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Research Article

IN VITRO MICROSCOPIC STUDY OF CALCIUM OXALATE MONOHYDRATE CRYSTALS GROWTH PATTERNS

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ABSTRACT

The purpose of the present study was to explore the possible morphological features and growth patterns of calcium oxalate monohydrate crystals. The study was carried out on a glass slide under microscope. The results showed that the calcium oxalate monohydrate crystals may be present in the form of arborescent, donut, dumbbell, needles, platy, prismatic, rosette, round edges, loose agglomerates and compact aggregates. The study provides detailed information about the morphology and aggregation patterns of calcium oxalate monohydrate crystals.

Keywords: Calcium oxalate monohydrate, crystallization, urolithiasis.

INTRODUCTION

The occurrence of urolithiasis in a particular area and population is typically relies on the topographical conditions, racial conveyance, financial status and dietary propensities. Territories of high humidity and raised temperatures are contributing geographic elements. Men are more frequently affected by urolithiasis than women, with a ratio of 3:1. Protein, sodium and oxalate rich diet, less fluid intake, lazy lifestyle, obesity, high blood pressure and familial occurrence are major clinical and epidemiological risk factors[1].

The risk of developing urolithiasis in adults appears to in the Western world is about 5–9% in Europe, 12% in Canada, 13–15% in the USA whereas in Eastern world is about 1–5%. However, the highest risks have been reported in Saudi Arabia as 20.1%[2]. Urolithiasis is a complex physicochemical process involving urinary super saturation, crystallization and calculogenesis[3]. In super saturation, there is no possibility of mineral or salt dissolution in urine. After super saturation, free ions in solution associate into microscopic particles to behave as nuclei for crystal formation. Epithelial cells, urinary casts, RBCs, and other crystals can act as nucleating centers in urine. These microscopic particles stick together to form larger particles known as aggregates. Movement of ions out of the solution towards growing crystals causes crystal growth. The aggregation of preformed crystals on the matrix coated surface of other crystals also plays an important role in crystal growth[4,5]. As a result of crystallization, freely moving crystals are harmlessly flush out through kidneys known as crystalluria or may remain attached in urinary tract cause urolithiasis and known as sedimentary crystals[6]. Uroliths or urinary calculi causes urinary tract lesions followed by infection and obstruction of the urinary passage leading to acute bacterial pyelonephritis[7,8]. Urinary calculi are composed of inorganic and organic materials. Inorganic phases include oxalate, phosphate and urate salts. Whereas, organic matrix consists of proteins, lipids, polysaccharides and cellular components, e.g., cystine, xanthine, calcium carbonate or hippuric acid serving as binding agent[4,8].

Uroliths are generally composed of calcium (75-90%), magnesium (10-15%), uric acid and urates (3-10%); and 0.5-1% is composed of cystine, hippuric acid, L-tyrosine and xanthine[1,2]. *In vitro* microscopic study of calcium hydrogen phosphate dihydrate[11] and mono sodium urate monohydrate[12] growth patterns have previously been reported by using reagents of single diffusion gel technique. In the present attempt, the authors observed growth pattern of calcium oxalate monohydrate by using reagents of double diffusion gel technique.

MATERIALS AND METHODS

Apparatus and Instruments

Nikon Eclipse E 400 binocular microscope, Japan ; Ricoh CX4 Digital Camera, Japan ; Microscope slides 25.4 x 76.2 (1 " x 3 ") Universal Health Care Products, China ; Whatman filter paper # 02, Whatman International Ltd., England.

Chemicals and Reagents

Acetic acid (glacial) 100 % anhydrous, calcium chloride dihydrate, magnesium acetate tetra hydrate, orthophosphoric acid 85 %, oxalic acid dihydrate and sodium silicate solution were purchased from Merck, Germany.

Method of crystal growth

Earlier reported method was used with some modifications[13]. The different stages of calcium oxalate monohydrate crystal growth were studied under a compound microscope. Crystals were grown on glass slide at 25 ± 2 °C. A drop of gel media (sodium metasilicate of 1.06 specific gravity and 3M acetic acid solution) at pH 5.02-5.17 was placed in the middle of glass slide and allowed to convert into gel. Gel formation occurs in about 5 min. Single drop of 1 M oxalic acid was dropped to the left and 1 M calcium chloride and magnesium acetate (1:1) solution was dropped to the right side of properly formed gel on the glass slide. As a result following reaction occurred:

