STONE FORMATION

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Keywords

- Urinary stones
- Urolithiasis
- Testicular microlithiasis
- Biliary stones
- Gallbladder stones
- Sialolithiasis
- Supragingival stones
- Pancreatic stones
- Broncholithiasis
- Pulmonary alveolar microlithiasis

The stone formation could be seen as a derangement in the process of biomineralization. Several chemical and physical conditions influence mineral formation and crystal growth in solutions. The necessary ions must be present at concentrations exceeding their normal solubility.

23.1. Urinary stones

The formation of stones in the urinary tract affects 5-10% of the population in Europe and North America [1]. An even higher frequency has been reported from other parts of world and there are only a few geographical areas in which stone disease is rare, e.g. in Greenland and in the coastal areas of Japan [2]. The annual incidence of stone formation in the industrialised world is generally considered to be 1500-2000 cases per million [2]. Although the chemical composition of stones varies widely a common denominator is the high risk of recurrent stone formation when the first stone is removed, although there is considerable variation among individuals.

23.1.1 Pathogenesis

Urolithiasis is a consequence of complex physical processes [3] and the pathogenesis of stone formation is a multifactorial process. A prerequisite for urinary stone formation is urinary crystal formation. For this, urine must be supersatured [4]. The crystallization potential of urine is related not only to the concentration of salt in question, but also to the presence or absence of other compounds, such as inhibitors, complexors or promotors [5]. Other factors are represented by anatomic abnormalities. The sequence of events leading to urinary stone formation is: saturation → supersaturation → nucleation → crystal growth or aggregation → crystal retention → stone formation. Some or all of these processes contribute to the development of a clinically significant stone.

The *supersaturation of urine* is considered the most important driving force behind stone formation. It is based on the binding of salts, which occurs after a certain concentration is obtained. A compound's thermodynamic solubility product (KSP) defines the saturation of a compound in a solution [6]. The KSP of a compound is equal to the product of a pure chemical in equilibrium between a solid and solvent in solution. If the salt concentration is less than the KSP, the compound remains in solution. However, if the salt concentration exceeds the KSP, the compound precipitates. This process is called homogeneous *nucleation*. Nuclei form the first crystals that do not dissolve and have a characteristic lattice pattern. In urine, nuclei usually form on existing surfaces, a process called heterogeneous nucleation. Epithelial cells, urinary casts, red blood cells and other crystals can act as nucleating foci in urine. The saturation needed for heterogeneous nucleation is much less than for homogeneous nucleation. Once a nucleus is formed, particularly if it is anchored, crystallization can occur at lower chemical pressures than required for the formation of the initial nucleus.

The initial nucleus can grow by the precipitation of additional salt on the lattice framework (*crystal growth*). The earliest site of stone formation in human beings is the papillary duct or the collecting duct tubule, where the diameter is 50 to 200 µm. The time to grow to a diameter of 200 µm depends on the state of supersaturation of urine.

Then, once nuclei are formed they bounce apart from each their, float freely, and become kinetically active. Under certain circumstances, these nuclei come in close contact and due to chemical or electrical forces can bind to each other, a process called *crystal aggregation*. Although it is impossible for crystal growth alone to give rise to a crystal large enough to occlude the lumen of the collecting duct, aggregates of crystals easily cal attain such a size [7]. The combination of crystal growth and crystal

aggregation can explain the genesis of urinary stones.

Although crystals may form, they generally do not become very large and they "wash out" before they become clinically significant, because of their short transit time within the urinary tract. However, an anatomic or functional abnormality can cause an obstruction to the flow of urine and the retention of urinary crystals (*crystal retention*). In fact, crystal aggregates are too fragile to occlude a collecting duct long enough to give rise to a stone. If a crystal is retained in the kidney, growth can occur for long periods of time whenever urinary supersaturation or aggregation of new crystals occurs [8]. Anatomical abnormalities in the kidney, such as meddullary sponge kidney or ureteropelvic junction, or even an increase in crystal epithelial adherence, can lead to crystal retention.

In normal urine, the concentration of calcium oxalate is many times more than its solubility. This is possible because in the urine are present many *inhibitors,* substances that modify or alter crystal growth, thus preventing stone formation; they allow higher concentration of calcium phosphate to be held in solution than in pure solvents. Although urine may be supersaturated with a salt, these inhibitors can prevent stone formation [9]. These molecules work by forming complexes with active surface compounds, which reduces their binding of calcium to oxalate. In fact many individuals who excrete increased calcium and oxalate do not form stones.

The list of compounds that can retard stone crystallization is extensive and includes metal ions as magnesium, simple compounds as citrate and macromolecules [10-12].

Citrate is the most important urinary stone inhibitor [13]. Citrate is the most abundant organic anion in human urine and by chelation of calcium ions, citrate efficiently lowers supersaturation, the driving force for crystallization [14]. In a supersaturation decay system is therefore expected to reduce induction time and rate of nucleation [15]. Urinary citrate permits base excretion without raising urine pH, which permits defence against alkali loads without precipitating calcium phosphate. Furthermore, citrate complexes calcium in a soluble form and prevents crystal growth of calcium phosphate and calcium oxalate in urine [16, 17].

