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DEPLETED ANTIOXIDANT VITAMINS AND ENHANCED OXIDATIVE STRESS IN UROLITHIASIS

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ABSTRACT

Background: Urolithiasis is the third most common urological disease with prevalence of 11% and recurrence rate of 50% and has a great impact on the economsically active population, representing significant health care cost burden. 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. Materials and Methods: In the present study 50 subjects presenting with urolithiasis were included. Serum levels of malondialdehyde, vitamin E and β-carotene were measured. These findings were compared with 50 age and sex matched control subjects. Unpaired't' test was applied for statistical analysis. Results: There was a significant increase in MDA ($p<0.001$) and significant decrease in Vitamin E ($p<0.01$) and β-Carotene ($p<0.001$) levels in patients. There was significant negative correlation MDA and vitamin E and between MDA and β-carotene. Conclusion: It appears that a role of lipid peroxidation and oxidative function exists in the pathogenesis of urolithiasis.

KEY WORDS

Oxidative stress, antioxidants, urolithiasis, vitamin E, β-carotene, malondialdehyde.

INTRODUCTION

Urolithiasis is calculus formation at any level in the urinary collecting system and is one of the most common diseases of the urinary tract which has tormented people throughout the ages[1]. It recurs at a very high rate without preventive measures. Most kidney stones are predominantly composed of calcium oxalate [2]. Despite enormous developments in nephrology and urology, we still do not know how to prevent them. 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. 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 [7]. Vitamin E and β-Carotene have proved to be efficient protectors to the membrane integrity. The present study was planned to quantitate the levels of serum malondialdehyde, Vitamin E and β-carotene 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, in collaboration of Department of Urology, J.J.M. Medical College attached to Bapuji and Chigateri hospitals, Davangere, Karnataka during the period April 2011 to April 2012.100 subjects were studied,

comprising of 50 healthy controls and 50 urolithiasis cases aged 15- 80 years that were diagnosed and confirmed by urologist. Patients with history of bowel disease, renal tubular acidosis, urinary tract anomalies, gout, diabetes mellitus and hypertension were excluded from the study. 50 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 and β- carotene levels were selected as controls. None of the subjects were on vitamin supplementation or used medications that could alter the study parameters.

6ml of venous blood was drawn under aseptic precautions into a plain bulb from selected subjects. Serum was separated by centrifugation at 3,000 rpm for 10 minutes and then Vitamin E was estimated by Baker and Frank method (8), serum β-carotene by Sobel and Snow method(9) and serum Malondialdehyde by Nadiger et al method (10) in all subjects within 24 hours of collection of the samples.

STATISTICAL ANALYSIS

Results are expressed as mean ± SD and range values for all the variables. Unpaired't' test is used to compare the mean values different biochemical parameters between cases and controls. Correlation analysis was performed to assess the relationship between different variables using Pearson's correlation co-efficient. For all the tests p value of < 0.05 was considered as statistical significant.

RESULTS

The general description of the study subjects are given in table 1. Of the 50 controls, 40 were males and 10 were females with a mean age of 37.1 ± 13.4 years. Among 50 urolithiasis cases, 41 were males and 9 were females with a mean age of 39.8 ± 13.6 years.

Table 2 shows comparative analysis of serum MDA, vitamin E and β-carotene levels between controls and urolithiasis cases.

In this study, a significant increase in plasma MDA level ($p<0.001$) was observed in patients compared to controls (**Table 2**) that were similar to findings of other investigators[11, 12, 13]. Highly significant value ($p<0.001$) in the level of mean serum MDA is seen among the patients (5.48±0.81 nmol/ml) when compared to the controls (2.26±0.84 n mol/ml). Levels of mean serum vitamin E are found to be lower in urolithiasis patients (0.70±0.27 mg/dl) when compared to controls (1.39±0.24 mg/dl) and the difference was found to be statistically significant ($p<0.001$). Similarly levels of mean serum β-carotene was found to be significantly lower ($p<0.001$) in urolithiasis patients (137.1±20.5 µg/dl) when compared to controls (203.5±25.4 µg/dl) (**Table 2**).

