CHAPTER 2
REVIEW OF LITERATURE

2.1 Urolithiasis
2.1.1 Overview

Kidney stone disease is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical and genetic risk factors. Nephrolithiasis, or kidney stone, is the presence of renal calculi caused by a disruption in the balance between solubility and precipitation of salts in the urinary tract and in the kidneys. The incidence is at peak among white males age 20 and 30 years old. It occurs both in men and women but the risk is generally high in men and is becoming more common in young women. The overall probability of forming stones differ in various parts of the world and is estimated as 1-5% in Asia, 5-9% in Europe, 13% in North America [25] and the recurrence rate of renal stones about 75% in 20 years span [26]. Continuous increase in cases reported in wake of GLOBAL WARMING. The researchers predict that by 2050, higher temperatures will cause an additional 1.6 million to 2.2 million kidney-stone cases, representing up to a 30 percent growth in some areas [27]. Kidney stones develop when urine becomes "supersaturated" with insoluble compounds containing calcium, oxalate (CaOx), and phosphate (CaP), resulting from dehydration or a genetic predisposition to over-excrete these ions in the urine. Kidney stones are of four types. The most common urinary stone types are calcium oxalate, calcium phosphate, uric acid, struvite (magnesium ammonium phosphate), and cystine.

2.1.2 Renal function

The physiological function of the kidneys is to excrete endogenous (e.g. creatinine, urea and oxalate) as well as exogenous (like drugs) waste products and maintaining body homeostasis. The kidneys purify toxic metabolic waste products from the blood in several hundred thousand functionally independent units called nephrons. Nephron is a functional unit of Kidney and human kidneys are composed of 1-2 million nephrons. The nephron is a specialized structure that is involved in concentration and dilution of primary urine. A nephron consists of one glomerulus and one double hairpin-shaped tubule that drains the filtrate into the renal pelvis. The glomeruli located in the kidney cortex are bordered by the Bowman's capsule. They are lined with parietal epithelial
Cells and contain the mesangium with many capillaries to filter the blood. The glomerular filtration barrier consists of endothelial cells, the glomerular basement membrane and visceral epithelial cells (also known as podocytes). All molecules below the molecular size of albumin (that is, 68 kDa) pass the filter and enter the tubule, which consists of the proximal convoluted tubule, the loop of Henle and the distal convoluted tubule as shown in Figure 2.1. An intricate countercurrent system forms a high osmotic gradient in the renal medulla that concentrates the filtrate. The tubular epithelial cells reabsorb water, small proteins, amino acids, carbohydrates and electrolytes, thereby regulating plasma osmolality, extracellular volume, blood pressure and acid–base and electrolyte balance. Non-reabsorbed compounds pass from the tubular system into the collecting ducts to form urine. The space between the tubules is called the interstitium and contains most of the intrarenal immune system, which mainly consists of dendritic cells, but also of macrophages and fibroblasts [28].

Figure 2.1 Structure of a kidney [28]
2.2 Crystalluria and stone morphology

It is well established that human urine is supersaturated with respect to ions and molecules, which can crystallize as clinical crystalluria with a potential for stone development. Regardless of the specific site of crystallization within the nephron, crystals can either pass from the kidney into the bladder and be excreted or attach to cells in the late collecting duct and grow into mature kidney stones. The crystalline composition of a stone reflects the urine chemistry and abnormalities in tubular physiology during the process of stone development. Crystalline material is the primary constituent of most human urinary tract stones. All stones contain macromolecules and other cellular components from the urine, termed matrix, and the amount of matrix normally approaches 2–5%, although some stones can be composed entirely of matrix. Most human stones contain more than one crystalline component and are termed multicomponent stones. The presence of multicomponent stones suggests multiple physiological 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 2.1.

The levels of urinary supersaturation of different solutes determine the specific types of stones [30-32]. Many of the crystals demonstrate birefringence so observation of growth morphology is often more definitive. Calcium oxalate monohydrate can be observed as ovals or dumbbells and calcium oxalate dihydrate as bipyramids. Apatite crystals usually appear as an amorphous precipitate, and frequently grow as clumps of very small crystallites. Struvite crystals grow in a characteristic coffin lid shape. Uric acid crystals appear as flat parallelepiped plates, and cystine crystals appear as hexagonal plates.
Table 2.1 Types of stones [29]

<table>
<thead>
<tr>
<th>Type</th>
<th>Frequency (%)</th>
<th>Gender (M: Male, F: Female)</th>
<th>Crystals shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium oxalate, mix</td>
<td>70-75</td>
<td>M</td>
<td>Hexagon; Dumbbell shaped; Bipyramidal envelope</td>
</tr>
<tr>
<td>Calcium phosphate (Brushite)</td>
<td>~10</td>
<td>F&gt;M</td>
<td>Amorphous: Alkaline urine</td>
</tr>
<tr>
<td>Uric acid</td>
<td>5-10</td>
<td>M=F</td>
<td>Diamond; Acid urine</td>
</tr>
<tr>
<td>Struvite (Magnesium ammonium phosphate)</td>
<td>~10</td>
<td>F</td>
<td>Coffin lid; Infection/urea splitter</td>
</tr>
<tr>
<td>Cystine</td>
<td>~1</td>
<td>M=F</td>
<td>Hexagon</td>
</tr>
</tbody>
</table>

2.2.1 Calcium oxalate stones

When CaOx concentration is 4 times above the normal solubility a crystal starts to form. If the CaOx concentration is 7 to 11 times higher than normal solubility the nucleation begins. In low urine volume, the presence of high calcium and high oxalate the supersaturation (SS) of CaOx is increased. Citrate in the urine forms soluble complex with urinary Ca. If urine has low citrate concentration SSCaOx is promoted to form CaOx stone. If urine pH is less than 6.5, proportion of divalent and trivalent ions are increased then SSCaP is favorable. The levels of urinary supersaturation of the different solutes determine the specific types of stones [30-32].

Dietary oxalate may be important in stone development; spinach, beets and rhubarb in particular, contain large amounts of oxalate and they may increase urinary oxalate excretion and predispose to the development of calcium oxalate stones. High dose vitamin C therapy can also lead to increased oxalate generation as vitamin C (ascorbic acid) is metabolized. Oxalate reabsorption in the colon is reduced by the formation of insoluble calcium oxalate [33-35].
These stones are frequently hard, dark brown, and often have a dull gray exterior. Pure dihydrate stones are usually small and spherical consisting of a tan or yellow cluster of platelets. The platelets are sharp and are arranged in various orientations. Admixed monohydrate/dihydrate stones frequently have many of the characteristics of a dihydrate stone because dihydrate most frequently appears on the exterior of the admixed stone. These stones are normally larger than pure dihydrate stones, are often spherical, and have a cluster of yellow platelets surrounding a hard dark brown interior.

2.2.2 Calcium phosphate stones
Stones that contain more than 50% CaP are uncommon [36]. The major determinants of CaP supersaturation are alkaline urine pH > 6.3 combined with hypercalciuria [37]. Treatment of CaP stones is similar to that of CaOx stones in that reduced dietary sodium and protein, high fluid intake, and thiazides are effective in our experience [38], although few trials deal specifically with this group of stone formers. The role of alkali therapy in the treatment of patients with CaP stone disease is highly controversial, as the balance of risks and benefits is likely patient-dependent. Potassium alkali can raise urine citrate levels, and may reduce urine calcium, with additional direct inhibitory effects on CaP crystal formation [39]. Citrate also inhibits individual crystal growth and aggregation, a beneficial effect not reflected in supersaturation calculations. These positive effects, however, are usually accompanied by a rise in urine pH which can predispose to CaP crystallization and worsening of stone disease. Which effect will be predominant in any given patient is difficult to predict. If treatment with alkali is undertaken, completion of follow up 24 hour urines to assess these differential effects is paramount.

Pure apatite stones are usually small, white in color with a very fine granular surface. Occasionally, these stones are also light brown with a smooth shiny surface. The most frequently occurring stone admixture of apatite or calcium oxalate monohydrate and calcium oxalate dihydrate is generally smooth, spherical, and has light brown platelets on the surface. Pure brushite stones are normally clusters of beige, nodular material surrounding a crystalline interior with a cauliflower-like growth pattern. Occasionally, the surface has a yellow or white tinge [52].
2.2.3 Struvite stones
Struvite stones (also sometimes known as triple phosphate stones or infection stones) are composed of calcium magnesium ammonium phosphate and form in the presence of upper urinary tract infections with urease-producing bacteria (most commonly *Proteus*, *Providencia*, and sometimes *Klebsiella*, *Pseudomonas*, and *enterococci*). Because of their potential for rapid growth and substantial morbidity, early detection and eradication are essential [40]. Normal urine is undersaturated with ammonium phosphate; struvite stone formation occurs only when ammonia production is increased and the urine pH is elevated, which decreases the solubility of phosphate. Bacterial urease is essential for the development of struvite stones because it leads to an elevation in ammonium, carbonate and urinary pH all at the same time. The stones may also occur on infected calcium, uric acid or cystine stones, especially after instrumental procedures. Struvite stones are three times more common in women than men, presumably because urinary tract infections are more common in women. They are typically very large and may be so large as to fill the renal pelvis (forming a "Staghorn calculus"). Their growth is rapid and they often grow back after surgical removal because infected fragments of stone have been left behind [41-43]. Struvite stones are difficult to treat, and require collaboration with an expert urologist. Treatment requires both removal of all stone material and effective antibiotic therapy. Antibiotic therapy should be guided by culture of the stone itself (or renal pelvic urine obtained at the time of surgery) as well as the bladder urine [44].

Pure struvite stones are usually off-white to light brown in color with a rough textured surface. Struvite stones frequently grow in a staghorn shape. Admixed struvite/apatite stones are usually light brown in color with a coarse, granular surface. The interior is normally intermixed with white and light brown layers [52].

2.2.4 Uric acid stones
The three major factors in the development of uric acid stones are low urine volume, hyperuricosuria, and abnormally acidic urine pH. However, low urinary pH is the principle determinant in uric acid crystallization [45]. At a urinary pH of less than 5.5, uric acid is poorly soluble, but solubility increases at a pH greater than 6.5. The solubility of undissociated uric acid is only 90 mg/L. Uric acid is a weak organic acid with a pKa of 5.5. Therefore, at low urine pH, undissociated uric acid precipitates to form uric acid stones [46]. A diet rich in animal protein, because of its high purine
content, which produces uric acid in its catabolism, may increase the risk of uric acid stone formation [47, 48]. The main treatment is to increase the solubility of uric acid in urine and to reduce its concentration. Urinary alkalinization is the cornerstone of medical management of uric acid stones. Patients with known uric acid stones without significant obstruction or infection can receive a trial of oral medical dissolution, which will also serve as preventative therapy. Both potassium and sodium alkali treatment can effectively raise urinary pH, but potassium citrate is preferred over sodium citrate because sodium loads increase urinary calcium excretion.

