THE ROLE OF SEX HORMONES IN THE FORMATION OF RENAL STONES WITH REFERENCE TO URINARY TRACT INFECTIONS OF IRAQI PATIENTS

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ABSTRACT

Background: As the prevalence of renal stones is different in males and females, some studies have focused on the possible role of sexual hormones and their receptors in renal calcium stone disease. The clinical as well as laboratory relevance of these observations with other related parameters in human subjects remains to be further elucidated.

Methods: Testosterone, progesterone and estrogen (estradiol, E2), Calcium, Phosphorus, Uric Acid, Magnesium, Vitamin D3 and parathyroid hormone concentrations in Serum were determined. The obtained renal stones were analyzed. Bacterial isolates causing urinary tract infections were identified from 150 urolithiasis patients.

Results: Sex hormones assaying revealed that 25 patients (15.6%) had elevated testosterone level in both male and female, and the serum levels of this hormone associated with the status of renal stone disease associated with urinary tract infections. A positive correlation was found between the total urinary testosterone concentrations and the activity of urokinase. 11 patients had abnormal estrogen values. This result represents (6.8%) of the total number of the patients in the study. Progesterone considered as risk factor in stone formation and the unadjusted RR (relative risk) for incident kidney stones in postmenopausal women was 30 % higher compared with premenopausal women. The level of vitamin D3 in males was higher than of the girls. Blood samples were obtained from the total of 150 patients and the means of S.Ca+2, S.Na+ and S.K+ were variable. Urinary tract infections were highly associated with testosterone with elevation in serum CaOx.

Conclusions: Some females had high level of progesterone associated with low level of estrogen deficiency increases the sensitivity of
bone to parathyroid hormone, leading to a net increase in bone resorption and increased urinary calcium excretion. This finding leads to renal stone formation and consequently urinary tract infections. Urinary levels of sodium and uric acid.

**Key Words:** UTI, Renal stone, Serum ions, PTH, Sex hormones.

**INTRODUCTION**

Urinary Tract Infection (UTI) is common bacterial infection across all age groups, and affects around 20% of women at some time during their life. UTI is the second most common clinical indication for antibiotic treatment in both primary and secondary care. It is also the most common hospital acquired infection in United States, accounting for 23% of all infection[1]. UTI represents the commonest genitourinary disease in children and is the second commonest infection which affect them[2]. Infection of the UTI may present in wide variety ways. This range from asymptomatic bacteruria, to sepsis with multi-organ dysfunction. Infection may arise from any part the UT such as the kidney as pyelonephritis, pyonephrosis, renal absceses or the bladder as cystitis, the prostate as prostatitis and prostate absceses or the urethritis[3]. A better understanding of pathogenesis of UTI and the role of host and bacterial factors have improved the ability of identify the patients at risk and prevent or minimize squeale. New diagnostic test and antimicrobial agent that achieve high urinary levels that can be administered orally and that are not nephrotoxic have significantly reduced the need for hospitalization for severe infection. Shorter coarse therapy and prophylactic antimicrobials have reduced the morbidity and cost associated with recurrent urinary tract infection[4]. As the prevalence of renal stones is different in males and females[5,6], some studies have focused on the possible role of sexual hormones and their receptors in renal calcium stone disease. In animal studies serum estradiol was associated with increased urinary citrate and decreased urinary oxalate excretion[5,7] and castration in males was also associated with lower urinary oxalate excretion. The clinical relevance of these observations in human subjects remains to be further elucidated. Different studies examined the role of sex hormones(estrogen and testosterone) on urinary oxalate excretion and kidney stone formation in an experimental model of urolithiasis. Adult male and female Sprague Dawley rats with different sex hormone modulations were given 0.75% ethylene glycol for 2 weeks to induce hyperoxaluria and kidney calcium oxalate crystal deposition[8]. Other study was conducted to extend our earlier study on the role of testosterone in the pathogenesis of urolithiasis and to further investigate the influence of sex hormone on the pathogenesis of calcium oxalate stone.
Estrogen has been demonstrated to inhibit bone resorption and increase renal absorption of calcium [9,10].

