

Calcium and Phosphate Homeostasis in Patients with Recurrent Nephrolithiasis

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Abstract

Objectives: The aim of the study was to evaluate the Calcium (Ca) and Phosphate (Ph) homeostasis and their association with plasma 25(OH)2 vitamin D3 (VitD3) and parathyroid hormone (PTH) in patients with recurrent nephrolithiasis.

Methods: A cross-sectional, involved 100 confirmed patients with renal stone (RS). Their serum levels of Ca, PTH, Ph, and VitD3 had assessed. Biochemical analysis of renal calculi and crystals had been investigated also. The summary measures had described as mean \pm SD for continuous variables and frequencies/percentage for nominal variables. The mean serum Ca, Ph, PTH, and VitD3 were (8.01 \pm 2.2 mg/dl, 2.9 \pm 1.2 mg/dl, 56.7 \pm 24.7 pg/dl, and 7.03 \pm 4.2 pg/ml), respectively.

Results: Almost all patients (97%) had a positive history of previous RS with a predominant family history in most instances (82%). There was a positive non-significant correlation between VitD3 with serum Ph and Ca. Whereas an inverse non-significant correlation between PTH with serum Ca and VitD3 had observed. Urinary crystals analysis revealed that uric acid represented 53% of the total crystals, followed by Ca-oxalate. Stone analyses revealed that around 3/4th of the cases had Ca-oxalate stone, followed by Ca-oxalate with uric acid, then Ca-phosphate, and the least type was mixed stone types.

Conclusion: There was a positive non-significant correlation between VitD3 with serum Ph and Ca. There was an inverse non-significant correlation between PTH with serum Ca and VitD3. Serum Ca, and Ph were non-significant predictors of renal stones and/or urinary crystals.

Keywords: Renal stone, parathyroid hormone, Vitamin-D, nephrolithiasis, calcium, phosphate

Introduction

Renal stone (RS) is a worldwide health problem worrying people for prolonged periods. The prevalence of RS in males is roughly 6–9% and in females, it is nearly 3–4%,^{1,2} and the predictable time risk of stone development is 5–12% in the USA and Europe.³ Preceding researches had exposed that elevated levels of active vitamin-D, is linked with higher calcium urine excretion.² Both parathyroid hormone (PTH) and 1,25-dihydroxy vitamin D3 (VitD3) have regarded as hormonal regulators of calcium metabolic balance. PTH release that is activated by hypocalcemia, rises plasma calcium (Ca) by activating bone resorption, kidney reabsorption, and intestinal calcium uptake indirectly via the kidney synthesis of VitD3.⁴ The serum levels of Ca and phosphate (P) in a research of 356 men with RS, were higher than in control subjects.⁵ Owing to their contribution to the Ca homeostasis and RS, PTH and VitD3 have gained much attention. Findings from a meta-analysis suggest that serum VitD3 level in kidney stone patients was significantly higher than that in control.⁶ Nevertheless, the precise roles of these factors in urolithiasis remain to be clarified.⁷ To assess the probable role of VitD3 and PTH as essential factors in the incidence of RS, we investigated their association with plasma calcium and phosphorus levels.

Patients and Methods

Study Strategy and Patient's Collection

This was a cross-sectional study conducted in Al-Sadiq teaching hospital, Babylon (middle of Iraq), for one-year (2018–2019). A total of 100 subjects (aged 45.8 \pm 17.7 years, 76 males and 24 females) presented with RS had enrolled in the study. All were attending urology consultation clinics (after being referred from specialty clinics) for followup or lithotripsy. The diagnosis of RS had completed by the urologists using radiographic and sonographic techniques. The demographic surveys had included data like age, gender, history of RS, history of diabetes, hypertension and other complications. The patients were known to have one of the ensuing problems had excepted from our study; urinary tract anomalies, urinary tract obstruction, hyperparathyroidism, and those on regular VitD3 therapy. An informed consent from all the participants had been gotten prior to be involved in the study.