There are other important stone formation inhibitors and the absence or reduction of these inhibitors can aid in the production of stone formation [18].

Although normal urine contains only small amounts of protein, proteins secreted by tubular epithelial cells are present in the final urine. These urinary inhibitor molecules include Tamm-Horsfall mucoprotein (THP) [19]. THP is one of the main components of urinary proteins. It is a glycoprotein produced and secreted by the thick ascendant limb of the loop of Henle. THP of normal subjects inhibits the aggregation but has little effect on nucleation and growth of CaOx crystals [20]. However, THP activity is influenced by its own concentration, urinary pH and ionic strength, playing a dual role in crystal formation depending on the environmental conditions. Moreover, THP isolated from the urine of recurrent stone formers sometimes becomes a promoter of calcium oxalate (CaOx) aggregation due to a tendency to self-aggregation, which removes it from effective interaction with CaOx monohydrate crystals [19].

Nephrocalcin, an acidic glycoprotein containing γ-carboxyglutamic acid, is another inhibitor and acts by impairing crystal nucleation, growth and aggregation.

Osteopontin (OPN) is a phosphorylated protein of wide tissue distribution that is found in association with dystrophic calcification including in the organic matrix of kidney stones [21-23]. It is a strong inhibitor of crystal formation and growth in vitro. There are some evidence that OPN is implicated in stone disease with the primary emphasis being on the interaction of OPN

with calcium oxalate, the major constituent of calcium containing stones [24].

Fibronectin is a multifunctional α2-glycoprotein distributed throughout the extracellular matrix and body fluid [25, 26]. Fibronectin is oversecreted from renal tubular cells as a result of CaOx crystal stimulation, and inhibits aggregation of CaOx crystals and their adhesion to renal tubular cells [27].

Substances that form soluble complexes with the lattice ions for specific crystals, such as CaOx, are called *complexing agents*. These decrease free ionic activity and thus reduce the level f saturation of the stone-forming substance. Pure *promotors* of stones are rare. Glycosaminoglycans promote crystal nucleation but inhibit crystal aggregation and growth [28]. THP may act as a promotor or an inhibitor of crystallization.

The presence of noncrystalline organic *matrix* in urinary stone is known from 1684 [29]. Chemical analysis of matrix revealed 65% hexosamine and 10% bound water. The matrix contains substances similar to uromucoid found in urine, except that the matrix lacks the 3.5% sialic acid found in the urinary uromucoid. Some Authors suggest that the matrix is nothing more than a coprecipitate with the crystals that form stone [30]. Considering the microscopic findings that demonstrate concentric lamellated structure, the matrix may be a ground substance for stone formation. Du Toit [31] suggested that a factor in stone formation is an alteration in the excretion of the enzymes urokinase and sialidare. According to this Author, decreased urokinase and increased sialidare in urine leads to the formation of mineralizable stone matrix. We know that *Proteus Mirabilis* and *Escherchia Coli* (*E. Coli*) decrease urokinase and increase sialidase activity. According to these findings, *E. Coli* may cause urolithiasis by producing matrix substances that in turn increase crystal adherence to the epithelium. Matrix calculi are predominantly found in individuals with recurrent infections of urease-producing organisms. They are radiolucent and may be mistaken for a uric acid stone, although the association with alkaline urine and infection is certainly helpful in this distinction. Animal and cellular studies have shown that exposure to high levels of oxalate and/or CaOx crystals produces cellular injury at the level of the proximal kidney tubular cells inducing cellular changes which may vary from cellular adaptation to cell death [32]. It has been demonstrated that injury produced by crystal oxalate is a predisposing factor to subsequent CaOx stone formation [33]. Oxalate in fact increases the availability of free radicals by inhibiting enzymes responsible for their degradation; reactive oxygen species can damage the mitochondrial membranes and produce a decrease in mitochondrial transmembrane potential [34]. These events are a known early process in the apoptotic pathways. It has been shown that exposure renal epithelial cells to oxalate produces increased DNA synthesis, altered gene expression and apoptosis. The role of free radicals in lithogenesis is also supported by the fact that vitamin E and selenium, known anti-oxidant agents, can prevent in vitro the lipid peroxidation of renal proximal tubular cells [35]. Additionally, oxalate injury can result in shedding of the microvillous brush border. Changes in membrane lipids manifest as an increase in lipid urinary contents [36].

As a consequence a significant increase in urinary excretion of proteins and lipids in stone formers may be indicative of a cellular damage resulting from prolonged exposure to oxalate and/or deposition of CaOx crystals in the tubules [37].

CaOx, struvite and uric acid stones contain 2 to 4 times more lipids than proteins [38]. The lipid matrix is a good nucleator of CaOx crystals from a metastable solution. Formation of a complex between calcium and acidic phospholipids is considered one of the initial steps in stone formation [39]. A number of studies have demonstrated that

lipids that participate in crystallization of calcium phosphate form complexes with calcium and bind tightly to the crystals [37]. Interestingly, some cells do not seem to respond to oxalate injury, suggesting that changes in gene expression could protect from apoptosis and consequently from lithiasis.

