

STUDY OF SOME BIOCHEMICAL CHANGES IN URINE AND SERUM OF EXPERIMENTALLY- INDUCED UROLITHIASIS RABBITS BY SODIUM OXALATE

Arwa , H.M.AL-Saeed

Department of chemistry , College of Science , University of Basrah ,Basrah , Iraq.
(Received 7 April 2013,Accepted 29 May 2013)

Keywords; Serum ,Rabbits ,Sodium oxalate.

ABSTRACT

The present study was undertaken to find the alteration in some urine and serum parameters of urolithiasis rabbits induced by sodium oxalate .Animals were divided into two groups six in each, first group animals received only normal saline (0.9%) as control group , while second group animals were treated with sodium oxalate (70 mg / Kg b.w intraperitoneal) daily for 15 days as treatment group . The results of urine analysis indicated that increased urine specific gravity , protein level and leukocytes , while decreased pH in treatment group as compared with control group , also observed that hemolysis in urine but there was no significant difference ($p > 0.05$) in urine level of glucose , Ketones , nitrite , urobilinogen and bilirubin in treatment group as compared with control group . The results of serum analysis indicated that there was no significant difference ($p > 0.05$) in serum level of calcium and phosphorus , while , increased serum level of sodium , potassium , uric acid and creatinine in treatment group as compared with control group . The study indicated that there was some changes in both urine and serum parameters in urolithiasis .

INTRODUCTION

Urolithiasis is the condition where urinary calculi are formed or located anywhere in the urinary system or the process of forming stones in the kidney , bladder or ureters [1] . Globally , urolithiasis is third most common urological disease affecting both males and females [2] .The most common stone is calcium oxalate , while the hardest stone is cystine monohydrate .Other stone composition include triple phosphate , ammonium , magnesium , urate and xanthine [3] . The pathogenesis of stone formation is a

multifactorial process. A prerequisite for urinary stone formation is urinary crystal formation for this, urine must be supersaturated [4]. The crystallization potential of urine is related not only to the concentration of salt, but also to the presence or absence of other compounds such as inhibitors, complexors or promoters [5]. Risk factors for urolithiasis include age, sex, diet, geographic location, genetic predisposition and urinary composition. Urinary composition determination stone formation based on three factors exceeding the formation product of stone formation components, the quantity of inhibitors (e.g citrate and promoters (e.g sodium, uratesetc.) in the urine [6].

The purpose of this study was to determine the alteration in urine and serum parameters of urolithiasis case.

MATERIALS AND METHODS

Experimental Animals :

Male domestic rabbits weighing about 1500gm were used for this study. The animals were divided into two groups six in each. The first group animals received only 0.9 % normal saline intraperitoneally consider control group, while the second group animals were injected intraperitoneally with sodium oxalate (70 mg / Kg b.w / day) for 15 days consider treatment group [7]. After 15 days rabbits from each group were placed in cages for 24 h for urine collection, blood samples were drawn from the heart of overnight fasted animals into plastic syringes. The serum was separated by centrifugation at 3000rpm for 10 minutes.

Urinalysis

Urine samples were analysed to know the changes in biochemical constituents of urine by using urine test strips [8]. After urine collection each sample was put in clean test tube and a test strip was immersed inside the urine sample for half a minute then taken out and then excess amount of urine was wiped on the rim of test tube then the strip let to dry for a minute and then comparison of colors of tests found in the test strip matched with the indicator scale color found in container of strips to know the intensity of change in color of the indicator which indicates the reaction of the urine content with the indicators found on the test strip in case of change of the biochemical components of urine.

Serum Analysis

The concentration of serum calcium , sodium , potassium , phosphorus , uric acid and creatinine were estimated by standard colorimetric procedures [9] , [10] , [11] , [12] , [13] and [14] .

Statistical Analysis

The data were expressed as mean \pm standard deviation , the statistical analysis of the data was performed by univariate analysis of variance and $p \leq 0.05$ was considered significant . Least significant different test (LSD) was used to test the difference between groups .

RESULTS

Table (1) indicated that urine samples showed negative results for glucose , ketones , nitrite and bilirubin in both control and treatment groups ,while we observed that significant increase ($p \leq 0.05$) in urine specific gravity and leukocytes , high significance increased ($p < 0.01$) in protein level in treatment group as compared with control group . The results indicated that hemolysis in urine samples in treatment group as compared with control group .Urine samples showed that altered pH value below 7, while the results indicated no significant difference ($p > 0.05$) in urobilinogen in treatment group as compared with control group .

