Original article

Ethylene Glycol Induced Calcium Oxalate Crystals and Oxidative Damage of Renal cells

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Abstract:

Introduction: : Urolithiasis is one of common renal disease that attributed to multiple factors. In other hand, calcium oxalate is the most common urine lithiasis and urinary calcium and oxalate oversaturation are both important to calcium oxalate stone formation. The percent study was undertaken to examine the possible role of oxidative stress and cell injury in stone formation.

Method: In this research 12 rats with average weight of (200-250) ±6 grams in two groups, accidentally were chosen. Stone group animals consume ethylene glycol 0.75% and ammonium chloride 1% for three days and then ethylene glycol 0.75% for 25 days to create stones in their kidneys. Control group rats maintained on regular food and drinking for 28 days. The status of oxidative stress induced by Calcium oxalate was evaluated by malondialdehyde and superoxide dismutase in renal tissue.

Findings: In present study, the body weight gain was small in stone group compare control group but kidney weight increased significantly. Many crystals deposit of Caox were seen in all regions of renal tubules in stone group. Calcium excretion increased after stone formation but magnesium decreased significantly. Urinary creatinine content was higher in control group. The serum creatinine, urea and uric acid were remarkably increased in urolithiasis induced group compare with control group. Superoxide dismutase activity decreased significantly in stone groups but MDA production increased in stone group when compare with control group.

Conclusion: Our results indicate that ethylene glycol produces oxidative stress in the kidneys as shown by increased tissue MDA significantly. The antioxidant agents like vitamins and etc. can reverse these defective cycles properly.

Keywords: Urolithiasis, Calcium Oxalate, Malondialdehyde, Superoxide Dismutase

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Introduction:

Urolithiasis is one of common renal disease that attributed to multiple factors (1). In other hand, calcium oxalate is the most common urine lithiasis and urinary calcium and oxalate oversaturation are both important to calcium oxalate stone formation (1-3). A number of mechanisms have been proposed to account for renal crystal formation (4). Administration of high dose oxalate may lead to calcium oxalate stone formation by renal cell injuries (5). Moreover, oxalate crystals can make a cell injury by oxidative stress (6). The main etiology of renal cell damage due to calcium oxalate crystal is still unknown. Studies confirm that subepithelial plaque on the renal papillae may induce damages (7). Cell injury increases the attachment of oxalate crystals to the injured renal epithelial cell (4). Ethylene glycol is a nephrotoxic compound that can induce hyperoxaluria. asselman in a study showed that calcium oxalate adherence occurred in injured renal cell induced by ethylene glycol (8). The percent study was undertaken to examine the possible role of oxidative stress and cell injury in stone formation.

Methods:

In this research 12 rats with average weight of (200-250) ±6 grams in two groups, accidentally were chosen. The study protocol was approved by the ethical committee of Kerman university of medical sciences (No.: IRKMU.REC.1395.592). These groups were “control”, “kidney stones”. Stone group animals consume ethylene glycol 0.75% and ammonium chloride 1% for three days and then ethylene glycol 0.75% for 25 days to create stones in their kidneys. Control group rats maintained on regular food and drinking for 28 days. At the end of study urine sample were collected and evaluated for urinary calcium, phosphor, magnesium, citrate, creatinine and PH. Animals were sacrificed by cervical decapitation under ether anesthesia and serum was collected and evaluated for serum creatinine, urea, uric acid, phosphors and calcium. Both kidney specimens sent to pathology laboratories for microscopic examination of renal tubular Calcium Oxalate crystals deposition. The status of oxidative stress induced by Calcium oxalate was evaluated by malondialdehyde (MDA) and superoxide dismutase (SOD) in renal tissue. Data analyzed by SPSS ver.22, using T Test for comparisons among two groups. P value less than 0.05 was considered significant.

Findings:

In present study, the body weight gain was small in stone group compare control group but kidney weight increased significantly. Many crystals deposit of Caox were seen in all regions of renal tubules in stone group (Figure 1). Calcium excretion increased after stone formation but magnesium decreased significantly. Urinary creatinine content was higher in control group. The serum creatinine, urea and uric acid were remarkably increased in urolithiasis induced group compare with control group. SOD activity decreased significantly in stone groups but MDA production increased in stone group when compare with control group (Table 1).