**MATERIALS and METHODS**

**Patients**

This study was conducted in the Urology Department in Tikrit Teaching Hospital. The number of the patients was 160 and they were recruited from November 2011 until July 2012. All having urolithiasis including 104 males and 54 females. Patients aged between 15 to 70 years. This study was a part of grant donated by University of Tikrit for medical and health research. The exclusion criteria were as follows: Patients who received antibiotics, Patients who underwent catheterization of urethra or ureter and Immunocompromised patients.

**Samples collection**

The samples that were collected from the patients were urine, blood and stone if available. Urine sample was collected for biochemical and microbiological examination. A mid stream urine (MSU) sample was collected (30 ml of urine) and transported separately to the laboratory within 30 minutes for microbiological study [11] Blood was withdrawn from the upper limb (median cubital vein) of the patient after the skin was disinfected with 70% alcohol using sterile disposable syringe and transferred into a sterile plane tube. Blood sample for calcium concentration determination was done without tourniquet and the patient in the sitting position. Five millimeters sample of blood was collected and centrifuged and only unhaemolysed serum was used. The serum was placed in a plane tube then labeled and freezed for further studies. Renal stone was collected from the patients if it was available and kept in a sterile screw-cupped containers or sterile sealed bags.

**Identification of bacteria**

The isolation and identification of pathogens were carried out according to methods done by Al-Jebouri and Madish [12].

**Chemical Analysis of Renal Stones**

The obtained renal stones were analyzed using BIOLABO kit (France) according to the manufacturer’s instructions.
Determination of calcium, phosphorus, magnesium and uric acid concentrations in serum
These were estimated using BIOLABO kit (France) according to the manufacturer’s instructions.

Determination of Parathyroid hormone (PTH) concentration:
PTH concentration was determined by using ACTIVE I-PTH ELISA kit from DSL(U.S.A.) and according to the manufacturer’s instruction.

Determination of Vitamin D$_3$ in Serum
Vitamin D concentration was determined using (IDS, U.K.) according to the manufacturer’s instruction.

Determination of Testosterone hormone concentration.
Testosterone hormone concentration was determined using BioCheck ELISA kit from DSL (U.S.A.) and according to the manufacturer’s instructions.

Determination of Progesterone hormone concentration
Progesterone hormone concentration was determined by Monobind ELISA kit from DSL (U.S.A.) and according to the manufacturer’s instructions.

Estradiol (E2) hormone concentration was determined using Monobind ELISA kit from DSL (U.S.A.) and according to the manufacturer’s instructions.

Statistical analyses
Also all statistic programs that used in present study done using F-test or (ANOVA) and Chi-square[13]. All means values well compared to determined the significant difference using Ducun’s Multiple Range With probability $P \leq 0.05$.

RESULTS
Urine culture result for the patients
The most common organism was $E. coli$ which was isolated from 14 patients with percentage of 31.1%. $E. coli$ was the most prevalent followed by $Proteus mirabilis$, $Pseudomonas aeruginosa$, $Staphylococcus aureus$, $Klebsiella pneumoniae$, $Staphylococcus Saprophyticus$ and $Serratia marcescens$ and the frequencies of isolation were 22.2% (10)
Figure 1). 15.5% (7), 11.1% (5), 7.4% (5), 13.3% (6), 4.4% (2) and 2.2% (1) respectively (Statistically, there was highly significant difference between males and females according to the distribution of the isolated bacteria (P value ≤ 0.05).

Figure 1. Percentage of isolated bacteria from urine culture of the patients.

Chemical analysis of the stones collected from the patients. In the present study, 100 stones were collected and analyzed. The most predominant stones collected were calcium oxalate, calcium phosphate and uric acid stones and their percentage were 65%, 70% and 45% respectively. The cations was calcium 85% followed by magnesium and their percentage was 7%, while the anions collected in this study were phosphate, oxalate and urate carbonate and their percentages were 70%, 65% and 6% respectively (Figure 2).

Figure 2. Chemical analysis of obtained stones.
Statistically, there was no significant difference in stone composition distribution between males and females (P value > 0.05) using Chi-square test.

3.4. The relationship between urinary tract infection and stone type.
Table 1 shows positive urine culture among patients who underwent lithotomy or passed their stones spontaneously. Forty five (28%) of the patients had positive urine culture, and the ratio of females to males was almost 1.6:1. The number of patients with infective stones who had positive urine culture was 7 (4.3%) and 4 (2.5%) patients of them were males. The number of the patients with non infective stone who had positive urine culture was 38 (24%) patients and 25 (16%) patients of them were females. Statistically, there was no significant difference in distribution of positive urine culture between the infection stones and the non infection stones according to sex using Chi-square test (P value ≥ 0.05).

