fifty five urinary stone formers in HUSM which were collected from January 2003 to December 2009. All stones removed from patients were placed in polyethylene dry bottles labeled with the name, identification number, hospital registration number, sex, age, date and ethnicity of the patient. The location of the stone and treatment were also recorded. Urinary calculi weighing more than 500mg were selected and prepared for the investigation. The specimens of urinary calculi were washed with distilled water, air-dried,

finely ground and pressed into powder and divided into 4 samples and prepared for analysis using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and X-ray Fluorescence (XRF). All tests were performed at the Science Labs, School of Material and Mineral Resources Engineering, Engineering Campus USM Transkrian Penang.

Results

A total 155 urinary calculi were included in our study based on the inclusion criteria. The median (IQR) age of our study population was 60.0 (21) years (range 6 to 86 years). The majority of patients were within 50 to 70 year old. Out of 155 patients, 124 patients (80%) were male and 31 (20%) were female. The male to female ratio is 4:1.The racial distribution of the study subjects were 151 (97.4%) Malay and only 4 (2.6%) were from other ethnic groups.

There were seventy six patients (47.8%) with lower urinary tract calculi, seventy five patients (47.2%) had upper tract calculi and eight patients (5.06%) had both upper and lower urinary calculi. One hundred and forty five patients (91.2%) had undergone minimal endourology procedures, twelve (7.5%) had open surgery and two patients (1.3%) had combined open and endourology intervention. Among the patients who underwent minimally invasive intervention, sixty five patients (41.1%), fifteen (9.4%), fifty eight (36.5%), four (2.5%) underwent Vesicolithotripsy, Urethrolithotripsy, PCNL and PCVL respectively.

Taking into account of the locality of the urinary calculi, there is significant association between the locations of the stone with age and gender of the patient (p-value < 0.001). There is no significant association between location of the urinary calculi with race and surgical intervention.

In the FTIR study, the most composition of the stone formers were uric acid stones 59 (38.1%). Forty two (27.1%) were struvite stones, followed by calcium oxalate monohydrate 32 stones (20.6%) and calcium oxalate dihydrate 17 stones (11.0%). The sum of oxalate stone was 49 stones (31.6%). Apatite stone were found in 5 patients (3.2%). There was no cystine or brushite caluli identified. However the numbers of the stone samples were small as both cystine and brushite stones may not be included in this study.

In XRD analysis, mixed calcium oxalates stones formed the majority of cases with sixty four patients (41.3%). Calcium oxalate dihydrate stones were found in twenty six patients (16.8%) and sixteen were calcium oxalate monohydrate (10.3%). The total number of calcium oxalate stone was 106 (68.4%). Twenty six of the analyzed stones were mixed stones (16.8%), fifteen were uric acid stones (9.7%), six (3.9%) were struvite stones and two were (1.3%) were calcium oxalate plus uric acid.

The results of scanning electron microscope micrograph study showed the morphology of urinary calculi. This method of urinary analysis suggests the presence of a monoclinic system and platy-like morphology of calcium oxalate monohydrate crystals, a monoclinic and platy-like shape of calcium oxalate dihydrate crystal, a monoclinic system and prismatic shape like of uric acid crystals and a monoclinic system of struvite crystals.

With regards to the agreement between FTIR and XRD analysis, only four compositions were detected by both methods. Thus, agreement between the two methods was obtained using Kappa statistic. Based on the results, there was moderate agreement between these two methods ($\kappa = 0.42$, p-value < 0.001) with regards to four urinary composition.

Conclusion

In conclusion, combination of at least two methods is beneficial and will give accurate result and necessary for the analysis of multi-component stones. Using single technique for urinary calculi analysis is observed to be inaccurate as different machine has its own advantages and disadvantages. We found that XRD and FTIR spectroscopic methods of analysis are currently two methods that should be considered for the accurate and complete characterization of crystals in urine and for the compositional analysis of urinary calculi. However, for clinical approach with consideration of cost and maintenance, the FTIR technique using library match spectroscopy would be a good tool for the analysis of urinary calculi constituents as it is accessible and available in HUSM. It is cheaper and has large collection of urinary calculi data which is helpful in the analysis of urinary calculi.

