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SCANNING ELECTRON MICROSCOPIC STUDY OF KIDNEYS OF DIABETIC NON-CALCULOGENIC RATS ON DIFFERENT DIETS

Dr. Dhanalekshmy T. G.*

Assistant Professor and H.O.D., Department of Zoology, All Saints' College, Thiruvananthapuram-695007.

***Corresponding Author: Dr. Dhanalekshmy T. G.**

Assistant Professor and H.O.D., Department of Zoology, All Saints' College, Thiruvananthapuram-695007.

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ABSTRACT

Urolithiasis is a painful urinary disorder that results in the excretion of tiny crystals in the urine. Kidney stones contain commonly, calcium in combination with phosphate or oxalate. Diet plays an important role in the pathogenesis of kidney stones as dietary ingredients is understood to influence the solute concentration of urine. The experimental work was done in wistar rats to study the morphology of the crystals seen in the sections of kidney of diabetic non calculogenic rats fed on different diets under Scanning Electron Microscope to understand whether these diets aggravate calculogenesis. Diabetes mellitus was induced in the experimental rats with alloxan monohydrate. Rats were fed on diet low in vitamin A and high in vitamin C for three months. At the end of the experiment, the kidneys were excised and fixed and later studied under different magnification power in the JEOL JSM-5600 LV SEM to understand the morphology of the crystals seen in these tissues. At magnification x170, kidneys of the diabetic non-calculogenic rats on high vitamin C diet showed approximately 3-4 crystals/field. The crystals were polymorphic. At x1300, single large crystals were seen. The kidneys of the diabetic non-calculogenic rats on low vitamin A diet at magnification x200 showed 3-5 crystals per field. Some of the crystals showed the typical shape of calcium monohydrate crystal. At magnification x900, large multifaceted crystals were seen. The significant number of polymorphic crystals and single large crystals seen in the kidneys of rats on high vitamin C diet indicates the tendency for crystal aggregation in these rats. The calcium oxalate monohydrate crystals and large multifaceted crystals seen in the kidneys of the diabetic non-calculogenic rats on low vitamin A diet indicate the propensity to promote crystal aggregation, which can lead to urolithiasis. The present study clearly point that diets high in vitamin C and low in vitamin A in can aggravate the calculogenic propensity in diabetic rats.

KEYWORDS: Diabetic non-calculogenic, Diabetes mellitus, Calcium oxalate monohydrate, Calculogenic propensity, Crystal aggregation, Scanning Electron Microscope.

INTRODUCTION

Urolithiasis, the calcification that forms primarily in the kidney or ureter may also migrate into the lower urinary system and is prevalent in relatively young age groups. There has been a marked increase in the prevalence and incidence of urolithiasis in the USA and worldwide over the past several decades.[1] Affluent societies have a higher rate of small upper tract stones whereas large struvite stones occur more commonly in developing countries. Bladder stones are more common in underserved countries and are likely related to dietary habits and malnutrition[2] The risk factors that contribute to a higher incidence of stone formation are sex, ethnic background, family history, medical history, diet, environment and medications. Diabetes mellitus is considered to be one of the major public health challenges in many Asian countries and in many other industrialized nations.[3] Diabetes mellitus is associated with long-term complications like cardiovascular disease, retinopathy, neuropathy, and nephropathy[4] and it is also known to influence risk of stone formation.[5] Diabetes may be a risk factor for urolithiasis, but findings from epidemiological studies are inconsistent.[6] Diet plays a crucial role in urolithiasis. Diet can contribute to the etiology, management or prevention of recurrence of kidney stone because dietary ingredients and fluid intake influence the volume, pH and solute concentration of urine. Dietary intake that is high in sodium, oxalate, fat, protein, sugar, unrefined carbohydrates, and ascorbic acid (vitamin C) and fluid intake consisting of drinking water high in minerals has been linked to stone formation.[7] Experimental studies in male rats have shown that a vitamin A-deficient diet caused important changes in the composition of the urine and prolonged vitamin A deficiency leads to the formation of renal calculi.[8] The dietary components influence the biochemical parameters such as oxalate, uric acid, calcium and sodium.[9] The main urinary risk factors are low urine volume, low pH, hypercalciuria, hyperoxaluria, hyperuricosuria and reduced urinary inhibitors for stone formation. A mild degree of hyperoxaluria may be much more important than

