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DEPLETED NITRITE AND ENHANCED OXIDATIVE STRESS IN UROLITHIASIS

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ABSTRACT

Crystal aggregation and retention are critical events for the formation of kidney stones. There is a close association between crystal development and free radical activity in vivo. In the present study 30 subjects presenting with urolithiasis were included. Serum levels of total lipid peroxides, nitric oxide (as nitrite), α-tocopherol, plasma ascorbic acid (vitamin C) and erythrocyte superoxide dismutase activity were measured. These findings were compared with 30 age matched control subjects irrespective of sex. Student's 't' test was applied for statistical analysis. There was a significant increase in lipid peroxides ($p<0.001$), where as significant decrease in nitrite ($p<0.01$) and α-tocopherol ($p<0.001$) levels were observed. Plasma ascorbate ($p>0.05$) and erythrocyte superoxide dismutase activity ($p>0.05$) was also found to be decreased but the difference was not statistically significant which suggests that oxidative stress is evident in urolithiasis with depletion in antioxidant status where as decrease in nitric oxide may be less abetting in disease condition.

KEY WORDS

Urolithiasis, Lipid Peroxidation, Nitric Oxide, Antioxidants.

INTRODUCTION

Urolithiasis is one of the most common disease of the urinary tract which has been afflicting human kind since antiquity. Urolith formation is a multifactorial process (1). For most human diseases, increased formation of reactive oxygen species is secondary to primary disease process (2). Similarly association of urolithiasis and free radicals has been reported (3). Experiments performed on animals (3,4), cultures (5) and human sera (6) have revealed that there is presence of enhanced oxidative stress in stone forming conditions. Oxalate is known to induce lipid peroxidation by unknown mechanism which causes disruption of the structural integrity of the membranes (3,7). Superoxide dismutase (SOD) is an inbuilt defense mechanism to fight back peroxidative stress. Along with SOD in response to the damaging peroxidative effect, α-tocopherol has proved to be an efficient protector to the membrane integrity (8). This positive role of α-tocopherol is synergistic with ascorbic acid to ameliorate the same (9).

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A combined study relating peroxidative stress, antioxidant capacity and the entity of nitric oxide in stone forming conditions in humans has not been cited yet. Therefore in light of the above concepts the present study was planned to quantitate the levels of serum malondialdehyde, nitrite, α-tocopherol, plasma ascorbate and erythrocyte superoxide dismutase and also to investigate their possible bearings in pathogenesis of urolithiasis.

MATERIALS AND METHODS

The present study was carried out in the Department of Biochemistry, Dr. V. M. Government Medical College, Shree Chattrapati Shivaji Maharaj General Hospital and Solapur Kidney Care Center, Solapur, which included a total of 60 subjects. These subjects were divided into two groups. Group I included control subjects and Group II included patients with urolithiasis (stone formers).

The study group (group II) for this project included 30 stone forming patients having obstruction at the ureteropelvic junction and/or vesico-ureteric junction between the age group of 22 – 60 years irrespective of sex. Out of these, 8 subjects were recurrent stone formers and the rest had first episode of stone formation. The presence of stone was diagnosed and confirmed by the urologist with the help of either ultra-

sonography, KUB, IVP or spiral CT report depending upon the size, shape, position, radiolucence and radiopacity of the stone. 30 age matched healthy subjects were selected as controls (Group I). These subjects were selected after careful screening for any history of diseases which may lead to increased oxidative stress such as diabetes mellitus, cardiovascular diseases, infectious diseases, inflammatory diseases etc. and were strictly excluded. None of the subjects from Group I and Group II were on vitamin supplementation or used medications that could alter the study parameters.

Five ml of venous blood was collected. 4ml of which was collected in a plain bulb and the separated serum used for the estimation of malondialdehyde as an index of lipid peroxidation (10), nitrite to assess formation of nitric oxide (11) and α-tocopherol (12). Remaining one ml blood was collected in a heparinized bulb for the assessment of plasma ascorbic acid (13) and erythrocyte superoxide dismutase (14).

The values are expressed as mean ± SD for patients and controls separately. Students 't' test was done for the comparison of data.

RESULTS AND DISCUSSION

Results of the study have been depicted as mean ± SD in Table 1.