CHAPTER 1

INTRODUCTION

INTRODUCTION

Urolithiasis or urinary calculi is a major health problem with its high morbidity, high cost of management and potential for end-stage renal disease Worldwide epidemiological data show an increase in incidence rates of stone disease. In Germany, for instance, the incidence rate rose during the last decade from 0.54% to 1.47% (Hesse *et al.*, 2003). In the United States of America, an increase in stone disease of 37% was observed over the last 20 years (Stamatelou *et al.*, 2003). The reasons are multifold such as lifestyle, dietary habits and improved medical care.

Sreenevason (1981) had conducted a study in Malaysia for a period of five years; from 1972 to 1976. He reported that average occurrence of urinary calculi registered in Malaysia Medical Office of Health were 221 cases per year. According to Sreenevason, the incidence for upper tract in Kelantan was 33.3 per 100000 and 3.6 per 100000 populations for lower tract calculi. In another study by Lim *et. al.* (1988) reported that the annual incidence of new patients undergoing stone surgery in Kelantan was 10.1 per 100000 residents. The rate for upper tract stone was 3.4 per 100000 and 6.8 per 100000 for lower stone.

Gender and age related composition of urinary stones have also been identified by Daudon *et al.* (1995). Etiological factors which are considered responsible for stone formation are metabolic disorder, ion transport within the intestinal track and kidney and diet including fluid intake. Geographical location, water quality, hot climate, occupation, stress, drug and

bacteria induced factors are all external factors causing urinary stones. Considerable research in this area is being done to understand the three stages of stone formation, heterogeneous nucleation, crystal growth and crystal aggregation, and the role of modifiers (inhibitors, promoters and complexor) of the same in the stone formation phenomena. (Menon *et al.*, 1997). In order to understand the influence of environmental and etiological factors on growth of stones, it is essential to know the constituents of it. The composition of stones varies from patient to patient and also with time within the same patient.

Nowadays, excellent options for interventional stone therapy, such as ESWL, URS and PCNL, warrant a comfortable stone management. The new methods are non-or minimally-invasive; they can be performed often without general anaesthesia in an outpatient setting; only some procedures require a short hospital stay. It is, therefore, not surprising that stone removal has become more attractive than elaborate metaphylactic measures (Hesse *et al.*, 2006).

In an epidemiological study, Ljunghall and Headstrand (1975) reported an approximately 50% natural recurrence after 5 years. As early as 1986, the annual recurrence rate after ESWL (consider stone-free rate at 3 months) was reported to be 8% (Ljunghall *et al.*, 1975). The recurrence rate after surgical intervention reported by Sutherland et al. (1982) was 40% after 11 years. There is a need to develop a cost-analysis model to compare the expenditure of treating recurrent stones versus the cost of prophylaxis against these stones to break the stone cycle and give a cost-effective solution to the patient.

In the daily routine, metabolic evaluation and metaphylaxis; which comprises metabolic therapy and secondary prevention of stone disease, have regrettably become less important. On the other hand, we have learnt from a lot of other diseases that prevention is superior to intervention. And, in times of financial pressure, it is more cost effective.

Stone metaphylaxis reduces the recurrence rate by some 40% thus lowered annual cost of stone removal. Medical treatment could lower the recurrence rate after ESWL, endourological procedures as well as open surgery. Hence, complete metabolic evaluation and stone composition, especially in high risk groups, is integral to the selection of the most appropriate intervention to prevent kidney stone recurrence (Motley *et al.,* 2001., Curhan *et al.*, 2001).

Kidney stones are a common disorder of the urinary tract. Knowing the composition of the calculus is thus important for a more complete evaluation of the metabolic study. The nature of the urinary calculus in fact helps the physician to find a convenient metaphylaxis consisting of both sanitary and therapeutic measures. Study of the composition of urinary calculi remains one of the most interesting aspects of the kidney stone pathology as every calculus contains some information concerning its composition and its structure which result from complex processes. Some of these leave their characteristic marks on the structure of the concretion. For this reason it is important to recognize the distinctive features of the calculus, its nature and composition (Saita *et al.* 1997).

Numerous approaches have been used for the analysis of renal stones. These include sophisticated techniques such as X-ray diffraction crystallography, infrared spectroscopy, scanning electron microscopy with energy dispersion, x-ray fluorescence, thermo-gravimetry, polarized microscopy or through to simple techniques such as wet chemical analysis.