Pure uric acid calculi are radiolucent on plain radiographs but visible on ultrasonography or computerized tomography (CT). Uric acid stones are spherical with a smooth yellow-orange surface. The surface of uric acid dihydrate stones is often dark orange and the stone is composed of small spherical regions [52].

2.2.5 Cystine stones

Cystine is an amino acid formed by the linkage of two cysteine molecules via a disulfide bond. The limited solubility of cystine can result in stone formation. Stones are generally composed of pure cystine although admixtures with calcium salts can occur rarely. Cystine solubility increases at higher urine pH [49, 50]. Urine dilution, alkalinization and chelating therapy have remained the cornerstone of the therapeutic approach. Cystine excretion may fall modestly on a sodium-restricted (<100 mmol/day) and protein-restricted (0.8 g/kg/day) diet. If stones recur despite adequate hydration and alkaline urine pH, a cysteine-binding drug should be added. Cysteine-binding drugs have sulfhydryl groups that allow them to form mixed disulfides with cysteine that are more soluble than the homodimer [51]. Recurrent stones should be analyzed, because therapy may need to be adjusted to prevent the formation of stones containing CaP due to the alkaline urine pH. Pure L-cystine stones are homogeneously composed of very small yellow spheroids.

2.2.6 Matrix

Matrix stones are noncrystalline and take on a variety of shapes and colors. The stones are composed of a variety of organic molecules including urinary macromolecules and membrane fragments [52].
2.2.7 Other
Other substances that have been reported in stones include the drugs sulfamethoxazole, crixivan, guaifenesin, triamterene and 5-fluorocytosine, xanthine, 2,8-dihydroxyadenine, gypsum, and silicates following antacid therapy. Growth morphology for these components is often variable [53].

2.3 Epidemiological risk factors for stone disease

2.3.1 Age, sex and racial differences
Men are at greatest risk of developing kidney stones with incidence and prevalence rates between two and four times that of women [54, 55]. Baker et al. found that the peak age for the development of calcium oxalate stones was between 50 and 60 years. Uric acid stones tended to occur in an older population with an average age of 60–65 years. Infection stones, however, occurred in younger people, most commonly in women between the ages of 20 and 55 years. A second peak is seen, particularly in men, between 55 and 70 years of age. This study also found that 70% of all stones analyzed were from men. Men were at greater risk of producing calcium oxalate stones (73% were in men) and uric acid stones (79% were in men). Women were at greater risk of infection stones (58% occur in women) [56]. Several groups have reported racial differences in the risk of developing kidney stones. Soucie et al. in a large cross-sectional survey in the United States found that the prevalence of kidney stones was highest among White people and lowest in Black people. Hispanic and Asian people had an intermediate prevalence [57].

2.3.2 Climate and season
Many epidemiological studies have recorded a geographic variability in the prevalence of stone disease. It has been postulated that this variability may be owing to variations in climate and sun exposure, although others have questioned the role of diet and water quality as well. The most convincing evidence to date, however, reveals temperature and sun exposure to play important roles in the geographic variability of stone disease. It has been well documented that the incidence of urinary stones is higher in countries with warm or hot climates, probably due to low urinary output and scant fluid intake. Also, in a given population, stone recurrence is higher in summer and fall than in winter and spring. It is believed that individuals living in hot climates have an increased lifetime prevalence of stone disease secondary to dehydration. Further, individuals
living in areas with increased sun exposure are likely to have absorptive Hypercalciuria secondary to elevated vitamin D synthesis [57, 58].

2.3.3 Geography
Kidney stone incidence varies in different parts of the world, high incidence areas are Scandinavian countries, Mediterranean countries, British Isles, northern Australia, central Europe, portions of the Malayan Peninsula, China, Pakistan and northern India whereas the incidence of kidney stone formation is lower in areas like Central and South America, some parts of Africa. In Asia stone-forming belt has been reported to stretch across Sudan, Saudi Arabia, the United Arab Emirates, the Islamic Republic of Iran, Pakistan, India, Myanmar, Thailand, Indonesia and Philippines [59]. In India, with a prevalence rate of 15%, two high incidence stone belts have been found to occur. The first belt starts from Amritsar in North and while passing through Delhi and Agra ends up in U.P. The other belt which starts from Jamnagar in west coast extends inwards towards Jabalpur in central India. Very low incidence areas have been in West Bengal and coastal areas of Maharashtra, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh [4, 7]. The effect of geography on the incidence of stone formation may be direct, through its effect on temperature, high temperatures increase perspiration, which may result in concentrated urine, which in turn promotes increased urinary crystallization.

2.3.4 Occupation
The role of occupation in stone formation is highly debated. Kidney-related complications are on the increase because of geographic factors: residence in the stone belt, occupation related lifestyle changes - in case of indoor occupation - sedentary habits, stress, unhealthy dietary plan in terms of healthy or over healthy food intake, irregular food habits and fluid intake (intake of juices and beverages instead of water) or the other spectrum of physical manual labor - involving working outside exposed to heat and sun, low socioeconomic status, malnutrition and reduced fluid intake. Some experts speculated that this increased risk might be due to a hormone called vasopressin, which is released during stress, which increases the concentration of urine. A report suggested that manual workers had a higher incidence of urolithiasis when compared to sedentary workers [60].
2.3.5 Nutritional aspects
An unbalanced diet or particular sensitivity to various foods in stone formers can lead to urinary alterations such as hypercalciuria, hyperoxaluria, hyperuricosuria, hypocitruria and excessive acid urinary pH. Over the course of time, these conditions contribute to the formation or recurrence of kidney stones, due to the effect they exert on the lithogenous salt profile. The fundamental aspects of the nutritional approach to the treatment of idiopathic nephrolithiasis are diet, water intake and body weight.

2.3.5.1 Diet
Diet has long been suspected to affect the incidence of stone disease. Specific dietary factors, which have been shown to have a role in stone disease, include animal protein, supplemental calcium, sodium, oxalate, and fruit juices. Excessive animal protein intake has been shown to lead to an increase in urinary excretion of calcium and uric acid, and a decrease in urinary citrate. Additionally, a recent study suggests that a diet rich in animal protein leads to an increase in urinary oxalate excretion in recurrent idiopathic calcium stone formers [61]. Animal protein induces stone formation, reports indicated operation of different mechanisms. (a) Protein contains amino acids composed of sulfur, such as cystine and methionine, which are more prevalent in animal protein. Sulfur is oxidized, yielding sulfate, which generates an acid load that is buffered by bone. The resultant osseous dissolution provides more calcium to be excreted [62, 63]. (b) Sulfate also forms a soluble complex with calcium in the nephron and limits the reabsorption of this cation. (c) Increased protein consumption augments glomerular filtration, thus delivering more calcium to the nephron [64-66]. (d) Animal protein has a high purine content, which explains the associated increase in uric acid excretion. This is a risk factor for the development of uric acid stones and may play a role in calcium stone formation. (e) Chronic metabolic acidosis induced by the increased acid load decreases calcium reabsorption within the nephron [67]. (f) The decreased urinary pH may potentiate uric acid lithiasis, and it enhances citrate reabsorption in the proximal tubules, thus decreasing the excretion of this important inhibitor of crystallization [68]. (g) The augmented oxalate excretion with increasing dietary protein reported by some investigators may be caused by generation of more glycolate, an oxalate precursor [69]. Studies investigating the role of calcium intake and stone formation differentiate dietary calcium intake from supplemental calcium, used most commonly by women [70]. In contrast to traditional belief, recent studies have
shown an inverse relationship between dietary calcium intake and the incidence of stone disease [71]. However, there also seems to be a direct relationship between the use of supplemental calcium in women and the incidence of urolithiasis. These studies hypothesize that dietary calcium binds to dietary oxalate and reduces the intestinal absorption of oxalate, thus reducing the risk for calcium oxalate stone formation. Based on this hypothesis, women are advised to take supplemental calcium only with meals. Other epidemiological studies implicate sodium and certain fruit juices with an increase in the incidence of nephrolithiasis. Increased sodium intake has been linked to increased urinary calcium excretion, and thus to increased calcium stone formation. Increases in sodium intake of 100 mmol may produce an increase in urinary calcium of 1 mmol [72]. An inverse relationship occurs between renal potassium and calcium excretion, which brings attention to the role of potassium-rich foods such as vegetables and fruits in the prevention of stone formation [73]. Potassium consumption augments renal tubular phosphate absorption, which inhibits the synthesis of 1,25-dihydroxyvitamin [74]. This results in decreased intestinal absorption of calcium, which reduces urinary calcium excretion. Another potential benefit is that foods high in potassium content are usually replete with alkali, which reflects the dietary intake of actual bicarbonate or potential bicarbonate that reduces net acid excretion and stimulates urinary citrate excretion [66].

2.3.5.2 Fluid Intake
Supersaturation of the urinary environment with stone-forming constituents is a prerequisite for calculus formation and increased fluid consumption results in excretion of higher volume of urine, which is less supersaturated with stone-forming constituents. High fluid intake is associated with a lower risk of developing kidney stones in men and women [75]. Certain beverages also appear to provide additional protection with coffee, tea, beer and wine consumption associated with reduced risk of kidney stones while grapefruit juice consumption was associated with an increased risk [75, 76]. Increased fluid intake has been demonstrated to have a positive effect on two urinary inhibitors, citrate and Tamm-Horsfall protein. Hydration augments urinary citrate excretion, which was thought to result from an increased fluid flux in the proximal tubule to the cells of this portion of the nephron. The ensuing intracellular alkalosis blunts citrate reabsorption, leading to increased excretion of citrate. Urinary dilution has been found to increase the inhibitory activity of Tamm-Horsfall protein in calcium oxalate crystal aggregation in the urine of stone patients [77, 78].
2.3.5.3 Body weight
Overweight condition and obesity was found in 59.2% of the men and 43.9% of the women and both these conditions were strongly associated with an elevated risk of stone formation in both genders due to increased urinary excretion of promoters but not inhibitors of calcium oxalate stone formation and further concluded that overweight and obese men are more prone to stone formation than overweight women. Excess body weight may be associated with various functional /structural lesions of the kidney and will lead to nephrolithiasis, glomerulomegaly, diabetic nephropathy, carcinoma of the kidney [79].

2.3.6 Hypertension
A modest association has been reported between hypertension and nephrolithiasis in both sexes. In prospective studies, people with a history of nephrolithiasis are more likely to develop hypertension [80, 81] and those with hypertension are more likely to develop kidney stones, especially when they are overweight [82].

2.3.7 Metabolic abnormalities
People who form kidney stones often have metabolic or other abnormalities detectable on urinary testing. The common abnormalities include low urinary volume, hypercalcuria (25–40%), hyperoxaluria (10–50%), hyperuricosuria (8–30%) and hypocitraturia (5–30%). There is, however, significant overlap with healthy controls who also often have biochemical ‘abnormalities’, albeit less frequently [83, 84].