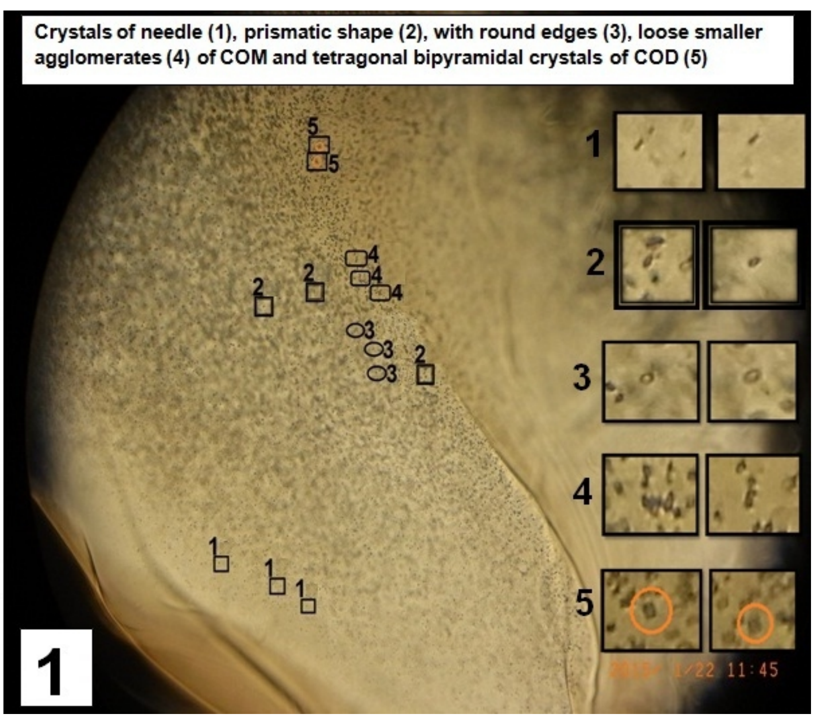
$$CaCl_2 + H_2C_2O_4 \longrightarrow CaC_2O_4 + 2HCl$$

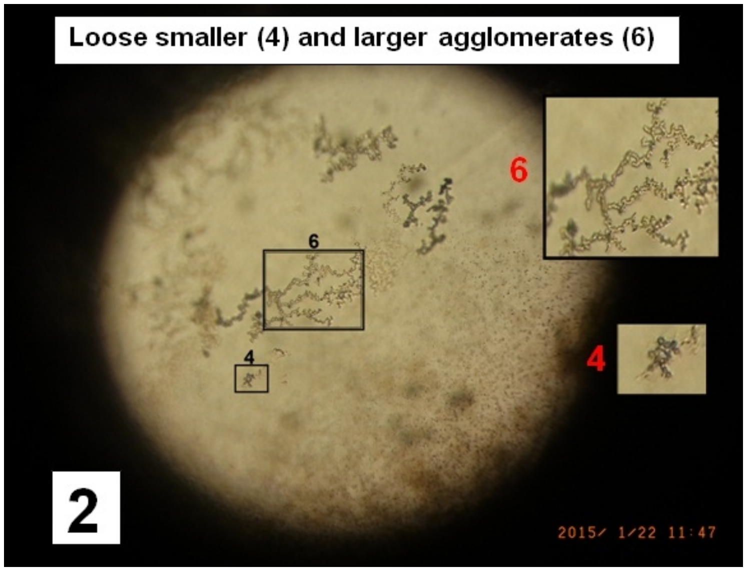
The increasing amount of HCl at growth site dissolves calcium oxalate crystals after a certain period of time and this dissolution was prevented by magnesium acetate solution along with $CaCl_2$. Thus formed crystals can be easily observed[13]. The glass slide was observed under microscope till it was completely dry.

RESULTS AND DISCUSSION

The formation of calcium oxalate monohydrate crystal was observed under microscope at 10x and 100x magnification. The arborescent, donut, dumbbell, needles, platy, prismatic, rosette and round edges crystals of calcium oxalate monohydrate were observed except X- shape crystals. Few tetragonal bipyramidal crystals of calcium oxalate dihydrate were also observed. The crystal-crystal collision results the formation of numerous agglomerates and aggregates which indicates major contribution of COM crystals in kidney stone formation. COM crystals were starting to grow from needles to platy and then to prismatic shape and other different behavior as crystals with rounded edges, donut and rosette before reaching towards equilibrium state. Loose small agglomerates were formed as a consequence of crystal-crystal collision which gradually increase in size and the void spaces of agglomerate fill to change into densely packed aggregates. Agglomerates also possessed composites of platy crystals arranged in the form of flower petals forming rosettes. Arborescent crystals were tree like platy crystals, with well-defined dendritic side branches in one or more direction from the central point. Dendritic crystals were observed for the first time as a growth phase of COM (Photograph-1a & b).

