

Oxidative stress and antioxidant vitamins status in patients with Urolithiasis from North Eastern Part of India

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Abstract

There is high prevalence of urolithiasis in the natives of the North Eastern part of India and required urgent attention. The present study was conducted with an aim to assess the state of oxidative stress and antioxidant vitamins status in patients with urolithiasis from North Eastern Region of India as there is no clinical data to date in the patients with urolithiasis from this region of India. In the present study 50 subjects diagnosed with urolithiasis were included after obtaining informed consent. Plasma levels of lipid peroxidation (LPO), vitamin-E (α -tocopherol) and vitamin-C (ascorbic acid), erythrocyte superoxide dismutase (SOD) and catalase (CAT) activities were measured. These findings were compared with 40 age matched control subjects irrespective of sex. A significant increased in LPO products as indicated by malonylaldehyde (MDA) level ($p < 0.001$), and significant reduction in SOD and CAT activities ($p < 0.001$) was observed in the patients subjects as compared to control. Whereas non significant decreased ($p > 0.05$) in the plasma vitamin-E and vitamin-C were also recorded in patients than those of the control. The result of the present study suggests that oxidative stress is an evident and bearings in pathogenesis of urolithiasis with depletion in antioxidant status.

Key words : Urolithiasis; Oxidative stress; Antioxidant Enzymes; Antioxidant Vitamins.

INTRODUCTION

Urolithiasis is the formation of stones in the kidney, bladder and/or urethra and is considered to be one of the most common urological disorders and multifactorial recurrent disease and has afflicted human since time immemorial. These stones may be persisted for long time leading to secondary complications causing serious consequences to the patient's life^[1]. For most human diseases, oxidative stress characterized by increased formation of reactive oxygen species and a state of damage caused by reactive oxygen species (ROS) is considered secondary to primary disease process^[2]. Similarly, an association of enhanced oxidative stress and stone forming conditions have been reported both in animals^[3] and human studies^[4]. One of the consequences of this is manifested in the formation of lipid peroxides in cell membranes, resulting in dysfunction of the same. There are studies in experimental animals which reported induction of lipid peroxidation and acute phase proinflammatory cytokines (IL-1, IL-6, and TNF- α) by oxalate and oxalate load which causes disruption of the structural integrity of the membranes^[5] and in which free radical plays a prominent role. Given the higher reactivity of the ROS, living things have been developed several efficient mechanisms that enable stabilization and disposal of them to mitigate their harmful effects^[6]. Among them are inbuilt antioxidant scavenging enzymes such as SOD, CAT and small molecular antioxidants such as reduced glutathione (GSH)^[7].

Along inbuilt antioxidant enzymes, the synergistic effect of α -tocopherol and ascorbic acid has proved to be an efficient protector to the membrane integrity in response to the damaging peroxidative effect^[8]. Such compounds can intercept free radical included chain reaction and prevent further oxidation. A combined study relating peroxidative stress and antioxidant capacity in stone forming conditions in humans were very few and needed to

quantitate their level in order to investigate their possible bearings in pathogenesis of urolithiasis. While oxidative stress is a well-known mechanism of action in the genesis of cell injury in different pathologies^[9] in the context of urolithiasis, most of the study supporting this hypothesis is experimental, with few clinical data available.

Mizoram, a state in the North-East corner of India can be said to fall in the broad belt area of stone disease covering south-east, middle-east, north-east Asia and facing an acute problem of this disease. Due to lack of research facilities, the remoteness, difficult geographical situations, the prevalence of urolithiasis are virtually unknown outside of the Mizoram. A preliminary survey from the laboratory highlighted the fact that urolithiasis is a major problem in this region and required urgent attention. It is commonly held that almost every family has a member afflicted with this disease. In view of the high prevalence of the urolithiasis and no clinical data to date from this region has prompted us to investigate for the first time oxidative stress and antioxidant vitamins status in the patients with urolithiasis with an aim to investigate their possible bearings in pathogenesis of urolithiasis in this living population. The result of the study is reported in the present paper.

MATERIALS AND METHODS

The present study was conducted in the Department of Zoology, Pachhunga University College, Aizawl, Mizoram in collaboration with Government Civil Hospital, Mizoram during the period of January 2014 to September, 2015 which included a total of 90 subjects after obtaining informed consent. These subjects were divided into two groups. Group I included control subjects and Group II included patients with urolithiasis (stone formers). The group II of this study included 50 stone forming patients having obstruction at the ureteropelvic junction and/or

vesico-ureteric junction between the age group of 22 - 62 years irrespective of sex. The presence of stone was diagnosed and confirmed by the urologist with the help of either ultrasonography or having radio-opaque stone demonstrable on abdominal roentgenograms who were admitted at urology ward at Government Civil Hospital, Aizawl, Mizoram, India. 40 age matched healthy subjects were selected as control. 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.

