Biochemical Evaluation of Metabolically Active Urinary Stones

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Abstract

The current study had been conducted to assess the clinical significance of lipid peroxidation (malondialdehyde (MDA) concentration), (total antioxidant capacity (TAC) and superoxide dismutase (SOD), and biochemical test of [urea, creatinine (Cr), uric acid (U.A), calcium (Ca^{+2}), potassium (K^{+}), sodium (Na^{+}), phosphorus (PO_4^{-2})] in serum of urinary stone patients and also assess biochemical test of [urea, creatinine (Cr), uric acid (U.A), calcium (Ca^{+2}), potassium (K^{+}), sodium (Na^{+}), phosphorus ((PO_4^{-2}) and pH] in 24 hour urine sample. Sixty patients with metabolic active urinary stones had been admitted to AL-Hilla Teaching Hospital, Urology Department during the period of November 2013 to April 2014. All patients underwent full history and physical examination including: age, gender, family history of urolithiasis, past history of recurrent stone and any current medical diseases. The urinary stones group composed of sixty patients (48 male and 12 female), the control group includes apparently healthy individuals (18 males and 12) females. A highest occurrence of urinary stones is in the age 31-40 years. The results of the present study showed non-significant difference in urea and Cr and the result showed a significantly increase in U.A. Also the study showed a significant increase in the level of Ca^{+2} and non-significant in the level of Na and K and a significant decrease in the level of PO_4^{-2} .This study showed significant increase in MDA concentration and significant decrease in TAC and SOD , however showed significant increase in concentration of urea. Cr, U.A, pH, Na; and decrease in the level of Ca^{+2} and P and non-significant in level of K in 24 hour urine in the urinary stones group compared to the control group. In conclusion, urinary stone disease is affected by lipid peroxidation and antioxidant enzyme.

الخلاصة

صمم البحث الحالي لتقييم الدهون المؤكسدة (المالون ثنائي الديهايد (*MDA*) والقدرة الكلية لمضادات الأكسدة (*TAC*) وانزيم السوبر اوكسيد دسميوتيز (*SOD*) واجراء الفحوصات الكيميائية (اليوريا والكرياتنين وحامض اليوريك والكالسيوم والبوتاسيوم والصوديوم والفسفور) في دم مرضى الجهاز البولي وكذلك تم اجراء الفحوصات الكيميائية (اليوريا والكرياتنين وحامض اليوريك والكالسيوم والبوتاسيوم والصوديوم والفسفور والدالة الحامضية) في الادرار المجموع خلال ٢٤ ساعة .ستون مريضا مصابون بحصى الجهاز البولي اثناء دخولهم في مستشفى الحلة التعليمي في مدينة الحلة للفترة ما بين تتشرين الثاني ٢٠١٣ الى نيسان ٢٠١٤.تم اخذ التاريخ المرضي الكامل لكل مريض والمتضمن (العمر والجنس والتاريخ المرضي للعائلة ووجود تكوين حصى متكررة لدى المريض والامراض المزمنة الاخرى).عدد المرضى المصابون بحصى الجهاز البولي (٤٢ذكر و١٨ انثى) والمجموعة الضابطة (١٨ ذكر و١٢ انثى) .كانت اعلى نسبه لحدوث حصى الجهاز البولي في الفئة العمرية ٣١-٤٠ سنه ..وأثبتت الدراسة عدم وجود زياده ملحوظة في مستويات اليوريا ،كرياتنين،ووجدت الدراسة زياده في مستوى حامض اليوريك. وكذلك وجدت الدراسه زياده ملحوظة في مستوى الكالسيوم بينما مستوى الصوديوم والبوتاسيوم لا توجد زياده فيه ونقص ملحوظ في نسبة الفسفور،ووجدت زياده في مستوى المالون داي الديهايد ونقص في مستوى مضادات الأكسدة الاجمالي (*TAC*)، انزيم الدبيسموتاز الفائق (*SOD*) . وأثبتت الدراسة زيادة ملحوظة في مستويات اليوريا، كرياتنين، يورك اسد ، صوديوم ،الدالة الحامضية ونقص ملحوظ في نسبة الفسفور والكالسيوم وعدم وجود زياده ملحوظه في مستوى البوتاسيوم في الادرار المجموع خلال ٢٤ ساعة للمرضى المصابين بحصى الجهاز البولي مقارنة بالمجموعة المسيطرة .نستنتج مما سبق ان مرض حصى الجهاز البولي تتأثر بمستويات الدهون المؤكسدة وأنزيمات مضادات الاكسدة.