23.1.2 Classification of stones

Kidney stones are made of different types of crystals. Most are CaOx or calcium phosphate, a combination of CaOx and calcium phosphate, uric acid, magnesium ammonium phosphate, also known as struvite or infection stones, cystine, and miscellaneous types such as occur with drug metabolites.

23.1.2.1 Calcium stones

Calcium stones [Fig. 1] are heterogeneous in composition and pathophysiology. CaOx and calcium phosphate are the most frequently encountered compounds, but CaOx stones are much more frequent than calcium phosphate stones. Nearly seven decades ago, Randall described plaque-like lesions in the renal papillae, which were invariably present in patients with CaOx stones, although sometimes also present in individuals who did not form stones [4]. Now called Randall's plaques, these lesions were believed to be the nidus upon which CaOx stones arose and grew. Microscopically, these plaques seem to arise from the basement membrane of the thin limbs of the loops of Henle, expand through the interstitium sometimes encasing the renal tubules and vas recta, and eventually protrude into the uroepithelium in the renal papillae. Composed of calcium phosphate, Randall's plaques seem to provide the platform for CaOx crystal to form through heterogeneous nucleation and grow to a nephrolith.

See Table 1 for the most common causes of calcium-containing stones. Hypercalciuria, hypercalcemia, and hyperoxaluria are three common metabolic disorders that lead to calcium stone formation.

Hypercalciuria

This common metabolic abnormality is encountered in 60% of stone formers. There are several metabolic causes of hypercalciuria that lead to stone formation [40]. *Absorptive hypercalciuria* is caused by an increased intestinal calcium absorption. This causes an increase in renal filtered calcium and produces hypercalciuria. Another problem is that renal calcium can produce a hypocalcemiac state, which causes a secondary hyperparathyroidism. Parathyroid hormone causes intestinal absorption and bone resorption of calcium, which leads to further calcium wasting and hypercalciuria. *Resorptive hypercalciuria* is caused by excessive bone resorption of calcium, primarily from hyperparathyroidism. A *renal phosphate leak* creates hyperphosphaturia, which can produce the excess 1,25-$(OH)_2$-vitamin D that produces hypercalciuria.

Hypercalcemia

Hypercalcemia is caused by hyperparathyroidism, sarcoidosis, steroids, malignancy, idiopathic causes, and immobilization. Immobilization is the second most common cause of stone disease. Immobilization causes the increased bone resorption of calcium which, in turn, increases renal filtration. This creates hypercalciuria and the precipitation of CaOx and calcium phosphate stones.

Hyperoxaluria

The primary hyperoxaluria is caused by an autosomal recessive disorder in oxalate biosynthesis that causes an increase in the hepatic production of oxalate. Secondary hyperoxaluria, enteric oxaluria, is caused by intestinal hyperabsorption of oxalate. Enteric hyperoxaluria is seen in inflammatory bowel disease, bowel resection, and small bowel bypass procedures. With these conditions there is an increase in bile salt and fatty acids that combine with calcium leading to increased oxalate available for absorption.

This effect of oxalate is dependent on the so-called calcium-oxalate interaction, whereby oxalate absorption from the bowel is affected by formation of poorly absorbed CaOx, and the amount of free oxalate in urine is affected by formation of a soluble complex of calcium and oxalate.

In fact, with increased intestinal absorption of oxalate, there is an increase in urinary oxalate leading to formation of CaOx stones because oxalate complexes with calcium within the renal tubules, precipitates, and aggregates to form stones.

The bile acids and fats within the lumen of the bowel bind and reduce the amount of free calcium, leaving an increased amount of oxalate to be absorbed from the intestine and excreted in the urine. These patients also have low urinary citrate and magnesium as a result of chronic metabolic acidosis due to chronic diarrhea. All these factors lead to CaOx stone formation.

23.1.2.2 Uric Acid Stones

Uric acid is an end product of purine metabolism. It's the same crystal that causes gout, an arthritic condition. Foods high in purines are red meat, fish, and chicken. High body-mass index, glucose intolerance, and overt type 2 diabetes are common in uric acid stone formers. See Table 2 for the conditions associated with uric acid nephrolithiasis.

The solubility of uric acid depends on the acidity or alkalinity of the urine. In acid urine, pH less than 5.5, uric acid crystals precipitate leading to stone formation. If urine is alkaline, uric acid remains soluble and doesn't precipitate out. Knowledge of this fact is the basis of the medical treatment of uric acid stones. Gout, myeloproliferative disorders, and chemotherapy are common causes of these stones. They are radiolucent on plain X-ray, but can be visualized on urography [Fig. 2] or CT scan.

