

Evaluation of biochemical parameters in calcium oxalate renal stone formers

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ABSTRACT

Background: Calcium oxalate renal stones are the most predominant cases of renal stones, their formation is more complex and no specific cause for the stone can be identified so called 'idiopathic'. This study was designed to analyze the metabolic and biochemical alterations in serum, urine and their relation to pathophysiology of calcium oxalate stone formation

Patients and Methods : In this study, individuals have been classified into three groups: Group (A) included (29) apparently healthy persons of non calcium oxalate stone formers aged (20-35 years), group (B) included (16) patients with calcium oxalate renal stone aged (20-35 years) and group(C) included (15) patients with calcium oxalate renal stone aged (40-70 years). Fasting serum, random urine and 24-hours urine samples were collected from all individuals to determine urine volume, creatinine clearance, serum and urine levels of calcium, phosphate ,uric acid ,zinc, copper and serum levels of total cholesterol, high density lipoprotein-cholesterol and urea .

Results: Calcium oxalate stone formers group (B) exhibited significantly decreased serum levels of uric acid ($P=0.015$),zinc ($P=0.031$) with increased serum level of total cholesterol($P=0.034$) when compared to similar age group of healthy control ,group (A). Urinary parameters in calcium oxalate stone formers also showed significantly increased levels of 24-hour urine calcium($P=0.05$) and urine calcium: creatinine ratio ($P=0.05$)when compared to healthy control. While, older age calcium oxalate stone formers, group (C) showed significantly decreased urine volume ($P=0.015$)with increased kidney stone size($P=0.03$) when compared to younger age calcium oxalate stone formers, group (B).

Conclusions: Level of urinary calcium and urine volume are the most important urinary factors in enhancing calcium oxalate stone formation. While the observed changes in biochemical measurements of serum in calcium oxalate stone formers may indicate a probable metabolic relation in pathogenesis of this disease.

Key words : Nephrolithiasis , 24-hours urine collection, stone formers , Calcium oxalate

الخلاصة

تعد حصى او كزالات الكالسيوم الكلوية من أكثر أنواع الحصى الكلوية انتشارا وتشكيلها الاكثر تعقيدا ولا يوجد سبب محدد لتكوينها، صممت الدراسة لتحليل التغيرات الايضية والكيميائية الحيوية في إدرار وأمصال الدم للمرضى المكونين لحصى او كزالات الكالسيوم الكلوية وعلاقتها بفسلجة مرضية تكوين تلك الحصى .
المشاركين وطرق العمل : تم تقسيم المشاركون في هذه الدراسة إلى ثلاث مجاميع : مجموعة (أ) وتشمل (29) شخص لا يكونون حصى او كزالات الكالسيوم الكلوية تتراوح أعمارهم بين (20-35 سنة)، مجموعة (ب) وتشمل (16) مصاب بحصى او كزالات الكالسيوم الكلوية تتراوح أعمارهم بين (20-35 سنة) ومجموعة (ج) التي تشمل (15) مصاب بحصى او كزالات الكالسيوم الكلوية تتراوح أعمارهم بين (40-70 سنة). وقد تم جمع عينات الإدرار، أمصال الدم بعد فترة صيام وكذلك جمع الإدرار لمدة 24 ساعة لجميع المشاركين لتقدير حجم الإدرار وطرح الكرياتينين و قياس مستوى الكالسيوم، الفوسفات، حامض اليوريك، الزنك والنحاس في أمصال الدم والإدرار وكذلك تقدير مستوى الكولسترول والكولسترول عالي الكثافة و اليوريا في أمصال الدم

النتائج: أظهرت مجموعة المصابين بحصى اوكزالات الكالسيوم الكلوية عند مقارنتها مع مجموعة السيطرة من نفس الفئة العمرية انخفاض معنوي في مستويات أمصال الدم لحمض اليوريك (مستوى الدلالة = 0.015) و الزنك (مستوى الدلالة = 0.031) مع ارتفاع معنوي في مستوى الكولسترول الكلي (مستوى الدلالة = 0.034). كما أظهرت متغيرات الادرار في المكونين لحصى اوكزالات الكالسيوم ارتفاع معنوي في مستوى الكالسيوم في الادرار المتجمع خلال 24 ساعة (مستوى الدلالة = 0.05) ونسبة الكالسيوم إلى الكرياتينين البولي (مستوى الدلالة = 0.05) عندما قورنت مع متغيرات الادرار لمجموعة السيطرة. بينما أظهرت مجموعة المصابين بحصى اوكزالات الكالسيوم الكلوية ذات الفئة العمرية الاعلى، مجموعة (ج)، تناقص معنوي ملحوظ في حجم الإدرار (مستوى الدلالة = 0.015) مع زيادة معنوية في حجم الحصى (مستوى الدلالة = 0.03) عندما قورنت مع المجموعة (ب).