Introduction

Urinary calculi are poly crystalline aggregates composed of varying amounts of crystalloid and a small amount of organic matrix. Stone formation requires saturated urine that is dependent on pH, ionic strength, solute concentration, and complexation [1]. Urinary stones are typically classified by their location in the kidney (nephrolithiasis), ureter (ureterolithiasi) or bladder (cystolithiasis), or by their chemical composition (calcium-containing, struvite, uric acid, or other compounds) [2].

Lipid Peroxidation and Urolithiasis

The process of calcium stone formationstartsas a precipitation of calcium phosphate either in theloopofHenleor in the distal part of the distal tubule [3].Althoughthe urine at these levels of the nephron might be critically supersaturated with calciumoxalatein patients withhyeroxluriaand in experimental animals following administration of ethyleneglycol, theion activity product of calciumoxalate crystalformation. Any crystallization, that occurs in this part of the nephron most certainly, is acilitated by promoters and it has been suggested that lipoprotein membranes from the brush border of proximal tubular cells might serve this purpose the brush border membrane might be injured by free radicals formed as the result of toxic effects on the cell [4,5]. This might lead to lipid peroxidation and cell death [6]. The released membrane fragments that are transported down the nephron there by can supply a suitable surface for deposition of the calciumoxalate and calcium phosphate. Studies revealed that the lipid peroxidation products, thiobarbituric acid reactive substances, hydroperoxide, and dieneconjugates were excessively released in tissues of urolithicrats and in plasma of rates as well as stone patients [7, 8].

The study aimed to estimated some biochemical changes to the urolithiasis patients, and to estimate the correlation of such condition with oxidative stress status in addition to 24 h urine analysis.

Materials and Methods

Patient and Control Subjects:

A total of sixty urolithiatic patients in the age group ranging from 9-66 years old, admitted to Al-Hilla Teaching Hospital, Urology Department from the period of 1st of November 2013 till 30th of April 2014. Patients have had a radio-opaque stone (s) demonstrable on plane film of kidney, ureter and bladder X-ray (KUB) and intravenous urography (IVU). Thirty apparently healthy individuals were taken as a control group. This group comprises of 18 males their age ranging from 11-65 years, and 12 females their age ranging from 14-60 years. The patients had the following criteria: Demonstrated Radio-opaque stone(s) or radio-lucent stone(s) with detection of the stone size, site and position by using intravenous urography (IVU) and/or CT scanning .The patients were free of medical diseases including diabetes mellitus, hypertension, and rheumatologic disease. Pregnant patients had been excluded from the study group. Also all patients underwent full history and physical examination including: age, gender, smoking, family history of urolithiasis, past history of recurrent stone and any current medical diseases. Each person who contributed in control group underwent full history and physical examination including: age, gender, smoking, past history of diseases and medications All tests have been performed on serum in Biochemistry Department of Collage of Medicine in University of Babylon.

Methods:

Five to eight milliliters of blood were obtained from urinary stone patients and controls, then collected in tube without anticoagulants and were left for 15 minutes at room temperature to clot. After that, the blood samples were centrifuged at 1000-2000 xg for approximately 15 minutes. Then the sera were aspirated and

stored at (-20^{O}C) until use. Also ten samples collected for 24-hour urine. Collection of urine began to in the morning after wake up .The first urinationis discarded and the collection of urine was started then after 24 hour .The last sample was included.

Determination of serum urea, creatinine, uric acid, calcium, potassium, sodium and phosphorus concentration in serum and urine were done. These estimated using BIOLABO kit (France) according to the manufacturer's instructions [9,10].

Determination of Serum Total Antioxidant was done using Bio Vision developed the TAC assay Kit, which can measure either the combination of both small molecule antioxidants and proteins or small molecules alone in the presence of our proprietary protein Mask [9,10].

Determination of malondialdehyde (MDA) was done as described previously [11] in brief; to 150 µl of serum, 1 ml of 17.5% Tri chloroaceticacid, 1 ml of 0.6% Thiobarbuturic acid and 70% TCA was added. The supernatant was measured at 532 nm against reagent blank [12].

