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\textsuperscript{2+}), potassium (K\textsuperscript{+}), sodium (Na\textsuperscript{+}), phosphorus (PO\textsubscript{4}\textsuperscript{3-})] in serum of urinary stone patients and also assess biochemical test of [urea, creatinine (Cr), uric acid (U.A), calcium (Ca\textsuperscript{2+}), potassium (K\textsuperscript{+}), sodium (Na\textsuperscript{+}), phosphorus (PO\textsubscript{4}\textsuperscript{3-}) 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\textsuperscript{2+} and non-significant in the level of Na and K and a significant decrease in the level of PO\textsubscript{4}\textsuperscript{3-}. 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\textsuperscript{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.

التاريخ

المبحث الحالي لتقديم الدهون المؤكدة (المالون ثنائي النايتيدايد TAC) والقدرة الكلية لمضادات الأكسدة (MDA) وازنزم السوبر-

SOD وأجزاء الفحوصات الكيميائية (البوريا والكتانيت والكالسيوم والبوتاسيوم والكيوليوم والفسفور) في دم مستوى حمض البولي والكالسيوم والبوتاسيوم والكيوليوم والفسفور والالتهاب الحضاري (لا) في الادوار المجموع خلال 24 ساعت. ستون مريضا مصابون بحبيص الجهاز البولي أثناء دخولهم في مستشفى الجلة التعليمي في مدينة الجلة للفترة ما بين 2013 إلى 2014. تم اتخاذ التاريخ المرضي الكامل لكل مريض والمصدم (العمر والجنس والتدخين والمرض) للسماح بالبحث. والمرضى المصابين بحبيص الجهاز البولي 42 ذكر و18 اثنا (18 ذكر و12 اثنا). كانت اتاحة نسبة للحبيص الحبيص البولي في الغلة العاملية 32.01

ستون. وأثبتت الدراسة عدم وجود زيادة ملحوبة في مستويات البوسيرة الكتانيت ووجود الدراسة زيادة في مستوي حمض البولي. وكذلك يوجد الدراسة زيادة ملحوبة في مستوي الكالسيوم بينما مستوي الصوديوم والبوتاسيوم لا يوجد زيادة فيه يوotic ومسحوق في مستوى الصوديوم ووجدت

SOD في مستوي الصوديوم يومي البولي وقص في مستوي مضادات الأكسدة الإنجالي (TAC)، أنزيم السوبر-فانتاز (SOD) في مستوي الصوديوم يومي البولي وقص في مستوي مضادات الأكسدة الإنجالي (TAC)، أنزيم السوبر-فانتاز (SOD) في مستوي الصوديوم يومي البولي وقص في مستوي مضادات الأكسدة الإنجالي (TAC). ووجدت الدراسة زيادة ملحوبة في مستوي البوتاسيوم في الادوار المجموع خلال 24 ساعه للمرضى المصابين بحبيص الجهاز البولي مضاربة بالجمعية المسيطرة. نستنتج مما سبق ان مرض حبيص الجهاز البولي تمثل بمستويات الدهون المؤكدة وأنزيمات مضادات الأكسدة.
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 (ureterolithiasis) 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 formation starts as a precipitation of calcium phosphate either in the loop of Henle or in the distal part of the distal tubule [3]. Although the urine at these levels of the nephron might be critically supersaturated with calcium oxalate, patients with hyperoxaluria and in experimental animals following administration of ethyleneglycol, the ion activity product of calcium oxalate crystallization. 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 calcium oxalate 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°C) until use. Also ten samples collected for 24-hour urine. Collection of urine began to in the morning after wake up. The first urination is 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% Thioarbarutic 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

<table>
<thead>
<tr>
<th>Gender</th>
<th>Patients (n=60)</th>
<th>Control (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>48</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>18</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Control Mean ± SD</th>
<th>Patient Mean ±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC mmol/l</td>
<td>18.769±1.645</td>
<td>6.148±1.868</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>MDA (μmol/L)</td>
<td>2.493±0.850</td>
<td>7.845±1.134</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>SOD U/ml</td>
<td>0.836±0.085</td>
<td>0.541±0.149</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Control Mean ±SD</th>
<th>Patient Mean ±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>30.225±0.030</td>
<td>30.175±0.030</td>
<td>P&lt;0.34</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.529±0.004</td>
<td>0.521±0.003</td>
<td>P&lt;0.08</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>5.109±1.356</td>
<td>6.28±1.264</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

**Sodium, potassium, Calcium and phosphorus:**

The results showed non-significant difference in \(\text{Na}^+\) and \(\text{K}^+\) concentration in sera of renal stones group compared with \(\text{Na}^+\) and \(\text{K}^+\) 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

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Control Mean ±SD</th>
<th>Patient Mean ±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.61±0.706</td>
<td>10.64±2.04</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>143.035±0.005</td>
<td>143.039±0.003</td>
<td>P&lt;0.48</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.057±0.006</td>
<td>4.055±0.004</td>
<td>P&lt;0.78</td>
</tr>
<tr>
<td>Inorganic phosphorus (mg/dl)</td>
<td>4.471±1.348</td>
<td>3.175±0.826</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

**Twenty Four Hours Urine**

The urinary urea, \(\text{Cr, U.A, PO}_4^{2-}\), \(\text{pH}\), \(\text{K}^+\) and \(\text{Na}^+\) concentration were persistently higher \((P<0.01)\), in patients when compared with control groups.

However a significantly decrease \((P<0.01)\) in \(\text{PO}_4^{2-}\) and \(\text{Ca}^{2+}\) 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

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Control Mean±SD</th>
<th>Patient Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/day)</td>
<td>1744.93±106.04</td>
<td>1850.2±67.66</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Creatinine (mg/day)</td>
<td>15.75±2.91</td>
<td>16.6±2.74</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>
Discussion
Results showed that there 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 dilution, 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 PO₄³⁻ 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 alklalization 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
35. Borghi L., Schianchi T., Meschi T. et al. (2002). "Comparison of Two