Table 1. Interrelationship between urinary tract infection and stone type.

<table>
<thead>
<tr>
<th>Type of the stones</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>Infection stones</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Non infection stones</td>
<td>13</td>
<td>25</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>28</td>
<td>45</td>
</tr>
</tbody>
</table>

$X^2 = 1.322, \ df = 3, \ P \text{ value} \geq 0.05 \text{ (not-significant)}$

3.5. Interrelationship between selected serum ions abnormalities and the chemical composition of urinary stones

Table 2. The interrelationship between serum values of selected ions and the chemical composition concerning urinary stones in patients who underwent lithotomy or spontaneous stone shedding and control subjects.

<table>
<thead>
<tr>
<th>Type of stone</th>
<th>No</th>
<th>S.U A M</th>
<th>S.Ca$^{2+}$ M</th>
<th>S.Mg$^{2+}$ M</th>
<th>S.PO4 M</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.CaOx</td>
<td>14</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CaPO4</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>CaPO4Ox</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>CaPO4OxUA</td>
<td>19</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>CaPO4UA</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Uric acid  | 4  | 2  | 0  | 0  | 0  | 0  | 0  | 0  | 2  
Struvite {AMP} | 5  | 0  | 0  | 0  | 0  | 0  | 1  | 0  | 4  
CaPO₄CO₃ | 5  | 1  | 0  | 0  | 0  | 0  | 2  | 0  | 3  
CaPO₄CO₃OxUA | 15 | 2  | 0  | 0  | 1  | 1  | 1  | 0  | 10 
CaPO₄OxUA AMP | 5  | 0  | 1  | 0  | 0  | 1  | 0  | 1  | 2  
Total of patients | 100 | 9  | 3  | 10 | 3  | 10 | 7  | 10 | 1  | 47 |

Note: Some patients have more than one metabolic abnormalities; Ox: oxalate ;Ca: calcium ; AMP: ammonium magnesium phosphate ; U.A: uric acid; PO₄:phosphate; CO₃:carbonate; S.Mg+²:Magesium;

The serum values abnormalities included an increase in S. UA, S. Ca+2 and S. P+5 and decrease in S. Mg+2. Table 2 and 3 show a comparison of the values of serum values of Ca+2, P+5, UA and Mg+2 with sex hormones. Statistically there was no significant difference in the distribution of S.UA, S. Ca+2, S. P+5 and S. Mg+2 between males and females in sex hormones testosterone, estrogen and progesterone (P value more than 0.05).

Table 3. The relationship between selected ions and sex hormone abnormalities.

<table>
<thead>
<tr>
<th>Serum ions</th>
<th>No.</th>
<th>↑ Testosterone</th>
<th>↓ Estrogen</th>
<th>↑ Progesterone</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.Ca</td>
<td>36</td>
<td>M 11</td>
<td>F 10</td>
<td>M 4</td>
<td>F 3</td>
</tr>
<tr>
<td>S.PO₄</td>
<td>19</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>S.UA</td>
<td>35</td>
<td>14</td>
<td>9</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>S. Mg</td>
<td>21</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>111</td>
<td>35</td>
<td>28</td>
<td>9</td>
<td>11</td>
</tr>
</tbody>
</table>

M: Male, F: Female; S.Ca: serum calcium; S.PO₄: serum Phosphorus; S. Mg: serum Magnesium; S.UA: serum Uric Acid; ↓: Low Level; ↑: high levels.
Table 4 shows a comparison of the means serum values of S. Ca$^{2+}$, S. PO$_4^{+}$, S.UA and S. Mg between patients and controls. Statistically, there was no significant difference in means serum values of Ca$^{2+}$ (p=0.223) and no significant difference in means Mg$^{2+}$ (p=0.181), while there was a highly significant difference in means of PO$_4^{+}$ (p=0.000) and UA(0.016) between patients and controls.

Table 4. Distribution of means of selected serum components among patients and controls.