23.1.2.3 Magnesium Ammonium Phosphate Stones, struvite or infection stones

Depending on their composition, these stones are also call triple phosphate, struvite, or infection stones. Infection stones are formed as a result of persistent infections caused by urease producing bacteria and urinary tract obstructions are frequently involved [41]. These stones are characterised by their exceptionally rapid growth. Infection stones consist of monoammonium urate, struvite (magnesium ammonium phosphate - MgNH4PO4·6H2O) and/or carbonate apatite [42]. Carbonate apatite starts to crystallise at a urine pH level of 6.8. Struvite precipitates only above a pH level of 7.2. Considering the pathogenesis of struvite stones, during an urinary tract infection due to urease-producing pathogens, which are often correlated with urine flow disturbances, the urea in urine is split into ammonia and CO2 catalysed by the bacterially produced enzyme (urease). This in turn raises the pH of the urine because of the steady ammonia and CO2 production so that after a short time the urinary pH levels out between 7.2 and 8.0. Ammonia continues to be hydrolysed and forms ammonia ions. Around the urease-producing bacteria, Mg^{2+}, a normal component of urine, is rendered insoluble at alkaline pH and precipitates as struvite due to the presence of NH_4^+ and PO_4^{3-}. Bacteria that produce urease act on the urea present in urine to form ammonia, bicarbonate, and carbonate ion. The most common bacteria associated with struvite stones is *Proteus* [41], but other bacteria such as *Klebsiella*, *Pseudomonas* and *Staphylococcus species* may also be implicated.

23.1.2.4 Cystine

Cystinuria is an autosomal recessive disorder caused by the defective transport of cystine and dibasic amino acids (lysine, ornithine and arginine) in the brush border of proximal renal tubules and intestinal tract. This leads to increased urinary excretion of these compounds, but the only one that forms

stones is the cystine: in fact, the low solubility of cystine induces its precipitation into the urinary tract. The phenotype is cystine stones because only cystine is soluble in urine [43]. Factors that should raise suspicion are young age of presentation, mildly radio-opaque stones, family history, and characteristic hexagonal cystine crystals.

23.1.3 Risk factors

We can distinguish the non-genetic factors from the genetic factors.

23.1.3.1 Non-genetic factors

23.1.3.1.1 Diet

No kidney stone disorder can be explained by nutrition alone. However, diet does play a crucial role in the pathogenesis of the most widespread forms of nephrolithiasis, for example calcium (CaOx and phosphate) and uric acid stones, triggering the formation of stones in people with a predisposition.

The first crucial point concerns *water* intake, the importance of which cannot be underestimated in the prevention of stone disease. The most effective way to increase urinary volume, which in stone-forming adults should always be superior to 2 liters/day, is to drink sufficient quantities of water [44].

Considering CaOx stones, there is still considerable controversy surrounding the various factors that determine the level of oxalate in urine. The majority (55% to 70%) of urinary oxalate is derived from metabolism of glyoxalate and ascorbic acid. The remainder results from the intestinal absorption of oxalate from dietary sources. Nevertheless, diet and absorption may provide the critical quantity of additional oxalate necessary to trigger off the formation of CaOx stones. There are some foods particularly high in oxalate content and they include rhubarb, spinach, beetroot, parsley, okra, soya beans and many soya products, yams, wheat bran, nuts, peanut butter, sesame seeds, black pepper, chocolate, chocolate drinks and tea. Most fruits, cereals and vegetables contain a small quantity of oxalate but if these are extracted and concentrated, such as in cranberry juice tablets, the resulting oxalate content can be very high. The percentage of oxalate absorbed may vary widely depending on the intake of oxalate and the composition of the remainder of the diet. It also depends on body size presumably as a function of the greater intestinal area available for absorption [11, 44].

Several risk factors have been implicated in the pathogenesis of uric acid nephrolithiasis, but the underlying mechanisms are not completely understood. In fact stones can develop as a result of congenital or acquired causes, or can be idiopathic. Considering the acquired causes, low-carbohydrate high-protein diets, such as the Atkins' diet commonly used for weight reduction, deliver an exaggerated acid load to the kidney, reducing urinary pH and increasing the propensity for uric acid stone formation. Several recent studies on the metabolic aspects of idiopathic uric acid nephrolithiasis have suggested a link between insulin resistance and low urinary pH in gouty diathesis. Individuals with gouty diathesis share many features of the metabolic syndrome, including hyperglycemia and hyperinsulinemia, hypertension, a high body mass index, and hypertriglyceridemia.

Through a variety of mechanisms, first and foremost an increase in the body's acid load, an excess of *animal proteins* induces multiple metabolic urinary alterations such as hypercalciuria, hyperoxaluria, hyperuricuria, hypocitraturia and excessive urinary acidification, exposing the subject to the risk of forming both calcium and uric acid stones. Diets high in animal protein have been demonstrated to raise the risk for kidney stone disease. Epidemiological studies have disclosed a strong association between stone disease and the more affluent members of industrialized societies. This has been suggested to be the result, in large part, of increased dietary animal protein. Several mechanisms have been suggested to explain

protein induced hypercalciuria. One theory suggests that excessive animal protein intake provides an excess of sulfur-containing amino acids (e.g., methionine) that can be metabolised to sulfuric acid. To help buffer this excess acid load, bone is resorbed to provide phosphate and carbonate for this purpose. The other product of this bone dissolution, namely calcium, is cleared by the kidney, resulting in increased urinary calcium excretion. According to an alternative mechanism for explaining the deleterious effects of animal protein intake on stone disease, the animal protein diet, when its electrolyte composition and quantity of protein were kept the same as for the vegetarian diet, conferred an increased risk for uric acid stones, but, because of opposing factors, not for CaOx or calcium phosphate stones [45].