Biochemical Assays

We measured the serum Ca, Ph, VitD3, and PTH levels in all the stone former patients. Both Vitamin D3 and PTH had measured by specific Elabscience ELISA-Kit. Serum Ca, and Ph concentrations had evaluated by spectrophotometric

method (Biolabo, France). Microscopic morphological examination of urinary sediments had performed to reveal the type of urinary crystals. The stone analysis had completed using a dipstick chemical test (DYBOW™). Vitamin-D consumption may change falsely (low or high) levels of VitD3, henceforth, participants had inquired whether they taken oral VitD3, which was excluded from the study. Serum VitD3 levels had analysed using the definitions recommended in the Kidney Disease Improving Global Outcome Initiative guidelines.^{8,9}

Statistical Analyses

Statistical analyses had performed on SPSS version-25 software package. The summary measures were described as mean \pm SD for continuous variables and frequencies with percentage for nominal variables. Multiple regression analyses were applied to predict PTH, and VitD3 from sex, patients' age, family history, size/site of the stone, BMI, serum Ca, and serum Ph. All statistical tests were performed at $P < 0.05$ significance level.

Results

The main characteristics of patients with recurrent urolithiasis were demonstrated well in Table 1. The mean age of the enrolled patients was 45.8 ± 17.7 years, with males representing 76%. The mean serum Ca, Ph, PTH, and VitD3 were (8.01 ± 2.2 mg/dl, 2.9 ± 1.2 mg/dl, 56.7 ± 24.7 pg/dl, and 7.03 ± 4.2 pg/ml), respectively. Of the total patients, 56% had a history of any chronic diseases (diabetes 10%, hypertension 40%, or ischemic heart diseases 6%). The pH of the urine was equally distributed 51% acidic and 49% alkaline.

Summarized in Table 2 below, were the main characteristics of the RS. Half of RS had a bilateral renal (left and right) location, 28% located in the left, 21% in the right, and only 2% had both renal and extrarenal location. Around 2/3rd of the RS was radiopaque, and almost all patients (97%) had a positive history of previous RS (within the last 3-years) with a predominant family history in most instances (82%). More than 2/3rd of RS had large size (>10 mm), 28% had 5–10 mm size,

Table 2. Biochemical and basal characteristics of urinary stones of patients with recurrent urolithiasis involved in the study

Characteristics	Percentage
Site of stone in the urinary system	
Left kidney	28%
Right kidney	21%
Left and Right kidney	49%
Kidney and ureter	2%
Stone radiolucency	
Radiolucent	34%
Radiopaque	66%
Recurrent stone	
Negative history	3%
Positive history	97%
Family history of renal stone	
	82%
Size of the urinary stone	
≤ 5.0 mm	4.9%
$> 5.0 - 10.0$ mm	28.0%
> 10.0 mm	67.1%
Low vitD3	
	73%
Normal or high vitD3	
	27%
Low or normal PTH	
	79%
High PTH	
	21%

VitD3: Vitamin D3; PTH: parathyroid hormone.

and less than 5% had small size (<5 mm). Around 3/4th of patients had a lower VitD3 serum levels, and around 1/4th had high PTH levels. Two patients had ureteral calculi besides the RS. No vesical stone had recorded. In 28 patients, 2 or more stones had recorded.

There were no gender-differences in terms of calcium, phosphorus, PTH, and VitD3. There were non-significant correlations of serum Ca and Ph, PTH, and VitD3 with the types of RS and/or urinary crystals. Likewise, there were a non-significant differences of study variables between those with low and those with high serum VitD3 levels. There was a positive non-significant correlation between VitD3 with serum Ph ($r = 0.064$, $P = 0.619$) and Ca levels ($r = 0.095$, $P = 0.653$). Whereas an inverse non-significant correlation between PTH with serum Ca ($r = -0.122$, $P = 0.302$), Ph ($r = -0.096$, $P = 0.544$) and significant correlation with VitD3 ($r = -0.25$, $P = 0.03$) had observed (Table 3). Multiple regression analyses had run to predict PTH, and VitD3 from sex, patients' age, family history, size/site of the stone, serum Ca, and Ph. They were non-significant predictors of PTH and VitD3. All variables add non-significant statistically prediction, $P > 0.05$.