Table 1
Serum MDA, α-tocopherol, nitrite, plasma ascorbate and erythrocyte SOD in different Groups

Parameters	Group I	Group II
Serum MDA (nmol/ml)	1.25 ± 0.35	3.61 ± 0.53 *
Erythrocyte SOD (Units/mg Hb)	2.82 ± 0.30	2.63 ± 0.41
Serum α-tocopherol (mg/L)	14.59 ± 2.38	10.21 ± 0.91 *
Plasma Ascorbate (mg/dl)	1.16 ± 0.15	1.11 ± 0.19
Serum Nitrite (mmol/L)	47.55 ± 6.51	41.29 ± 8.32 **

Statistical comparison was done between Group I and Group II:
* $p < 0.001$; ** $p < 0.01$; rest not significant.

There was a significant elevation in the levels of serum malondialdehyde (MDA) ($p<0.001$) in patients as compared to controls. Further, we did not observe much variation in MDA levels between patients with recurrent stone formation and first episode of stone formation. These results are coherent with previous workers (6,15). It has been reported that there is no significant difference between MDA levels of recurrent stone formers and first episode stone formers (15). In urolithiasis oxalate has been reported to induce lipid peroxidation. Both in vivo and in vitro studies have revealed that the mechanism of induction of LPO by oxalate may be involved through the inhibition of catalase activity (4,16) and this may be the reason for elevated level of LPO in study group. It has been reported that the conditions which enhance peroxidation and depletion of thiol content increase the oxalate binding activity, which in turn promotes nucleation and aggregation property of stone matrix protein fractions. This behavior is also associated with peroxidized mitochondria and nuclei, suggesting that the peroxidation can be a causative factor for the initial stage of stone formation (17). Furthermore depletion in antioxidants either enzymatic or nutritional, shall add up to the progression of lipid peroxidation (18).

A non-significant decrease ($p>0.05$) as also stated by other workers (15,19,20) in the level of erythrocytic superoxide dismutase was observed in the present study. However, some authors have observed a significant decrease in SOD level hypothesizing that SOD must be over stretching itself to dismutate $O^{\cdot}_2$ to H_2O_2 (6). Superoxide dismutase is the only antioxidant enzyme which effectively dismutates superoxide radical ($O^{\cdot}_2$) and retards the impact of free radical damage (18). Along with antioxidant enzymes, the nutritional antioxidants (such as α-tocopherol and ascorbate) also play a pivotal role in scavenging free radicals. In corroboration with above concepts a significant decrease in the level of α-tocopherol was observed in patients with urinary stones ($p<0.001$) which is in agreement with other results (6,20). Significantly lower level of α-tocopherol is indicative of restricted antioxidant function and enhanced lipid peroxidative action resulting in probable damage to the renal tubular cells. This type of damage causes increased crystal adherence and may promote aggregation of stone. α-tocopherol is a major lipid soluble chain-breaking antioxidant. In addition, it has also demonstrated positive effect not only in restoring antioxidant status but also in preventing crystal deposition during oxalate challenge (5,16).

The previous process also leads to the formation of tocopherol radical. The regeneration of α-tocopherol from tocopherol radical involves synergistic reaction between α-tocopherol and ascorbate. This recycling reaction leads to the formation of dehydroascorbate which is further reduced to ascorbate by a non-enzymatic reaction with reduced glutathione. Depletion in ascorbate level is expected in the process of regeneration of α-tocopherol (18). However, a non-significant decrease ($p>0.05$) in plasma ascorbate level was

observed in the present study which is in accordance with other authors (20).

There was a significant decrease in nitric oxide measured in terms of serum nitrite ($p<0.01$) as compared to that of their control counterparts. The diminution of nitric oxide may find an answer with regard to the activity of osteopontin (OPN). Osteopontin being impressively multifunctional molecule has been documented to down regulate the expression of nitric oxide synthase (NOS) (21). Reports suggest that osteopontin directs calcium oxalate crystallization to the calcium oxalate dihydrate phase, which is significantly less adherent to the renal tubular epithelial cells than the calcium oxalate monohydrate phase (22). Recent studies report that osteopontin expression in the kidneys and a concomitant increase in its urinary excretion is significantly increased after deposition of calcium oxalate crystals in the kidneys (23). In relation to the action of osteopontin with nitric oxide Hwang et al. have observed that on exposure to reactive oxygen species (ROS) such as H_2O_2, NOS-mRNA level decreases simultaneously with an increase in OPN-mRNA level (21) which may be a possible reason for decrease in nitric oxide in stone forming conditions that shall require further confirmation.

Therefore in light of above discussion it may be concluded that, oxidative stress is functional in urolithiasis as evident from increased lipid peroxidation and decreased antioxidant status where as, decreased nitric oxide may be less abetting in stone forming conditions. However, exact biochemical interplay among these molecules in the disease process needs further elucidations.

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