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Discussion:

In present study, administration of ethylene glycol revealed that calcium crystals deposition increased significantly. Calcium oxalate induced damage is one of the important events in progression of stone formation and stone disease (8). Stone formation process initiated by crystals adhesion and internalization (4). Internalization of crystals activates the inflammatory response and induces renal epithelium injury (9). The oxidative stress will increase due to cell structural injury and inflammation (10). SOD is an antioxidant enzyme that located in cytoplasm (SOD1), mitochondria (SOD2) and extracellular space (SOD3) (11, 12). Then, cells structural damage results in SOD depletion and elevation of reactive oxygen species (6). MDA is a marker for oxidative stress (13). In our study, calcium oxalate crystals significantly increased in all renal tubular space and cell injury proved by MDA elevation. In other hand, the tissue parameters showed reduction in SOD levels and it was reflected in the kidney sections with tubular damage. Stone formation results in decreased GFR due to renal tubules obstruction and renal function decreased significantly due to crystals deposits that destruct renal tubules (14, 15). In our study, serum creatinine, urea and uric acid increased in urolithiasis induced group significantly. Rise in calcium level is due to renal tubular injury and acidosis, while phosphate accompanies hypercalciuria (16, 17). In the present study, urinary calcium increased in stone group. Although, no clear evidence has been reported regarding decrease magnesium level but supplementation of exogenous Magnesium has results in reduction in crystal formation (18). As present study, the level of magnesium was reduced in stone group rats. Our results indicate that ethylene glycol produces oxidative stress in the kidneys as shown by increased tissue MDA significantly. The antioxidant agents like vitamins and etc. can reverse these defective cycles properly.

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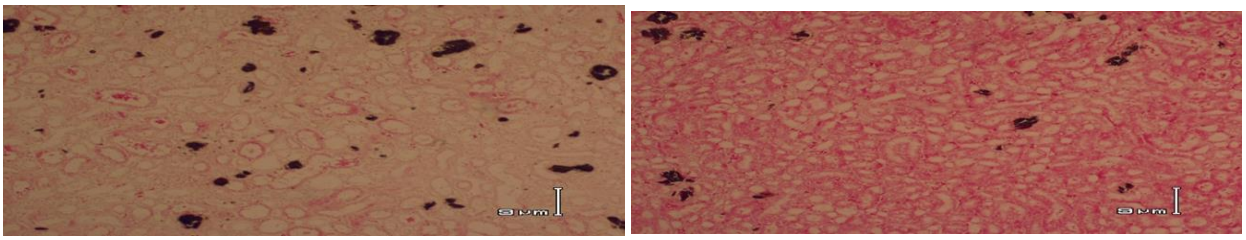
Tables and Charts:

Table 1: Comparison of stone group with control group.

			Stone group	Control group
Weight	Body weight (g)	Before	236.17±15.63	190.17±9.52
		After	251.83±19.42	242.17±10.41
		Weight differences	15.66*	52.00
	Kidney weight (mg)	Left	1149.40±47.72*	797.78±25.10
		right	1216/30±91.43*	828±17.45
Urine parameters	Phosphorus (mmol/L)		143.52±25.4	114.20±36.70
	Calcium (mmol/L)		4.79±0.38*	3.68±0.96
	Creatinine (mmol/L)		106.44±14.56*	181.10±5.84
	Magnesium (mmol/L)		0.73±0.26*	2.21±0.36
	Citrate (mmol/L)		1.96±0.64	2.05±0.33
	pH (nmol/L)		6.84±0.35	6.48±0.32
Serum parameters	Phosphorus (mmol/L)		9.56±0.27	8.95±0.20
	Calcium (mmol/L)		10.37±0.10	10.21±0.13
	Urea (mmol/L)		66.33±5.16*	45.00±2.57
	Uric acid (mmol/L)		1.87±0.22*	1.25±0.06
	Creatinine (mmol/L)		0.60±0.05*	0.47±0.02
Renal tissue	Number of CaOx deposits		18.42± 1.96*	5.68±1.21
	SOD (U/μg protein)		0.20±0.02*	0.36±0.06
	MDA (μmol/μg protein)		9.85±1.30*	6.26±0.58
***p<0.05**				

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Figure 1. Calcium oxalate crystals deposits (left; kidney tissue in ethylene glycol ammonium chloride induced urolithiasis, right; kidney tissue in control group)



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