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Biochemical and Molecular studies on the role of Androgens in the Pathogenesis of renal calcium stones

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Abstract: Although kidney stone disease has been recognized by the medical community, the mechanisms behind stone development and formation are still largely unclear. The recent technological developments have been applied in the surgical management of kidney stones.

The goal of the current research is to understand how androgens contribute to the development of renal calcium stones.

In this prospective, controlled trial and Egyptian male patients between the ages of 18 and 60 are included. All clinical data was collected including history, clinical examination, and standard laboratory testing. The hormones testosterone (T), free testosterone (FT), and dihydrotestosterone (DHT) were detected in plasma samples using immunoassay is an analytical technique.

Following a diagnosis of renal stones, 23 patients were included in the trial between June 2020 and June 2021. They were hospitalized for endoscopic treatment with PNL (percutaneous nephrolithotomy) or RIRS (retrograde intrarenal surgery), and their outcomes were compared to 23 controls. The control group's mean age was 60.2±6.1, while the SFG group' s was 54±10.3

There was a noticeable difference between the control group and the stone patients in terms of blood testosterone, free testosterone, and dihydrotestosterone. Male kidney stone patients considerably exhibited greater serum testosterone concentrations than age-matched stone-free people, according to the results of the ELISA test. Male kidney stone patients exhibited greater serum testosterone concentrations than age-matched stone-free people.

In conclusion, Increasing sex hormone levels were positively connected with CaOx(Calcium Oxalate) renal stones..

Keyword: kidney stones, calcium oxalate, testosterone.

Introduction

One of the first disorders recognized by medicine was nephrolithiasis or urolithiasis, widely known as kidney stone disease. According to estimates, 1 to 15% of people will have kidney stones at some point in their lives [1], and the prevalence and incidence of kidney stones are both said to be rising globally [2]. Urinary supersaturation and crystallization, which are the main contributors to intrarenal crystal precipitation, are the main causes of hereditary or acquired diseases associated with renal function impairment. Urinary supersaturation and crystallization can also be influenced by urine pH and specific

concentrations of compounds like CaOx, CaP, uric acids and urates, struvite, amino acids (cysteine), purines (2,8-dihydroxyadenine and xanthine), and drugs like atazanavir, sulfamethoxazole, amoxicillin, and ceftriaxone. [3]. Additionally, numerous modulator molecules, including receptors, promoters, and inhibitors have an impact on crystal growth. [4].

CaOx and calcium phosphate (CaP); calcareous and radio-opaque stones, are the most prevalent forms of kidney stones in humans [5, 6]. Randall's plaques (RPs), a CaP base that extends from the renal papillary surface's narrow limbs of the loop of Henle's basement membrane, is where kidney stones develop [7]. Males are more likely to experience CaOx and urate stones, whereas females are more likely to experience carbapatite and struvite stones [8, 9]. The importance of gender variations in the pathophysiological processes behind urinary stone disease is still not well understood.

Oxalate excretion in the urine, plasma oxalate content, and the deposition of CaOx crystals in the kidneys have been reported in previous research, to increase with androgens and decrease with estrogens.

The relationship between sex and kidney stone formation may also be due to increased androgen signalling [10]. In order to increase oxalate biosynthesis and androgen receptor (AR) signalling has the potential to directly upregulate hepatic glycolate oxidase, which may ultimately lead to kidney stone development [11] and at the transcriptional level, AR may directly up-regulate hepatic glycolate oxidase, and kidney epithelial (NADPH) oxidase subunit p22-PHOX. [12].

The purpose of this study is to investigate the relationship between male kidney stone patients and blood levels of free testosterone, dihydrotestosterone, and testosterone.

Patients and methods

Patients

This is a prospective, controlled study that included Egyptian male patients who were diagnosed with renal stones and received active stone intervention. all patients who were diagnosed with renal stones and managed with standard percutaneous nephrolithotomy (PNL) or retrograde intrarenal surgery (RIRS). Retrieved stones were submitted for stone analysis, and calcium oxalate (CaOx) stone formers were depicted. Patients with known hyper-thyroidism, hyperparathyroidism, recurrent urinary tract infections, renal and liver diseases, intestinal malabsorption, or metabolic syndromes were excluded from the study. In addition, patients with known structural or functional abnormalities of the urinary tract like vesico-ureteric reflux, neuropathic bladder, or pelviuretral junction obstruction were also excluded from the study.

Hormone analysis

For hormone analysis, all samples of blood were collected, and each sample was centrifuged at 3000 xg for 15 min. The separated plasma fractionated and stored at

-20 C until hormone assay for testosterone (T) (cat no. ALPCOR "In Vitro Diagnostic), free testosterone (FT) (cat no. ALPCOR "In Vitro Diagnostic), and dihydrotestosterone (DHT) (cat no. The Eagle Biosciences).