Stone and Crystal Analyses

Urine crystals analysis revealed that uric acid represented 53% of the total crystals. Then followed by Ca-oxalate (41%), and the least represented by other types (6%) of urinary crystal (Figure 1). Renal stone analysis revealed that Ca oxalate represented 3/4th of the total RSs. Then followed by Ca-oxalate and UA represents about 1/5th, and the least represented by other types of urinary stone (Figure 2).

Table 1. Main characteristics of patients with recurrent urolithiasis involved in the study

	Minimum	Maximum	Mean \pm Std. deviation
Age/years	5	19	45.8 ± 17.7
Sex No. (%)		Males 76 (76%) Females 24 (24%)	
Serum Calcium, mg/dl	4.2	15.9	8.01 ± 2.2
Serum Phosphorus, mg/dl	0.5	6.7	2.9 ± 1.2
Serum Vitamin D3, pg/ml	0.9	29.56	7.03 ± 4.2
Serum PTH, pg/dl	32.9	184.1	56.7 ± 24.7
Diabetes No. (%)		10 (10%)	
Hypertension No. (%)		40 (40%)	
Ischemic heart diseases No. (%)		6 (6%)	
Urinary pH		Acidic 51 (51%)	

No: number; PTH: parathyroid hormone.

Table 3. **Correlation of vitamin D3, Parathyroid Hormone, Serum Calcium and Phosphorus in patients with recurrent urolithiasis involved in the study**

		Serum Calcium	Serum Phosphorus	Vitamin D
Vitamin D	Pearson correlation	0.059	0.064	–
	Significance	0.653	0.619	
PTH	Pearson correlation	–0.122	–0.069	–0.25
	Significance	0.302	0.544	0.03

Urinary Crystals

■ Calcium oxalate
■ Others
■ Uric acid

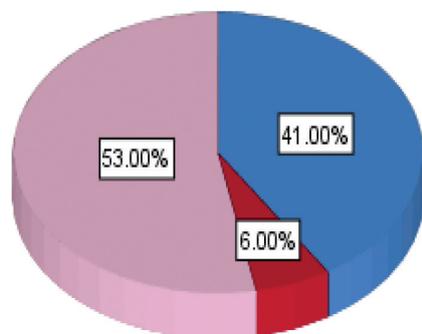


Fig. 1 Pie chart counts & percentage of urinary crystal.

Types of Renal Stone

■ 1 Calcium oxalate
■ 2 Calcium phosphate
■ 3 Calcium oxalate+UA
■ 4 Mixed stones

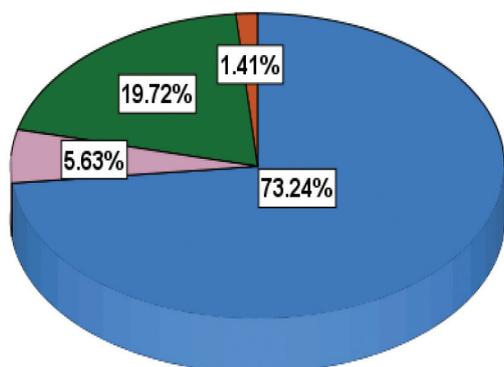


Fig. 2 Pie chart distribution of stone types among patients with renal stone.

Discussion

Urolithiasis is the 3rd common urinary disorder after prostatic diseases and urinary infections. Around 80–85% of RS are made up of calcium.⁷ The commonest type of urolithiasis is Ca-oxalate, then mixed Ca-oxalate/phosphate, struvite, uric acid, Ca-phosphate, and cysteine RS.² However, blood alterations in Ca, Ph, PTH, and VitD3 metabolism have not been

hitherto studied in patients with recurrent RS in Babylon. This study revealed that patients with RS had lower levels of serum VitD3, which consistent to other researches that had exhibited VitD3 insufficiency and deficiency are common in patients with RS.^{2,9,10} Although, all patients with RS had serum VitD3 fell within the reference range in another study.¹¹

