SCANNING ELECTRON MICROSCOPY OF CALCIUM OXALATE CRYSTALS GROWN IN-VITRO ON ADDING URINE SAMPLE OF EXPERIMENTAL RATS

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Abstract - Urolithiasis involves the formation of calcification in the kidneys, ureters and the bladder. Several risk factors are recognized to increase the potential of a susceptible individual to develop stones. Reduced fluid intake may increase the risk of stone formation in addition to increased consumption of sodium, oxalate, fat, protein, sugar, unrefined carbohydrates and vitamin C. It is now understood that obesity and type 2 diabetes mellitus, significantly increase the incidence of urolithiasis. Renal calculi are formed when the urine is supersaturated with salt and minerals such as calcium oxalate, struvite, uric acid and cystine and about 60-80% of stones contain calcium. Crystallogenesis is the first and essential step in stone formation. Crystal growth in the silica gel medium is widely used to evaluate crystallization of urinary crystals. Urine samples of the non-diabetic sodium oxalate induced calculogenic and diabetic sodium oxalate induced calculogenic rats were added separately on the top of the gel in requisite amount. Crystal growth set up was maintained for 30 days. The crystals obtained were studied under different magnification power in the JEOL JSM-6000 LV SEM to understand the morphology of the crystals. The crystals grown on adding the urine samples of the non-diabetic sodium oxalate induced calculogenic rats viewed under x700 were calcium oxalate monohydrate and were seen adherent to each other. Under x7000, stepping of the crystals were observed. The crystals grown on adding the urine samples of the diabetic sodium oxalate induced calculogenic rats viewed under x700 showed clustering and at x7000 interlocking of the crystals in the form of interpenetrant twinning was evident. Predominant number of calcium oxalate monohydrate crystals seen adherent to each other and the stepping of the crystal surface in the crystals of non-diabetic sodium oxalate induced calculogenic rats indicate the tendency for promoting crystal aggregation. Clustering and twinning of the crystals of the diabetic sodium oxalate induced calculogenic rats indicates the tendency for crystallization. The results conclude that diabetic rats with calculogenesis have more tendency for crystallization and aggregation of crystals which can lead to lithiasis.