الاستنتاج: يعتبر مستوى الكالسيوم البولي وحجم الادرار من اهم عوامل الادرار المؤثرة في تكوين حصى اوكزالات الكالسيوم الكلوية، بينما تشير المتغيرات المقاسة في امصال الدم الى وجود علاقة ايجابية محتملة في تكوين تلك الحصى.

Renal stone formation is a common disease with increasing incidence and prevalence worldwide¹. Climate, changing life style and dietary choices are the most probable causes of increasing incidence and prevalence². The annual incidence in Europe and North America is roughly of 0.5% with prevalence of 5.2% and 50% of patients may develop a new episode³. However, data from some developing countries showed similar figures to those described in western countries⁴.

Different studies related the renal stone formation to different metabolic disturbances. Pack et al (2004) proposed that serious changes in urinary factors may lead to formation of kidney stone⁵. Also, Maghbooli et al (2007) study exhibited a strong association between renal stone and metabolic bone disease⁶, while Mossetti et al (2008) study showed that metabolic syndrome and nephrolithiasis have a common background⁷.

According to chemical composition, renal stones can be classified into different types. Calcium oxalate renal stone is the most predominant type of renal stones in Iraq and different part of the world representing about 75% of cases.^{4,8,9} The formation of calcium oxalate stones is more complex and not yet completely understood and in most cases no specific cause for the stone

formation can be identified and so called 'idiopathic'.¹⁰

Most studies on renal stone didn't specify type or size of the stone. The present study included idiopathic calcium oxalate stone formers of relatively small size stone to analyze biochemical and metabolic alterations that affecting the pathophysiology of renal stone, and excluding the relatively harmful mechanical effects or kidney damage produced by other types of stones or relatively larger stones.

The aim of the present study was to investigate the possible metabolic changes that could be associated with idiopathic calcium oxalate renal stone formation.

Patients and Methods

Sixty individuals shared in this study during the period from April /2011 to December /2011. The individuals were divided into three groups: Group A (control) included (29) apparently healthy individuals without renal stone aged (20-35years), while group B and group C were of renal stone formers, who attended AL-Jamhuri Teaching Hospital/Center of Extracorporeal Shock Wave Lithotripsy. Group B involved (16) renal stone formers aged (20-35years) and group C involved (15) renal stone formers aged (40-70 years).

The detection of calcium oxalate stone is confirmed by patient history

,ultrasound, and radio image, all stones seems to be located in left and/ or right kidney with stone size of less than 15 mm³. Patients with acute or chronic kidney disease, Diabetes Mellitus, hypertension or patients on thiazide diuretics, calcium supplements were excluded from the study.

Sample collections and measurements: Random urine samples were taken to test for: types of crystals, presence of protein (individuals with no urinary protein were included). Concerning group B and group C, only those exhibited urinary calcium oxalate crystals were included that confirm radiological testing and previous patient history.^{11,12} The selected individuals agree to do fasting blood collection and 24- hours urine collection to measure serum and urine levels of calcium, phosphate, uric acid, creatinine, zinc and copper in addition to serum levels of total cholesterol, HDL-C and urea. Calcium was determined according to O-Cresol Phthalin Complexone (CPC) method using kit manufactured by Biolabo (France), phosphate was determined by reaction with ammonium molybdate in acidic medium a method without deproteinisation using kit manufactured by Biolabo (France), uric acid was determined by uricase method using Biolabo kit (France), creatinine was determined by Jaffe's reaction and measured kinetically using Biolabo kit (France). Whereas, zinc and copper were determined by Shimadzu AA 670 spectrophotometer

using atomic absorption technique, the absorbance measurement of zinc was at 213.9 nm wavelength and copper at 324.8 nm, cholesterol was measured by enzymatic method using Biolabo kit (France), HDL-c was determined by separation of HDL from serum then determination of cholesterol present in HDL by enzymatic method using kit purchased by Biolabo (France) and urea was measured by enzymatic colorimetric method using Biolabo kit (France).