There is now growing consensus that stone analysis is essential for successful prophylactic management of renal stone disease. Accurate stone analysis together with relevant blood and urine investigations are essential requirements for this approach to patient management, allowing possible causes to be defined and logical treatment strategies to be implemented. It is of note that calculi analysis is poorly performed, if at all, in many centers and this has fuelled the debate of the usefulness of this test. (Henderson MJ. 1995).

Stone analysis complements, but does not replace, blood and urine analysis in overall metabolic assessment of the stone former. It may be viewed as a 'biochemical biopsy' of the urinary environment at the time of crystal deposition and so allow identification of risk factors and/or specific diseases leading to its formation. Thus, the finding of a stone constituent such as cystine points to a specific diagnosis. Finding calcium oxalate and calcium phosphate raises the possibility of a number of disorders in the handling and metabolism of calcium (primary hyperparathyroidism, hypercalciuria, renal tubular acidosis) or oxalate (primary hyperoxaluria, gastrointestinal disorders) (Kasidas *et al.* 2004).

Apart from that, the stone analysis can help in demonstrating epidemiological trends in stone composition and distinguishes genuine stones from a range of artefactual materials. Differentiation of these materials of dubious origin from genuine renal stones prevents further unnecessary urological investigations and waste of resources.

In Malaysia, no systematic study on the composition of urinary stone in this region has been done using physical methods analysis. Today, advent of analytical tools has made it possible to examine and or identify the urinary stone constituents using various physical techniques.

We have done a study on the analysis of urinary stones from various patients in HUSM, Kelantan with the aim that the data from these patients may provide some idea about the compositions, associated factors, which causes high urinary stone disease of this region. We report analysis of one hundred and fifty five samples obtained from different patients using four physical techniques; powder X-ray Diffraction, Fourier Transform Infra-Red Spectroscopy, X-ray Fluorescence and Scanning Electron Microscopy in this dissertation.

1.1 Objectives

1. To determine the composition of urinary calculi in HUSM by physical methods.
2. To correlate the urinary calculi composition using various techniques under physical methods.
3. To determine the association of demographic features and location of the urinary stone formers.
4. . To determine the agreement between these methods.

Physical Methods:

FTIR : Fourier Transform Infrared Spectroscopy

XRD : X-Ray Diffraction Crystallography

SEM : Scanning Electron Microscopy

XRF : X-Ray Fluorescence

CHAPTER 2

LITERATURE REVIEW

LITERATURE REVIEW

2.1 Epidemiology of Urinary Calculi

Urinary tract stone disease is a frequent event that affects the population in all countries. Nevertheless, the overall probability of forming stones considerably differs in various parts of the world (Tiselius HG. 2000; Stamatelou *et al.*, 2003). In recent years kidney stone disease has become more widespread in developed countries. In industrialized countries the incidence of urinary tract stones is about 1,500–2,000 cases per million inhabitants (Tiselius HG. 2000). Stone disease has increased by 37% in the United State, reaching an incidence of 1.24% in the general population (Stamatelou *et al.*, 2003) and by 27% in Germany with the incidence rising from 0.54 to 1.47%. In Italy at present the incidence is 1.7% (Hesse *et al.*, 2004). Although 12% of the general population is affected by stone disease, the prevalence varies with geographical location, diet, environment and climate. Prevalence is highest in Saudi Arabia (20% of the population) (Robertson *et al.*, 1995), followed by Canada (12%), the USA (13%), Europe (5–9%) and Asia (2–5%) (Ramello *et al.*, 2000; Lewandowski *et al.*, 2004).

India, Pakistan and southern China make an important part of the urinary calculi belt in Asia and the rest of the stone world. It is believed that the general poor standard of living (food and vitamin intake especially) in this region accounts for the higher incidence of stone disease. India occupies a strategic location in Asia, looking across the seas to Arabia and Africa on the west and to Burma, Malaysia and the Indonesian Archipelago on the

east. A large population in India and the neighboring countries is still poorly nourished, living on a high alkaline ash farinaceous diet with negligible vitamin intake. In southeastern Asia Indonesia and Thailand are two countries where stone formation are high. Bladder calculi is a relatively common disease of childhood in western Sumatra with an incidence of 8.3/100,000 population per year. The majority of these are from poor families with a diet low in protein and phosphate. The composition of the calculi is primarily ammonium acid urate. This is consistent with the excretion of a high acid content due to both an acidogenic rice diet and diarrhea, combined with a low level of phosphate (Ansari *et al.,* 2003).