As a fat-soluble, VitD3 has multiple metabolic activities. It has a crucial role in the homeostasis of Ca and Ph, besides, has a vital role in the pathogenesis of RS. VitD3 regulate Ca-homeostasis by acting on three body organs which are kidney, intestine and bone.² The biochemical alterations disposing to lithogenesis are not always apparent and in many instances, the stones' type and the metabolic causes remain indefinite. In the existing study, a positive association (although nonsignificant) of VitD3 plasma levels with Ca and Ph had shown. In the same line, there was an obvious decrease of VitD3, in patients with RS, but with no much changes in Ca and Ph metabolism and bone metabolism between patients with RSs and healthy controls in another study.¹² Added, in a survey including 160 RS vis 217 controls, Netelenbos et al.,¹³ have showed a non-significant differences in serum VitD3 levels. Hence, it seems that high VitD3 values, by inducing hypercalcemia followed by hypercalciuria, may contribute to lithogenesis in some patients.¹⁴ The VitD3 is a powerful stimulus of intestinal Ca-absorption in recurrent stone formers has been stressed by many authors. In addition, owing to it has renal and bone action, further elevates serum Ca-levels.⁷ Furthermore, due to enhanced intestinal Ca-absorption, might reduce the intestinal binding of Ca to oxalate that in turn, encourages much oxalate absorption, thereby induce the exacerbation of hyperoxaluria.^{15,16}

The locations and sites of the stone in our work are consistent with other reports from Turkey and Iran, nonetheless the frequency of two-sided, presence of several RS and the size of RS were varied.^{14,17} In the current study, no bladder calculi had noticed similar to the Iranian report.¹² Similar to our outcomes, no correlation of the study variables with size of the RS, had noticed by Yun S. et al.¹⁸ Gender is also a recognized risk factor for lithogenesis. Unlike our study, there have been a few epidemiological studies that comment upon gender differences in terms of Ca, Ph, PTH, and VitD3 differences among patients with RS.¹⁹ However, based on a meta-analysis of 20 case-control types of research only 2-works could be divided into subtypes based on sex, unsatisfactory to create a reliable link. Thus, gender subgroups should be focused on future studies.¹⁹

History of RS was strongly positive in of the first/second degree relatives in the present study that is consistent with what had reported by the Iranian study of the infants with RS,¹⁴ though other studies had shown less rates^{9,16} of genetic factors.¹⁴ Several meta-analyses have shown different genes responsible for inherited lithogenicity. However, some genetic loci seem to share a minor role to RS like single nucleotide polymorphisms in osteopontin and vitamin D receptor genes and others.^{20–22}

The recurrence of the stones in about 26% of the patients which less than the recurrence of other studies, which was (35–50%) of the patients.^{16,23} Most of the patients with renal stone (80%) were drinking many soft drinks frequently which containing oxalate that increasing the risk of formation of renal stone.²³ The explanation of the relationship between VitD3 and PTH is multifaceted.^{9,24} There was a negative correlation between the PTH and VitD3 concentrations in the sera of our patients. This is concordant to the outcomes of other reports that revealed a negative correlation between the levels

of PTH and VitD3.^{2,10} Likewise, two prior researchers have displayed a strong association between PTH and VitD3 in patients with primary hyperparathyroidism.^{25,26} However, no significant relations had observed in a study conducted earlier among infants¹⁴ and adults.^{7,18}

Though still debated, prior reports from tertiary referral centers verified VitD3 insufficiency induce hypocalcemia that, in-turn, increase PTH release or “secondary hyperparathyroidism”. Higher levels of PTH in-turn elevate blood Ca. Regardless of raised Ca-reabsorption by the kidney, hypercalciuria is eventual result that place all patients at an higher risk of RS.^{2,27} Worth mentioning, in the existing study there is an alteration in PTH, VitD3, and Ca normal levels. The lack of inhibition of PTH levels by elevated VitD3 and Ca values in RS patients suggests a subtle variations of the parathyroid gland to inhibitory signals.²⁸ A current genome wide-association-reports have caught up about 6-loci in the regulation of serum Ca that might explain this phenomenon.²⁹