2.3.8 Inheritance and family recurrence
Autosomal recessive inheritance was defined for cystinuria and primary hyperoxaluria. The reported prevalence for cystinuria is 1–5% of all patients with urolithiasis and much lower for primary hyperoxaluria (~2 per million populations) [85]. Cystinuria and primary hyperoxaluria, as well as renal tubular acidosis and Dent’s disease, are some of the different monogenic conditions that have been identified to date as etiologies for urolithiasis. However, all of these rare conditions probably account for less than 2% of renal stones. A familial occurrence has also been suggested for hypercalciuria, one of the main risk factors for idiopathic urolithiasis. However, familial recurrence does not necessarily imply an inherited transmission, as it may be an effect of environmental factors shared by family members, mainly those related to dietary habits [86].
2.4 Pathophysiological risk factors for stone disease
The basis for calcium stone formation is supersaturation of urine with stone-forming calcium salts. A number of dietary factors and metabolic abnormalities can change the composition or saturation of the urine so as to enhance stone-forming propensity. Among the metabolic conditions are hypercalciuria, hypocitraturia, hyperoxaluria, hyperuricosuria, and gouty diathesis as shown in Table 2.2, and these conditions are reviewed in detail. Dietary factors also play a role in stone occurrence, and are discussed with regard to their role in preventing stone formation [87].

Table 2.2 Classification of underlying conditions in calcium stone formers [87]

<table>
<thead>
<tr>
<th>Condition</th>
<th>Metabolic/environmental defect</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypercalciuria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absorptive hypercalciuria</td>
<td>Increased GI calcium absorption</td>
<td>20-40%</td>
</tr>
<tr>
<td>Renal hypercalciuria</td>
<td>Impaired renal calcium reabsorption</td>
<td>5-8%</td>
</tr>
<tr>
<td>Resorptive hypercalciuria</td>
<td>Primary hyperparathyroidism</td>
<td>3-5%</td>
</tr>
<tr>
<td><strong>Hyperuricosuric calcium nephrolithiasis</strong></td>
<td>Dietary purine excess, uric acid overproduction or overexcretion</td>
<td>10-40%</td>
</tr>
<tr>
<td><strong>Hypocitraturic calcium nephrolithiasis</strong></td>
<td>GI alkali loss</td>
<td>-</td>
</tr>
<tr>
<td>Chronic diarrheal syndrome</td>
<td>Impaired renal tubular acid excretion</td>
<td>-</td>
</tr>
<tr>
<td>Distal RTA</td>
<td>Hypokalemia and intracellular acidosis</td>
<td>10-50%</td>
</tr>
<tr>
<td>Thiazide-induced</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hyperoxaluric calcium nephrolithiasis</strong></td>
<td>Genetic oxalate overproduction</td>
<td>-</td>
</tr>
<tr>
<td>Primary hyperoxaluria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary hyperoxaluria</td>
<td>Excessive dietary intake</td>
<td>2-15%</td>
</tr>
<tr>
<td>Enteric hyperoxaluria</td>
<td>Increased GI oxalate absorption</td>
<td>-</td>
</tr>
<tr>
<td><strong>Gouty Diathesis</strong></td>
<td>Low urine pH</td>
<td>10-30%</td>
</tr>
</tbody>
</table>

2.4.1 Hypercalciuria
Hypercalciuria is the most common pathophysiologic risk factor in calcium stone formation. Urinary calcium raises ionic calcium concentration and increases urinary saturation of stone-forming calcium salts (CaP and CaOx). In addition, complexation of calcium with urinary inhibitors such as citrate and glycosaminoglycans reduces urinary inhibitory activity, thereby increasing stone risk. Hypercalciuria can be more precisely
classified according to the site of primary metabolic derangement, whether intestine, kidney, or bone. Accordingly, hypercalciuria can be divided into three distinct subtypes: (1) absorptive Hypercalciuria (AH), characterized by intestinal hyperabsorption of calcium; (2) renal Hypercalciuria (RH), resulting from impaired renal tubular calcium reabsorption; or (3) resorptive hypercalciuria, caused by bone demineralization [88].

Although hypercalciuria is classified according to the site of the primary defect in calcium transport, secondary changes can occur at other sites; that is, renal calcium leak leads to secondary hyperparathyroidism, which results in bone resorption and increased intestinal calcium absorption. Hypercalciuria has a rich genetic predisposition. Nearly half of patients who have Hypercalciuria have a family history of stone disease [89].

### 2.4.1.1 Absorptive hypercalciuria

This condition, previously referred to as idiopathic hypercalciuria, results from intestinal hyperabsorption of calcium. This increases the renal calcium load resulting in hypercalciuria and formation of stones predominantly composed of calcium oxalate or mixed calcium oxalate and calcium phosphate [90]. The disease is heterogenous and multifactorial. The positive calcium balance suppresses parathyroid hormone (PTH) secretion and increases the renal filtered load of calcium, leading to increased urinary calcium excretion. AH is classified as Type I or II, according to the response to dietary calcium restriction. AH Type I is diet-unresponsive, whereas in AH Type II, urinary calcium normalizes in response to a low calcium diet.

### 2.4.1.2 Renal hypercalciuria

RH is caused by impaired renal tubular reabsorption of calcium. Renal loss of calcium reduces serum calcium and secondarily stimulates PTH secretion. Consequently, increased intestinal calcium absorption caused by enhanced 1,25-[OH]2D synthesis and mobilization of calcium from bone caused by increased PTH lead to hypercalciuria. Serum calcium remains normal because the loss of calcium in the urine is offset by enhanced intestinal calcium absorption and bone resorption. The pathogenesis of renal calcium leak is unknown. Several factors have been implicated in RH, including salt abuse and excessive urinary prostaglandins [91]. RH is relatively uncommon, occurring in approximately 9% of stone formers.
2.4.1.3 Resorptive hypercalciuria
Resorptive hypercalciuria is a rare cause of stone disease that is most commonly associated with primary hyperparathyroidism. Excessive PTH secretion from a parathyroid adenoma leads to bone resorption, increased renal synthesis of 1,25-[OH]2D (calcitriol), and enhanced intestinal absorption of calcium [92].

2.4.2 Hypocitraturia
Citrate is the most abundant organic anion in human urine, and is a well-recognized inhibitor of stone formation. Hypocitraturia is defined as urinary citrate excretion of less than 320 mg daily, although this is a somewhat arbitrary cutoff, because the acid-base status of the patient strongly determines total citrate excretion. Hypocitraturia is a well-known risk factor for calcium nephrolithiasis, and has been identified in 20% to 60% of calcium stone formers [93].

The protective effect of citrate is threefold, arising from its buffering capacity, its ability to complex with calcium in solution, and its inhibitory activity [94]. The buffering action of citrate is manifest during an alkali challenge, such that only a small rise in urinary pH occurs with an alkali load, thereby mitigating against calcium phosphate precipitation. Secondly, as an anion, citrate forms a soluble complex with calcium, reducing the ionic activity of calcium and decreasing urinary saturation of stone-forming calcium salts (CaOx and CaP). Finally, citrate directly inhibits crystallization, aggregation, and agglomeration of CaOx and CaP, thereby further reducing stone formation.

A variety of pathologic states associated with acidosis leads to hypocitraturia. Distal renal tubular acidosis (RTA) is associated with systemic acidosis, and is characterized by high urine pH 6.8 and low serum bicarbonate and potassium [95]. Chronic diarrheal states are also associated with systemic acidosis because of alkali loss in the stool. Excessive animal protein provides an acid load that promotes bone loss and causes hypocitraturia [96]. A recent study investigating the effects of a high protein, low carbohydrate diet, typified by the Atkins’ diet, demonstrated a significant reduction in urine pH and citrate during both the induction and maintenance phases of the diet. Other causes of acidosis associated with hypocitraturia are thiazide-induced hypokalemia, which produces intracellular acidosis, and vigorous exercise, which produces lactic acidosis [97]. Finally, idiopathic hypocitraturia may represent an isolated abnormality, unrelated to an acidic state.
2.4.3 Hyperoxaluria

Hyperoxaluria is defined as urinary oxalate excretion of greater than 40 mg daily. Hyperoxaluria is thought to increase the risk of stone formation by increasing urinary saturation of CaOx. The effect of oxalate on stone formation depends on the interaction between calcium and oxalate that takes place in the intestine and urine. In the intestine, oxalate absorption is modulated by dietary oxalate and the formation of a poorly absorbed calcium-oxalate complex. In the setting of dietary calcium restriction, calcium-oxalate complex formation is reduced, thereby increasing luminal free oxalate that is absorbed from the intestine and excreted in the urine. In the urine, calcium-oxalate interaction results in formation of a soluble complex that lowers ionic oxalate concentration.

Hyperoxaluria can be associated with primary disorders in biosynthetic pathways as shown in Figure 2.2 (primary hyperoxaluria), malabsorptive states (enteric hyperoxaluria), excessive dietary oxalate intake (dietary hyperoxaluria), or high substrate levels (excessive vitamin C). Primary hyperoxaluria Type 1 is caused by a rare inherited autosomal recessive disorder in glyoxalate metabolism by which the normal conversion of glyoxalate to glycine is prevented due to the deficiency of peroxisomal enzyme AGT (Alanine Glyoxylate Aminotransferase), leading to oxidative conversion of excess glyoxalate to oxalate in the peroxisome by glycolate oxidase or by lactate dehydrogenase in the cytoplasm. Primary hyperoxaluria type 2 is a rare monogenic disorder caused by a deficiency of the enzyme glyoxylate reductase GRHPR (D-glycerate dehydrogenase or hydroxypyruvate reductase), which catalyzes the conversion of glyoxylate to glycolate. When GRHPR is deficient, more glyoxylate is oxidized to oxalate. Systemic oxalosis ensues, and leads to excretion of markedly high levels of urinary oxalate, increasing urinary saturation of CaOx and causing stone formation and nephrocalcinosis.

Oxalate-degrading bacteria such as Oxalobacter formigenes have been shown to colonize the intestine of normal individuals, and may reduce intestinal oxalate. Absence of these bacteria has been linked to increased urinary oxalate levels and higher rates of stone formation in stone formers [98]. The contribution of oxalate-degrading bacteria to CaOx stone formation has not been fully elucidated.
Figure 2.2 Oxalate biosynthetic pathway. Type 1 primary hyperoxaluria is caused by a deficiency in peroxisomal alanine glyoxalate aminotransferase. Type 2 primary hyperoxaluria is caused by a defect in cytosolic glyoxalate reductase/hydroxypyruvate reductase. AGT, alanine-glyoxalate aminotransferase; GRHPR, glyoxalate reductase-hydroxypyruvate reductase, GO, glycolate oxidase; LDH, Lactate dehydrogenase [87]

2.4.4 Hyperuricosuria

Hyperuricosuria can lead to CaOx stone formation by heterologous nucleation on the surface of monosodium urate crystals [99]. Hyperuricosuria is defined as urinary uric acid exceeding 600 mg daily. The most common cause of hyperuricosuria is increased dietary purine intake, because uric acid is the end product of purine metabolism. Numerous other acquired and hereditary diseases can lead to hyperuricosuria, however, including gout, myelo- and lymphoproliferative disorders, multiple myeloma, hemolytic disorders, and hemoglobinopathies.