In a study done by Sreenevason (1981), the first study in Malaysia into the incidence of urinary calculi over 15 year period from 1962 to 1976 demonstrated the same pattern as in western and other industrialized countries. The incidence changed considerably from the first five years to third 5-year (224 per 100000 to 346 per 100000). In Malaysia as whole, upper tract stone were more common than lower tract stone. In Kelantan, for a period of five tears; from 1972 to 1976, Sreenevason reported that the incidence for upper tract was 33.3 per 100000 and 3.6 per 100000 populations for lower tract calculi. Lim *et. al.* (1988) has reported that the annual incidence of new patients in Kelantan, undergoing stone surgery was 10.1 per 100000 residents. The rate for upper tract stone was 3.4 per 100000 and 6.8 per 100000 for lower stone.

Stone disease is two to three times more common in males than in females (Dajani *et al.* 1988; Hassan and Dubbagh 1988). Ljunghall *et al.* (1977) also reported that stone disease

is more frequent in men (15%) than in women (6%). It occurs more often in adults than in elderly people and more often in elderly people than in children (Dajani *et al.* 1988). Whites are affected more often than Asian ethnicity. In addition, urolithiasis occurs more frequently in hot, arid areas than in temperate regions (Andrew and Chandru 2001).

With regards to stone recurrence, five years after the first episode 30–50% of patients suffer a recurrence (Johnson et al., 1979, Ljunghall *et al.,* 1984). Calcium oxalate stones are most likely to re-form and recurrence rates are 40% at 3 years, 74% at 10 years and 98% at 25 years (Lewandowski *et al.,* 2004). The recurrence rate for cystine stones ranges from 30 to 73% (Pareek *et al.*, 2005). The prevalence of upper renal tract stone disease in the Western world is progressively rising and in addition recurrence rates are unacceptably high; 10% after the first year of stone presentation, 35% at 5 years and 50% at 10 years (Milliner 1995).

The main types of stones have remained the same over many thousands of years. The oldest known urolith (≈4,800 BC) was yellow and comprised of uric acid nucleus and concentric laminations of calcium oxalate and struvite. This stone was found by Elliot Smith in 1901 in a prelustaric tomb at El Amrah, Egypt (Desnos E., 1972). In the past centuries, infection-induced stones (struvite) and uric acid stones were predominant. The share of calcium oxalate stones has increased with the beginning of the industrialized era. In the 1970s, prior to the introduction of ESWL, struvite stones represented an average of 10% of all cases. However, Japanese study showed a rate as high as 22% (Takasaki E.

1975). Comparable studies conducted in Japan and Germany indicates a struvite incidence of between 3% and 7% (Yoshida *et al.*, 1990; Schubert G 1996).

No uniform tendency can be observed in the occurrence of uric acid stones. A very good therapeutic option is chemolysis which involve alkalization of urine. A reduction in uric acid excretion through the use of allopurinol in which antagonize with a high intake of purine, leading to an average increase in serum uric acid concentration. In Germany, there was a sharp decrease in the occurrence of uric acid urolithiasis, whereas in the United States and Japan an increase was observed. A sharp increase in calcium oxalate urolithiasis was seen in all regions examined. More than 76% of all urinary stones consist of calcium oxalate and show an even increase in the occurrence of both whewellite and weddellite.

Earlier studies carried out in the United States showed a high incidence of calcium oxalate stones. Mandel and Mandel (1996) stress this increase in the number of calcium oxalate stones. Calcium oxalate stone diseases have to be approached case by case; thus, a given group of patients must be addressed with a comprehensive diagnostic program and specific metaphylactic measures.

2.2 Theories and Physicochemical of Urinary Calculi

Water is an important element in digestion, circulation, elimination and regulation of body temperature. The maintenance of normal composition and body fluid is a critical function of the urinary system. This is accomplished by glomerular filtration, tubular reabsorption

and tubular secretion of soluble and filtration plasma componenets. Under normal circumstances, urine will not contain solid particles (stones). Why do humans suffer from urinary stone disease? Although urinary stones have been noted in human remains as old as 7000 years (Menon *et al.,* 2002), the underlying etiology of stone formation remains a mystery. The development of urinary calculi is most likely a multi-factorial process and cannot be fully addressed by current theories.

