

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) \pm 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 Ca₂(C₂O₄) 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

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) \pm 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 Ca_{ox} 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).

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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References:

1. Hyams ES, Munver R, Bird VG, Uberoi J, Shah O. Flexible ureterorenoscopy and holmium laser lithotripsy for the management of renal stone burdens that measure 2 to 3 cm: a multi-institutional experience. *Journal of endourology*. 2010;24(10):1583-8.
2. Robertson W, Peacock M, Nordin B. Calcium oxalate crystalluria and urine saturation in recurrent renal stone-formers. *Clinical science*. 1971;40(5):365-74.
3. Robertson W, Peacock M, Nordin B. Calcium crystalluria in recurrent renal-stone formers. *The Lancet*. 1969;294(7610):21-4.
4. Tsujihata M. Mechanism of calcium oxalate renal stone formation and renal tubular cell injury. *International Journal of Urology*. 2008;15(2):115-20.
5. Khan S. Calcium oxalate crystal interaction with renal tubular epithelium, mechanism of crystal adhesion and its impact on stone development. *Urological research*. 1995;23(2):71-9.

6. Selvam R. Calcium oxalate stone disease: role of lipid peroxidation and antioxidants. *Urological research*. 2002;30(1):35-47.
7. Khan SR. Role of renal epithelial cells in the initiation of calcium oxalate stones. *Nephron Experimental Nephrology*. 2004;98(2):e55-e60.
8. Hovda KE, Guo C, Austin R, McMartin KE. Renal toxicity of ethylene glycol results from internalization of calcium oxalate crystals by proximal tubule cells. *Toxicology letters*. 2010;192(3):365-72.
9. Moriyama MT, Suga K, Miyazawa K, Tanaka T, Higashioka M, Noda K, et al. Inhibitions of urinary oxidative stress and renal calcium level by an extract of *Quercus salicina* Blume/*Quercus stenophylla* Makino in a rat calcium oxalate urolithiasis model. *International journal of Urology*. 2009;16(4):397-401.
10. Khan SR. Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. *Urological research*. 2005;33(5):349-57.
11. Khand F, Gordge MP, Robertson W, Noronha-Dutra A, Hothersall J. Mitochondrial superoxide production during oxalate-mediated oxidative stress in renal epithelial cells. *Free Radical Biology and Medicine*. 2002;32(12):1339-50.
12. Hirose M, Yasui T, Okada A, Hamamoto S, Shimizu H, Itoh Y, et al. Renal tubular epithelial cell injury and oxidative stress induce calcium oxalate crystal formation in mouse kidney. *International journal of urology*. 2010;17(1):83-92.
13. Thamilselvan S, Khan SR, Menon M. Oxalate and calcium oxalate mediated free radical toxicity in renal epithelial cells: effect of antioxidants. *Urological research*. 2003;31(1):3-9.
14. Marangella M, Bruno M, Cosseddu D, Manganaro M, Tricerri A, Vitale C, et al. Prevalence of chronic renal insufficiency in the course of idiopathic recurrent calcium stone disease: risk factors and patterns of progression. *Nephron*. 1990;54(4):302-6.
15. Kristensen C, Parks JH, Lindheimer M, Coe FL. Reduced glomerular filtration rate and hypercalciuria in primary struvite nephrolithiasis. *Kidney international*. 1987;32(5):749-53.
16. Nouvenne A, Meschi T, Prati B, Guerra A, Allegri F, Vezzoli G, et al. Effects of a low-salt diet on idiopathic hypercalciuria in calcium-oxalate stone formers: a 3-mo randomized controlled trial-. *The American journal of clinical nutrition*. 2009;91(3):565-70.
17. Bushinsky DA, Asplin JR, Grynepas MD, Evan AP, Parker WR, Alexander KM, et al. Calcium oxalate stone formation in genetic hypercalciuric stone-forming rats. *Kidney international*. 2002;61(3):975-87.
18. Ettinger B, Pak CY, Citron JT, Thomas C, Adams-Huet B, Vangessel A. Potassium-magnesium citrate is an effective prophylaxis against recurrent calcium oxalate nephrolithiasis. *The Journal of urology*. 1997;158(6):2069-73.

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				

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