Renal stones can be classified into two groups: calcium oxalate monohydrates are papillary stones usually attached to the papillary wall whereas calcium phosphates are sedimentary stones formed in renal cavities with poor urodynamic efficacy resulting sedimentation. Glycosaminoglycan is the protective layer of inner renal wall preventing the adhesion of calcium phosphate, uric acid and calcified organic matter. In case of damaged glycosaminoglycan, these solid particles adhered to the inner papillary wall and invite the attachment of calcium phosphates, glycoprotein aggregates and cellular debris to serve as nuclei during early stages of COM papillary stone formation[14]. Calcium oxalate monohydrate stones are formed due to excessive oxalate concentration[15]. Oxalates are toxic to renal epithelial cells causing cell membrane injury mediated by lipid and protein peroxidation through oxygen free radical's generation. Thus provide binding sites for calcium oxalate crystals and subsequent growth into renal calculi[16]. Calcium oxalate monohydrate are thermodynamically stable, monoclinic crystals with a *P*21/*n* space group and unit cell parameters of *a* 9.976 Å, *b* 14.588 Å, *c* 6.291 Å, and *β* 107.05°. COM are found with different morphological features as needles, prismatic, platy or platelet shaped, round edges, donut shaped, loose smaller, larger agglomerates and compact aggregates rosettes, prismatic, X-shaped crystals[17-22]. The present study was an attempt to observe the morphology and aggregation patterns of calcium oxalate monohydrate crystals and to develop an *in vitro* model for COM crystal growth.




Loose smaller (4) and larger agglomerates (6)
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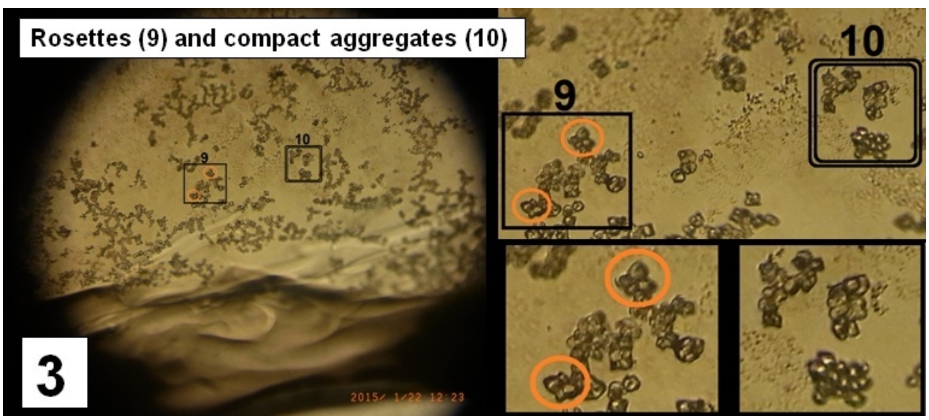
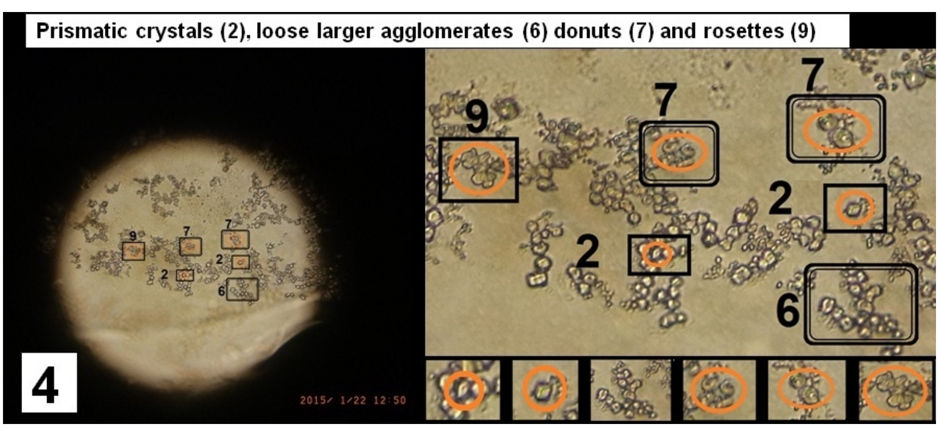