The research protocol for this study was reviewed and approved by Scientific Committee of Department of Pharmacology/College of Pharmacy-Mosul and the Research Committee of Ninavaha Health Directorate. All patients were informed about the research study .

Statistical analysis: The variables were evaluated for statistical significance by chi-square and t-test, the differences are considered to be statistically significant if $P \leq 0.05$. The analysis was performed with the statistical package SPSS collection (version 16).

Results

Group (A) was compared to group (B) to show the differences between normal healthy individuals and those with renal stones, also group (B) was compared to group (c) to show the effect of age on stone formation; as both of them, group (B) and (C), consist of renal stone formers but of different age groups.

Table 1. A Comparative description of healthy control and calcium oxalate stone formers(group A and group B) and a comparative description of calcium oxalate stone formers of different ages(group B and group C)

Parameters	Group A (n=29)	Group B (n=16)	<i>p</i> -value	Group B (n=16)	Group C (n=15)	<i>p</i> -value
Age(years)	26.1±6.3	28.1 ±3.89	NS	28.1±3.89	54.1±8.86	0.00 **
Percentage of eating red meat	90%	85%	NS	85%	83%	NS
Percentage of drinking beverage	60%	42%	NS	42%	58%	NS
Mean of stone size(mm ³)				6.78±2.48	10.14±3.4	0.03 *

Parametric data represented as mean±SD, non-parametric data represented as percentage

NS=(not significant) *p* value≥0.05, * significant difference (*p* value ≤ 0.05), ** significant difference(*p* value ≤ 0.005)

Table 2. Some serum biochemical measurements of healthy control (group A), calcium oxalate stone formers(group B)and calcium oxalate stone formers (group C) represented as mean±SD

Biochemical Parameters	Group A (n=29)	Group B (n=16)	<i>p</i> -value	Group B (n=16)	Group C (n=15)	<i>p</i> -value
Calcium (mmol/L)	2.28±0.267	2.2±0.267	NS	2.2±0.267	2 ±0.40	NS
Phosphate (mmol/L)	1.067±0.36	0.944±0.13	NS	0.944±0.13	1.215±0.6	NS
Uric acid (mmol/L)	0.33±0.092	0.28±0.052	* 0.015	0.28±0.052	0.26±0.049	NS
Urea (mmol/L)	5.19±1.3	4.8±1.06	NS	4.8±1.06	5.6±5.3	NS
Creatinine (µmol/L)	65.9±16.9	71.55±18.8	NS	71.55±18.8	79.13±22.8	NS
Urea:creatinine ratio	82.21±12.7	70.08±17.28	NS	70.08±17.2	75.48±22.8	NS
Total cholesterol (mmol/L)	5.15±0.49	5.68±0.81	* 0.034	5.68±0.81	5.3±0.14	NS
HDL-C (mmol/L)	1.7±0.31	1.52±0.27	NS	1.52±0.27	1.72±0.014	NS
Zinc (µmol/L)	16.09±4.57	12.45±4.39	* 0.031	12.45±4.39	12.77±4.48	NS
Copper (µmo/L)	15.56±4.49	16.5±4.49	NS	16.5±4.49	18.05±7.6	NS

NS (not significant), *significant difference (*P* value≤0.05)

if the range of urea :creatinine ratio= 40-100,this mean there is no renal injury.

Table 3 : Some urinary biochemical measurements in healthy control(group A), calcium oxalate stone formers(group B)and calcium oxalate stone formers group (C) represented as mean±SD

Biochemical Parameters Measured in Urine	Group A (n=29)	Group B (n=16)	p-value	Group B (n=16)	Group C (n=15)	p-value
urine volume (ml)	1765.2±551	2003±507.7	NS	2003±507.7	1529±408.5	* 0.015
Urine PH	5.4±0.24	4.4±0.37	NS	4.4±0.37	5.2±0.8	NS
Specific gravity	1.026±0.004	1.025±0.005	NS	1.025±0.005	1.022±0.007	NS
Cr.Cl (ml/sec/m ²)	1.09±0.11	1.13±0.3	NS	1.13±0.3	1.06±0.2	NS
Calcium (mmol/24h)	5.14±0.64	6.61±1.13	* 0.05	6.61±1.13	7±1.28	NS
Urine Ca:Cr	0.17±0.016	0.2±0.05	* 0.05	0.2±0.05	0.2±0.13	NS
phosphate (mmol/24h)	24.08±8.8	24.45±4.58	NS	24.45±4.58	27.84±7.29	NS
Uric acid (mmol/24 h)	3.21±1.29	3.49±0.9	NS	3.49±0.9	3.19±1.43	NS
Zinc (µmo/24h)	2.75±0.38	2.86±0.3	NS	2.86±0.3	2.84±0.3	NS
Copper (µmol/24h)	0.376±0.05	0.376±0.064	NS	0.376±0.064	0.34±0.042	NS