Excessive intake of *carbohydrates*, especially the simple ones, can generate a state of hyperinsulinism, leading to reduced renal tubular re-absorption of calcium, and hence to hypercalciuria. On the other hand, there are carbohydrates among the precursors of the endogenous synthesis of oxalate, so hyperoxaluria can be generated by this route too. This is the reason why a limited intake of simple sugars is recommended in the diet.

Excessive *fat* intake, except for foods containing high quantities of omega-3 fatty acids, has also been proven to increase oxaluria, perhaps due to an increase in the intestinal absorption of oxalate [46]. The traditional advice given to stone formers to reduce foods containing calcium by eliminating milk and milk derivatives is no longer accepted as valid. In fact, a low-calcium diet can reduce calciuria in hyperabsorbers of calcium, but can trigger a negative calcium balance and a loss of bone calcium.

Unfortunately, very few trials have evaluated the impact of *diet change* on stone recurrences in a controlled manner and for a sufficiently long period of time (at least 3 years). Indeed the famous low-calcium diet that has been applied for years throughout the world to reduce calciuria in hypercalciuric subjects and to attempt to prevent stone recurrences, has never been tested for prolonged periods in controlled conditions [47]. For this reason, in 1993 Borghi et al. [48] started a randomised trial in hypercalciuric stone-forming males, aimed at comparing the effects of the 'traditional' low-calcium diet (400 mg/day) with an 'anti-stone-forming' diet consisting of a normal calcium intake, a low animal protein and salt intake and a high potassium intake. The results of this trial demonstrated that calcium excretion dropped significantly with both diets, to a mean value of approx. 170 mg/day. In contrast, urinary oxalate increased in patients following the 'traditional' low-calcium diet (on average 5.4 mg/day) and decreased in those treated with the 'anti-stone-forming' diet (on average 7.2 mg/day). After 5 years, 12 of the 60 (20%) hypercalciuric subjects following our 'anti-stone-forming' diet had recurrences, compared to 23 of the 60 (38.3%) patients following the 'traditional' low-calcium diet [49].

Vitamin E, an anti-oxidant, has been shown to prevent the lipid peroxidation of renal proximal tubular cells in vitro. *Selenium*, acting as an anti-oxidant is also involved. *Vegetables and fruits* increase the urinary excretion of the stone-inhibiting citrate. The consumption of foods with a high oxalate content (spinach, rhubarb, beetroot, chard and nuts) should always be kept to a minimum or combined at the same time with foods providing a plentiful supply of calcium (e.g. spinach with a cheese gratin), which prevents the absorption of large quantities of oxalate from the intestine which would lead to an increase in it's excretion in the urine [46].

Some Authors [50] have demonstrated that *Green tea* have been shown to have antioxidant effects. In fact, green tea treatment increased SOD activity compared with the stone group. The degree of

apoptosis in the stone group was significantly increased compared with the drink and powder groups.

23.1.3.1.2 Body size

As body weight increases, excretion of calcium, oxalate and uric acid also increases in normal subjects and in stone formers [51]. In contrast, even a small drop in body weight in subjects with calcium stone disease is associated with a considerable reduction of lithogenous salts in their urine, and vice versa. In conclusion obesity and weight gain increase the risk of kidney stone formation. The magnitude of the increased risk may be greater in women than in men [52].

23.1.3.1.3 Environment

There is a growing body of evidence from NASA and the Russian space program showing that humans exposed to the microgravity environment of space have a greater risk for developing renal stones [53]. Increased bone resorption and the attendant hypercalciuria and hyperphosphaturia contribute significantly to raising the urinary state of saturation with respect to the calcium salts, namely CaOx and calcium phosphate. However, other environmental and dietary factors may also adversely affect urine composition and increase stone formation risk. Reductions in urinary volume have been consistently observed in short-duration flights [54-56].

23.1.3.2 Genetic factors

Family history is a known risk factor of urolithiasis [57, 58]. Genetic factors have been postulated to play an important role in the risk of urolithiasis, as demonstrated by the evidence that positive family history is a well-known risk factor for urolithiasis. Stone formation seems to be inherited with a polygenic mechanism [Tab. 3]. In the majority of children with urolithiasis a metabolic cause of stone formation can be identified, and several metabolic disorders have been elucidated (i.e. cystinuria or primary hyperoxaluria). In contrast, a metabolic cause is more rarely found in adults.

Approximately half of the patients who are labelled as having idiopathic hypercalciuria have a positive family history of kidney stones [59]. Familial idiopathic hypercalciuria has been described as an autosomal dominant trait in the earlier literature, but this is clearly an oversimplification of the genetics of familial hypercalciuria.