<table>
<thead>
<tr>
<th>Ions</th>
<th>Groups</th>
<th>Sample size</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca$^{2+}$</td>
<td>Patient Control</td>
<td>106 40</td>
<td>9.0 - 10.5 9.0 -10.5</td>
<td>10.206 9.1325</td>
<td>±1.140 ±0.4790</td>
<td>0.223 N. S.</td>
</tr>
<tr>
<td>PO$_4$</td>
<td>Patient Control</td>
<td>106 40</td>
<td>2.5 -4.5 2.5-4.5</td>
<td>3.8136 3.2325</td>
<td>±0.7850 ±0.6220</td>
<td>0.000 H.S.</td>
</tr>
<tr>
<td>S.UA</td>
<td>Patient Control</td>
<td>106 40</td>
<td>3 - 7 3 - 7</td>
<td>6.506 4.830</td>
<td>±5.282 ±1.006</td>
<td>0.016 H.S.</td>
</tr>
<tr>
<td>S.Mg$^{2+}$</td>
<td>Patient Control</td>
<td>106 40</td>
<td>1.6 -2.4 1.6 - 2.4</td>
<td>2.0127 2.1325</td>
<td>±0.4262 ±0.2515</td>
<td>0.181 N.S.</td>
</tr>
</tbody>
</table>

N.S.: Non significant; H.S.: Highly significant; SD, standard deviation ; the unit of serum values was mg/dl.

Table 5 It shows that urinary tract infection was more prevalent among females with relation to abnormalities in testosterone, progesterone and estrogen hormones. About 25 patients(14 females and 11 males) had abnormality in testosterone level, 11 patients( 7 females and 4 males) had abnormality in estrogen level, while progesterone level showed 24 patients(16 females and 8 males) had abnormality level . Statistically, there was a significant difference between males and females according to chi-square test (P value ≤ 0.05).

Table 5. Shows the interrelationship between selected hormonal abnormalities (testosterone ,estrogen, progesterone) and UTI.

<table>
<thead>
<tr>
<th>Hormonal abnormalities</th>
<th>UTI</th>
<th>No UTI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Testosterone</td>
<td>12 (48%)</td>
<td>8 (32%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Estrogen</td>
<td>4 (36.3%)</td>
<td>2 (18%)</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>8 (33%)</td>
<td>2 (8.3%)</td>
<td>8 (33%)</td>
</tr>
</tbody>
</table>
UTI: urinary tract infection

Note: Some patients had more than one metabolic abnormalities
Table 6 shows the means , standard error and significant difference of sex hormones between patients and controls . Testosterone hormone revealed a high significant difference with p=0.052 while in estrogen hormones show no significant difference concluded with p=0.901, but progesterone hormone showed a high significant difference with p=0.014

Table 6. Distribution of means of sex hormones among patients and controls(normal controls and UTI controls).

<table>
<thead>
<tr>
<th>Test Groups</th>
<th>Testosterone</th>
<th>Estrogen</th>
<th>Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>3.300 ± 0.458</td>
<td>45.72 ± 3.57</td>
<td>3.491 ± 0.333</td>
</tr>
<tr>
<td>Patients</td>
<td>4.868 ± 0.289</td>
<td>42.73 ± 3.21</td>
<td>4.987 ± 0.807</td>
</tr>
<tr>
<td>UTI’s</td>
<td>4.452 ± 0.667</td>
<td>45.55 ± 3.50</td>
<td>3.567 ± 0.456</td>
</tr>
</tbody>
</table>

* UTI’s : urinary tract infections.