NS (not significant),* significant (p value \leq 0.05). Cr.Cl (Creatinine Clearance),Ca:Cr (Calcium Creatinine ratio)

Calcium oxalate stone formers when compared with healthy control showed a significant decrease in serum uric acid and serum zinc levels while showed a significant increase in serum total cholesterol with ($p < 0.05$) (table 2), while other serum measurements showed no significant differences (table 2). Meanwhile, 24-hours urine parameters showed no significant difference except for 24-hours urine calcium and urine calcium: creatinine ratio which was significantly higher in group B (stone formers) when compared to group A (healthy control) with ($p \leq 0.05$) (table 3). However, comparison of different age groups of stone formers (between group B and group C) group C showed lower urine volume than group B, but other

biochemical parameters exhibited no significant differences (table 3).

Discussion

The selected individuals were not on any type of vitamin or minerals and apparently to be of no significant differences in consuming red meat and carbonated or soda beverages, which have been reported to exert their effects by changing urinary parameters^(13,14) especially calcium, phosphate, uric acid and urinary PH (table 1).

Concerning biochemical parameters measured in serum, phosphate level showed no significant difference between healthy individuals and calcium oxalate stone formers (table 2), this result was not

agree with Polo et al study¹⁵, may be because Polo's study incorporated patients with sever lithogenic activity and high recurrence rate with renal stone size more than 3 cm³.

The present study also showed a significant decrease in serum uric acid in calcium oxalate stone formers, group B when compared to healthy control ($p=0.015$) (table 2), this decrease has been observed by Scholz et al study¹⁶ and Gyawali et al study¹⁷ which may be related to a decrease in antioxidant activity in stone formers.¹⁸ However, Gyawali et al study explained the result by multifactorial etiology of urinary stone and some genetic variation in Nepalese patients.¹⁷

Calcium oxalate stone formers group B showed a significant increase in total serum cholesterol level but there is no significant changes in HDL-C level when compared to healthy control group A ($P=0.034$) (table2). This result is not in agreement with Polo et al study¹⁵ who found no significant changes in total serum cholesterol and HDL-C. However, it has been proposed that lipid play a significant role in calcium oxalate growth and the presence of high lipids in membrane of tubular cells provides a site for initial nucleation events¹⁹, because the free radicals generated from lipid peroxidation would facilitated the fixation of calcium oxalate crystals.²⁰ New evidence suggested that nephrolithiasis and atherosclerosis shared common systemic risk factors and/or pathophysiology.²¹ Therefore, there is a low incidence of renal stone in Eskimos and Japaness due to increase in consumption of polyunsaturated fatty acids (like omega-3) which have reported effects in enhancing lipid status.²² Also, it was found that serum cholesterol was significantly increased in calculogenic rats and administration of α -lipoic acid to rats counter act

calcium oxalate crystallization, in addition to antilipemic activity.²³

The lower value of serum zinc in stone formers when compared to healthy control ($p\leq 0.031$) (table2) is in agreement with Atakan et al study²⁴ as well as Ranqnekar and Gaur study²⁵, that may be attributed to higher oxidative properties which lead to decrease antioxidant status in calcium oxalate stone formers¹⁸ as zinc is considered as a potent antioxidant.²⁶

In accordance of urinary biochemical parameters measured in this study, there was no significant difference in 24-hours urine phosphate levels (table3), this result is not in agreement with Wikstrom et al study²⁷ who suggested a decrease in renal phosphate handling in calcium stone formers. However, Wikstrom may incorporated in his study patients with larger stone size especially that, in the present study, phosphaturia was developed in one patient in group C his stone size was 10 mm³.