Supersaturation

Urine contains many electrically active ions and small and macromolecular organic components. Some urinary constituents, referred to as inhibitors of crystal formation, enable the urine to retain more ions in solution than at the level of saturation (Menon *et al.,* 2002). For example, nephrocalcin can inhibit the precipitation of calcium oxalate crystals in urine (Worcester, 1996). The term supersaturation defined as a solution that contains more of the dissolved material than could be dissolved by the solvent under normal circumstances. The level of supersaturation of a salt is expressed as the ratio between the actual ion-activity product (AP salt) and the solubility product (SP salt). The ion-activity of a salt is calculated from the free ion concentrations and the activity coefficients corresponding to the charge of the ions in the salt. The point at which saturation of a solution is reached, and crystallization begins is commonly known as thermodynamic solubility product (Ksp).

Figure 1 illustrates the states of saturation as a general example of urinary stone components. If the concentration product is less than the solubility product, the solution is termed undersaturated, and crystal nucleation will not occur.

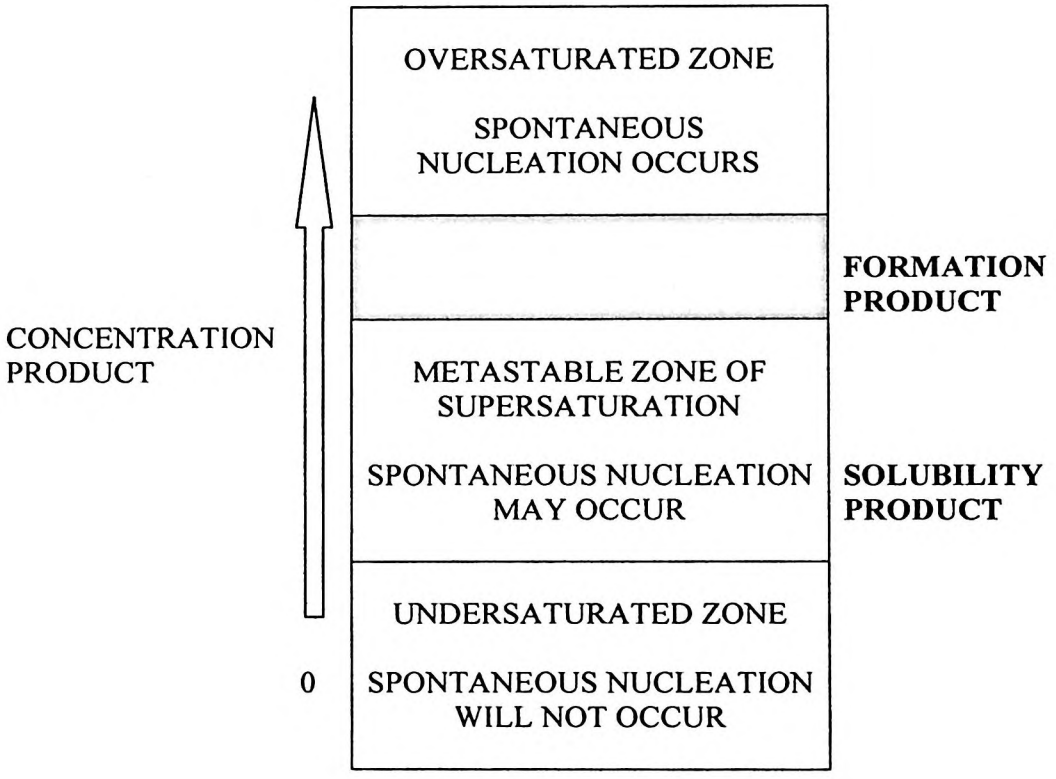

Figure 1: States of saturation

Nucleation

Although the precise pathogenesis of urinary stone formation is unknown, most believe it is related to crystal formation, especially in the early stages. Many crystalline substances have been identified in urinary stones. The principal components are calcium oxalate

monohydrate, calcium oxalate dihydrate, basic calcium hydrogen phosphate, calcium hydrogen phosphate, magnesium ammonium phosphate, uric acid, and cystine (Tiselius, 1996). Urine is often saturated with respect to the most common stone components.