The pathophysiology of hyperuricosuric CaOx nephrolithiasis is intimately related to urinary pH. At pH less than 5.5, poorly soluble undissociated uric acid precipitates, leading to uric acid or CaOx stone formation. At pH greater than 5.5, uric acid is found predominantly in its dissociated form, increasing urinary saturation of monosodium urate and promoting CaOx stone formation through heterogeneous nucleation [100]. Furthermore, monosodium urate has been shown to bind to urinary inhibitors, thereby
reducing urinary inhibitory activity and indirectly promoting CaOx crystallization [101].

2.4.5 Gouty diathesis
Gouty diathesis refers to uric acid stone formation associated with primary gout [102]. The invariant feature of this disorder is low urine pH, which promotes the precipitation of the sparingly soluble, undissociated form of uric acid, leading to uric acid stone formation. Gouty diathesis is also a risk factor for CaOx stone formation, however. Although uric acid, which is favored at low pH, is not as efficient as monosodium urate in promoting CaOx stone formation, it too leads to CaOx crystallization by way of heterogeneous nucleation [87].

2.5 Mechanism of renal stone formation
Formation of calcium oxalate kidney stones is the result of cellular as well as extracellular events. The interplay between renal epithelial cells and Ox and/or CaOx crystals alters renal cell functions, changes the extracellular environment and plays a significant role in the formation of CaOx stones [10]. The formation of renal stones is a consequence of increased urinary supersaturation with subsequent formation of crystalline particles. Since most of the solid particles crystallizing within the urinary tract will be excreted freely [11]. However, when solid particles are retained within the kidney, they can grow to become full-size stones. Crystals can be retained at many sites in the kidneys and undergo the size-enhancing process of growth and aggregation. In order for stones to be formed, not only do crystals need to be retained within the kidney, but they must be located at sites from which crystals can cause ulceration at the papillary surface to form a stone nidus. It is thought that renal tubular injury plays an important role at this point. Khan hypothesized that renal tubular injury promotes crystal retention and the development of a stone nidus on the renal papillary surface [12]. In addition, renal tubular injury enhances crystal nucleation at low supersaturation [13]. Crystal–cell interaction is the next step, and is also promoted by renal tubular injury. The crystals that are internalized in the interstitium undergo growth and aggregation, and develop into renal stones. Persistent mild hyperoxaluria by itself or through crystallization of CaOx is injurious to the renal epithelium [103].
2.5.1 Extracellular events
Extracellular events include supersaturation, crystal nucleation, growth and aggregation and occur in renal tubular lumens and renal pelvises.

2.5.1.1 Urinary supersaturation and crystallization
The formation of kidney stones or nephrolithiasis is a result of crystal formation in the kidneys. The driving force for crystallization is the development of supersaturation with respect to the precipitating salt. Stone formers tend to excrete urine that is more supersaturated than that of non-stone formers [105-107]. It has been suggested that with a transit time across the kidney of 5–10 min, residence time is too short for crystals to nucleate and grow large enough to be trapped. The inner diameter of the various
segments of the renal tubules ranges from 15 to 60 µm. CaOx crystals, growing at the rate of 1–2 µm/min, cannot grow larger than a few microns and are therefore excreted with urine without causing stone development. In tubular fluid and urine, crystallization processes are largely dependent on solution composition. Human urine is a complex solution containing not only Ca and Ox but also other ions and macromolecules that can interact with Ca and/or Ox and modulate crystallization because of their activity as chelators. For instance, by forming soluble complexes with calcium and oxalate, respectively, citrate and magnesium reduce free ion activity and the relative supersaturation of calcium oxalate [108]. Crystals can precipitate in the urinary tract when the urine is supersaturated, i.e. when the concentration of salts is higher than what can be kept in solution. CaP can precipitate already in the loop of Henle or distal part of distal tubules, while CaOx supersaturation usually occurs later in the nephron, in the collecting ducts. CaP that is precipitated in the earlier parts of the nephron dissolves as it travels through the tubules to the collecting ducts, where the pH is lower. At that site, CaP can act as nucleation site for CaOx crystals [109-111]. On the basis of supersaturation, CaOx nucleation can first occur in the loops of juxtamedullar nephrons [112].

2.5.1.2 Crystal nucleation
The initial step in the transformation from a liquid to a solid phase in a supersaturated solution i.e. when the concentration product of the ions of the stone components is greater than the formation product, ions begin to cluster close together to form the earliest non soluble crystal structure, is called nucleation. This process begins with the coalescence of stone salts in solution into loose clusters that may increase in size by addition of new components or clusters [113]. There are two types of nucleation: homogeneous and heterogeneous. When the process occurs spontaneously in a pure solution, homogeneous nucleation results. Because impurities are always present in human urine, homogeneous nucleation is unlikely to occur in vivo. The surfaces provided by the impurities can serve as a nidus in the nucleation process, leading to heterogeneous nucleation. 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. Formed crystals either can be excreted in the urine as crystalluria or grow and/or aggregate to become clinically significant stones. Finlayson and Reid calculated the
required time for free crystals to nucleate and grow, and concluded that before free calcium oxalate particles could grow large enough to be trapped within the renal tubules, they would be excreted in the urine rather than develop into calculi [114]. Based on the concentration profile of calcium and oxalate in the urine, tubular fluid and renal tissue, it was suggested that interstitium of the inner medulla had the highest Ox concentration and the best chance of being the primary nucleation site for CaOx [115]. CaOx crystals can, however, migrate from tubular location to the interstitium [12]. 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 [116].

*In vitro* and *in vivo* studies have shown that renal tubular cell injury can promote crystallization of CaOx crystals by providing substances for their heterogeneous nucleation. *In vitro* cell degradation following renal tubular cell injury produces numerous membrane vesicles, which have been shown to be good nucleators of calcium crystals. *In vivo* crystals observed in the renal tubules of hyperoxaluric rats are always associated with cellular degradation products [13, 117]. The stone matrix contains both membrane vesicles and lipids. Phospholipids of the cell membranes are proposed to help crystal nucleation [118]. Lipids isolated from the kidney stone matrix also promoted the nucleation of CaOx crystals. Interestingly, membranes of injured but intact cells also showed the capacity to nucleate CaOx crystals. Direct nucleation on cell surface can also promote crystal retention within the tubule [119]. Although crystal nucleation is thought to be one of the prerequisites for urolithiasis, it 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.

### 2.5.1.3 Crystal growth

Crystal growth, defined as the rate of deposition of ions onto a crystal nidus, occurs at the interface between the liquid and solid phases and involves both solute transfer and interfacial processes [120]. Growth of crystals is influenced by the rate of diffusion of various solutes and by properties of the crystal surface, including surface charge. Moreover, analysis of extracts of kidney stones confirms that the urinary macromolecules are indeed incorporated into the crystal lattices of stones [121-124].
These considerations suggest that the kinetics of crystal growth in vivo likely differ from those in vitro when growth occurs in pure solutions of inorganic chemicals. Moreover, the kinetics in vivo may differ in different segments of the nephron since urine concentrations and composition vary in different parts of the nephron [125, 126] and since different macromolecules are produced in different regions. For example, crystals formed in the early portion of the nephron may be coated with nephrocalcin, which is produced only in proximal tubules and in the thick ascending limb of the loop of Henle [127], or with inter-alpha-inhibitor (bikunin), found in both proximal and distal tubules [128, 129]. Crystals forming later in the nephron may interact with Tamm-Horsfall glycoprotein [77], urinary prothrombin fragment 1 [130, 131] and/or osteopontin [130, 132], which are produced in the thick ascending limb of the loop of Henle and/or in the distal convoluted tubules. Since the rate of CaOx crystal growth is low and the transit time of tubular fluid through the kidney amounts to only several minutes, it has been calculated that the probability of a single particle achieving a pathophysiologically relevant size by the process of crystal growth alone is extremely low, even if growth proceeds at an uninhibited rate of 2 µm per minute [112].

### 2.5.1.4 Crystal aggregation

The process whereby crystals in solution stick together to form larger particles is called aggregation. According to Randolph and Drach, aggregation is the grouping of two or more particles held together by strong intermolecular forces, which cannot be dispersed by shear forces [133]. Robertson et al. found that recurrent calcium stone formers tend to excrete larger calcium oxalate crystals (10–20 µm in diameter) than those (3–4 µm in diameter) in nonstone formers [134]. Although crystal growth is definitely a step in CaOx renal stone formation, the process of growth is so slow that crystals cannot become large enough to obstruct the renal tubules and be retained there by this mechanism alone, as several minutes are required for the tubular fluid to pass through the kidney. For this reason, the more critical step is thought to be crystal aggregation. Kok et al. 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 [135]. 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 but the speed of aggregation is rapid enough to allow development of significantly sized particles within
seconds. These observations reveal the potential role of crystal aggregation in urinary stone formation. Ultrastructural examination of crystal aggregates in the kidneys, as well as urine, displays membranous cellular material closely associated with the crystals [118]. It is our understanding that cell debris, formed as a result of exposure to high concentration of Ox and CaOx crystals, collects with the crystals resulting in the formation of larger particles. Membrane lipids with properly aligned calcium-binding head groups bridge crystals together and promote crystal aggregation.

2.5.1.5 Crystal retention
Randall proposed that the calcific deposits originate 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 [136]. 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 [137]. It has been postulated that the crystal adherence reaction is mediated through cell surface substances, termed crystal-binding molecules [138]. Several compounds, including phosphatidylserine (PS) [139, 140], sialic acid [141], collagen type IV [142], osteopontin 143], and hyaluronan [138], have been shown to be candidates of crystal-binding molecules. The crystal–renal cell interaction is supported by clinical observations and laboratory studies [144]. For example, endocytosis of calcium oxalate crystals was observed in a patient with type 1 primary hyperoxaluria [145]. In studies using monkey renal epithelial cells as a model of the distal tubular epithelium, COM crystals were endocytosed by the cells and cellular proliferation was induced [146, 147]. Crystals can reside in the kidneys by crystal formation in the renal interstitium, aggregating with other crystals, attachment to the renal epithelial cells after their formation in the renal tubules and not moving with the urinary flow, and growing large enough to be trapped.

