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LIPID PEROXIDATION AND ANTIOXIDANT VITAMINS IN UROLITHASIS

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ABSTRACT

The present, study was conducted to determine the level of malondialdehyde (MDA) as an index of free radial induced lipid peroxidation and antioxidant vitamins – vitamins A, vitamin C and vitamin E in 75 confirmed cases of urolithiasis. Significantly high level of MDA ($p<0.001$) with significantly low levels of vitamin E ($p<0.001$) and vitamin A ($p<0.001$) with no significant decrease in vitamin C ($p > 0.05$) were observed in the plasma of urolithiasis cases as compared to normal controls. In conclusion, it appears that a role of lipid peroxidation and oxidative function exists in the pathogenesis of urolithiasis. But, the exact mechanism how this occurs remains to be elucidated.

KEY WORDS

Lipid peroxidation, Malondialdehyde (MDA), Antioxidant Vitamins, Urolithiasis.

INTRODUCTION

Lipid peroxidation represents oxidative tissue damage caused by hydrogen peroxide, superoxide anion and hydroxyl radicals, resulting in structural alteration of membrane with release of cell and organelle contents, loss of essential fatty acids with formation of cytosolic aldehyde and peroxide products. Malondialdehyde is a major end product of free radical reaction on membrane fatty acids. Although the cell is endowed with several antioxidant systems to limit the extent of lipid peroxidation, under certain conditions protective mechanism can be overwhelmed, leading to elevated tissue levels of peroxidation products. Antioxidant can be classified as preventive and chain breaking antioxidants. Antioxidant vitamins such as alpha tocopherol (vitamin E), vitamin A and ascorbic acid (vitamin C) belong to the second category. Such compounds can intercept free radical induced chain reaction and prevent further oxidation. Many studies on the etiology of stone disease have focused on the properties of urine that effect crystal nucleation and growth. Crystal adherence to the surface of injured renal epithelial cells is considered initiating events in the genesis of urolithiasis (1). Factors leading to initiation of calcium oxalate formation are still not known. However the oxidant (free radical production) and antioxidant imbalance may be one of the major factors leading to the process of crystal deposition in renal tissues (2). Manipur is a small State in the North Eastern corner of India where the incidence of urolithiasis is very high among the natives who are different in food habits, and also socially, culturally and ethnically from the people of the mainland of India. The present study is therefore taken to evaluate the role of lipid peroxidation and antioxidant vitamins in the etiology of stone formation in population of Manipur.

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MATERIALS AND METHODS

The case-control study was conducted in the Department of Biochemistry in collaboration with Department of Urology, Regional Institute of Medical Sciences, Imphal, India during the period December 2002 to November 2003. A total of seventy five urolithiasis patients in the age group ranging from 20 to 50 years and above, who were having radio-opaque stone demonstrable on abdominal roentgenograms and who were admitted in Urology Ward, irrespective of age, sex and socio-economic status were included in the study. The study group comprised of 51 males and 24 females. Twenty five healthy subjects, age and sex matched from public at large including medical undergraduate and post graduate students free from any history of smoking, alcoholism and co existence of any such disease which can also lead to similar changes in plasma levels of MDA, vitamin E, vitamin A and vitamin C

Table 1 : Comparison of mean ± SD of plasma malondiadehyde (MDA), plasma vitamin A, plasma vitamin E and plasma vitamin C levels between study and control groups.

Parameter	Study group (n=75)	Control group (n=25)	d.f	t	p
Malondialdehyde (nmol/ml)	5.12 ± 1.24	3.27 ± 0.36	98	7.31	< 0.001
Vitamin A (mg/dl)	13.18 ± 7.95	34.99 ± 11.40	98	10.60	< 0.001
Vitamin E (mg/dl)	0.66 ± 0.23	1.10 ± 0.23	98	8.11	< 0.01
Vitamin C (mg/dl)	1.08 ± 0.28	1.011 ± 0.31	98	1.03	< 0.05

levels were selected as controls.

Venous blood samples were collected from study and control subjects who 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. For estimation of vitamin A, blood samples were collected in a separate, clean and sterile vial without any anticoagulant. Estimation of MDA (3), vitamin A (4), vitamin E (5) and vitamin C (6) were carried out within 24 hours of collection of the samples.