Considering uric acid stones there are distinct geographical and ethnic variations in the incidence of this kind of stone development compatible with environmental or genetic susceptibility in some populations: for example although the incidence of uric acid stones in the United States is reported to be 5–9.7% of all kidney stones analysed, it is 25% in Germany and as high as 39.5% in Israel [60]. Recently, Ombra et al. identified a possible genetic basis for this increased risk by studying a homogeneous population with a high incidence of uric acid stones in Sardinia [61]. The majority of the patients evaluated had low urinary pH with high titratable acidity. In addition, a third of the individuals were hyperuricosuric, with a uric acid excretion greater than 700 mg per 24 h. The major abnormality in this population is therefore high urine acidity. The investigators used multi-step linkage and allele-sharing analysis to identify a locus on chromosome 10q21–22 associated with increased susceptibility to uric acid stone formation. Subsequent investigation further identified a candidate gene, named ZNF365 [62].

23.2. Other urological stones: Testicular microlithiasis

Testicular microlithiasis is an uncommon condition characterized by calcifications within the seminiferous tubules. The true prevalence in a normal population has not been defined. It was first described radiologically by Priebe and Garret in 1970 [63]. Morphologically, the microliths consist

of degenerated intratubular cells which form a calcified core. In a histopathological classification two different forms of testicular microlithiasis have been described by Renshaw [64]. The most frequent type is represented by *laminated calcifications* that occurred in association with testicular malignancies, in cryptorchid testes, and also in normal testes. The second type consists of *hematoxylin bodies* that are exceptionally encountered in connection with testicular malignancies [65]. A prognostic value of testicular microlithiasis concerning the development of testicular cancer cannot be derived from the published data [66] and the clinical significance of testicular microlithiasis remains unclear [67, 68].

23.3. Biliary and gallbladder stones

Biliary stone disease is a common disorder, usually associated with stones in the gallbladder and can cause significant complications. Formation of stone usually occurs in the presence of the following factors: abnormalities of the bile constituents, bile stasis and the presence of nidus for stone formation [69].

The cause of *gallbladder stone* is very complicated, various factors are involved [70]. Three conditions must be met to permit the formation of cholesterol gallstones: 1. Bile must be supersaturated with cholesterol; 2. Nucleation must be kinetically favourable; 3. Cholesterol crystals must remain in the gall bladder long enough to agglomerate into stones. Most patients have cholesterol gallstones. Nucleation of cholesterol crystals is considered the essential initial step in cholesterol gallstone formation [71]. Cholesterol cannot dissolve in water, but it can dissolve in bile. The concentration of cholesterol in bile is about 10-20 mmol/L, this concentration is about 106 times denser than that of the solubility of cholesterol in water [72].

From the viewpoint of crystallogeny, in a system of solution, the precondition for solute to be separated out and to form crystal is that the solute in the system must be in the state of super-saturation, which is an unstable state in thermodynamics [73]. Only in this state, can the solute be crystallized [74]. It also causes imbalance between nucleation-leading factors and antinucleation factors and abnormal function of the gallbladder and so on [75].

Excessive cholesterol may be kept in vesicles (i.e. spherical bilayers of cholesterol and phospholipids, without bile salts), provided that enough phospholipid is available. When relatively low amounts of phospholipids are present, cholesterol crystal formation occurs in supersaturated bile, which is the beginning of gallstone formation. Primary bile salts are synthesized from cholesterol in the liver (i.e. cholate and chenodeoxycholate). Secondary bile salts (mainly deoxycholate) are formed from primary bile salts in the intestine by bacterial transformation [76].

Cholesterol crystallization is promoted by hydrophobic bile salts (chenodeoxycholate, deoxycholate)

and by phospholipidswith unsaturated acyl chains. Iron deficiency has been shown to alter the activity of several hepatic enzymes, leading to increased gall bladder bile cholesterol saturation and promotion of cholesterol crystal formation [77].

23.4. Miscellaneous

23.4.1 Sialolithiasis

Sialolithiasis or the formation of sialoliths or salivary stones, typically occurs in the ducts of the submandibular and parotid glands of middle-aged adults. Sialolithiasis is the most common cause of salivary gland obstruction [78]. It can be complete or partial and may show recurrence. Sialolithiasis is the most common disease of salivary glands. It is estimated that it affects 12 in 1000 of the adult population [79]. Males are affected twice as much as females [80]. Children are rarely affected but a review of the literature reveals 100 cases of submandibular calculi in children aged 3 weeks to 15 years old [81]. The retained saliva applies retrograde

pressure on the salivary gland, the chyma and the ductal system. Salivary calculi develop due to pathologic formations of calcareous deposits in salivary ducts or glands, whereby minerals form around an organic matrix. Many theories have been put forward to explain salivary calculi formation, such as calcification around foreign bodies, desquamated epithelial cells and microorganisms in the duct. Although the exact cause of these stones is unknown, some stones may be related to dehydration, which thickens the saliva; decreased food intake, which lowers the demand for saliva; or medications that decrease saliva production, including certain antihistamines, blood pressure drugs and psychiatric medications.

23.4.2 Dental stones

Dental calculus is calcified dental plaque and it is primarily composed of mineral as well as inorganic and organic components. Supragingival and subgingival calculus contain 37% and 58% mineral content by volume, respectively [82]. Dental calculus contains both total phospholipids and acidic phospholipids in much higher concentrations than parotid saliva [83]. In addition, the concentration of phospholipids in the saliva of heavy calculus formers is significantly higher than that of light calculus formers. These findings suggest that phospholipids play an important role in calculus formation [84]. Theoretically, supersaturation of saliva, especially plaque fluid, with respect to calcium phosphate salts is the driving force for dental plaque mineralization. Although calculus can be induced in germ-free animals, human calculus development invariably involves plaque bacterial calcification.