Determination of pH was done using PH meter

Determination of superoxide dismutase (SOD) was done as described previously [13] in brief; to 50µL of serum,75 mM of tris-HCL buffer, 30 mM of EDTA and 2mM of pyrogallol were added. An increase in absorbance was measured at 420nm for 30 min. The activity of SOD is expressed as u\ml of serum.

Statistics:

Student's t-test was used to determine the significant difference between two groups at p=0.05 level. When multiple means have been compared, significance (p=0.05) has been determined by analysis of variance (ANOVA), followed by Fisher's protected least significant difference test (LSD). While the correlation between two variables have been estimated by Pearsons's correlation coefficients at 0.05 level

Results

Urinary Stones Sampling Characteristic

1-Gender and Renal Stones

Among 60 patients with urinary stones who contributed to this study, there were (48) males and (12) females (Table-1).

Table 1: The sampling characteristics of study groups

Gender	Patients (n=60)	Control (n=30)
Male	48	12
Female	12	18

Biochemical studies

1. Total antioxidant, Malondialdehyde and Superoxide dismutase

The results in table 2 showed significant difference (p<0.01) in oxidant and antioxidant in sera of patients when compared with control group.

Table 2: Oxidants and antioxidants in sera of urinary stones and control group

Biochemical test	Control Mean± SD	Patient Mean ±SD	p-value
TACmmol/l	18.769±1.645	6.148±1.868	P<0.01
MDA(µmol/L)	2.493±0.850	7.845±1.134	P<0.01
SOD U/ml	0.836±0.085	0.541±0.149	P<0.01

2. Estimation Function of Kidney:

Blood urea and creatinine found to be non-significant between patients and control group (P<0.34, P<0.08) respectively in table (3).Uric acid showed significantly difference in urinary stone patients in comparison with control groups (P<0.01).

Table 3: Mean and standard deviation of biochemical changes in sera of urinary stone patients and control group

Biochemical test	Control Mean ±SD	patient Mean ±SD	p-value
Urea(mg/dl)	30.225±0.030	30.175±0.030	P<0.34
Creatinine(mg/dl)	0.529±0.004	0.521±0.003	P<0.08
Uric acid(mg/dl)	5.109±1.3566	6.28±1.264	P<0.01

Sodium, potassium, Calcium and phosphorus:

The results showed non-significant difference in Na^+ and K^+ concentration in sera of renal stones group compared with Na^+ and K^+ concentration in sera of the control, (P<0.48) (P<0.78) respectively (Table 4).

The result showed a significant difference (p<0.01) in ionized calcium concentration in sera of renal stone group compared with ionized calcium concentration in sera of the control group .On the other hand the current results showed significant difference (p<0.001) in phosphorus concentration in sera of renal stones group compared with phosphorus concentration in sera of the control (Table 4).

Table 4: Mean and standard deviation of biochemical changes in sera of urinary stones and control group

Biochemical test	Control Mean ±SD	patient Mean ±SD	p-value
Calcium(mg/dl)	9.61±0.706	10.64±2.04	P<0.01
Sodium(mEq/L)	143.035±0.005	143.039±0.003	P<0.48
Potassium(mEq/L)	4.057±0.006	4.055±0.004	P<0.78
Inorganic phosphorus(mg/dl)	4.471±1.348	3.175±0.826	P<0.01

Twenty Four Hours Urine

The urinary urea, Cr,U.A, PO_4^{-2}, pH, K^+ and Na^+ concentration were persistently higher (P<0.01), in patients when compared with control groups. However a significantly decrease (P<0.01) in PO_4^{-2} and Ca^{+2} were observed in stone formers when compared with control groups.