5 ml of venous blood was collected in a plain bulb from group-I control and group-II patients with urolithiasis were placed in reclining position for a minimum period of 10 minutes before sampling. EDTA vials were used to collect blood samples for estimation of plasma MDA, vitamin-E and vitamin-C. Estimation of MDA as an index of lipid peroxidation^[10], vitamin-E^[11] and Vitamin-C^[12] were carried out within 24 hours of collection of samples. Remaining 1 ml blood was collected in a heparinized bulb for the assessment of erythrocyte SOD^[13] and CAT^[14]. The values are expressed as mean \pm SD for patients and controls separately. Students't test was done for the comparison of data and Pearson's correlation was analysed to see correlation coefficient between MDA, SOD, CAT, vitamin-E and vitamin-C using SPSS version 20.01.

RESULTS

The average age of the study group was 40.27 ± 12.91 , of which 51 were men and 39 women. From the present study, it reveals that group-II patients with urolithiasis has a state of increase oxidative stress and weak antioxidant defence system as demonstrated by a highly significant value ($p < 0.001$) in the level of mean plasma MDA (4.06 ± 0.693 nmol/ml) as compared to control group-I (1.94 ± 0.456 nmol/ml).

Since the group-II patient with urolithiasis have increased lipid peroxidation, as expected the activities of SOD (7.25 ± 1.87

Unit/mg Hb) and CAT (56.5 ± 4.98 μ mol/l) were significantly decreased ($p < 0.001$) in them than those of group-I control (12.17 ± 1.21 Unit/mg Hb of SOD and 71.4 ± 6.23 μ mol/l of CAT) indicating that group-II patients with urolithiasis are under the oxidative stress (Tables 1). A positive correlation was recorded between MDA and SOD activity ($r = 0.180$, $p > 0.05$) while a negative correlation between MDA and CAT activity ($r = -0.161$, $p > 0.05$), however, the correlations were not significant (Tables 2). Along with antioxidant enzymes, the nutritional antioxidants such as vitamin-E and vitamin-C also play a pivotal role in scavenging free radicals. A non significant decreased ($p > 0.05$) in the Vitamin-E (1.59 ± 0.24 mg/dl and Vitamin-C (0.44 ± 0.05 mg/dl) were observed in the group- II patients as compared to the control group-I (1.66 ± 0.23 mg/dl of vitamin- E and 0.47 ± 0.05 mg/dl of vitamin-C) (Tables 1). A negative correlation was observed between MDA and vitamin-E ($r = -0.46$, $p > 0.05$) and also between MDA and Vitamin-C ($r = -0.116$, $p > 0.05$), however, the correlation is not significant (Tables 2).

DISCUSSION

Crystal aggregation and retention are critical events in the formation of stones in the urolithiasis. There is close association between crystal development and free radical activity in vivo. In this study, the relation between urolithiasis and oxidative stress could be corroborated in the group-II patients studied, which shows elevated levels of LPO with significant decrease in the activities of the antioxidant enzymes. The activity of the inbuilt antioxidant enzymes SOD and CAT were significantly lowered in the group-II patients with urolithiasis as compared to control group-I and this could mean an exaggerated oxidative stress due to formation of stones in the kidney/or ureter. These results are coherent with previous workers^[15-17] who reported that oxalate stone formation induced lipid peroxidation in urolithiasis. This may be the reason for the elevation of LPO products in the plasma of group-II patients with urolithiasis as it have been revealed by in vivo and in vitro studies that oxalate can induced LPO through inhibition of antioxidant enzymes^[18-20]. Upon oxalate binding, the condition of peroxidation enhances and increase along with the depletion of thiol content which may in turn promotes nucleation and aggregation property of stone matrix protein fractions. This

Table 1: Comparison of mean \pm SD of plasma malondialdehyde (MDA), plasma vitamin-A, plasma vitamin-E and plasma vitamin-C levels between control group-I and group-II patients with urolithiasis.