Table 1 : Results of urine analysis

Parameters	Control group	Treatment group
Specific gravity	1.015 \pm 0.01	1.030 \pm 0.05 *
pH	8 \pm 1.00	5 \pm 1.50*
Glucose (mg/ dl)	-	-
Protein (mg/ dl)	-	30.40 \pm 5.10**
Ketones (mg/ dl)	-	-
Blood (RBC/ μ l)	-	10 \pm 2.10*
Nitrite	-	-
Leukocytes (WBC/ μ l)	-	25 \pm 1.05*
Urobilinogen (mg/ dl)	0.1 \pm 0.02	0.3 \pm 0.05
Bilirubin (mg/ dl)	-	-

Mean \pm SD , *P \leq 0.05 , **P \leq 0.01

The results of serum analysis indicated that high significance increased ($p < 0.01$) in serum level of sodium, uric acid and creatinine , significance increased ($p < 0.05$) in potassium level in treatment group as compared with control group ,While no

significance difference ($p>0.05$) in serum level of calcium and phosphorus in treatment group as compared with control group as shown in table 2 .

Table 2 : Results of serum analysis

Parameters	Control group	Treatment group
Calcium(mmol/L)	2.90±0.11	3.12±0.20
Sodium(mmol/L)	135.21±3.51	148.31±4.50**
Potassium (mg/dl)	5.21±0.35	8.41±0.53*
Phosphorus (mmol/L)	3.51±0.12	2.88±0.31
Uric acid (mg/dl)	2.65±0.25	5.50±0.34**
Creatinine (mg/dl)	1.52±0.31	3.12±0.42**

Mean±SD , *P≤ 0.05 , **P ≤ 0.01

DISCUSSION

The specific gravity (S.G) reagent on the strip is sensitive to the number of ions in the urine specimen and is a measure of urine concentration , in the present study we observed that increased urine specific gravity in treatment group may be due to high concentration of salts in urine as refer by [15] increased specific gravity in renal disease .PH is a measure of hydrogen ion concentration of the urine , so the results indicated that pH value below [normal range 8.2] this result may be promotes the formation of calcium oxalate as refer by [16] . Typically , glucose does not appear in the urine until the plasma level exceeds (180-120 mg / dl) , so the results indicated that glucose test is negative . The urine strip is very sensitive to intact red blood cells and even more so to free hemoglobin , the results indicated that hemolysis in urine , this may be due to tubular disorders or bladder stone [17] . Nitrite in the urine normally negative , as show in the present study , because the presence of nitrite usually indicates a urinary tract infection caused from members of the gram-negative rods such as E.coli which are able to reduce dietary nitrate to nitrite [18] , so this result refer that no presence of bacteria in urinary tract . The results indicated that presence of leukocyte this result may be due to the inflammatory process in the urinary tract because of presence of stones [19] . In the present study we observed that there is no significant difference ($p>0.05$) in bilirubin and urobilinogen level in the urine , these pigments that are cleared by the liver .

The results of serum analysis indicated that no significant difference ($p>0.05$) in serum level of calcium and phosphorus in treatment group as compared with control group ,while the results indicated there is high significant increase ($p<0.01$) in serum level of sodium , uric acid and creatinine , significant increase ($p<0.05$) in potassium level in treatment group as compared with control group , this may be due to administration sodium oxalate results in hyperoxaluria [20] which consider a major risk factor in the pathogenesis of renal stones [21] , so that the glomerular filtration rate decreases due to the obstruction of the outflow of the urine by stones in kidney . The waste products , particularly creatinine and uric acid accumulate in the blood [22] . which indicated that there was a marked renal damage .

دراسة بعض التغيرات البايوكيميائية في الأدرار ومصل الدم للأرناب المصابة بالحصى البولية المستحدثة بواسطة أوكزالات الصوديوم

أروى حميد محمود السعيد

قسم الكيمياء ,كلية العلوم , جامعة البصرة , البصرة , العراق.

الخلاصة

أجريت هذه الدراسة لمعرفة التغير في بعض معايير الأدرار و مصل الدم للأرناب المصابة بالحصى البولية المستحدثة بواسطة أوكزالات الصوديوم . قسمت الحيوانات الى مجموعتين (سنة لكل مجموعة) حيوانات المجموعة الاولى أعطيت المحلول الملحي الطبيعي (0,9%) كمجموعة سيطرة بينما عوملت حيوانات المجموعة الثانية مع أوكزالات الصوديوم (70 ملغم / كغم من وزن الجسم) يوميا لمدة 15 يوم كمجموعة معاملة . أظهرت نتائج تحليل الأدرار زيادة الكثافة النوعية , مستوى البروتين وكريات الدم البيضاء بينما أنخفضت الدالة الحامضية في مجموعة المعاملة مقارنة بمجموعة السيطرة كما لوحظ حدوث تحلل كريات الدم الحمراء في الأدرار لكن لم يحدث فرق معنوي ($p>0.05$) في مستوى الكلوكوز , الكيتونات , النايتريت , يوروبلنوجين و البلروبين في مجموعة المعاملة مقارنة بمجموعة السيطرة . أظهرت نتائج تحليل مصل الدم عدم وجود فرق معنوي ($p> 0.05$) في مستوى الكالسيوم و الفسفور بينما لوحظ ارتفاع مستوى الصوديوم , البوتاسيوم , حامض اليوريك , و الكرياتينين في مجموعة المعاملة مقارنة بمجموعة السيطرة. أظهرت الدراسة الحالية حصول بعض التغيرات في معايير الأدرار ومصل الدم في حالة الحصى البولية .