Even though all urinary stones are made of crystals, not every stone former has crystalluria in their voided specimen (Cifuentes, 1983). Conversely, crystalluria is very common in nonstone formers (Werness *et al.,* 1981; Robertson *et al.,* 1972; Robertson *et al.,* 1969; Elliot *et al.,* 1976; Elliot *et al.,* 1980; Fleisch, 1978). There are overlaps of the severity of crystalluria between stone formers and nonstone formers. Robertson and Peacock, however, showed that stone formers tend to excrete larger crystal clumps than nonstone formers (Robertson *et al.,* 1972). In addition, stone formers generally had calcium oxalate dihydrate crystalluria, whereas nonstone formers generally had calcium oxalate monohydrate crystalluria. The clinical significance of these findings is still unknown.

Calcium oxalate is a major component of renal calculi. Calcium oxalate may exist in the monohydrate (COM), dihydrate (COD), or trihydrate (COT) configurations. Calcium oxalate monohydrate is the most stable and the COT is the most unstable thermodynamically.

It is intriguing that calcium oxalate crystalluria is typically in the dihydrate form whereas, in renal stones, it is most commonly present in the monohydrate form.

There is supporting evidence that the COT is the first nucleated crystal phase of calcium oxalate. Subsequently, COT is converted to COD and then to COM (Gardner., 1976;

Tomazic *et al.*, 1979; Tomazic *et al.*, 1980). COT is so unstable that it is rarely detected in urine and urinary stones. COD can be transformed to COM in a relatively short period of time (3.5 d) (Nakai *et al.*, 1996). Their solubility differs with COT being the most soluble, COD intermediate, and COM being the least soluble.

Despite the fact that COM is the most stable crystal phase, COD can exist in urine for a prolonged time; why this is so is unknown. Several factors in urine, however, favor the presence of the COD. For example, the rate of transformation from COD to COM is reduced by magnesium (Hesse *et al.* 1976; Berg *et al.*, 1976). Polyphosphate markedly inhibits the crystallization of COM but has a much smaller effect on the formation of COD and COT (Tomazic *et al.*, 1980). Some macromolecules in urine favor formation of COD crystals (Wesson *et al.*, 1996).

A large proportion of urinary stones contain a mixture of calcium oxalate and calcium phosphate. Crystallization of calcium oxalate is thought to start through the process of heterogeneous nucleation, which is facilitated by a good fit for the calcium oxalate lattice. Calcium phosphate may act as a nidus in this example (Pak, 1969; Lanzalaco *et al.*, 1988; Meyer *et al.*, 1975). It has been demonstrated that seed crystals of hydroxyapatite have the ability to induce the growth of COM crystals from a metastable supersaturated solution of calcium oxalate (Meyer *et al.*, 1975).

Different types of crystals tend to nucleate in different parts of the nephron. Crystal nucleation of calcium carbonate, calcium phosphate, and calcium oxalate are more likely to

occur in the loop of Henle, the late distal tubule, and the collecting ducts, respectively (Coe *et al.,* 1990; Luptak *et al.* 1994). Although crystal nucleation is thought to be one of the prerequisites for urolithiasis, this alone cannot explain stone disease, in which the stones are much larger than the crystal nuclei. There must, therefore, be other mechanisms for crystals to grow and be retained in the kidney to form stones before they are excreted in the urine.

Crystal aggregation and growth

Crystal nuclei will bind to each other to form larger particles. This process is called aggregation or agglomeration. When one large particle is broken into many small particles in solution, energy must be consumed to overcome the forces holding the small particles together. Conversely, the aggregation of small particles into large aggregates is favored from a thermodynamic perspective (Garten, 1996). According to Randolph and Drach, aggregation is different from agglomeration (Randolph *et al.,* 1980). Aggregation, by their definition, is the grouping of two or more particles held together by strong intermolecular forces, which cannot be dispersed by shear forces. The definition of agglomeration, on the other hand, is the grouping of two or more particles held together by weak cohesive forces, which can be broken up by dispersion with shear forces or water solvents. Most authors use the term aggregation, when referring to the binding of urinary crystals.

Aggregation is influenced by the saturation of the solution, although it can occur in supersaturated as well as undersaturated solutions (Blomen, 1982). Crystal aggregation

likely plays an important role in urinary stone formation. Several pieces of evidence support this theory. Crystalline clumps are frequently seen in the center of calculi, particularly in whewellite/weddellite, uric acid, and brushite stones (Mandel *et al.,* 1987).