They were analyzed by IMMULITE® 2000 Immunoassay Systems. The immunoassay is an analytical technique that employs antibodies and/or antigens to detect and measure a wide variety of analytes, including hormones

Statistical Analysis

Continuous variables were presented as mean ± SD for normally distributed and as median and range for non-normally distributed. Variables that were ordinal and nominal were shown as frequency and percentage. Both the Mann-Whitney test for asymmetric continuous variables and the Student's t-test for regularly distributed continuous variables were used to compare the study and control groups. Data storage and analysis were performed using the SPSS programme.

Results

Patient's characteristics

The patients (n = 23) were enrolled in the study after diagnosis with renal stones and they were hospitalized for endoscopic treatment with PNL or RIRS and they were compared against 23 healthy subjects. The mean age was 60.2±6.1 in control group but stone formers

group (SFG) was 54±10.3 (Table 1). The frequency of the stone is described in table 1and Figure 1.

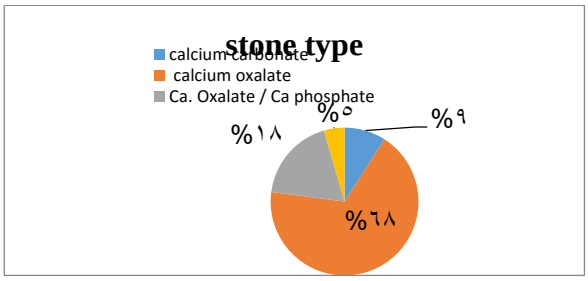

Fig 1: The distribution of stones between the patients

Table 1: Patients characteristics

Item	Control group (n=23)	Stone patients (n=23)
Age (mean ± SD)	60.2609± 6.14386	54.0435± 10.38565
Stone type, n (%) calcium carbonate calcium oxalate Ca. Oxalate / Caphosphate Ca.oxalate / magnesium ammonium phosphate Ca.oxalate / Ca.phosphate / calcium hydrogen phosphate	–	2 (8.7%) 15(65.2%) 4(17.4%) 1(4.3%) 1(4.3%)

Hormonal analysis

Regarding serum testosterone, free testosterone, and dihydrotestosterone, a significant difference was observed between the control group and the stone patients. Using the ELISA test, male kidney stone patients had significantly higher serum testosterone concentrations than age-matched stone-free individuals (table 2).

Table 2: The difference in hormonal concentration between SFG and control group

	Controlgroup (n=23)	Stonepatients (n=23)	Pvalue
testosterone (2.62-16.0ng/ml)	2.5161±.77131	13.7430± 3.62968	0.000
Free testosterone (10.0-40.0pg/ml)	16.3370±4.93644	31.2317±7.37181	0.000
DHT (25.0-90.0ng/dl)	30.7435 ±5.72989	47.6870±14.74863	0.000

Discussion

Renal stones have major economic and medical burdens due to the cost of treatment, sick leave from work, risk of renal impairment, and renovascular hypertension [13]. Another significant issue with stone diseases is the risk of recurrence; 50% of patients with kidney stones have a 50% chance of developing another episode of stone disease within 7 years [14].

CaOx stones form as a result of a complex and multifactorial process that includes underlying genetic and metabolic abnormalities, lifestyle patterns, obesity, and a hot climate [15]. Recently, a strong association was observed between sex hormones and kidney stones. Testosterone and dihydrotestosterone hormones were found to be a potential promoting factor in the occurrence of CaOx urolithiasis, while estrogen was found to be protective against urolithiasis [16].

Testosterone is known to increase the hepatic levels of glycolic acid oxidase (GAO) and thus may lead to an increase in the hepatic synthesis of this enzyme, resulting in hyperoxaluria, which in turn may be responsible for the increased predisposition to calcium oxalate urolithiasis. It promotes stone formation by suppressing renal osteopontin expression and increasing urinary oxalate excretion. Active dihydrotestosterone (DHT) is produced from testosterone by the cytosolic enzyme 5a-reductase and has been believed to be partially responsible for exaggerated hyperoxaluria observed in the rat ethylene glycol model of urolithiasis [17,18].

In this study, Testosterone, Free testosterone and dihydrotestosterone were found to be significantly higher in SFG compared to the control group. These results go in parallel with the previous result of Naghiiet al. [19]. These findings suggest that testosterone, free testosterone, and dihydrotestosterone may all play a key role in the pathogenesis of renal stones.