REFERENCES

- 1- **Pearle M.S; Calhoun, E.A and Curhan, G.C. (2007).**Chapter 8: Urolithiasis . In Litwin, MS; Saigal, CS. *Urologic Diseases in America (NIH Publication No. 07–5512)*. Bethesda, Maryland: US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. pp. 283–319.
- 2- **Stamatiou, K.N; Karanasion ,V.I. ; Lacrois, R .E. ; Kavouras , N.G .; Papadimitriou , V.T. and Chlopsios , C.(2006) .** Prevalence of urolithiasis in rural Thebes, Greece. *Rural Remote Health.* 6:610.
- 3- **Materazzi, S. ; Curini, R. ; D'Ascenzo, G. and Magri , A.D. (1995).** TG-FTIR coupled analysis applied to the studies in urolithiasis: characterization of human renal calculi. *Termochimica Acta*, volume 264, 75--93.
- 4- **Randall, A. (1937) .** The origin and growth of renal calculi. *Ann.Surg.* 105: 1009-1020.
- 5- **Elliot, J. S. (1973) .** Structure and composition of urinary calculi. *J. Urol.(Baltimore)* 109, 82–83.
- 6- **Unal , D. ; Yeni , E .; Verit, A. and Karatas O.F. (2005) .** Prognostic factors effecting on recurrence of urinary stone disease: a multivariate analysis of everyday patient parameters. *Int Urol Nephrol.*;37:447-52.
- 7- **Bhaskar, K. and Selvam, R. (1985) .** *Arogya–J. Health Sci.*, 11, 48.
- 8- **Khudayer , A . N . and Hassan , B.F.(2011) .** Effect of age and some reproductive states on biochemical constituents of cows urine and relation with some disease conditions . *Basrh J.of Agri.Sci.*
- 9- **Barnett, R.N. ; Skodon, S.B. and Goldberg , M.H.(1973).** Performance “ of Kits used for clinical chemical analysis of calcium in serum . *Amer.J.Clin. Pathol.* 59, 836-845
- 10- **Gitelman, H. (1967) .** *Anal . Biochem .* 20 , 521 .
- 11- **Henry, R. J. (1974) .** *Clinical Chemistry . Harper and Row New York , Sec. Edit.* 643 .
- 12- **Tietz , N.W. Fundamentals of Clinical Chemistry . (2006).** Saunders Philadelphia , 4th Edit. 984
- 13- **Fossati, P. ; Prencipe , L. and Berti, G. (1980) .** Use of 3,5 –dichloro-2- hydroxy benzenesulfonic acid / 4-aminophenazone chromogein system in direct enzymic assay of uric acid in serum and urine .*Clin. Chem.* 261 : 227-273.

- 14- Bartels , H. ; Bohmer , M. and Heierli , C. (1972) .** Serum creatinine determination without protein precipitation . Clin. Chim. Acta. 37 : 193-197 .
- 15- Kumar A.(2004) .** Urine examination for calculogenic crystals--a newer approach using refrigeration. Trop Doct.; 34:153-155.
- 16- Robertson , W.G. and Peacock , M. (1980).** The cause of idiopathic calcium stone disease: hypercalciuria or hyperoxaluria.Nephron . 26 :105-10.
- 17- Santer , R. ; Kinner , M. and Lassen ,C.L .(2003).** Molecular analysis of the SGLT2 gene in patients with renal glucosuria. J Am Soc Nephrol .14(11):2873–82.
- 18 - Vehaskari ,V.M; Rapola , J. and Koskimies , O. (1979).** Microscopic hematuria in school children: epidemiology and clinicopathologic evaluation. J Pediatr;95(5 Pt 1):676 –84.
- 19- Gorelick, M.H .and Shaw, K.N.(1999) .** Screening tests for urinary tract infection in children: a meta-analysis. Pediatrics;104(5):E54.
- 20-Parks , J. H. ; Fredric , L.C. and Asplin, J. R. (1992).** The pathogenesis and treatment of kidney stones . N.Engl. J. Med. 327 : 1141 .
- 21-Tiselius , H.G.(1997) .** Risk formulas in calcium oxalate urolithiasis, World J Urol;15:176-85.
- 22- Divakar, K. ; Pawar , A .T; Chandrasekhar , S. ; Dighe, S. and Divakar ,G.(2010) .** Protective effect of hydroalcoholic extract of *Rubia cardifolia* root against ethylene glycol induced urolithiasis in rats. Food Chem Toxicol .48:1013-8.