A negative correlationship was observed between MDA and vitamin E ($r = -0.540$, $p< 0.01$) and also between MDA with β-Carotene ($r = -0.380$, $p<0.01$) (**Table 3**). It means that decrease in the levels of these vitamins accelerate the lipid peroxidation thereby generating more MDA.

DISCUSSION

Oxalate the major stone forming constituent has been reported to induce free radical generation, which results in peroxidative injury to renal epithelial cells. In this study, a significant increase in plasma MDA level ($p<0.001$) was observed in patients compared to controls (**Table 2**) that were similar to findings of other investigators [7, 11, 12, 13, 14]. 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 E ($p<0.001$) and serum β-carotene ($p<0.001$) were observed in urolithiasis cases when compared to controls (**Table 2**) which is similar to findings of others [6, 7, 11, 15, 16]. The decreased levels of serum Vitamin E, β-carotene in urolithiasis patients appears to be a consequence of increased oxidative stress due to oxalate induced free radical mediated lipid peroxidation[6]. Negative correlationship between MDA and antioxidants such as vitamin E and β- carotene (**Table 2**) suggest that the imbalance caused by the levels of these parameters may be the major factor leading to crystal adherence on the surface of renal epithelial cells thereby leading to genesis of urolithiasis. Study was done with manual methods. Correlation between parameters and size, anatomical position of stone was not included. More studies may be required to substantiate the results and also to identify new antioxidant molecules which may well prove to be better preventive factors.

Table 1: Age and sex wise distribution of subjects

		Controls	Cases
Number of subjects		**50**	**50**
Age(years)	Mean± S.D	37.1± 13.4	39.8±13.6
	Range	18 - 67	15 - 75
Gender	Male	40	41
	Female	10	9

Table 2: Comparison of serum Malondihaldehyde, SOD activity, Vitamin E and β – Carotene in controls and urolithiasis cases

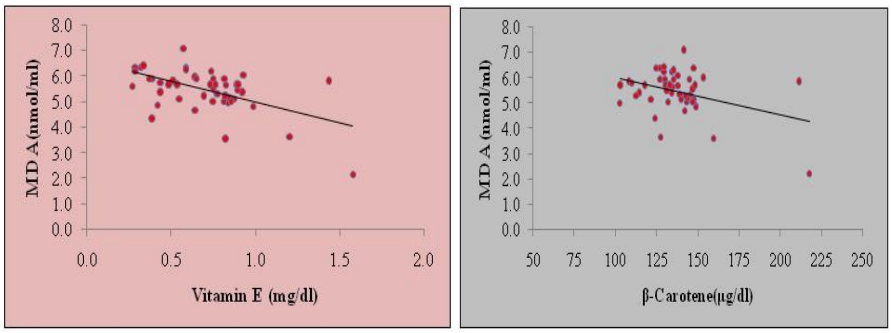
		MDA (nmol/ml)		Vitamin E (mg/dl)		β -carotene (µg/dl)	
		Range	**Mean ± SD**	**Range**	**Mean± SD**	**Range**	**Mean± SD**
Controls	50	0.51 –4.77	2.26±0.84	0.69 – 1.69	1.39±0.24	105.8–248.1	203.5±25.4
Cases	50	2.20 – 7.11	5.48±0.81	0.27–1.58	0.70±0.27	102.6–218.0	137.1±20.5
Mean Diff.		3.22		0.69		66.4	
t-value *		19.56		13.33		14.38	
p-value		<0.001, HS		<0.001, HS		<0.001, HS	

***Unpaired t-test; HS: Highly Significant**

Table 3: Relationship between serum MDA, SOD activity, vitamin E and β-carotene among the urolithiasis cases

	Pearson's correlation coefficient 'r' value	Significance p – value
MDA and vitamin E	– 0.54	<0.01, S
MDA and β -carotene	– 0.38	<0.01, S

Graph 1: Relationship between serum antioxidants and serum MDA in patients.



CONCLUSION

Oxidative stress is functional in urolithiasis as evident from increased lipid peroxidation and decreased antioxidants namely serum vitamin E and β-carotene in urolithiasis patients when compared to controls. From this study, it appears that a role of lipid peroxidation and nutritional antioxidants exists in the pathogenesis of urolithiasis as observed from the negative correlation between the serum MDA and other antioxidants. Further it emphasizes the importance of antioxidants in preventing stone formation.

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