2.5.2 Cellular events to oxalate exposure
Cellular events occur in cells of the renal epithelium and interstitium. These include management of acid base balance, urinary citrate, oxalate, calcium, magnesium, and pH as well as response of renal epithelial cells to the changing urinary environment, particularly oxalate overload and the presence of calcium oxalate crystals.
2.5.2.1 Crystal–cell interaction

Before crystals can turn into an actual stone, they have to be retained in the kidney. Crystals grow and aggregate to the point at which they become too large to pass through the tubular lumen and become trapped. Crystal retention in the kidney could be dependent on the interaction between crystals and the epithelium lining the renal tubules, even when crystals are small [110, 111, 114, 148]. It is hypothesized that this occurs when the epithelium lining the renal tubules becomes susceptible to crystal binding. Under pathological conditions, crystal binding molecules that are normally absent from the cell surface might be expressed, enabling crystal-cell interaction. The interaction is influenced by the type of crystal, the presence of a crystal coat, the type of cell surface, and the surface electric charge [141, 149]. Once adhered, the crystals are subsequently internalized into the epithelial cells through endocytosis; altered gene expression, cytoskeletal alterations, and cellular proliferation can then occur [144]. The process of attachment or endocytosis of crystals to renal tubular cells is what is generally meant by crystal–cell interactions. The structural characteristics of the binding and uptake of COM crystals by BSC-1 cells have been characterized by scanning electron microscopy (SEM). Microvilli on the apical cell surface appear to make initial contact with the crystal before its internalization. Transmission electron microscopy (TEM) confirmed that endocytosis of COM crystals by BSC-1 cells occurs as early as 30 min after exposure. These structural and functional studies of crystal–cell interactions in culture indicate that COM crystals rapidly adhere to microvilli on the cell surface and are subsequently internalized [150, 154].

2.5.2.2 Renal epithelial injury and crystal nucleation

Both animal models as well as tissue culture studies indicate that exposure to high levels of oxalate and CaOx crystals is injurious to renal epithelial cells [12, 155]. These effects are additive and concentration dependent leading to both apoptosis and necrosis [156]. Death, degradation, and detachment of many epithelial cells are results of cell injury. Dead epithelial cells disintegrate into membranous vesicles. In vivo, injury causes exposure of the basal lamina, which often becomes a site for crystal attachment. Urinary crystals are frequently associated with the membranes of cellular degradation products suggesting their involvement in crystal formation. Cell membranes are further implicated in crystallization by in vitro studies, which show production of CaOx crystals by incubating vesicles of isolated renal brush border membrane in metastable
solutions [157]. Cellular degradation products may also be involved in crystal retention by slowing urinary movement through the renal tubules as well as by promoting crystal aggregation and increasing the size of crystal aggregates.

2.5.2.3 Attachment of CaOx crystals to renal tubular epithelial cells

*In vivo* and *in vitro* studies have provided evidence for crystal retention within the kidneys via attachment to renal epithelial cells. A number of studies have demonstrated that renal epithelial cell injury promotes crystal attachment as a consequence of changes in the surface properties of affected cells and/or unmasking of attachment sites beneath or between cells. The animal model studies showed that CaOx crystals attach to cellular surfaces and basement membranes [12]. The reasons for their attachment include crystal-PS interaction and crystal-basement membrane interaction. The crystal–PS interactions could occur as a result of redistribution of PS on the cell surface of renal cells [158] that have lost their membrane lipid asymmetry as depicted in Figure 2.4. Studies indicated that redistribution of the phospholipid, phosphatidylserine (PS), to the surface of the cell can promote crystal binding. This phospholipid is normally restricted to the inner leaflet of the membrane via an ATP-dependent process that may involve the actin cytoskeleton [159]. When cells are damaged, membrane PS redistributes to the surfaces of cells, where it can serve as a binding site for CaOx crystal attachment and recognition signal for engulfment and removal by macrophages [160].

![Figure 2.4 Schematic representation of loss of membrane phospholipid asymmetry following cell injury][140]
The second mechanism of the crystal interaction with basolateral or basement membrane components could be a result of the loss of cell polarity, as illustrated in Figure 2.5. This type of injury may allow cell membrane components that are usually sequestered to the basolateral surface or in the tight junction region to migrate to the apical surface of the cell. Studies by Mandel and his collaborators [161-163, 139, 140] were among the first to demonstrate this linkage, showing an increase in crystal binding following treatments that disrupt tight junctions, allowing crystal access to membrane constituents normally restricted to basolateral membranes [161]. When primary cultures of inner medullary collecting duct cells were exposed to crystals of CaOx, uric acid or hydroxyapatite, crystals preferentially adhered to cells with impaired tight junctions. Recently, similar conclusions were made when MDCK-b1 cell monolayers were first physically injured by removal of a strip of cells and then exposed to CaOx crystals. Crystals specifically adhered to residues on the growth substrate and surfaces of injured and regenerating cells. It was concluded that both mature and immature cells surfaces express crystal binding molecules but, while they are available on surfaces of immature cells, in mature cells these molecules become available only after injury. These results strongly support the suggestions that epithelial damage promotes crystal adherence to the renal epithelium. Molecules, which become available on cell surfaces on exposure to high Ox and CaOx crystals, include phosphatidylserine, CD44, osteopontin, hyaluronan. All of them have been shown to promote crystal adherence to renal epithelial cell surfaces. Khan et al. observed crystal attachment to the brush border of proximal tubules in rats. Some urinary macromolecules have an inhibitory effect on CaOx crystal attachment. Lieske et al. reported that diverse polyanionic molecules in urine, such as specific glycosaminoglycans, glycoproteins, and citrate, block the binding of COM crystals to the cell membrane. One common feature of molecules that inhibit COM crystal adhesion to cells is their polyanionic character. They mentioned that although polyanions present in tubular fluid may coat crystals and thereby inhibit their adhesion to tubular cells, a distinct and separate set of signals acts on the cells to regulate their response to crystals that do bind [164, 165]. Related studies suggested that the crystal attachment may also involve extracellular matrix proteins and/or cellular binding sites for these proteins, which are normally masked in intact monolayers Studies by Lieske and colleagues [141, 146] demonstrated an attenuation of crystal attachment in BSC-1 cells by treatment with arginine-glycine-aspartic acid-serine (RGDS, a tetrapeptide that bind to integrins), or by pretreatment with
fibronectin, a connective tissue protein containing this peptide sequence. Similarly, Verkoeien et al. [166] demonstrated that crystal attachment to wounded MDCK monolayers could be attenuated by enzymatic removal of hyaluronic acid [167], expressed by subconfluent cultures of renal epithelial cells in vitro and by damaged renal epithelial cells in vivo [168].

Figure 2.5 Schematic representation of loss of cell polarity following cell or tissue injury [140]

2.5.2.4 Endocytosis of CaOx crystals by renal tubular epithelial cells and cell proliferation

CaOx crystals binding to renal epithelium often leads to endocytosis followed by DNA synthesis and cell proliferation as indicated by an increase in the incorporation of thymidine into DNA, increase in cell number and enhanced expression of immediate early genes c-jun, c-myc, Egr-1 and nur 77 [12, 147, 169]. The proliferative response could serve to promote further crystal attachment as the dividing cells round up and detach, unmasking new attachment sites on the underlying basement membrane [147]. In particular, both in vivo [170] and in vitro [146, 147, 171-173] studies provided evidence that the adherence of crystals may activate endocytic pathways that bring about an internalization of attached crystals. This uptake involves an active engulfment of surface particles, following attachment to specific sites on the cell surface. After attachment, the crystals are internalized, eventually moving to a basolateral location where they become anchored to the basement membrane. It was suggested that disruption of tight junctions, which leads to migration of adhesive basolateral molecules to cell surfaces, increases crystal binding [174]. Internalized crystals are
transported into the lysosomes where they appear to dissolve because of extremely low pH [175] or released at the basolateral surface, a process that may account for the appearance of crystals in the renal interstitium in experimental stone disease [170]. This may promote crystal retention by exposing attachment sites on the ensuing immature cells as well as the basal lamina. CD44 is generally expressed at the basolateral membrane of the confluent renal epithelial cells and at their apical membrane during proliferation [176]. Cells are susceptible to crystal adherence during proliferation but lose this feature after the development of functional tight junctions and attaining confluency. Tubular epithelial cells of the injured kidneys also express CD44 in vivo. In addition to CD44, its two ligands, OPN and hyaluronic acid (HA), are also implicated in crystal binding. Crystal binding is significantly reduced by enzymatic removal of HA. Pretreatment of MDCK cells with polyclonal antibodies against OPN as well as transfection of NRK-52E cells with antisense OPN cDNA also reduces adhesion of CaOx crystals [177]. Other molecules, which support CaOx crystal attachment to the renal epithelial cells include PS, sialic acid containing glycoproteins, collagen, and nucleolin and/or glycosaminoglycans such as HA [149, 167]. However, pretreatment with annexin V significantly reduces the attachment by annexin binding to superficial PS [178]. Neuraminidase pretreatment reduces crystal attachment indicating a role for sialic acid containing glycoproteins [179]. In addition, the presence of free bikunin, osteopontin, fibronectin, heparan sulfate and matrix Gla protein inhibits crystal attachment.

The presence of nephrocalcin inhibits DNA synthesis [147]. Both attached and internalized crystals promote adhesion of additional crystals to the cell surface. Crystal endocytosis is an active process and can be enhanced by treatments that stimulate cell migration and/or proliferation, such as ADP and cytokines [146] and inhibited by agents that interact with cell adhesion sites including Tamm-Horsfall Protein (THP), tetrapeptide RGDS, fibronectin, heparin and transforming growth factor (TGFß2) [146, 171, 172]. The inhibitory effect of arachidonic acid and its metabolites PGE1 and PGE2 on crystal endocytosis is mediated by cAMP. In addition, endocytosis is inhibited by an increase in intracellular calcium. There is generalized reorganization of intermediate filaments and concentration of F actin at the sites of internalized crystals [173]. Furthermore, prevention of cytoskeleton assembly by cytochalasin B and colchicine inhibits CaOx crystal internalization by MDCK cells. Crystal endocytosis is also attenuated by treatments that inhibit protein kinase C or that disrupt the
cytoskeleton [173]. Lieaske et al. reported that the internalization of CaOx crystals by BSC-1 and MDCK cells is a regulated event that can be modified by various signals [146]. In addition, they reported that the adsorption of nephrocalcin, a urinary glycoprotein of renal cell origin, to COM crystals prevented attachment of the crystal to the plasma membrane, engulfment, or both, and thereby prevented mitogenic effects.