hypercalciuria in the genesis of calcium oxalate stones. Oxalate is considered a major risk factor in renal stone disease because of its poor solubility and possible toxicity. The idea that ionized oxalate is harmful to kidney cells was derived from studies that were performed in animals[10] and cell culture.[11] Evidences suggests that the assumed oxalate-induced cell injury in fact often is caused by crystals. [12] Approximately 80% of urinary oxalate is synthesized within the liver and a small percentage (20%) from dietary intake. Overindulgence of diets rich in oxalates can contribute to hyperoxaluria through intestinal absorption of oxalate. This includes foods such as rhubarb, green leafy vegetables, spinach, cocoa, beer, coffee or tea, or excess ascorbic acid (vitamin C) intake.[7] Low urinary citrate, the inhibitor causes the urinary environment to become supersaturated with calcium salts, promoting nucleation, growth, and aggregation, resulting in stone formation. Renal calculi are formed when the urine is supersaturated with salt and minerals such as calcium oxalate, struvite, uric acid and cystine.[13] Effective kidney stone prevention can be done by identifying risk factors for stone formation and incorporating dietary changes.

MATERIALS AND METHODS

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. The urine and blood samples of these rats were collected and the presence of sugar was noted. Experimental rats were fed on diet low in vitamin A and high in vitamin C diet and water ad libitum. The low vitamin A diet was prepared for the experimental rats by mixing 5 kilogram of ground raw rice with one kilogram of casein and 25 milliliter of kidicare syrup and ten numbers of powdered B complex tablets, the food stuffs known to be deficient in vitamin A. The high vitamin C diet was prepared by mixing 5 kilogram of rat feed with 10 grams of vitamin C. This low vitamin A diet and high vitamin C diet was given to the experimental rats for three months to see whether these diets aggravates the calculogenic propensity. At the end of the experiment kidneys of the rats were collected and fixed in 10% formalin for scanning electron microscopic study.

RESULTS

The kidneys of the experimental rats namely diabetic non-calculogenic rats on low vitamin A diet and diabetic non-calculogenic rats on high vitamin C diet were studied under different magnification power of scanning electron microscope to understand the morphology of the crystals seen in these tissues.

The kidneys of the diabetic non-calculogenic rats on high vitamin C diet showed significant number of crystals of different shapes. Under magnification x170 approximately 3-4 crystals per field were seen. The crystals were polymorphic (Figure.1). At magnification x1300 single large crystals were seen (Figure.2). The crystals measured 8.22 x 6.41µm.

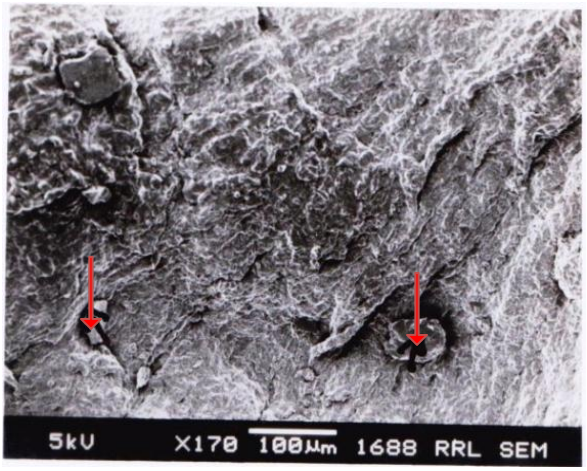

Figure 1. (x170)

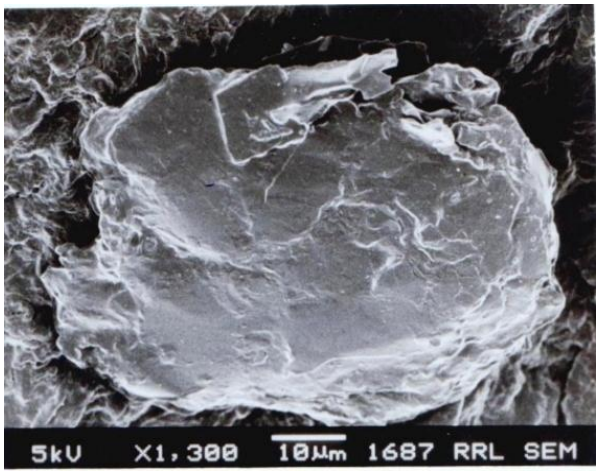

Figure 2. (x1300)

Scanning Electron Microscopic view of the kidney of diabetic non-calculogenic rats on high vitamin C diet

The kidneys of diabetic non-calculogenic rats on low vitamin A diet under magnification x200 showed approximately 3-5 crystals per field. Some of the crystals showed the typical shape of calcium oxalate monohydrate crystal (Figure.3). Most of the crystals did not show definite shape. Under magnification x900, single large crystals were seen. The crystals were multifaceted and were partly broken (Figure.4). The crystals measured 136 x 96 µm.

