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A Study on the Status of Antioxidant Vitamins (Vitamin-A and Vitamin-E) in Urolithiasis Patients

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Original Research Article

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Abstract: Aetiology of urinary stone disease is a function of multiple factors. Besides physicochemical aspects of crystal aggregation and stone formation, studies on the role of oxidative stress in urolithiasis are also getting attention in recent years. The objective of the present study is to trace the status of antioxidant vitamins viz., vitamin-A and vitamin-E, in urinary stone disease. Twenty five urolithiasis patients, 20 male and 5 females in the age group of 20 to 55 years, were selected for study. Twenty normal healthy persons, 14 male and 6 females in the age group of 20 to 55 years were also selected for study as controls. Serum retinol (vitamin-A), and serum α-tocopherol (vitamin-E) levels of subjects (patients) and controls were estimated. Results revealed that the levels of vitamin-A and vitamin-E in the stone patients were significantly lower ($p < 0.03$ for vitamin-A & $p < 0.006$ for vitamin-E) than that of the normal healthy persons. As such, urinary stone disease seems to be associated with a low antioxidant vitamins' status. This might be due to their (vitamins') increased utilisation to fight the oxidative stress created by the injury of renal tubular cells, which might have been induced by the crystals of stone.
Keywords: Urolithiasis, Nephrolithiasis, Urinary stone disease, Vitamin-A, Vitamin-E, Antioxidant vitamins, Oxidative stress.

INTRODUCTION

Important events in urinary stone formation are, the formation of crystals, and their retention in the renal tubules [1]. The retention of crystals in the renal tubules to grow, over the time, into a stone is rather a more important event in urolithiasis [2].

Thus, there may be a role of renal epithelium in the genesis of urinary stone disease. The most important and stubborn crystal constituent of urinary stones is calcium oxalate. A number of studies have shown that exposure to high levels of oxalate and/or calcium oxalate produces cellular injury at the level of the proximal renal tubular cells [1]. It has even been demonstrated that the cellular injury produced by oxalate crystals is a predisposing factor to calcium oxalate stone formation [3]. As a result of membrane damage of renal tubular cells, the urinary content of lipids increases [4]. The urinary lipids would also serve as a good nucleating matrix for the deposition and growth of calcium oxalate crystals from a metastable solution [5]. It has also been proposed that the exposure of renal epithelial cells to oxalate produces increased DNA synthesis, altered gene expression and apoptosis through the activation of p38 mitogen-activated protein kinases [6].

Thus, the newer strategies in urolithiasis research would be to cope up with the oxidative stress and renal cell membrane damage, caused by the free radicals. Vitamin-A, vitamin-E and vitamin-C are the chain breaking antioxidants. They can intercept free radical induced chain reactions and prevent further oxidation. α-tocopherol (vitamin-E) is a naturally occurring lipid soluble antioxidant. It is an important vitamin, implicated with a protective role in many diseases owing to its free radical scavenging action. In fact, vitamin-E and selenium has been found, in-vitro, to prevent the lipid peroxidation of renal proximal tubular cells [7, 8]. The major function of vitamin-E is as an antioxidant for unsaturated fatty acyl moieties of lipids within membranes. In the mammal, the decreases in liver microsomal drug hydroxylation and the increases in net synthesis of xanthine oxidase have been found in vitamin-E deficient animals [9]. Along with superoxide dismutase, in response to the damaging peroxidative effect, α-tocopherol (vitamin–E) has proved to be an efficient protector to the membrane integrity [10].This protective role of α-tocopherol is synergistic with ascorbic acid to ameliorate the peroxidatve damage [11]. Vitamin-A and vitamin-C are also antioxidant vitamins. The antioxidant properties of the carotenoids are thought to prevent the development of diseases, such as cancer and cardiovascular diseases, in which the action of free radicals is implicated. The

role of vitamin-C in urolithiasis, however, is controversial. There have been speculations that low ascorbate levels observed in urolithiasis patients is probably due to its conversion into oxalate, which in turn, induces free radical generation, thereby causing renal stones [12,13]. Thus, on the whole, the level of antioxidant defence of the body assumes importance in the extent of oxidative damage to the renal cells in urolithiasis.

With the above views in mind, we have presently studied the serum retinol (vitamin-A) and serum α-tocopherol (vitamin-E) levels of some urolithiasis patients, and also of some normal healthy persons. Our aim is to probe the role of these antioxidant vitamins in affording protection from crystal/stone induced oxidative stress in urinary stone disease.

MATERIALS AND METHODS

Selection of subjects for study

Twenty five urolithiasis patients, 20 males and 5 females, in the age group of 20 to 55 years, were selected for study. The stone patients were selected at random from among those attending the local clinics and were confirmed for suffering from urolithiasis, as diagnosed by ultrasonography or X-ray. Care was taken to see that the selected patients were not suffering from any other additional disease.

Twenty normal healthy persons, 14 male and 6 females, in the age group of 20 to 55 years, were also selected for study as controls. Care was taken to see that the normal healthy persons, selected for study, were free from the habit of smoking, alcoholism or any such disease that would lead to oxidative stress. All subjects (stone patients and healthy persons) were in the dietary habit of occasional non-vegetarian food.