There was no significant differences in the levels of 24-hours urine uric acid (table 3), that agrees with Gurhan and Taylor study²⁸ who incorporated 3350 renal stone formers, challenging the prevailing belief of that uric acid in urine increases the risk of calcium oxalate stone formation.²⁹ However, the protective action of (Allopurinol)® to calcium oxalate stone may be related to improve endothelial dysfunction and reducing oxidative stress.^{30,31}

Also there was a significant increase in 24-hours urine calcium in calcium oxalate stone formers when compared to healthy control ($p=0.05$) (table3), which is in agreement with Polo et al study¹⁵ who measured 24-hour urine calcium in general calcium stone formers with sever lithogenic activity and found a significant hypercalciuria in stone formers with ($p=0.000$). However, Conte et al³² study explained that, calcium

oxalate stone is more frequent in normocalciuric patients, while mixed calculus of calcium oxalate and phosphate associated with marked hypercalciuria. Moreover, this study showed a significant increase in urine calcium: creatinine ratio in calcium oxalate stone formers when compared to controls (table3), indicating that there is higher calcium leak from kidneys of stone formers than kidneys of control, which may attributed to the presence of interstitial papillary deposits that may affect on calcium reabsorption.³³

This study also exhibited no differences in urinary zinc between different groups (table3), which is in contrast with Atakan et al study²⁴ who found a higher urinary zinc in healthy individuals while Ranqnekar and Gaur study²⁵ as well as Ozgurtas et al study³⁴ found a higher urinary zinc in stone formers, suggesting that urinary zinc possess no inhibitory activity to calcium oxalate stone formation. Also this study exhibited no differences in serum and urine copper (table2, table3) that agrees with Ozgurtas et al study³⁴ suggesting that there is no effect of urinary copper on formation of kidney stone.

Lastly, concerning urine volume, the lower urine volume in older age group calcium oxalate stone formers when compared to younger age group calcium oxalate stone formers (group C and group B) ($p \leq 0.015$) (table3) may be attributed to a lesser consumption of water. However, the lower urine volume can increase concentration of lithogenic substances in urine³⁵ and may result in significant increase in stone size of this group of stone formers (group C) when compared to (group B) ($p \leq 0.03$) (table 1).

In conclusion: level of urinary calcium and urine volume are the most important urinary factors in enhancing calcium oxalate stone formation. While

the observed changes in biochemical measurements of serum in calcium oxalate stone formers may indicate a probable metabolic relation in pathogenesis of this disease.

References

- 1- Knoll T. Epidemiology, pathogenesis and pathophysiology of urilithiasis. *European Urology Supplements* 2010;9:802-806.
- 2- Chen YK, Lin HC, Chen CS, et al. Seasonal variations in urinary calculi attacks and their association with climate: a population based study. *J Urol* 2008;179:564-569.
- 3- Moe O W. Kidney stones: pathophysiology and medical management. *The Lancet* 2006;367:333-334.
- 4- Trinchieri A. Epidemiology of urilithiasis :an update. *Clin Cases Miner Bone Metab* 2008;5:101-106.
- 5- Pack C Y C, Huet B A, Poindexter J R, et al. Relative effect of urinary calcium and oxalate on saturation of calcium oxalate. *Rapid Communication. Kidney International* 2004 ;66 :2032-2037.
- 6- Maghbooli Zn, Hossein –Nezhad A, Adibi H, et al. Association between Renal stone ,Bone Mineral Density and Biochemical Parameters. *Iranian J Publ Health* 2007:45-50.
- 7- Mossetti G, Rendina D, Filippo G, et al. Metabolic syndrome and nephrolithiasis : can we hypnotize a common background?. *Clin Cases Miner Bone Metab* 2008;5:114-117.
- 8- Daudon M, Donsimoni R, Hennequin C, et al. Sex and age related composition of 10617 calculi analysed by infrared spectroscopy. *Urol Res* 1995;23:319-326.