Table 4: Mean and standard deviation of biochemical changes in sera of renal stones and control group in 24 hour urine

Biochemical test	Control Mean±SD	Patient Mean±SD	P value
Urea(mg/day)	1744.93±106.04	1850.2±67.66	P<0.01
Creatinine(mg/day)	15.75±2.91	16.6±2.74	P<0.01

Uric acid(mg/day)	480.01±149.51	528.63±126.92	P<0.01
Sodium (mEq/day)	109.396±55.88	113.425±57.80	P<0.01
Potassium(mEq/day)	76.09±28.47	86.04±23.81	P<0.01
Inorganic Phosphorus(mg/day)	809.53±223.68	782.59±217.94	P<0.01
Calcium(mg/day)	213.80±51.25	200.45±54.32	P<0.01
PH	5.62±0.52	7.84±0.55	P<0.01
Volume of urine(ml)	1065±368.21	1100±386.58	P<0.01

Discussion

Results showed that those were an increment in the rate of males compared to females, which is consistent with other studies Guest *et al*. [14]. The endogenous estrogen and estrogen treatment in postmenopausal women may decrease the risk of stone recurrence by lowering urinary calcium and calcium oxalate saturation. Estrogen may also help to prevent the formation of calcium stones by keeping urine alkaline and raising protective citrate levels. Experiments in animals demonstrated that testosterone promoted crystal growth by suppressing osteopontin expression in the kidney and increasing urinary oxalate excretion while estrogen possibly inhibited stone formation by increasing osteopontin expression in the kidney and decreasing urinary oxalate excretion [15]. The lower serum testosterone level may contribute to some of the protection of women and children against oxalate stones. This factor could lead to the higher incidence of urinary stones cases in males than females observed in same study [16]. A significant increase in MDA concentration has an agreement with previous studies [17]. While other workers had detected a significant increase in MDA concentration in plasma, erythrocytes [18] and urine of urolithiatic patients [19]. However, this elevation has been supported by experimental rat studies which have been reported an elevation in lipid peroxidation in induced calcium oxalate nephrolithiasis rats administered sodium oxalate [20].

The condition which enhance peroxidation and depletion of thiol content increase the oxalate binding activity, which in turn promotes nucleation and aggregation property of stone matrix protein fractions. This behavior is also associated with peroxidized mitochondria and nuclei, suggesting that the peroxidation can be a causative factor for the initial stage of stone formation [21]. Suresh et al. [22] suggested that the statistically decrease in total antioxidant in kidney stone patients as compared to controls. Increased levels of TAC indicate to absorption uric acid and the activation of antioxidant enzymes as an adaptation to the oxidative stress [23]. In addition, high concentration of a number of metabolites, including uric acid can lead to pro-oxidant effects, introducing a further decrease of the antioxidant capacity [24]. Which agree with the result of other study done by Gyawali [25]. Waste products are normally present in the blood, and the concentration of each varies with in a normal range .It may be concluded that the kidneys are excreting these wastes at normal rates [26] and urinary stones cannot cause a blockage in the flow of urine .Furthermore, uric acid showed a significantly increased ($p<0.01$) in urinary stone patients in comparison with control groups which agreed with the results of Brian, [27]. Uric acid are very sensitive to dietary influences; High intake of dietary protein can causes increase level of uric acid. The results showed no significant differences in Na^+ concentration in sera of

renal stones group compared with Na concentration in sera of the control, as in table (4), which agree with the results of the study of Jafari *et al*. [28].

Dietary sodium increases the risk of urolithiasis. Salt intake expands intravascular volume, which can increase urinary calcium level, likely by decreasing renal tubular calcium reabsorption. Increase salt intake can induce mild systemic metabolic acidosis, which can lower urinary citrate level, and increase the risk of calcium precipitate in kidneys. The potassium, in the present study, showed no significant difference ($P<0.08$) which agree, with the results of Mert *et al.* [29]. The result showed a significant increase ($p<0.01$) in ionized calcium concentration in sera of renal stone group compared with ionized calcium concentration in sera of the control group as in table (3)**.**and in agreement with David [30] and Deska and Timothy [31]. The availability of calcium for stone formation depends ultimately on the dietary intake, intestinal absorption, excretion in the faeces transport across cell membranes from the extracellular to the intracellular components of the body fluids and renal tubular reabsorption of calcium. The study of Shaker *et al.* [32] found that the mean ± SD of phosphorus concentration in renal stone and control group were 2.10 ± 0.86 and 3.39 ± 0.75 mg/dl, respectively.

The present study agrees with Ahmed *et al.*, which showed the low urinary volume [33], despite the evidence that the urine volume was an important risk factor for all parameters evaluated. The study by Borghi *et al.* confirms that urine volume is a real risk factor in urolithiasis and increase in fluid intake to at least 2 L/day is the initial therapy for the prevention of stones recurrence [34]. It is generally believed that an increased amount of fluid intake associated with reduced stone formation probably through dilation, which consequently reduced the urinary saturation levels [35].