23.4.3 Pancreatic stones

Stone formation in the pancreatic duct system is common in chronic pancreatitis [85]. Plugs formed by the precipitation of the protein within the interlobular and intralobular ducts are one of the earliest findings in chronic pancreatitis and the protein plugs subsequently perpetuate inflammation of the gland through repeated obstruction of the pancreatic duct system. Lactoferrin may play a role in the formation of the protein plugs frequently seen in chronic pancreatitis because of its ability to produce an aggregation of a large acidophilic protein, such as albumin, called Pancreatic stone protein. This protein can play an inhibitor role in the process of calcium carbonate precipitation in pancreatic juice.

23.4.4 Broncholithiasis and Pulmonary alveolar microlithiasis

Broncholithiasis is defined as a condition in which calcified or ossified material is present within the bronchial lumen [86]. A broncholith is usually formed by erosion by and extrusion of a calcified adjacent lymph node into the bronchial lumen. Other causes of broncholithiasis include *(a)* aspiration of bone tissue or in situ calcification of aspirated foreign material; *(b)* erosion by and extrusion of calcified or ossified bronchial cartilage plates; and *(c)* migration to a bronchus of calcified material from a distant site, such as a pleural plaque or the kidney (via a nephrobronchial fistula) [87].

Pulmonary alveolar microlithiasis is a rare idiopathic disease characterized by the diffuse presence in the alveoli of innumerable minute calculi called microliths [88]. This disease can also present in a single area of the lung as a secondary localized disease in the presence of adenocarcinoma, tubercular remnants or pleural mesothelioma [89-91]. The aetiology and pathogenesis are still unknown [92, 93].

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Fig. 1: Plain X-ray (left) and urography (right) showing an ureteral CaOx stone

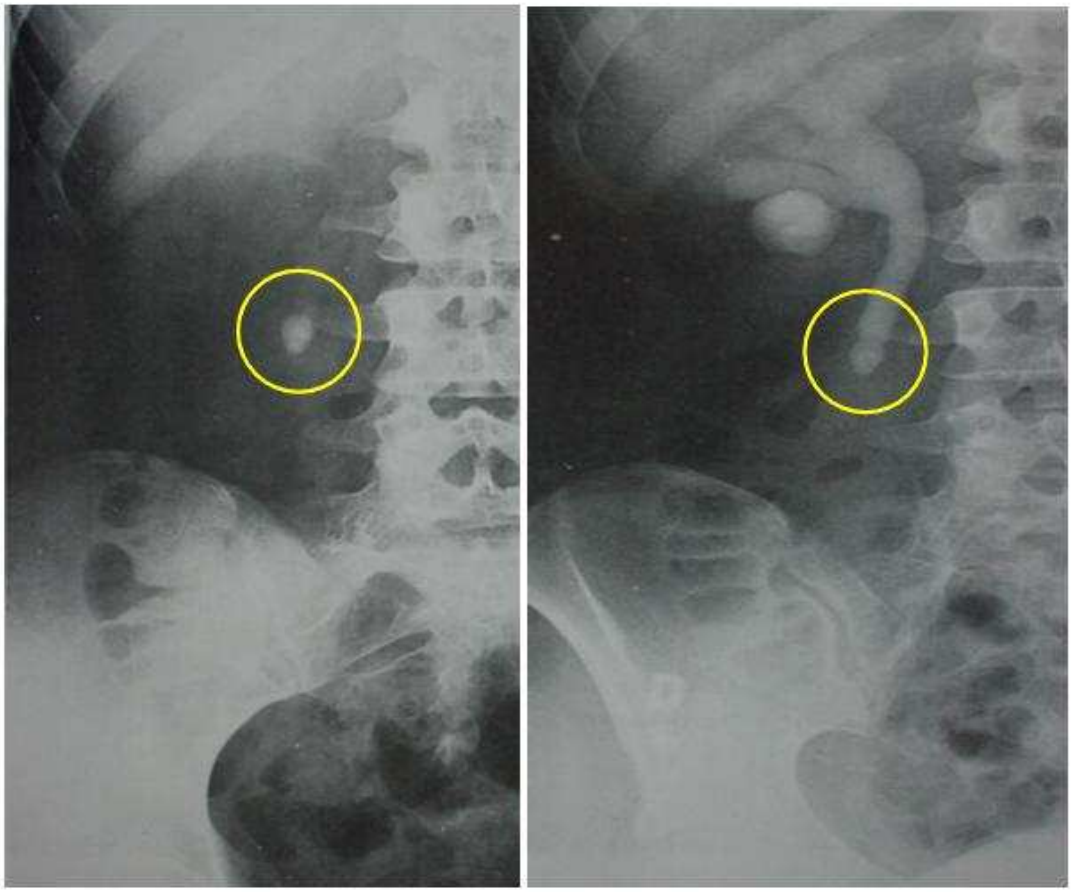
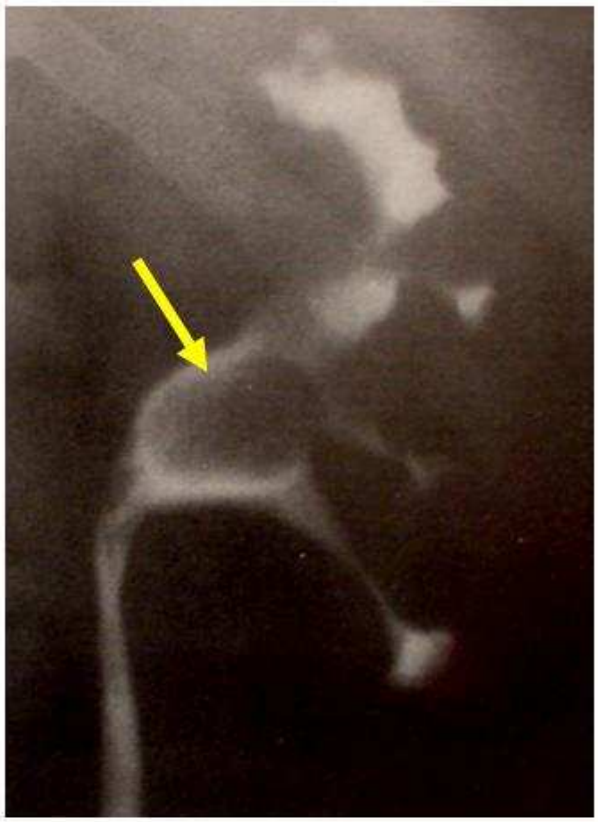