Key words: Diabetic calculogenic rats, twinning, crystallogenesis, in-vitro

I. INTRODUCTION

Urolithiasis is one of oldest and the most frequent global health problem with high recurrence rate. Epidemiological studies revealed that urolithiasis is more common in men than in women and is more prevalent between the ages of 20 to 40 in both sexes [1]. Uroliths are generally composed of calcium as calcium oxalate monohydrate and calcium hydrogen phosphate dihydrate (75-90%), magnesium as ammonium magnesium phosphate hexahydrate (10-15%), uric acid and urates (3-10%); and 0.5-1% is composed of cystine, hippuric acid, tyrosine and xanthine. Calcium containing uroliths are known as brushite, whewellite, weddellite, whitlockite and carbonate apatite [2]. Urolithiasis is a multistep bio-chemical process. Crystallogenesis is the first and essential step in stone formation which is based on three steps nucleation, growth and aggregation. Crystallisation occurs when the crystal forming materials, such as calcium oxalate, calcium phosphate, uric acid reach their upper meta stable limits, which result in first solid phase precipitations, then grow, aggregate, and finally form stones [3]. Research on the pathogenesis of calcium oxalate stone formation has indicated that the calcium oxalate induced generation of oxidative stress may be the initial trigger of a vicious cycle of urolithiasis formation [4]. Excessive quantities of calcium oxalate crystals could trigger the production of reactive oxygen species in kidney tissues which leads to renal epithelial injury and apoptosis, which provide sites for crystal attachment and eventual retention within the kidneys [5]. In vitro models provide the study of renal stone formation and thus are significantly and effectively used to evaluate prophylactic management. The crystal growth in the silica-hydro gel is used to evaluate crystallization of urinary crystals. This technique provides systematic studies on the growth of urinary crystals and hence provides the information about mechanism of urinary stone formation [6]. Scanning electron microscope is widely used to identify the crystalline structure and orientation of materials making up the sample. It is now understood that obesity and type 2 diabetes mellitus, significantly increase the incidence of urolithiasis [7]. It is established that rise in urine oxalate concentrations elevate the potential for stone formation [8] but recent studies have deemed oxalate and calcium is equally responsible for forming calcium oxalate stones [9]. Oxalate plays an important role in kidney stone formation and hence hyperoxaluria, one of the main risk factors for kidney stone formation should be treated by both dietary oxalate restriction and drugs.
MATERIALS AND METHOD
The experimental work was done in male rats of wistar species each weighing 200-250 gm. Six rats each were included in the group and the experiment was conducted for a period of three months. Diabetes was induced in the experimental group of rats by injecting 3% aqueous solution of alloxan monohydrate in a dose of 150mg/kg body weight prepared by weighing the requisite dose of the drug and dissolving it immediately in distilled water and administering to the rats. Calculogenesis was induced by giving orally ad libitum 0.1% of sodium oxalate to the experimental rats. In-vitro growth of calcium oxalate crystals was done in Hane’s tubes in silica gel medium by single diffusion method. 20 ml of sodium metasilicate solution of density 1.03g/cm$^3$ was taken and the pH was adjusted to 6 using 3M acetic acid. To this, 5ml of 1M calcium chloride was added. The solution was mixed well and set aside overnight for gel formation. Two sets, each consisting of five tubes with gel were arranged for each experiment. Next day, 5ml of 1M oxalic acid was added on top of the gel followed by 5ml of the urine sample of the experimental groups. Crystals appeared as a cloudy precipitate. At the end of the 30th day, the crystals were cleared of the gel by repeated washing with distilled water and filtered through micropore filter paper. Crystals were air dried and kept in clean dry bottles. The dry calcium oxalate crystals were studied under different magnification power of the JEOL JSM-5600 LV SEM to understand the morphology of the crystals.

RESULT
The crystals grown on adding the urine samples of the non-diabetic sodium oxalate induced calculogenic rats viewed under x70 were calcium oxalate monohydrate. The crystals were seen adherent to each other (Fig.1). Under x700, stepping of the crystals were observed (Fig.2).

DISCUSSION
Kidney stone disease is a common chronic disorder seen in humans and the most common type of renal stone is made of calcium oxalate. Crystallisation represents the first phase of urinary stone formation. Kok and Khan using modern computational methods have proposed that aggregation of free crystals can result in urinary microliths large enough to occlude collecting ducts by free particle nucleation [10]. Predominant number of calcium oxalate monohydrate crystals adherent to each other at x70 and stepping of the crystal surface at x70 seen in the crystals of the non-diabetic sodium oxalate induced calculogenic rats indicate the tendency of these rats to promote crystal aggregation. Calcium oxalate stones are made of calcium oxalate monohydrate and calcium oxalate dihydrate crystals. Calcium oxalate monohydrate, the thermodynamically most stable form, is observed more frequently in clinical stones than calcium oxalate dihydrate. Calcium stone formation involves different phases of increasing accumulation of calcium oxalate and calcium phosphate by nucleation, crystal growth, crystal aggregation and crystal retention [11]. The calcium oxalate monohydrate crystals seen clustering at x70 and...
twinning at x700 of the diabetic sodium oxalate induced calculogenic rats indicates the tendency of these diabetic calculogenic rats to promote crystallization. The crystal aggregation and attachment of crystals or aggregates to a nidus such as renal epithelial cells can enhance crystal nucleation which promotes the processes of stone formation.

CONCLUSION

Kidney stone formation is the result of crystal formation, aggregation and retention in the kidney during crystalluria. The results conclude that diabetic rats with calculogenesis have more tendency for crystallization and aggregation of crystals which can provoke stone formation.

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