2.5.2.5 Migration of inflammatory cells into the interstitium
Diverse inflammatory cells are present in the interstitium next to the tubules that contain crystals [180]. Interstitial crystals are surrounded by inflammatory cells positive for CD45 (antigen which identifies all leukocytes), ED1 (specific for monocytes and macrophages) and MHCII (major histocompatibility class II antigen). Some crystals are seen inside multinucleated giant cells which are also positive for CD45, ED1 and occasionally for MHCII as well. In addition, global assessment of gene expression in kidneys of hyperoxaluric rats has shown differential regulation of many genes linked with immune response and inflammation [181]. Renal tubules, the interstitium, and crystal-associated material also stain positive for the monocyte chemoattractant protein-1 (MCP-1), a key regulator of the inflammatory response known to attract cells of the inflammatory cascade such as monocytes. Renal epithelial cells express MCP-1 mRNA and protein, and their levels are increased following exposure to Ox and CaOx crystal [182]. Many crystallization modulators, whose production is upregulated by exposure to Ox and CaOx crystals, are also participants in the inflammatory and repair processes. OPN is a specific monocyte chemoattractant for renal interstitium and its production is increased prior to monocyte infiltration. Acute inflammatory conditions are known to up- or down-regulate transcription of inter-α-inhibitor (ITI) genes. Bikunin, a plasma protease inhibitor, is associated with inflammation and stabilizes the extracellular matrix. THP is seen in the renal interstitium in several forms of tubulointerstitial diseases. The administration of THP is shown to produce interstitial inflammation and scarring, can activate alternate pathways, interact with neutrophils and bind to certain cytokines. Prothrombin is the precursor of thrombin and fragments 1 and 2. Thrombin is involved in platelet aggregation and blood coagulation and plays a major role in the recruitment and activation of infiltrating immune cells. Other studies have provided evidence for the activation of renin-angiotensin system during the development of tubulointerstitial lesions of CaOx crystals [183, 184]. Reduction of angiotensin production by inhibiting
angiotensin-converting enzyme as well as blocking angiotensin receptor reduced crystal deposition and ameliorated the associated inflammatory response. We have recently shown that CaOx crystal deposition in rat kidneys activates the renin-angiotensin system and increases renin expression in the kidneys and serum.

2.5.2.6 Activation of Phospholipase A₂

However, both the process of endocytosis and the processes regulating membrane phospholipid asymmetry involve the actin filament lattice, which is intimately linked to a number of membrane bound signaling molecules, including tyrosine kinases, phospholipases, phosphoinositide kinases, etc. [159]. These signaling molecules control multiple pathways within cells including those regulating the activity of cytosolic phospholipase A₂ (cPLA₂), an enzyme that hydrolyzes the acyl group from the sn-2 position of phospholipids. This enzyme produces a number of active byproducts including arachidonic acid and assorted lysophospholipids that can, in turn, stimulate other signaling pathways within cells [185]. Activation of PLA₂ has been observed in a number of pathologies involving injury of renal epithelial cells [186-188]. In addition, patients with active stone disease show elevations in plasma arachidonate levels and in the arachidonate content of red cell membranes [189, 190], suggesting a role for PLA₂ in the etiology of stone disease. Thus, it was of interest to discover that cPLA₂ is also activated in renal epithelial cells following exposure to elevated concentrations of oxalate [191, 192]. Studies on MDCK cells demonstrated that oxalate produces a time- and concentration-dependent increase in the activity of cPLA₂ [191] that may be responsible for many of the other cellular actions of oxalate. Indeed activation of this enzyme may be responsible for the induction of a number of immediate early genes that are involved in cellular proliferation, since inhibition of PLA₂ activity blocked the oxalate-induced increases in Egr-1, c-jun and c-myc mRNAs [192]. The induction of several early response genes appears to be mediated by specific lysophospholipid byproducts of PLA₂ activation, since lysophosphatidylcholine (Lyso-PC), but not arachidonic acid or lysophosphatidic acid, mimicked the effects of oxalate on gene expression [192]. Lysophospholipids released by PLA₂ activation have also been implicated in the proliferative responses of OK cells [193], vascular smooth muscle cells [194] and mouse renal proximal tubular cells [195].
2.5.2.7 Activation of Neutral Sphingomyelinase

Renal cell exposure to oxalate also increases the cellular levels of ceramide, another lipid signaling molecule [196, 197]. Oxalate-induced increase in ceramide are of interest because in other cells, ceramide has been shown to mediate a variety of responses, including proliferation, differentiation, cytotoxicity and death [198]. The nature of the stimulus for neutral sphingomyelinase activation in response to oxalate exposure is not completely understood. Oxalate may exert a direct effect, but it is also likely that oxalate works indirectly, via PLA₂ activation in renal epithelial cells, since pretreating MDCK cells with AACOCF₃, a selective inhibitor of cytosolic PLA₂, blocked the oxalate-induced increase in ceramide production [197]. Moreover, arachidonic acid, a lipid signal generated by PLA₂ activation, mimics oxalate actions on ceramide production in renal cells [197]. A similar link between the PLA₂ and the ceramide pathways also occurs in HL-60 (human leukemia) cells [199]. Several studies in neural cells have suggested that the cross-talk between these two pathways may also occur in the opposite direction, namely, ceramide may enhance the activation of PLA₂ [200, 201]. This possibility has not yet been confirmed in renal epithelial cells. Irrespective of the trigger(s) eliciting ceramide production, it is clear that increased availability of this lipid can exert multiple effects on cellular function, including apoptosis [198, 202]. Several studies have linked oxalate toxicity to apoptotic renal cell death [196, 203], although the precise links between ceramide and renal cell death have not yet been completely elucidated.

2.5.2.8 Alterations of mitochondrial function and cellular redox state

Two lines of evidence support the notion that alterations in mitochondrial function are responsible for many of the effects of oxalate (and by extension for the effects of its lipid mediators). First, many studies indicate that mitochondria are the major source of oxidants in mammalian cells. Second, in whole kidney and in isolated kidney cells there is evidence supporting an increase in oxidant stress following oxalate exposure [204]. Early evidence came from studies on experimental animals which found that experimental increase in urinary oxalate loads also increased the excretion of lipid peroxides [205-207] and decreased the levels/activities of renal antioxidant enzymes [208, 209]. To determine whether oxalate exposure could elicit a direct, acute oxidant stress in renal cells, a number of studies have used monolayer cultures of renal epithelial cells to identify oxalate-induced changes in mitochondrial production of
reactive oxygen molecules. Many studies have observed that oxalate exposure in a variety of kidney cell lines increases free radical production [210, 216] and induces toxicity [196, 211, 212, 216-219]. In other studies, oxalate treatment of renal cell cultures increased production of lipid peroxides and increased the release of intracellular enzymes [212]. Moreover, oxalate-induced toxicity and free radical production could be attenuated by pretreatment with various antioxidants [211-213, 217, 219], by treatments that disrupt electron transport in mitochondria [210], and by genetic manipulations that enhance expression of bcl-2 [196], a protein that modulates mitochondrial permeability [214].

Recent studies have examined mitochondrial responses to oxalate and its lipid mediators in more detail. Treatment of renal epithelial cells with oxalate, ceramide, arachidonic acid or lyso-PC evoked a number of changes in mitochondrial function, including depolarization of the mitochondrial membrane [220]. The changes in mitochondrial membrane potential were accompanied by an increase in the production of reactive oxygen molecules in isolated mitochondria, by an increase in the oxidation of mitochondrial thiols and by an increase in the peroxidation of mitochondrial membranes [210]. These findings provide support for earlier studies in which oxalate exposure was shown to increase the permeability of the inner mitochondrial membrane that led to release of mitochondrial factors cyto-c required for activation of caspases, cysteine proteases that are involved in apoptotic cell death [215] and to increase the oxidation of mitochondrial glutathione [208]. Interestingly, the effects on mitochondrial membrane potential could be blocked by AACOCF3, an inhibitor of cPLA2. Thus, it seems likely that the increase in renal oxidant stress caused by exposure to high levels of oxalate in vitro [211, 212] or in vivo [205-207, 209] are due to changes in mitochondrial function mediated by lipid signaling molecules. Moreover, Angiotensin II induces oxidative stress by activating membrane-associated nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase), which leads to the production of superoxide. Reactive oxygen species (ROS) generated through the activation of NADPH oxidase are also involved in the production of MCP-1, which causes the recruitment of monocytes and/or macrophages to the interstitium.
2.5.3 Adaptive response to oxalate

2.5.3.1 Increased expression of early response genes and cellular proliferation
Oxalate exposure to renal cells also elicits adaptive responses that may enhance the survival of the remaining renal epithelial cells. For example, oxalate exposure activates pathways leading to proliferation of renal cells (a process that facilitates replacement of damaged/dead cells), producing increased expression of several immediate early genes (IEGs). Many of these IEGs are transcription factors that, in turn, regulate expression of downstream genes involved in proliferation, increased DNA synthesis, and increased number of renal epithelial cells [220].

In cultured kidney cells, oxalate exposure leads to rapid increases in expression of several IEGs. For example, in LLC-PK1 cells (a line of porcine proximal tubular epithelial cells), oxalate exposure leads to an increase in c-myc expression and an increase in cellular proliferation [216]. Treatments that block expression of the c-Myc protein (e.g., treatment of cells with an antisense oligonucleotide directed against c-myc) abolished the oxalate-induced increases in c-Myc expression, DNA synthesis, and cell number [216]. Other IEGs induced by exposure to oxalate or to calcium oxalate crystals, include Egr-1, c-jun, and nur-77 [219, 221]. Oxalate-induced upregulation of IEGs was attenuated by concomitant exposure to antioxidants [219], suggesting that this process was dependent upon prior generation of reactive oxygen species. Lipid-signaling molecules may initiate this process, since inhibition of PLA2 selectively blocks the effects of oxalate on IEGs induction [191].

2.5.3.2 Increased expression of proteins regulating crystal binding
When renal epithelial cells are exposed to oxalate ions and CaOx crystals, there is an increase in gene expression and production of several urinary macromolecules, which modulate the nucleation, growth, aggregation and retention of crystals in the kidneys. The calcium binding property of these molecules enables them to interact with calcium containing crystals. Some of them, such as OPN, Fibronectin, have specific domains to interact with cell membranes, which facilitate their immobilization and promotion of crystal attachment. Almost all of the modulators are produced by the kidneys and excreted in the urine [222, 223].

Fibronectin (FN) is one of the macromolecules over secreted from renal tubular cells as a result of stimulation by COM crystals [224]. It has also been reported that FN protected against renal tubular cell injury caused by oxalate and COM crystals, as
Chapter 2—Review of Literature

shown by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide] assay and morphological examination. We speculate that FN has an inhibitory effect on the adhesion of COM crystals in renal tubular cell injury [225]. The adaptive effects of oxalate, like the toxic effects, appear to be mediated by PLA₂ and subsequent ROS production [220].