Parameters	Group-I (Control, n=40) Mean \pm SD	Group-II (Patients with Urolithiasis, n=50) Mean \pm SD	P value
Plasma MDA (nmol/ml)	1.94 ± 0.456	4.06 ± 0.693	<0.001
Erythrocyte SOD (Unit/mg Hb)	12.17 ± 1.21	7.25 ± 1.87	<0.001
Erythrocyte CAT (μ mol/l)	71.4 ± 6.23	56.5 ± 4.98	<0.001
Vitamin -E (mg/dl)	1.66 ± 0.23	1.59 ± 0.24	>0.05
Vitamin- C (mg/dl)	0.47 ± 0.05	0.44 ± 0.05	>0.05

Table 2: Correlation coefficient between MDA, SOD, CAT, Vitamin-E and Vitamin-C

Plasma level	MDA(r)	P value
SOD	0.180	>0.05
CAT	-0.161	>0.05
Vitamin-E	-0.046	>0.05
Vitamin-C	-0.116	>0.05

type of activity is also associated with peroxidized mitochondria and nuclei, suggesting that the peroxidation can be a causative factor for the initial stage of stone formation^[21]. In addition to this, reduction in either nutritional antioxidants or enzymatic antioxidants shall also enhance to the progression of lipid peroxidation^[22].

The production of lipid peroxidation products depends on activities of antioxidant enzymes such as SOD and CAT which registered a significant reduction in erythrocyte of group-II patients with urolithiasis. A positive correlation was recorded between MDA and SOD activity while a negative correlation between MDA and CAT activity, however, the correlations are not significant. There is conflicting data on the status of the antioxidant enzymes in the subjects with urolithiasis with some observed higher while other reported significant decreased as compare to healthy control. The positive correlation between MDA and SOD in this study may be due to increase activity of SOD in response to increased lipid peroxidation product. Our result is in agreement with those reported by Singh and Barjatia^[23], who hypothesized that SOD must be over stretching itself to dismutate $O^{\cdot -}$ to H_2O_2 . SOD is the only antioxidant enzyme which effectively dismutates $O^{\cdot -}$ to H_2O_2 and which in turn convert to water by CAT to retards the impact of free radical damage. Therefore, reduction in the plasma level of SOD and CAT here can be explained due to consumption of antioxidant enzymes by increasing lipid peroxidation.

Along with antioxidant enzymes, α -tocopherol play a pivotal role in scavenging free radicals. In corroboration with above concepts a decrease in the level of vitamin-E was observed in group-II patients with urinary stones ($p>0.05$) and a negative correlation was observed between MDA and vitamin-E which is in agreement with reported by others^[24-25].

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^[26]. 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 vitamin-E. 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.

In this study vitamin-C level in plasma is not significantly different in group-II patients with urolithiasis as compared to control group-I ($p>0.05$, Table-1). A negative correlation is also observed between MDA and Vitamin-C, however, the correlation is not significant. In this study, there is possibility that ascorbic acid is endogenously converted to oxalate and appears to increase the absorption of dietary oxalate which in turn induces free radical generation thereby causing renal stones^[27]. However, it is not very clear whether the reduction in plasma levels of vitamin-E and Vitamin-C is because of reduced dietary intake or consequence of the disease because of the increased utilization during oxidative stress. Lower level of α -tocopherol and ascorbate is an indicative of restricted antioxidant function and enhanced lipid peroxidative action resulting in probable damage to the renal tubular cells.

The study has been carried out in the Mizo tribal population Mizoram, a North eastern state of India ethnically different from the rest of the population of India and have different dietary habits. Herein, we found significant increased in the oxidative stress parameters and non significant decreased in levels of vitamin-E and vitamin-C between group-I control and group-II patients with urolithiasis in this population. It is not clear whether the negative co-relationship between MDA and antioxidant enzymes like CAT and antioxidant vitamins like vitamin-E and vitamin-C (Table-2) are result or the cause of this disease, however, it suggests that the imbalance caused by the level of these parameters may be the major contributing factor leading to crystal aggregation and adherence on the surface of renal epithelial cells thereby leading to genesis of urolithiasis. Further evaluation need to be done in other tribe population to find out the level oxidative stress indicators and vitamin-E and Vitamin-C which can contribute to the genesis of the urolithiasis.

CONCLUSION

The result of the present study suggests that increased oxidative stress and decreased in antioxidant vitamins may be the major contributing factors to genesis of urolithiasis in the living population of North Eastern Part of India. Thus, the role of lipid peroxidation and oxidative function is an evident and bearings in pathogenesis of urolithiasis along with decrease level of antioxidant vitamins.

Declaration of Conflicts of Interest by Authors

We declared that public there is no conflict of interests between us.

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