Oxalate excretion in the urine, plasma oxalate content, and the deposition of CaOx crystals in the kidneys have all been increased with androgens and decreased with estrogens. Furthermore, the link between sex and kidney stone production may be caused by increased androgen signalling. Androgen receptor (AR) signalling can directly influence hepatic glycolate oxidase and kidney epithelial nicotinamide adenine dinucleotide phosphate

oxidase to enhance oxalate synthesis and ultimately lead to kidney stone development (NAPDH) [20].

One of our study limitations is the small sample size, so we recommend further study in large sample size.

In conclusion, CaOx renal stones were found to positively correlate with changes in sex hormone levels. More studies are recommended to prove the actual role of androgen in CaOx stone formation.

References

1. Romero V, Akpinar H and Assimos DG: Kidney stones: A global picture of prevalence, incidence, and associated risk factors. Rev Urol **12**: e86-e96, 2010.
2. Morgan MS and Pearle MS: (2016) Medical management of renal stones. BMJ 352: i52,.
3. Daudon M, Frochot V, Bazin D and Jungers P: Drug-induced kidney stones and crystalline nephropathy: Pathophysiology, prevention and treatment. Drugs **78**: 163-201, 2018.
4. Rodgers AL: (2017) Physicochemical mechanisms of stone formation. Urolithiasis **45**: 27-32,.
5. Parmar MS: Kidney stones. BMJ **328**: 1420-1424, 2004.
6. AggarwalKP, NarulaS, KakkarM and Tandon C: (2013) Nephrolithiasis: Molecular mechanism of renal stone formation and the critical role played by modulators. Biomed Res Int: 292953, 2013.
7. Khan SR, Pearle MS, Robertson WG, Gambaro G, Canales BK, Doizi S, Traxer O and Tiselius HG: (2016) Kidney stones. Nat Rev Dis Primers 2: 16008,.
8. Ye Z, Zeng G, Yang H, Li J, Tang K, Wang G, Wang S, Yu Y, Wang Y, Zhang T, et al: (2020) The status and characteristics of urinary stone composition in China. *BJU Int* **125**: 801-809,.
9. Sun X, Shen L, Cong X, Zhu H, He L and Lu J (2011): Infrared spec-troscopic analysis of 5,248 urinary stones from Chinese patients presenting with the first stone episode. Urol Res **39**: 339-343,.
10. Fuster DG, Morard GA, Schneider L, Mattmann C, Lüthi D, Vogt B and Dhayat NA: (2020) Association of urinary sex steroid hormones with urinary calcium, oxalate and citrate excretion in kidney stone formers. Nephrol Dial Transplant: Dec 9,
11. Yoshihara H, Yamaguchi S and Yachiku S: (1999) Effect of sex hormones on oxalate-synthesizing enzymes in male and female rat livers. *J Urol* **161**: 668-673,.
12. Liang L, Li L, Tian J, Lee SO, Dang Q, Huang CK, Yeh S, Erturk E, Bushinsky D, Chang LS, et al: (2014) Androgen receptor enhances kidney stone-CaOx crystal formation via modulation of oxalate biosynthesis & oxidative stress. MolEndocrinol **28**: 1291-1303,.
13. Rule AD, Bergstralh EJ, Melton LJ, Li X, Weaver AL, Lieske JC (2009). Kidney stones and the risk for chronic kidney disease. Clinical *Journal of the American Society of Nephrology*.: 4:804-11
14. Sutherland J, Parks J, Coe F. (1985) Recurrence after a single renal stone in a community practice. Mineral and electrolyte metabolism.: 11:267-9
15. Xu H, Zisman AL, Coe FL, Worcester EM. (2013)Kidney stones: an update on current pharmacological management and future directions. Expert Opinion on Pharmacotherapy.: 14:435-47
16. Gupta K, Gill GS, Mahajan R. (2016) Possible role of elevated serum testosterone in pathogenesis of renal stone formation. International *Journal of Applied and Basic Medical* Research.: 6:241
17. Zhu W, Zhao Z, Chou F, et al. (2019) Loss of the androgen receptor suppresses intrarenal calcium oxalate crystals deposition via altering macrophage recruitment/M2 polarization with change of the miR-185-5p/CSF-1 signals. Cell death & disease.: 10:1-19
18. Fan J, Glass MA, Chandhoke PS (1998) Effect of castration and finasteride on urinary oxalate excretion in male rats. Urol Res **26(1)**:71–5.
19. Naghii, Mohammad Reza, MnasourBabaei, and Mehdi Hedayati.

(2014)"Androgens involvement in the pathogenesis of renal stones formation." PLoS One **9.4**: e93790

20. Z. Wang, Y. Zhang, J. Zhang, Q. Deng, and H. Liang, (2021) “Recent advances on the mechanisms of kidney stone formation (Review),” *Int. J. Mol. Med.*, vol. **48**, no. 2, pp. 1–10, Aug., doi: 10.3892/IJMM.2021.4982/HTML
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