Rosettes (9) and compact aggregates (10)
9
10
9
10
3


Prismatic crystals (2), loose larger agglomerates (6) donuts (7) and rosettes (9)
9
7
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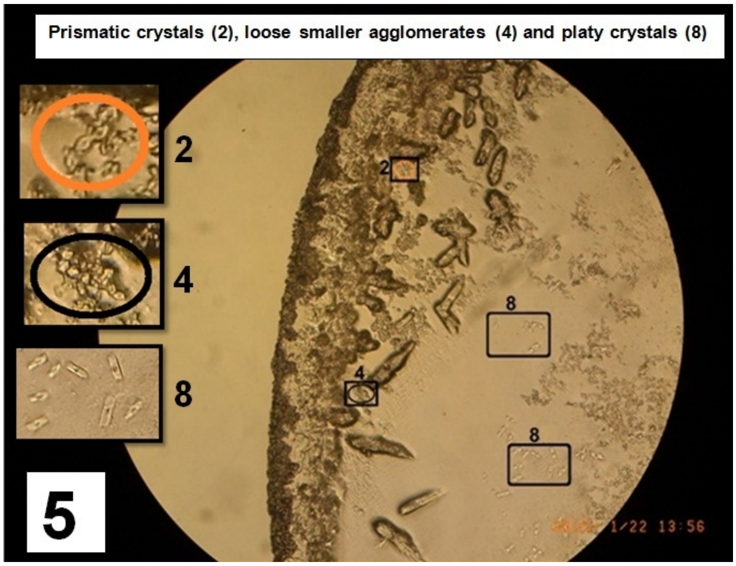
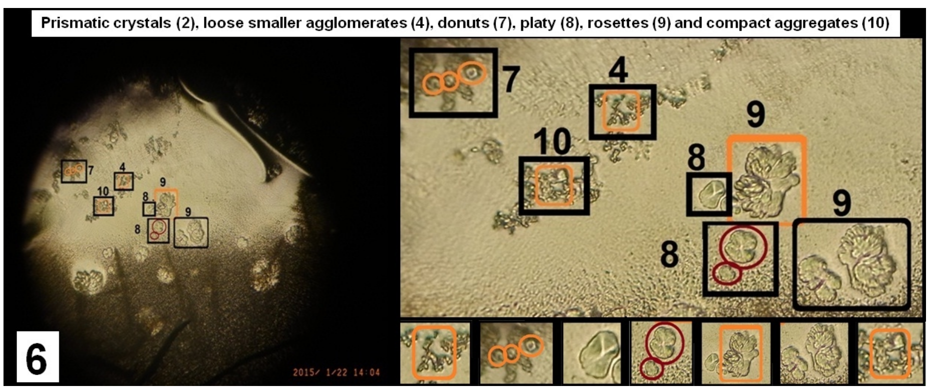
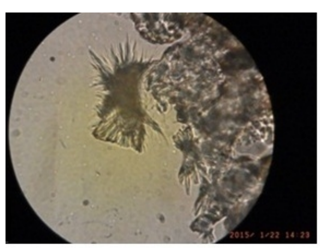


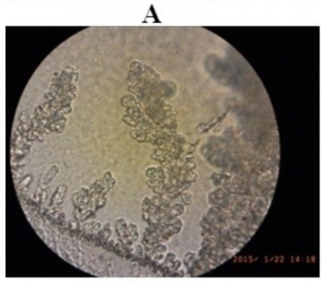


Photograph-1(a): COM crystal formation under compound microscope (at 10x magnification) needles(1), prismatic(2), round edges(3), loose smaller agglomerates(4), tetragonal bipyramidal COD (5); loose larger agglomerates (6), donut (7), platy or platelet shaped(8), flower shape / composite particles(9) and compact aggregates (10).

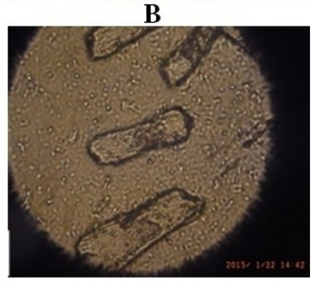


A

B



C



D

Photograph-1(b): COM crystal formation under compound microscope (at 100x magnification); A: Needle aggregates; B: Platy or platelet shape and prismatic; C: Arborescent or dendritic; D: Platy or platelet shape.

CONCLUSION

Calcium oxalate monohydrate crystals may be formed as arborescent , donut, dumbbell, needles, platy, prismatic, rosette and round edges crystals. It was a preliminary but to our knowledge first study of this type which not only provides the growth pattern, but also different morphological characteristics of calcium oxalate monohydrate crystals. Now, the authors are focusing on other scientifically based authentic aspects of the same study.

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