RESULTS

Subjets with urolithiasis were ranging in age from 31 to 40 years (30.6%), followed by the age group of 21-30 years constituting 28.0%. Males (68.0%) are affected more predominantly than females (32.0%). Statistically 2.1 males are affected for every female. Highly significant value (p<0.001) in the level of mean plasma MDA is seen among the study group (5.110 ± 1.243 n mol/ml) when compared to the control group (3.269 ± 0.364 n mol/ml). However levels of mean plasma vitamin A are found to be lower in the study group (13.175 ± 7.946 mg/dl) than the control group (34.99 ± 11.398 mg/dl) and the difference is found to be statistically significant (p<0.001). Similarly levels of mean plasma vitamin E level is also found to be significantly lower (p<0.001) in the study group (0.664 ± 0.233 mg/dl) when compared to control group (1.098 ± 0.228 mg/dl). No significant difference (p>0.05) in the levels of mean plasma vitamin C was found between the study group (1.078 ± 0.277 mg/dl) and the control group (1.010 ± 0.310 mg/dl) (Table 1). A negative correlationship was observed between MDA and vitamin A (r = -0.231, p< 0.05) and also between MDA with vitamin E (r = -0.374, p<0.05). It means that decrease in the levels of these vitamins accelerate the lipid peroxidation thereby generating more MDA. A negative correlation between MDA and vitamin C is also observed. However, the correlationship is not significant (r = -0.176, p<0.05: Table 2).

Table 2 : Correlation Coefficient between MDA, Vitamin A, Vitamin E and Vitamin C among the study groups.

Plasma level	MDA (r)	P value
Vitamin A	- 0.321	< 0.05
Vitamin E	- 0.376	< 0.05
Vitamin C	- 0.176	> 0.05

DISCUSSION

In this study, urolithiasis was found to be most predominant in the age group of 31-40 years comprising of 30.6%. Other investigators also got similar findings (7). Age group of early twenties to late forties is physically most active period in life. Increased physical activities have been shown to induce a several fold increase in plasma xanthine oxidase that could induce oxidative stress to the filtrating renal tissue (8). Another possible mechanism may be due to increased level of serum testosterone in age group of 21 – 40 years, which resulted in increased production of oxalate by liver from its endogenous precursors (9). Oxalate the major stone forming constituent has been reported to induce free radical generation, which results in peroxidative injury to renal epithelial cells (10). Finalyson B (11) reported that lower serum testosterone level may contribute to some of the protection women and children have against oxalate stones. This factor could lead to the higher incidence of urinary stone cases in males (68%) than females (32%) observed in the study.

In this study, a significant increase in plasma MDA level (p<0.001) was observed in patients compared to controls (Table 1) that were similar to findings of other investigators (12,13). Increase in MDA levels observed could be due to increased oxidative stress in kidney from various sources or decrease in antioxidant defense mechanism and vice-versa.

Lower levels of serum vitamin A (p<0.001) and plasma vitamin E (p<0.001) were observed in study cases compared to controls (Table 1) which is similar to findings of others (14,15,16). It is not very clear whether the reduction in serum

levels of vitamin A and vitamin E is the cause (because of reduced dietary intake) or consequence of the disease (because of increase utilization during oxidative stress). In this study, vitamin C level in plasma is not significantly different in the patients with urolithiasis as compared with control group ($p>0.05$: Table 1). A negative correlation was observed between plasma MDA level and plasma vitamin C but result is not statistically significant, ($r = -0.176$, $p>0.05$) (Table 2). Chalmer AH et al (17) reported lower excretion rate of ascorbate in urolithiasis. It is possible that Ascorbic acid is endogenously converted to oxalate and appears to increase the absorption of dietary oxalate which inturn induce free radical generation thereby causing renal stones (18). Hyperoxaluria found in urolithiasis patients induces calcium oxalate crystal deposition in kidney. Oxalate crystals are the main component of renal stones (19). It is not clear whether negative correlationship between MDA and antioxidants such as vitamin A and vitamin E (Table 2) are result or the cause of disease, however, it suggests that the imbalance caused by the levels of this parameters may be the major factor leading to crystal adherence on the surface of renal epithelial cells thereby leading to genesis of urolithiasis. The study has been conducted in small populations of Manipur comprising of various ethnic groups having different dietary habits. We found no difference in levels of plasma vitamin C between urolithiasis cases and normal controls among these population. Further evaluation has to be done in different populations to find out the level of plasma vitamin C in urolithiasis cases and to see whether any reduction is found in the levels of vitamin C which can contribute to the genesis of urolithiasis.

From this study, it appears that a role of lipid peroxidation and oxidative function exists in the pathogenesis of urolitiasis as observed from the negative correlation between the MDA and plasma vitamin A and also between MDA and plasma vitamin E. But the exact mechanism how this occurs remains to be elucidated.

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