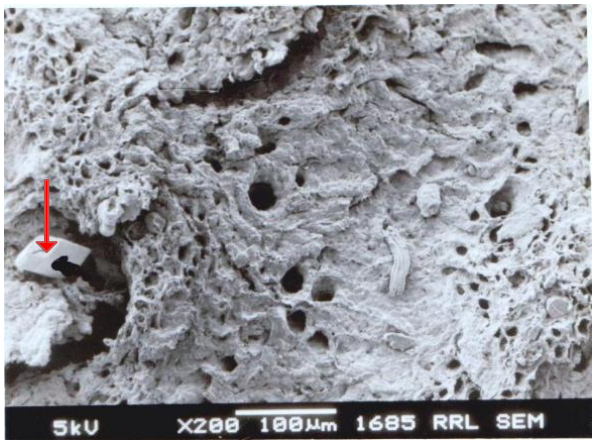

Figure 3. (x200)

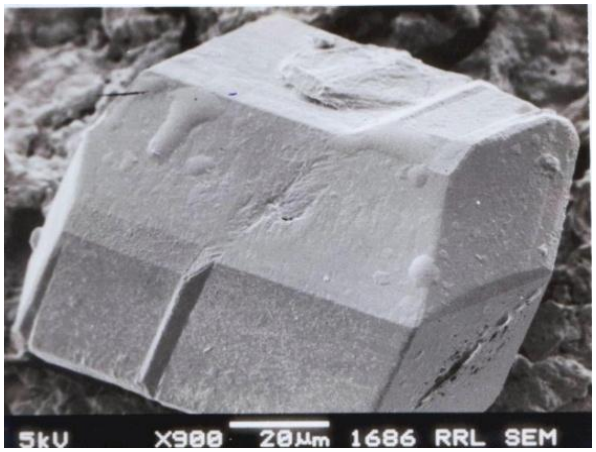

Figure 4. (x900)

Scanning Electron Microscopic view of the kidney of diabetic non-calculogenic rats on low vitamin A diet

DISCUSSION

Nephrolithiasis can be defined as the result of formation and retention of crystals within the kidneys[14] where during crystalluria, stone formation mainly seems to occur by crystal aggregation.[15] The kidneys of the diabetic non-calculogenic rats on high vitamin C diet showed significant number of polymorphic crystals and single large crystals. These single large crystals along with irregularly shaped small crystals can cause cell injury and also promote the tendency for crystal aggregation. The principle that crystal retention in the kidney leads to renal calcification is indisputable. Randall developed already in 1937 the theory that calcium stones can start by calcium oxalate growth initially fixed on papillary calcifications which are called Randall's plaques. [16] This mechanism allows crystal aggregates to grow to stones of a critical size where they cannot anymore be washed out of the urinary tract by the urine flow. Studies usually are performed with calcium oxalate monohydrate because this is the most common crystalline phase of kidney stones.[17] Significant number of crystals with typical shape of calcium oxalate monohydrate and large multifaceted crystals in the kidneys of diabetic non-calculogenic rats on low vitamin A diet indicates the propensity to promote crystal aggregation.

CONCLUSION

Significant number of polymorphic crystals and single large crystals in the kidneys of the diabetic non-calculogenic rats on high vitamin C diet indicate the tendency for crystal aggregation in these rats. The significant number of crystals with typical shape of calcium oxalate monohydrate crystal and large multifaceted crystals in the kidneys of diabetic non-calculogenic rats on low vitamin A diet indicates the propensity to promote crystal aggregation, which can lead to urolithiasis. It is now understood that dietary modifications of oxalate, animal protein, and minerals may play an important role in decreasing the calcium oxalate saturation and in long term they can have an important role in the management of stone disease.

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