Urine pH found to be increased significantly ($P<0.01$) in patient as in table (5). The formation of various type of kidney stones strongly influenced by urinary pH. An alkaline pH favours the crystallization of calcium and phosphate containing stones .Where an acidic urine pH promotes uric acid or cysteine stones [36]. Moreover change in the systemic pH hemostasis as seen in the form of chronic metabolic acidosis alter urine concentration of substances that contribute to crystal formation (e.g. Ca^{+2}, PO_4^{-2}) as well as of substance that may prevent stone formation (e.g. citrate, magnesium) [37].

Urine Ca^{+2} found to be decreased significantly ($P<0.01$) in patient, as in table (5), while other study shows increase in the Ca^{+2} concentration in patients group [38]. There is a close relationship between sodium and calcium excretion, sodium excretion depend on the intake of sodium containing salt, mainly sodium chloride .As each gram of NaCl contains 17 mmol of both Na and Cl. The relationship between calcium excretion and calcium intake are complex. Sodium and protein can affect calcium excretion even more than calcium intake itself. The low frequency of hypercalciuria may be due to the presence of stones fragments in the urinary tract that attract urinary calcium to participate in the crystallization process as many researchers have recommended metabolic evaluation to be performed in control groups [39]. It is reported in most published series as Laube *et al.* [40] that high dietary calcium intake decrease the absorption of oxalate in the intestinal lumen by making complexes with calcium. It seems probable that in patient group, the increase oxalate excretion is due to a fall in the intraluminal calcium concentration. This fact could be attributed to the poor consumption of calcium rich food or it might be the consequences of high intake of oxalate food [41]. The present study showed a significant decrease in PO_4^{-2} concentration in urine with ($P<0.01$) as shown in table (5.), while other study

shows an increase in the concentration of PO_4^{-2} as Siener *et al.* [42]. On the other hand, the study showed a significant increase in Na^+ concentration in urine with ($P<0.01$), as shown in table (5.). This result agrees with the results of Ahmed et al. [33]. Also it showed that a significant increase in K^+ concentration in urine with ($P<0.01$) as shown in table (5), and showed a significant increase in amount of urea in urine with ($P<0.01$) as well as Cr. This was agreed with the result demonstrate by Hivre *et al.* [43]. There was a significant increase in level of uric acid in urine with ($P<0.01$). The chances of stones formation increase with increasing serum uric acid levels and urine excretion rates. Higher sodium excretion rates also increase uric acid excretion and decrease urinary citrate excretion [44]. The management of uric acid stones involves with low protein/purine diet, large amount of fluids, and alkalization of urine [45].

Animal protein is also the major dietary source of purines, the precursors of uric acid. Excessive intake of animal protein is therefore associated with hyperuricosuria, a condition present in some uric acid stone formers. More importantly, uric acid solubility is largely determined by the urinary pH. As the pH falls below 5.5 to 6.0, the solubility of uric acid decrease, and uric acid precipitate, even if hyperuricosuria is not present. One last important link is between dietary protein and stones which is the decrease in calcium oxalate solubility caused by uric acid .As a result, hyperuricosuria is also associated with calcium stone formation [46]. Uric acid may provide heterogeneous nuclei for calcium oxalate stone formation [47]. In patients with urinary stones, metabolic evaluation and intervention should be considered to prevent the recurrence of stones [48].

References

1. Valerie CS. and Tina S.; (2010). "Essentials of physiology and Anatomy". 6th.ed. Philadelphia. p:421-424.

2. Al-Saadi S M.S.C. "Biochemical and gentic study in urinary stone patients in Babylon city. M.Sc Thesis University of Babylon "Medicine Collage. (2014).

3. Aspline JR., Mandel NS. and Coe FL.; (1996)."Evidence or Calcium Phosphate Super saturation in the LOOP of Henle". Am J Physiol.270.P:604-617.

4. Davison AM., Gameron JS., Grunfeld J. and et al. (2005)."Oxford Text Book of Nephrology".3 rded. Oxford University Press. P:1199-1210.

5. Khan SR.; (1996). "Calcium Oxalate Crystal Interaction with Renal Epithelium, Mechanism of Crystal Adhesion and its Impact on Stone Development". J Urolo Res.23.P:71-79.