4.Discussion
As shown in Figure 1, the commonest organism isolated from the urine in this study as E.coli (31.1%). This results was almost similar with reported elsewhere[14].E.coli was followed by Proteus mirabilis(17.9%) ,it was agreed that the Proteus mirabilis is the second most common cause for UTI[15]. Proteus mirabilis and Klebsiella pneumoniae are associated with phosphate stones while E.coli are commonly associated with ca-oxalate stones which were relatively similar to other findings reported somewhere else[14,15,16]. Vitamin D concentration in the present study was found with mean 16.7ng/ml. These results were almost similar to Van der Wielen et al[17] and Krader[18 ]findings. Half of patients had an elevated PTH level and all the vitamin D-deficient patients with elevated PTH levels had
hypercalciuria and normal S.Ca$^{2+}$. These data support conducting prospective trials to investigate the effects of vitamin D repletion as a mean to correct hypercalciuria and prevent stone formation$^{18}$, excessive urine Ca$^{2+}$ is linked to stone formation$^{[19]}$. In the present study, three serum components were measured and compared, which showed that was 25 patients (15.6%) had elevated testosterone level in both male and female, and the serum levels of this hormone associated with the status of renal stone disease. These results were almost similar to that by Lee el al $^{[20]}$. In comparison with females formed the rationale for hypothesizing a possible role for sexual hormones in urinary stone pathogenesis. Animal studies have found that stone incidence severely decreases from 71 down to 14% after castration in ethylene glycol-treated male rats$^{[20,21]}$. A human study reported higher levels of testosterone in stone forming males relative to the controls $^{[22]}$. The current study showed the level of estrogen in 160 patients with renal stones and from the 160 patients only 11 patients had abnormal estrogen values. This result represents (6.8%) of the total number of the patients in the study as shown in table (5). All 11 patients showed have low level of estrogen hormone, These results were almost similar with those of Carol et al $^{[23]}$. Most females in current study had low level of estrogen belong to the postmenopausal phase, who were almost similar with those of Carol et al $^{[24]}$. This association between free estradiol concentration and renal calcium handling may be a significant factor in the relation between endogenous estrogen concentration and fracture observed in elderly women $^{[25]}$. In this study, the effect of estrogen on the kidney was to enhance the tubular reabsorption of calcium in response to PTH. However, loss of estrogen's antagonism of PTH activity can be inferred by the development of postmenopausal hyperparathyroidism$^{[26,27]}$. Although the beneficial effects of estrogen therapy in primary hyperparathyroidism are well documented in the literature $^{[28]}$, with risks associated with estrogen use have also been well publicized $^{[29]}$. In addition, the amount of estrogen required to reduce the serum calcium in primary hyperparathyroidism is higher than most tolerate, although some positive results have been observed with lower doses$^{[30]}$. Progesterone considered as risk factor in stone formation and the unadjusted RR (relative risk) for incident kidney stones in postmenopausal women was 30 % higher compared with premenopausal women. It was noted no association between menopause and incident kidney stones. There was, however, a 30% increase in the multivariate-adjusted risk with surgical menopause$^{[31]}$. Current study showed the level of progesterone in 160 patients, (16 females and 8 males) had high level of progesterone hormone. This result revealed 15 % of the total number of the patients in the study as shown in table (5).
females in current study in menopause phase associated with an increase in urinary calcium excretion, and these result almost similar to those of Nordin et al[32]. Similar differences in urinary calcium excretion between premenopausal and postmenopausal women were found in an unmatched study [33]. Also some females had high level of progesterone associated with low level of estrogen deficiency increases the sensitivity of bone to parathyroid hormone, leading to a net increase in bone resorption and increased urinary calcium excretion, and these result almost similar to those of Riggsa et al [34]. The menopausal changes in urinary calcium excretion may prevent hypercalcemia, but they could theoretically also yield an increased risk for bone loss and possibly kidney stone formation. However, with natural menopause, the loss of estrogen leading to increased calcium excretion may be too gradual to noticeably elevate the risk of kidney stone formation. High levels of progesterone, aldosterone, and human chorionic gonadotropin lead to changes in cardiac output that are responsible for increasing the glomerular filtration rate (GFR) and renal plasma flow (RPF) by approximately 25% during pregnancy. As a result, there is a large increase in excretion of urinary metabolites. Urinary levels of sodium and uric acid that potentiate stone formation become elevated. In contrast, citrate, magnesium, and glycosaminoglycans inhibit stone formation. Urinary calcium excretion is raised 2 to 3 times as a result of increased production of 1,25-dihydroxycholecalciferol by the placenta[35]. Elevated vitamin D levels, in turn, promote absorptive hypercalciuria. Interestingly, the elevated calcium levels are countered by raised excretion of stone inhibitors and increased urine output as a result of elevated GFR and RPF[35].

5. CONCLUSION
The present study showed that more than 15%(25/160) of the patients had elevated levels of testosterone, while almost 7%(11/160) of the patients had lower levels of estrogen. The present study also revealed that 15% of the patients had elevated levels of progesterone. These findings were associated with hypomagnesaemia which might led to development of urinary stone which was due to induction of autonomous hyperparathyroidism and its consequence of increment urinary calcium excretion and consequently urinary tract infections might occur.

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