2.5.3.3 Apoptotic and necrotic cell death promote crystal nucleation and attachment

Perturbations in mitochondrial function are often accompanied by an increase in mitochondrial permeability and a release of pro-apoptotic factors. These factors in turn trigger the activation of cellular caspases, serine proteases that have been linked to apoptotic cell death [226]. Exposure to high levels of oxalate in vitro [196] and in vivo [203] leads to an increase in the abundance of apoptotic (and necrotic) renal epithelial cells by a process involving increased oxidant stress [219]. Damaged cells exhibit membrane alterations that can promote adherence of crystals to the cell surface. These membrane alterations include exposure of annexin binding phosphatidylserine to the cell surface. Clusters of negatively charged headgroups of phosphatidylserine attract calcium and can act as sites for attachment of calcific crystals to cell surfaces. Such clusters on the surface of apoptotic bodies and membranous cellular degradation products can promote crystal nucleation [117]. In addition, damaged cells can foster crystal growth and deposition in another way, by providing cellular debris for crystal nucleation. Crystals formed in vivo all contain an organic core with a composition similar to that found in cellular membranes [227], and the addition of cellular membranes to artificial urine has been shown to increase crystal formation in vitro [228]. Therefore, cellular damage that leads to shedding of dead cells and the generation of cellular debris within the tubular lumen would foster crystal nucleation. It would also foster the growth of crystals via agglomeration.

2.5.4 Signaling pathways

- Ox and CaOx crystals induce oxidative stress in the kidneys, a condition in which either more ROS such as superoxide and H₂O₂ are produced that can be dealt with or endogenous antioxidant defenses are depleted. Renal cell cultures exposed to oxalate leads to a reduction in the activity of antioxidant enzymes, an increased production of lipid peroxides [219] and caused apoptotic and necrotic cell death.
Exposure to oxalate elicits a number of membrane changes, including a redistribution of phosphatidylserine (PS), altering the membrane surface in such a way to enhance crystal binding. Attached crystals may then be internalized by endocytosis.

Oxalate-induced membrane perturbations also causes activation of at least two lipid signaling cascades, one involving phospholipase A\(_2\) (and resulting in increased release of arachidonic acid and formation of lysophospholipids [191] and the other involving sphingomyelinase (and resulting in increased levels of ceramide and decreased levels of sphingomyelin) [197].

NADPH oxidase is also a major source of ROS in the kidneys [229] and Reactive oxygen species (ROS) may be produced by the activation of NADPH oxidase located at the plasma membrane or by the activation of PLA\(_2\) and neutral sphingomyelinase (N-Smase).

Even though mitochondria have been shown to be a source of free radicals and ROS [230]. Lipid signals (arachidonic acid, lysophospholipid and ceramide) act on mitochondria, disrupting mitochondrial membrane potential, promoting mitochondrial dysfunction, increasing oxidation of mitochondrial thiols, increasing peroxidation of mitochondrial membranes, increasing oxidation of mitochondrial glutathione and increasing permeability of the inner mitochondrial membrane that led to release of mitochondrial factors cyto-c required for activation of caspases, cysteine proteases that are involved in apoptotic cell death [208, 220, 230] and thereby, increasing production of reactive oxygen species (ROS).

Many signaling molecules such as protein kinase C (PKC), c-Jun N-terminal kinase (JNK) and p38 mitogenactivated protein kinase (MAPK), and transcription factors such as NF-\(\tilde{\text{B}}\) and activated protein-1 (AP-1), are activated by ROS. They lead to upregulation of early response genes (\(c\)-\textit{jun}, \(c\)-\textit{myc}, \textit{Egr-1}, \textit{nur 77}) to replace damaged cells, and proteins such as MCP-1, OPN, fibronectin, bikunin and TGF-\(\beta\)1 to modulate crystal formation, that may serve as adaptive functions [231].

ROS also promote cell damage which may unmask additional crystal binding sites. Attached crystals may form centers for nucleation of new crystals, which would favor stone development. Crystal uptake by endocytosis may exacerbate cell damage; alternatively crystals may dissolve within lysosomes or re-emerge at the basolateral surface, again providing centers for stone growth in the renal interstitium [231].
Cell death produced by oxalate exposure may leave cellular debris that forms a nidus for additional crystal growth, also promoting stone formation [231].

**Figure 2.6 Oxalate action on renal cells [231]**

### 2.6 Clinical diagnosis of kidney stones
Non-obstructing kidney stones produce no symptoms or signs apart from hematuria. However, the kidney stone may cause severe pain, usually accompanied by nausea, vomiting and hematuria (renal colic) when it passes into the ureter. Patients may also complain of urinary frequency and urgency. These signs and symptoms lead to many emergency department visits and hospitalization. The pattern of the pain from stone depends on its location: a stone in the upper ureter leads to pain in the flank that may radiate to the upper abdomen. When the stone is in the lower ureter, pain may radiate to the ipsilateral testicle in men or labium in women. If the stone is lodged at the ureterovesical junction, the main symptoms will be urinary frequency or urgency. Symptoms quickly improve after passing the stone. On physical examination, the patient is often in excruciating pain, and is unable to achieve a comfortable position. Ipsilateral costovertebral angle tenderness may also be present.
Laboratory tests may show a leukocytosis which may be due to a stress response or infection. Serum creatinine is often elevated if the patient is volume depleted, or if there is bilateral ureteral obstruction or unilateral obstruction in a patient with a solitary kidney. The urinalysis will have red blood cells, white blood cells and occasionally crystals. However, because of the often non-specific physical examination and laboratory findings, imaging studies are critical in making the diagnosis.

Initial evaluation includes obtaining a non-contrast helical CT, which can accurately visualize the size and location of the stones. A kidney, ureter and bladder (KUB) film, although it is insensitive to uric acid stones since they are radiolucent and therefore are not visualized. However, it can visualize calcium – containing, struvite and cystine stones in the kidney or ureter. Complete ureteral obstruction and upper urinary tract infection (UTI) are indications for stone removal by ESWL or surgery [36, 232]

2.6.1 Medical and nutrition evaluation of kidney stones
A comprehensive history should be taken by one of the health care providers, and the following items should be covered: prior kidney stones, composition of prior stones if known, dietary history including an estimate of typical daily fluid intake, social history including details regarding occupation and lifestyle, and family history. The medical history should focus on identifying diseases that increase stone risk including conditions that lead to hypercalciuria, gout, chronic diarrhea and malabsorptive gastrointestinal disorders.

2.6.2 Interpretation of biochemical and urine tests
2.6.2.1 The 24-hour urine collection
The best way to evaluate stone risk is a 24-hour urine collection and analysis [232]. Two 24-hour urine collections are recommended for the initial evaluation for an accurate analysis and to determine variability [233]. The 24-hour urine collection should be several weeks after any procedures (i.e. 6-8 weeks after lithotripsy) in order to minimize the risk of result being influenced by infection or presence of blood due to these causes. Infection can change the pH and citrate levels.

It is very important that patients continue with their usual diet and activities during the collection period. The 24-hour urine creatinine excretion can give information about the adequacy of the urine collection. In general, adult males produce 18–24 mg creatinine/kg/d and females 15-20 mg/kg/d [232]. 24-hour urine collection is not
accurate as the urinary creatinine levels will be higher than normal for over collection and lower than normal for under-collection [233].

The 24-hour urine sample should include volume, and the solutes calcium, phosphorus, oxalate, citrate, pH, creatinine and uric acid to provide an estimate of supersaturation and the risk of stone formation. Creatinine is tested to ensure full collection and to normalize solute excretion to the more constant amount of creatinine. Dietary factors include sulfates which are mostly from animal protein and sodium since they are related to calcium, potassium, and magnesium excretion. Urea nitrogen is used to estimate protein catabolic rate (PCR). The PCR is usually indicative of dietary protein intake in an individual who is not in a catabolic state. The relationship between urinary nitrogen appearance rate and estimated dietary protein intake is then calculated. The value of the 24-hour urine is to evaluate dietary nutrients and fluid intakes and to provide guidance for the patient’s management. For example, normal urinary calcium levels are <250 mg/d for men and <200 mg/d for women. High urinary calcium may be caused by idiopathic hypercalciuria, or diet high in sodium or protein. Low urinary calcium is often due to malabsorption or underlying bone disease. A normal urinary oxalate level is 20-40 mg/d. High levels are due to high oxalate diet, increased endogenous production, high vitamin C consumption and irritable bowel disease. Normal urinary citrate levels are >450 mg/d for men and >550 mg/d for women. High animal protein diets and renal tubular acidosis (RTA) can increase acid production affecting urinary pH so that it declines citrate levels.

2.7 Treatment of kidney stones
Urgent surgical intervention is indicated in a patient with an obstructed, infected urinary tract, worsening renal function, intractable pain or vomiting or obstruction of a solitary or transplanted kidney. Analgesia is essential and parenteral Non-Steroid Anti-Inflammatory Drugs (NSAIDs: Ketorolac) are as effective as narcotics. NSAIDS are less likely to cause nausea, but should be avoided if the patient has impaired renal function. Pain is due to renal capsule dilatation, and so intractable pain may require decompression of the obstruction. If urgent intervention is not required, the treating physician needs to decide if the stone can be passed spontaneously. The likelihood of spontaneous passage decreases as the size of the stone increases and stones >5-6 mm are not likely to pass spontaneously.
Patients who are having repeated stone attacks should be instructed to strain their urine and submit the stone for composition analysis. Repeated imaging (plain abdominal radiography (KUB) for radiopaque stones and CT for radiolucent stones) is warranted to confirm stone passage. If follow-up imaging reveals no movement after a month, urologic intervention is generally warranted [234].

2.7.1 Surgical treatment
Larger and more proximal ureteral stones are less likely to pass spontaneously and usually require urologic evaluation. If the stone does not pass rapidly, the patient can be sent home with oral analgesia and instructions to return for fever or uncontrollable pain. Infection in the setting of obstruction is a surgical emergency and mandates emergency drainage.

2.7.1.1 Extracorporeal shock wave lithotripsy
The introduction of shock wave lithotripsy in the early 1980s revolutionized the treatment of nephrolithiasis. A shock wave is generated by a source external to the patient that propagates through the body before being focused on a kidney stone. Shock waves cause stone fragmentation directly by producing mechanical stresses or indirectly by the collapse of cavitation bubbles which pass spontaneously days or weeks later [235]. Although shock wave lithotripsy is the most common treatment for urolithiasis, it can have side effects. In human and animal models it can cause acute renal injury [236]. Obese patients may not be effectively treated with ESWL. Cystine stones are very hard and are often not effectively treated with ESWL.