6. Thamilselvan S. and Khan SR.; (1998)."Oxalate and Calcium Crystal are Injurious to Renal Epithelium Cells: Results in Vivo and in Vitro Studies". J Nephro l11.P:66-69.

7. Selvam R.; (2002)."Calcium Oxalate Stone Disease:Role of Lipid Peroxidation and Antioxidants". J Urol Res., 30 (1): 35-47.

8. Singh PP. and Barjatiya MK.; (2002)."Peroxidation Stress and Antioxidant Status in Relation to Age in Normal and Renal Stone Formers". Ind J Nephro l12. P:10-15.

9. Herspring K J.; (2008). "Appl Physiol".105.P:1889-1896.

10. Hirai D.; (2011) "Acute antioxidant supplementation and skeletal muscle vascular conductance in aged rats: role of exercise and fiber type". Am J physiol Heart Circphysiol. 300: H1536-H1544.

11. Yagi K.; (1987) "Lipid peroxides and human diseases". Chem Phys Lipids. 45. P: 337–351.

12. Magnani L., Gaydou M., Hubaud JC. (2000). "Spectrophotometric measurement of antioxidant properties pf flavones and flavones against superoxide anion". Anal. Chim. Acta 411, 1-2, 1. P: 209-216.

13. Marklund S.; (1974). "Involvement of the superoxide anion radical in the auto-oxidation of pyrogallol

and a convenient assay for superoxide dismutase" .Eur.J.Biochem47.P:469-474.

14. Guest AR., Cohan RH., Korobkin M., et al.(2001)."Assessment of the clinical utility of the rim and comet-tail signs in differentiating renal stones from phleboliths". AJR Am J Roentgenol; 177 (6). P: 1285-91.

15. Abbagani S., GundimedaS. ,Varre S. and *et al*. (2010). "Kidney Stone Disease: Etology AND Evaluation". Int. J. App. Biol, Pharmaceut. Technol., (1). P:175-182.

16. Cavendish M.; (2008). "Kidney disorders". Diseases and Disorders 2 (1st ed.). Tarrytown, New York: Marshall Cavendish Corporation. P:490-3

17. Bet VV., Deshpande KH., Suryakar An and *et al.* (2006)."Depleted Nitrite and Enhanced Oxidative Stress in Urilithiasis". Ind J Clin Biochem., 21(2): 177-180.

18. Buxi J., Sharma K., Mehta and *et al.* (1994). "Superoxide Dismutase and Malondialdehyde Levels in Urinary Disorders ".Ind J Clinc Biochem 19(1): 47-49.

19. Tungasanga K., Sriboonlue P., Futrakul P. and *et al.* (2004). "Renal Tubular Cell Damage and Oxidative Stress in Renal Stone Patients and the Effect of Potassium Citrate Treatment". J Urol Res.33 (1): 56-69.

20. Relvam R. and Bijikurien T.; (1987). "Induction of Lipid Peroxidation by Oxalate in Experimental Rat Urolithiasis". J Biosce 12(4): 367-373.

21. Thamilselvan S. and Mani M.; (2005)."Vitamin E Therapy Pevents Hyperoxaluria Induced Calcium Oxalate Crystal Deposition in the Kidney Improving Renal Tissue Antioxidant Status". B J U Intern. 96 (1): 117-126.

22. Suresh C., Wilma D., Rajni A.; (2008). " Lipid Peroxidation and Total Antioxidant Capacity in Patients with Chronic Renal Failure". Asian Journal of Biochemistry3.P: 315-319.

23. Elif D., Arisan S., Murat K .;(2007)."Antioxidant enzyme, advance in moleculer Biology". (1).P:41-46.

24. Khan S., Thamilselvan S. (2000). Nephrolithiasis: a consequence of renal epithelial cell exposure to oxalate and calcium oxalate crystals. Mol Urol, 4.P: 305–312.

25. Gyawali PR, Joshi BR, Gurung CK. (2011). "Correlation of calcium, phosphorus, uric acid and Magnesium Level in Serum and 24 Hours Urine of Patients with Urolithiasis" Kathmandu University medical journal 9(2):45-56.