Highly aggregated structures are noted in microscopic examinations and ultrastructural analyses of renal stones. Iwata et al. developed a partial dissolution method and used a scanning electron microscope to examine the internal architecture of COM urinary calculi (Iwata *et al.* 1985). They found all calculi have three distinct zones. A core zone is composed of randomly aggregated platelike crystals with frequent rosette formations. An intermediate area is composed of radially arranged piles of sheetlike crystals. The peripheral layer shows concentric laminations and each layer of lamination is composed of minute crystals. Khan and Hackett also studied architectural details and intercrystalline associations of urinary calculi and demonstrated that all stones have a central nucleus region and a peripheral zone (Khan *et al.,* 1993). The former contains an aggregate of discernible individual crystals. The peripheral region, on the other hand, consists of concentric layers of either discrete crystals or solid striated material.

In a study, measuring the effects of urines from healthy subjects and from calcium oxalate stone formers, Kok *et al.* (1990) demonstrated that the urines from stone formers had a similar effect on the solubility, but a significantly lower ability to inhibit the crystal growth and the crystal aggregation. They concluded that defective inhibition of the kinetic process of crystal aggregation constitutes a major physiochemical mechanism of calcium oxalate renal stone formation, which appears to be modulated by urinary citrate concentrations.

Subsequent studies have suggested that Tamm-Horsfall protein (THP) from stone formers bear functional abnormalities resulting in decreased ability to inhibit calcium oxalate crystal aggregation (Wiggins, 1987; Hess *et al.* 1991). Furthermore, a single crystal can never reach a size large enough to be retained within the kidney by crystal growth alone but the speed of aggregation is rapid enough to allow development of significantly sized particles within seconds (Blomen, 1982). These observations reveal the potential role of crystal aggregation in urinary stone formation.

Epitaxy

Multicomponent urinary stones are frequently observed. These stones, when sectioned, have distinct concentric rings. Different layers contain different components but each layer has a homogeneous crystalline composition (Mandel *et al.*, 1987). Although the mechanism of growth in these multicomponent stones is not fully understood, epitaxy has been suggested to play a significant role.

Epitaxy describes the process of oriented growth of one crystalline lattice over another crystalline lattice whose dimensions are similar (Mandel *et al.*, 1990). In general, epitaxy can be considered a special type of heterogeneous nucleation. The major forces holding these structures together can be explained by analyzing the crystal structures of the common components of renal calculi.

Matrix

Analysis of urinary stones reveals that they are not simply composed of crystals but rather contain a noncrystalline protein-like component, termed matrix. The percentage of matrix, by weight, in urinary stones varies. In general, most solid urinary stones have a matrix content of about 3% by weight (Boyce *et al.*, 1959). Matrix stones, however, may contain up to 65% of matrix (Allen *et al.,* 1966). Although the matrix can be found throughout a urinary stone, it is not evenly distributed. Warpehoski and colleagues reported that matrix from the core of calcium oxalate stones weighed an average of 2.7% but total matrix weight was 5.7% when the outer zone of the stones was analyzed (Warpehoski *et al.* 1981).

Many studies have been conducted to identify the role of matrix in urinary stone formation (Roberts *et al.*, 1986; Nishio *et al.,* 1985). Although several encouraging findings have been reported, there is still no satisfactory theory to explain the importance of urinary stone matrix in the development of urinary stones.

Inhibitors and Promoters

This review of the pathogenesis of urinary stone formation would be incomplete without mentioning inhibitors and promoters. Urine contains substances that influence crystallization processes, and therefore regulate stone formation. Substances that reduce the crystallization are called inhibitors whereas those that increase crystallization are

termed promoters. Numerous molecules that have been mentioned that inhibit crystallization in vitro but many stone formers have normal levels of these substances; others will continue to develop stones despite replacement of these known inhibitors. The formation of urinary calculi requires a complex combination of factors, both promoting and inhibiting stone formation (Table 1).