Collection of blood samples and estimation of serum retinol and α-tocopherol levels:

Informed consent was taken from all the subjects (stones patients and controls) before taking their blood samples. Blood was collected with the help of a pathologist. Five ml of venous blood was collected in a plain bulb, taking all necessary precautions. The separated serum was used for the estimation of retinol and α-tocopherol. The liquid chromatography tandem mass spectrometry (LC-MS/MS) method was used for the estimations. All the precautions, required for the estimations, were taken. The value of serum retinol levels and serum α-tocopherol levels were compared separately between the stone formers and the controls. The data were expressed as mean ± standard deviation. Statistical analysis of the data was performed by student's t-test.

RESULTS

The results obtained in the present work are recorded in Table-1. The mean serum retinol and α-tocopherol levels of urolithiasis patients were found to be significantly lower ($p<0.03$ for retinol and $p<0.006$ for α-tocopherol) than that of the normal healthy persons (controls).

Table-1: Serum retinol (vitamin–A) and serum α-tocopherol (vitamin-E) levels of urolithiasis patients and normal healthy persons

Parameter	Normal healthy persons. (n=20)	Urolithiasis patients. (n=25)	p-significance
Retinol (μg/dL)	66.5±20.5	47.6±11.8	significant ($p<0.03$)
α-tocopherol (mg/dL)	1.06±0.33	0.61±0.24	significant ($p<0.006$)

The data are expressed as mean ± S.D. Statistical analysis was performed by student's t-test.

DISCUSSION

Studies on oxidative stress in urolithiasis disease and the related renal cellular damage have been receiving attention in recent years. Level of antioxidant defence system of the body to cope-up with the oxidative stress, created by the crystals/stones, has also been forming an interesting/important subject of study. The most stubborn stone forming mineral, calcium oxalate increases the availability of free radicals by inhibiting the enzymes responsible for their degradation. The reactive species can damage the mitochondrial membranes and produce a decrease in mitochondrial trans-membrane potential. Reactive oxygen species also promote cell membrane damage by unmasking the additional crystal binding sites. Attached crystals at these sites form centres for nucleation of new crystals, favouring stone development. Crystals up-taken by endocytosis exacerbate cell damage. Alternatively the crystals may dissolve within lysosomes or re-emerge at the basolateral surface, again providing centres for stone growth in the renal interstitium. Cell death produced by oxalate exposure may leave cellular debris that forms a nidus for additional crystal growth, also promoting stone formation [14]. Antioxidant defence of the body such as superoxide dismutase, antioxidant vitamins are of utmost importance to cope-up with the reactive oxygen species and afford protection from renal cellular damage. Enhanced free radical stress in urolithiasis patients, indicated by an increased serum malondialdehyde, a decreased erythrocyte superoxide dismutase and a low serum antioxidant vitamins' levels, has been observed in some studies [15, 16]. However, more studies are required for unequivocal conclusions.

Presently, we have found (Table-1) the mean serum retinol level of urolithiasis patients at 47.6±11.8 µg/dL which was significantly low ($p<0.03$) as compared to that (66.5±20.5 µg/dL) of the normal healthy persons. Mean serum α-tocopherol level of urolithiasis patients was found to be at 0.61±0.24 mg/dL. This was also found to be significantly lower ($p<0.006$) than that (1.06±0.33 mg/dL.) of the controls.

Thus, it is clear that both the antioxidant vitamins (vitamin-A and vitamin–E) are in significantly lower values in urolithiasis patients. A decrease in these (vitamin level) values might have been due to their increased participation in antioxidant activity, in order to counteract the free radicals and thus, overcome the oxidative stress being created by the oxalate/calcium oxalate of the stone.

Normal serum level (reference interval) of vitamin–A for the adults is 30 to 80 µg/dL [17]. All of our present subjects and controls were adults. A study of Table-1 shows that the mean serum vitamin-A level of normal healthy persons (controls) was found near the upper value of the reference Interval. The patients, however, were found to have mean vitamin –A levels much towards the lower limit of the normal value for that age group. Similarly, the normal value (reference interval) of serum α-tocopherol (vitamin–E) is 0.5 to 1.8 mg/dL for adults [17]. Once again, it is observed (Table-1) that the controls have mean serum vitamin-E levels near to the upper limit of the normal values, but that of patients was almost touching the lower limit of the reference interval. In fact, some of the patients had a very low vitamin-E level, which was much below the normal value. Almost all of our study subjects (patients as well as controls) belong to a middle income group. Their socioeconomic status and food habits are mostly the same. In fact, some of the controls were even related to the patients and living in the same house and consuming the same food. Their nutritional intake, quite expectedly, would almost be similar. Despite this, a low vitamin-A and vitamin-E levels in the patients, indicate to the fact that these low values are somehow related to the incidence of urolithiasis in them.

Thus, it is seen that the urinary stone disease leads to depletion in antioxidant vitamin levels, probably as a result of oxidative stress.

CONCLUSION

Our present studies suggest that the level of oxidative stress in urolithiasis patients, particular in the chronic and recurrent stone formers, should be assessed. The antioxidant vitamin levels, particularly vitamin-A and vitamin-E levels should be assessed in the patients. If need be, the physicians/surgeons may consider supplementation of these antioxidant vitamins, particularly vitamin-E, as a strategy towards the medical management of the oxidative stress and related renal cell injury, in the disease. The supplementation should, however, be carefully planned so as to avoid hypervitaminosis.

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