26. Scanlon V. and Sanders T.; (2011)."Essential of Anatomy and Physiology ".6th ed .p:462.

27. Brian RM.; (2010)."Medical evaluation and management of urolithiasis". 2(1). P:3-9.

28. Jafari H., Barzin M., Salehifar E., et al., (2014)."Etiologic and Epidemiologic Pattern of Urolithiasis in North Iran; Review of 10-Year Findings" 24(1) :69-74.

29. Mert I., Ergene B., Suntar I. *et al.* (2014). "Preclinical Evaluation of Antiurolithiatic Activity of Viburnum Opulus L. On Sodium Oxalate –Induced Urolithiasis Rat Model". 578103.p:10.

30. David S.; (1996)." Laboratory Test Handbook". 4th ed. Lexi-Comp Inc. p: 798.

31. Deska K., and Timothy JP; (1998). Diagnostic and Laboratory Test Reference. St. Louis: Mosby-Year Book, Inc. p: 1154-1155.

32. Shaker RA. Al-Gazally ME Al-Jaff EH.2012.' The Measurement of Intact Parathyroid Hormone and Serum Electrolytes and Their Relation with Renal Stones in Hilla City" MJB. 9(1).P:159-156.

33. Ahmed I., PanasotaMS., Tariq M., *et al.* (2013). "Frequency of metabolic Abnormalities in urinary stones patients". Pak. J. Mea. SCI 29 (6):1363-1366.

34. Borghi L., Mesch T., AmacoF. *et al.* (1996). "Urinary Volume, Water and Recurrence in Idiopathic Calicum Nephrolithiasis": 5-Year Randomized Prospective Study. J. Urol. 155 (839):43.

35. Borghhi L., Schianchi T., Meschi T. et al. (2002). "Comparison of Two

Diets for Prevention of Recurrent Stones in Idiopathic Hypercalciuria". N Engl J Med., 346.P:77-84.

36. Wagner CA., Mohebbi N. (2010). "Urinary pH and Stone Formation ". J of Nephrology, 23(16): 165-169.

37. Devuysto O. and Pirson X. (2007). "Genticsof Hypercalciuriua Stone Forming Disease", Kidney Lat. (72): 1065-1072.

38. Gyawali PR.; (2011). "Correlation of Calcium, Phosphate,Uric Acid and Magnesium in Serum and 24 Hours Urine of Patents With Urolithiasis". Kathmandu Univ Med J., 34(2).P:54-6

39. Pak CY., Odvina CV., Pearle MS, et al., (2005) "Effect of Diatery Modifcation on Urinary Stone Risk Factors". Kidney Int. 68(5). 2264.P:73.

40. Laube N., Pullmann M., Hergarten S. *et al.* (2003). "Influences of Urinary Stones on the composition of 24-hour urine sample". Clin Chem. 49 (281). P:5.

41. Liebman M., Costa G. (2000). "Effect of Calcium and Magnesium on Urinary Oxalate Excretion After Oxalate Loads". J Urol. 163(1565).P:9.

42. Siener R., Eber D., Nicolay C., Hesse A. (2003). "Dietary Risk Factor for Hyperoxaluriaformers". Kidney Int. 630 (1037): 43.

43. Hivre MD., Bhale DV., Bujurge AA., Mahat RK. (2013)"A study of 24 hour urinary Calium, phosphorus and uric acid levels in normal subjects and patients with urolithiasis". Int. J. Recent Trends in Sci. Technol., 9 (2):179-198.

44. Nayan M., Elkoushy M., Andonian S. (2012). "Variations between Two 24-Hour Urine Collections In Patients Presenting To a Tertiary Stone Clinic ". 6(1): 30-3.

45. Goldfarb D. and Coe R. (1999). "Prevent of recurrent nephrolithiasis". American Academy of Family Physicians; 60(8): 2269-2276.

46. Malhotra KK. (2008)."Medical aspect of renal stones". Indian Academy of clinical medicine. 9(4): 282-286.

47. Heilberq IP. And Schor N. (2006). "Renal stones disease: causes, evaluation and medical treatment" Araq Bras Endocrinol Metab 50(4): 823-831.

48. Elfadil GA., Ibrahim ME., Ahmed SA. (2010). "Metabolic Constituents of Urinary Stone Composition in Sudanese Children". Egypt Acad J Sci.2 (2): 21-25.