Table 1: Stone promoting and inhibiting factors

Promoting factors	**Inhibiting factors**
Calcium	**Inorganic**
Sodium	Citrate
Oxalate	Magnesium
Urate	Pyrophosphate
Cystine	
Low urine pH	**Organic**
Tamm-Horsfall protein	Tamm-Horsfall protein
Low urine flow	Urinary Prothrombin fragment 1
	Protease inhibitor: inter a inhibitor
	Glycosaminoglycans
	Osteopontin (Uropontin)
	Renal lithostathine
	Other Bikunin, Calgranulin
	High urine flow

Crystal retention

Previous sections in this chapter have explained theories about crystal precipitation, growth, and aggregation. None of these elements would result in urinary stone formation if the nucleated crystals were flushed out by urinary flow. Crystal retention is therefore a key factor. In urolithiasis, crystal retention will occur if the crystals grow large enough to be trapped in renal tubules or if they adhere to urothelium before excretion. The earliest evidence of crystal retention was the finding of macroscopic plaques of subepithelial deposits of calcium crystals in renal papillae by Randall (Randall, 1940). He proposed that these calcified deposits originated in damaged renal tubule epithelial basement membranes and later erode into the urinary collecting system. These plaques, now known as Randall's plaques, are thought to serve as a nidus for urinary stone formation (Stoller *et al.*, 1996; Low *et al.*, 1997).

Many groups have investigated the concept of crystal retention leading to our current understanding. Normal urothelium has been shown to bear a protective mechanism against nucleation and adhesion of calcium oxalate crystals (Gill *et. al.*, 1980). Chemically injured urothelium will lose this property resulting in the adherence of calcium oxalate crystals (Gill *et al.*, 1980; Gill *et al.*, 1979). Gill and associates have also demonstrated that heparin, a sulfated heteropolysaccharide of glucuronic acid and glucosamine, could restore the anticrystal adherence property of injured urothelium (Gill *et. al.*, 1982).

Other sulfated polysaccharides, sulfated glycosaminoglycans, and monosaccharides were, however, not effective in the restoration of protection against calcium oxalate crystal adhesion. The glycosaminoglycan layer of normal urothelium plays an important role against bacterial adherence and is also responsible for preventing crystal adherence (Parsons *et al.,* 1975; Parsons *et al.,* 1977). The exact mechanism of action is unknown but it has been postulated that the sulfur groups on glycosaminoglycan molecules will bind large quantities of water molecules forming a "water barrier" on the cell surface thereby inhibiting calcium oxalate crystal and bacterial adherence (Parsons *et al.,* 1979).

In conclusion, the key element, therefore, appears to be inhibition of the steps in calculogenesis (supersaturation, nucleation, crystal growth, aggregation, and crystal/stone retention). Urolithiasis will not develop if any one of these steps is blocked.

2.3 Components of Urinary Calculi

Urinary calculi are solid biogenous formations of the urinary system. They mainly have a crystalline structure and the size is more than 1 mm. About 95% of stones are crystalline components and 5% are organic components, the so-called matrix or proteins (Gernot, 2006). Most human stones contain more than one crystalline component and are called multi-component calculi. The presence of multi-component calculi suggests multiple physiologic conditions that must be unraveled in the process of defining the optimal medical management and the avoidance of stone recurrence. The major crystalline components of human urinary tract stones are listed in Table 2.

Table 2: The Chemical Name, Composition, Mineral Name, and Abbreviation of the Most Common Components of Human Urinary Tract Calculi

Chemical name	*Mineral name*	*Chemical formula*	*Abbr*
Oxalates			
• Calcium oxalate monohydrate	Whewellite	$CaC_2O_4 \cdot H_2O$	COM
• Calcium oxalate dihydrate	Weddellite	$CaC_2O_4 \cdot (2+x)H_2O$	COD
Phosphates			
• Basic calcium phosphate	Apatite	$Ca_5(PO_4)_3(OH)$	AP
• Calcium hydrogen phosphate	Brushite	$CaHPO_4 \cdot 2H_2O$	BR
• Magnesium ammonium phosphate hexahydrate	Struvite	$MgNH_4PO_4 \cdot 6H_2O$	STR
Purines			
• Uric acid	Uricite	$C_5H_4N_4O_3$	UA
• Monosodium urate monohydrate		$NaC_5H_3N_4O_3 \cdot H_2O$	MSU
Other			
• L-Cystine		$(-SCH_2CHNH_2COOH)_2$	CY

Mandel IC, Mandel NS (2006)