- 9-Qaader DS, Yousif SY, Mahdi LK. Prevalence and etiology of urinary stones in hospitalized patients in Baghdad. *East Mediterr Health J* 2006;12:853-856.
- 10-Bushinsky D A. Recurrent hypercalciuric nephrolithiasis—Does diet help?. *New England Journal of Medicine* 2002;346:124-125.
- 11- Daudon M, Jungers P. Clinical Value of Crystalluria and Quantitative Morphoconstitutional Analysis of Urinary Calculi. *Nephron Physiol* 2004;98:31-36.
- 12- Kaid-Omar Z, Daudon M, Attar A, et al. Correlations between crystalluria and composition of calculi. *Prog Urol* 1999;9-Sep.:633-641.
- 13- Weiss GH, Sluss PM, Linke CA. Changes in urinary magnesium, citrate and oxalate levels due to cola consumption. *Urology* 1992;39:331-333.
- 14- Hesse A, Siener R, Heynck H, et al. The influence of dietary factors on the risk of urinary stone formation. *Scanning Microsc* 1993;7:1119-1127.
- 15- Polo M A, Martin M A, Haro-Muñoz T, et al. Biochemical Determinants of severe lithogenic Activity in patients with idiopathic calcium Nephrolithiasis. *Endourology and Stone* 2012;79: 48-54.
- 16- Scholz D, Schwille PO, Ulbrich D, et al. Composition of renal stones and their frequency in stone clinic: relationship to parameters of mineral metabolism in serum and urine. *Urol Res* 1979 ;7:161-170.
- 17-Gyawali PR, Joshi BR, Gurung CK. Correlation of calcium ,phosphorus ,uric acid a magnesium level in serum and 24 hours of patients with urolithiasis. *Kathmanda Univ Med J* 2011;34:54-56.
- 18 –Thamilselvan S, Khan S R, Menon M. Oxalate and calcium oxalate mediated free radical toxicity in renal epithelial cells:effect of antioxidant. *Urol Res*2003;31:3-9.
- 19- Talham D R, Backov R, Benitez I O, et al. Role of lipids in urinary stones:studies of calcium oxalate precipitation at phospholipid Langmuir monolayers. *Langmuir* 2006;22:2450-2456.
- 20-Selvam R. Calcium oxalate stone disease .role of lipid peroxidation an antioxidant. *Urol Res* 2002;30:35-47.
- 21- Alexander P, Kahn A, Elsner B H, et al. Kidney stones and subclinical Atherosclerosis in young adults:The CARDIA study. *The Journal of Urology* 2011;185: 920-925.
- 22- Baggio B. Genetic and dietary factors in idiopathic calcium nephrolithiasis: what do we have ,what do we need?. *J Nephrol* 1999;12:371-374.
- 23- Sumathi R, Jayanthi S, Uaralakshmi P. Impaired lipid metabolism in calcium –oxalate stone forming rat and DL alpha-lipoic acid supplementation. *Nutr Res* 1995;15:59-70.
- 24- Atakan I H, Kaplan M, Seven G, et al. Serum ,Urinary and stone zinc, iron, magnesium and copper levels in idiopathic calcium oxalate stone patients. *International Urology and Nephrology* 2007;39:351-356.
- 25- Ranqnekar GV, Gaur MS. Serum and urinary zinc levels in urolithiasis. *Br J Urol* 1993;71:527-529.
- 26- Powell S R. The Antioxidant Properties of Zinc. *Journal of Nutrition* 2000; 130:1447-1454.
- 27- Wikström B, Backman U, Danielson BG, et al. Phosphate metabolism in renal stone formers.(II): Relation to renal tubular function and calcium

- metabolism .Scand J Urol Nephrol 1981;61:1-26.
- 28- Curhan GC and Taylor EN. 24 –hr uric acid excretion and th risk of kidney stones. *Kidney Internatinal* 2008;73:489-496.
- 29-Ettinger B. Does hyperuricosuria play a role in calcium oxalate lithiasis ?. *J Urol* 1989;141:738-741.
- 30- Guthikonda S, Sinkey C, Barenz T, et al. Xanthine oxidase inhibition reverses endothelial dysfunction in heavey smokers. *Circulation* 2003;107:416-421.
- 31- Farquharson C A, Bulter R, Hill A, et al. Allopurinol improves endothelial dysfunction in chronic heart failure. *Circulation.* 2002; 106:221-226.
- 32- Gonte A, Genestar C, Grases F. Relation between calcium oxalate hydrate form in renal calculi and some urinary parameters. *Urol Int* 1990;45:25-27.
- 33- Kuo RL, Lingeman JE, Evan AP, et al. Urine calcium and volume predict coverage of renal papilla by Randall's plaque. *Kidney International* 2003; 64: 2150-2154.
- 34-Ozgurtas T, Yaku G, Gulec M, et al. Role of urinary zinc and copper on calcium oxalate stone formation. *Urol Int* 2004;72:233-236.
- 35-Goldfarb S. The role of diet in the pathogenesis and therapy of nephrolithiasis. *Endo Metab Clin North Am* 1990;19:805-820.