Fig. 2: Urography showing a renal radiolucent uric acid stone



Tab 1: Common Underlying Causes of Calcium- Containing Calculi

- Hypercalciuria (50%)
Idiopathic hypercalciuria (90%)
Primary hyperparathyroidism
High-sodium diet
High-protein diet
Medications (loop diuretics, calcium supplements)
- Hyperoxaluria (10%–20%)
Crohn's disease
Chronic pancreatitis
Celiac sprue
High vitamin C intake (in some patients)
Calcium restriction
Primary hyperoxaluria
- Hypocitraturia (10%–40%)
Idiopathic (90%)
Metabolic acidosis (diarrhea)
Distal renal tubular acidosis
Potassium depletion
- Hyperuricosuria (10%–20%)
Excessive dietary purine intake
- Other
Genitourinary abnormalities (medullary sponge kidney)

Tab. 2: Conditions associated with uric acid nephrolithiasis

1. Congenital conditions

- Disorders causing uric acid overproduction
  - Hypoxanthine guanine phosphoribosyl-transferase deficiency
  - Phosphoribosylpyrophosphate synthetase over-activity
  - Glucose-6-phosphatase deficiency
- Urate transporter defects
  - Congenital hypouricemia with hyperuricosuria

2. Acquired conditions

- Volume depletion
  - Excessive dehydration
  - Chronic diarrhea
- Increased purine states
  - Myelo/lymphoproliferative disorders
  - Other malignancies with or without chemotherapy
  - Hemolytic disorders
  - High animal protein intake
- Uricosuric drugs
  - Probenecid
  - High-dose salicylates
  - Radiocontrast agents

3. Idiopathic

- Gouty diathesis
- Primary gout

Tab. 3: Gene defects connected to stone formation

Increased excretion	Disease/Syndrome	Defect	Gene
Calcium	Familial idiopathic hypercalciuria		
	Familial hypocalcemia with hypercalciuria	Calcium-sensing receptor	3q13.3-21
	Dent's disease	Mutation of CLCN5-gene	Xp 11.22
	X-linked recessive nephrolitiasis type I	Mutation of CLCN5-gene	Xp 11.22
	X-linked-recessive hypophosphatemic rickets (XLRH)	Mutation of CLCN5-gene	Xp 11.22
	Distal renal tubular acidosis	Mutation of RTA-1 gene ?	
	Bartter's syndrome	Na-K-2Cl cotransporter	
	William's syndrome	Delection of the elastingene(ELN)	7q11.23
		calcitonin receptor gene (?)	7q21.3
	Wilson's disease	Copper transporting protein	13p14.1-21.1
Cystine	Cystinuria type I	rBAT / D2H (SLC3A1)	2p21
	Cystinuria type III (type II)		19q13.1
Oxalate	Primary hyperoxaluria type 1	Alanine:glyoxylate-aminotransferase	2q27.3
	Primary hyperoxaluria type 2	Glyoxylate-reductase/ D-Glycerate dehydrogenase	
Uric acid	Lesch-Nyhan syndrome	Hypoxanthine-guanine phosphoribosyltransferase	Xq26-27.2
	Phosphoribosyl-pyrophosphate-synthetase superactivity	Phosphoribosyl-pyrophosphate-synthetase	Xq22-24
	Glycogen-storage disease type 1	Glucose-6-phosphatase	17q21
2,8 Dihydroxy-adenine	Dihydroxyadeninuria	Adenine-phosphoribosyltransferase	16q22.2-22.3
Xanthine	Xanthinuria	Xanthin-oxidase	2p23-22

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