2.7.1.2 Ureteroscopy
Ureteroscopy involves retrograde visualisation of the collecting system using a rigid, semi-rigid, or flexible endoscope. Improved fibreoptics and deflectability and the reduced size of ureteroscopes have expanded the use of ureteroscopy for stones in the upper urinary tract. The ureteroscope has a working channel that allows the introduction of a variety of instruments for stone fragmentation and removal. A retrospective study showed that ureteroscopy is useful when lithotripsy fails; when complex or lower pole renal calculi are present; or when patient factors such as pregnancy, coagulopathy, or morbid obesity preclude lithotripsy. One disadvantage of ureteroscopy is that a ureteral stent, which causes considerable discomfort in some
patients, is often necessary to prevent obstruction from ureteral oedema or stone fragments [237].

2.7.1.3 Percutaneous nephrolithotomy

Percutaneous nephrolithotomy involves creating an access tract into the renal collecting system through which nephroscopy can be performed. The nephroscope has a working channel through which an intracorporeal lithotripsy device (lithotrite or laser) can be introduced. Stone fragments are removed using suction, graspers, or basket extraction. The technique enables stones to be retrieved for analysis, and all stone material can be removed so that the patient does not have to pass any fragments, as is common with shock wave lithotripsy and ureteroscopy. Although percutaneous nephrolithotomy is thought to be more invasive than other treatments, a large meta-analysis has demonstrated its safety and efficacy, particularly when stones are large, multiple, or complex [237].

2.7.1.4 Open surgery

Open surgery for renal stone disease has decreased considerably because of the adoption of non-invasive and minimally invasive techniques. The commonest current and acceptable indications for open surgery include complex stones in kidneys with a dilated collecting system, failure of percutaneous, endourological or ESWL, and stones in a kidney with anatomical abnormalities, e.g. PUJ obstruction, infundibular stenosis, ureteric strictures and concomitant open surgery [238].

2.7.2 Dietary management

Dietary modifications could play an important part in the management of stone disease in the region; keeping in perspective the social and cultural environment of the stone-formers, the following modifications should be advised in the long term.

- High fluid intake: a minimum of 10-12 glasses of water (250 mL each).
- Oxalate restriction: restrict the use of spinach, okra, green vegetables, tea, and green and black pepper.
- Fat-rich foods: reduce the consumption of oily or fat-rich food, especially animal-fat products.
- Sodium: avoid the use of salt shakers and salty food.
• Increase citrus fruits: promote the consumption of lemon juice, orange juice, and especially potassium-rich products.
• Increase the fibre intake, e.g. bran bread.
• Increase calcium intake by having at least two cups of milk/milk products per day.

2.7.3 Medical treatment
Medical treatment should be used on assessing 24 hour urinary metabolic abnormalities. Drug treatment is advised after a high fluid intake (>3 L/day) and dietary modifications in the long term fail to correct abnormalities or prevent recurrence. In cases of Hypercalciuria with normal parathyroid hormone levels, the treatment is thiazide diuretics and potassium citrate, with a reduction of sodium in the diet [239]. Patients with hyperoxaluria not related to diet should investigated for underlying bowel disease. Therapy should include a reduction in oxalate-rich diet, with pyridoxine supplements and lemon juice. For severe hypocitraturia investigations should be directed to detect gastrointestinal disorders and renal tubular acidosis. Here the mainstay of treatment is lemon juice or potassium citrate. Hyperuricosuria with hyperuricaemia is treated by allopurinol 300 mg/day and potassium citrate or orange juice as an alternative [240]. Most patients with stone disease present with more than one risk factor; in our studies, 10% presented with several metabolic or environmental risk factors. Medical therapy thus requires a combination, depending on the individual patient profile and the type of stone formation.
2.8 Phytotherapy

Treatment and prevention of kidney stones has considerably evolved during the last two decades by combination of dietary procedures, surgical treatments and medicaments, side effects of these methods and recurrence remain as problems to overcome. Thus, an adjunct to these conventional methods, phytotherapy is highly recommended. Medicinal plants have been known for millennia and are being used as a rich source of therapeutic agents worldwide. WHO reported that ~75% global population, most in the developing world, depends on botanical medicines for their basic healthcare needs with around 800 plants being used in indigenous system of medicines [241]. The use of herbal medicine is becoming popular due to toxicity and side effects of allopathic medicines.

Urolithiasis has been a matter of concern to clinicians since the time of Hipocrates. Many remedies have been employed during the ages to treat urinary stones. In the traditional system of medicine, most of the remedies were taken from plants and they proved to be useful though the rationale behind their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs. The various marketed composite antiurolithiatic herbal formulations, Cystone (Himalaya Drug Company, India), Calcury (Charak Pharmaceuticals, Mumbai, India), Chandraprabhabati (Baidyanath, India), Neeri (Aimil Pharmaceuticals, India), Uriflow (BioNeutrix Healthcare, USA), Uriflush (Global Pharmaceuticals, India) have been used worldwide [242].

2.8.1 Proteins: potent antiurolithiatic biomolecules

Until recently, pharmaceuticals used are being largely synthesized by organic chemistry. As knowledge about sources of many diseases and how body fights these diseases is available, focus is on developing the therapeutics that mimic or enhance the actions of body’s arsenal. Protein based drugs, as proteins are one of the main macronutrients in food, are one of the most important and rapidly growing segments of the pharmaceutical market with reduced immunogenicity, improved safety and greater effectiveness [242]. Insulin [243], plant lectins, Lunasin from soy [244], Bromelain from Pineapple [245], MAP30 (*Momordica* anti-HIV protein of 30 kDa) and GAP31 (*Gelonium* anti-HIV protein of 31 kDa) [246], are few bioactive plant protein and peptides being explored.
Till date not many reports are available about antilithiatic plant proteins and peptides, even though urolithiasis has afflicted mankind since antiquity and there are many herbal formulations available in market. The antilithiatic plant proteins isolated, purified and characterized till date are anionic, rich in acidic amino acids and have EF Hand domain, a characteristic feature of various calcium binding protein like calgranulin, osteopontin [247]. Acidic amino acids interact with calcium ions thus making them unavailable for oxalate to bind. A 98 kDa dimeric antilithiatic protein was purified from seeds *Dolichos biflorus* having abundant acidic amino acids. This protein showed similarity with Calnexin of *Pisum sativum* [248]. A CaOx growth inhibitor with two EF hand domains was purified and characterized from seeds of *Trachyspermum ammi* [249]. The protein maintained renal functioning, reduced renal injury and decreased crystal excretion in urine and retention in renal tissues [250]. A CaOx growth inhibitory protein isolated from *Tribulus terrestris* (~60 kDa), anionic with EF hand domain, was found to be cytoprotective in comparison to cystone [251]. These proteins and peptides can be produced on large scale using recombinant DNA technology, taking into consideration potential toxicity, allergenicity and stability of peptides.

### 2.9 *Terminalia arjuna*

**Classification:**
- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Myrtales
- Family: Combretaceae
- Genus: *Terminalia*
- Species: *arjuna*

**Common name:**
- Arjuna, Dhavala, Kakubha, Nadisarja, Veeravriksha, Partha, Indradru

**Part used:** Bark

### 2.9.1 Botanical description

The tree is about 60-80 feet height. Arjuna is large, evergreen with a spreading crown and dropping branches. The bark of *Terminalia arjuna* is smooth, pinkish-grey from
outside and flakes off in large, curved and rather flat pieces. The histology of *Terminalia arjuna* bark reveals the presence of single layered epidermis with hair like projections and few scattered lenticels. Underlying the epidermis is a thin layer of cortex. Periderm and secondary phloem are present in the old bark. Leaves are simple, borne sub-opposite coriaceous, often crenulating, oblong or elliptic. Their upper face is pale or dark green and the lower face is pale brown. The tree bears white sessile flowers arranged in short axillary spikes or in terminal pannicule. The flowers are bisexual. Linear, lanceolate-like bracteoles are present. Calyx is glabrous. Its fruit is a drupe, 2.5–5 cm long, ovoid or oblong, fibrous-woody, smooth-skinned with five hard angles or wings. The lines of the wings are oblique and curved upwards [252].

### 2.9.2 Geographical distribution

*Terminalia arjuna* (Roxb.) Wt. and Am. is a large evergreen tree distributed throughout the greater part of the Indian Peninsula along rivers and found in Sub-Himalayan tract, Chota Nagpur, Orissa, Bihar, Madhya Pradesh, West Bengal, Punjab, Deccan and Konkan.

### 2.9.3 Traditional uses

In the Indian system of Medicine, its bark decoction is being used as an astringent, cooling, urinary astringent, cardiotonic, in fractures, ulcers, spermatorrhoea, leucorrhoea, diabetes, cough, tumour, excessive perspiration, asthma, inflammation and skin disorders, dyslipidemia, hypertension, angina pain and congestive heart failure. Bark stem possesses diuretic, inotropic and chronotropic. Its useful phytoconstituents are: Triterpenoids, β-sitosterol, flavonoids and glycosides. Triterpenoids and flavonoids are considered to be responsible for its beneficial antioxidant cardiovascular properties. Chakradatta, the great ancient physician, recommended it to be given as a decoction of bark with milk or as a ghrita (a preparation with ghee or butter). Decoction of the bark has been used as ulcer wash, while bark ashes have been prescribed for snakebite and scorpion sting. When bark powder boiled in water and inhaled, cure headache and to kill worms in teeth. The fruit paste was also used on wounds. Fresh leaf juice is used for the treatment of earache and bark powder for treating heart ailments. Dried bark along with rice washed water was also used to treat blood in urine. Moreover, the fresh bark was chewed and swallowed the juice as an antacid [252].
2.9.4 Pharmacology

*Terminalia arjuna* bark helps maintain a healthy heart and reduces the effects of stress and nervousness (Himalaya herbal health care). It also promotes effective cardiac functioning and regulates blood pressure. *Terminalia arjuna* therapy for two weeks leads to significant regression of the endothelial abnormality amongst smokers [21]. The bark of *Terminalia arjuna* is also reported to inhibit nitric oxide production in murine macrophages [22] and is traditionally used to prevent kidney stone formation. Aqueous extract of the bark of *Terminalia arjuna* is shown to protect the liver and kidney tissues against CCl₄-induced oxidative stress probably by increasing antioxidative defense activities [23]. Casuarinin extracted from *Terminalia arjuna* attenuates H₂O₂-induced oxidative stress, decreases DNA oxidative damage and prevents the depletion of intracellular GSH in MDCK cells [24]. In a recent study, both alcoholic and aqueous extracts of the bark attenuated H₂O₂-mediated reactive oxygen species generation in human monocytic cells by promoting catalase and glutathione peroxidase activities and by sustaining cellular reducing power. Moreover, the extracts inhibited lipid peroxidation and 3-hydroxy-3-methyl-glutaryl-CoA, but had no effect on lipoprotein lipase. Arjunolic acid has been found to prevent the decrease in the levels of superoxide dismutase, catalase, glutathione peroxidase, ceruloplasmin, α-tocopherol, reduced glutathione, ascorbic acid, lipid peroxide and myeloperoxidase.