In the current work, there was a positive non-significant association between VitD3 levels with levels of Ca and Ph in the blood, which is compatible with the previous studies.^{2,18} While, a negative association of serum Ca with VitD3 had reported by a scholar.²⁸ On the other hand, no correlation observed between the level of serum VitD3 with Ca by another scholar.¹⁰

In contrast, in this work, there were negative correlations among serum PTH levels with Ca, Ph, and VitD3, consistent with prior study from the USA.²⁸ There was no correlation between PTH and Ph levels in a study published at 2017,² and a negative associations between serum Ca and PTH by study approved by “Mayo Clinic Institutional Review Board” at 2015.²⁸ In a cohort of RS subjects, high normal blood Ca and low normal PTH values had documented.²⁸

Renal stone analysis revealed that Ca-oxalate represented 3/4th of the total RSs. Likewise, urine crystals analysis revealed that uric acid represented 53% followed by Ca-oxalate (41%). The same results almost reported by several studies from Turkey, Spain and Italy.³⁰⁻³² Studies recommended that the frequency of monogenic RS in patients attending urologists is about 15%. For subjects without a monogenic origin of RS, the heritability of RS and hypercalciuria is >45% and >50%, individually.³³ These results are not away from our outcomes.

Hyperoxaluria is a hereditary disorder that is presented with high oxalate production with excess urinary oxalate, and a higher risk of calcium oxalate RS. Primary hyperoxaluria have three types: type-1, is the most severe, due to enzyme-mutation of “alanine-glyoxylate and serine-pyruvate aminotransferase”; type-2, due to enzyme-mutation of “glyoxylate and hydroxy-pyruvate reductase”, which is slowly progressed to ESRD; and type-3, due to enzyme-mutation in “4-hydroxy-2-oxoglutarate aldolase-1”, is least expected to progress to ESRD.³⁴ On another side, hereditary hyperuricosuria due to a defect of the enzyme “hypoxanthine-guanine phosphoribosyl-transferase”, and overactivity of “phosphoribosyl pyrophosphate synthase”.³⁵ Finally, renal phosphate wasting and hypophosphatemia caused by inhibited “fibroblast growth factor-23 (FGF23)”, a hormone that adjusts phosphate homeostasis and enhances VitD3 breakdown. Lower FGF23 levels and hypophosphatemia inhibit “CYP24A1-expression” and then enhance sensitivity to VitD3. Thus, vitamin D supplement in such subjects results in hypercalcemia and higher risk of RS and should be used with caution.³⁶ Transforming growth factor-beta (TGFβ) is released from different cells including

fibroblasts, macrophages, platelets and has multicellular impact. TGFβ essentially, belongs to TGFβ-superfamily.³⁷⁻³⁹ Currently, the researchers have exposed a specific role of TGFβ in the process of nephrolithiasis, as well it had reported that therapy with “anti-TGFβ-IgG antibody” prevents calcium oxalate-crystallization and interstitial fibrosis in nephrocalcinosis-associated CKD.⁴⁰

We think that the environmental factors contributing a crucial role in etiopathology of RS. Several factors affect subjects’ susceptibility for RS including food intake,⁴¹ individual renal GFR,¹⁸ genetic constitution,^{29,34-36,42} sunlight exposure,^{17,18} latitude and seasons of the year.^{23,24}

Further systematic investigations are desired to better realize and illustrate the exact biochemical interrelationships of Ca, Ph, PTH, and VitD3, both in the blood and urine principally respecting the metabolic, renal and skeletal disease. As well, little data are available with concerning other consequences of VitD3 insufficiency or normalization in a patient with RS.

Conclusions

There was a positive non-significant correlation between VitD3 with serum P and Ca. There was an inverse non-significant correlation between PTH with serum Ca and VitD3. Serum Ca, and Ph were non-significant predictors of and renal stones and/or urinary crystals.

Our study had conducted on a restricted number of patients. We recommend investigating Ca, P, oxalate, and uric acid values of urine in the upcoming analogous works. Second, biochemical analyses from a control individual had not obtained to perceive alterations or likenesses between the two groups. Thirdly, our outcomes had gained from a single center and perhaps could not been generalized. Finally, our conclusions should be interpreted vigilantly as the study not covering all seasons with various exposing periods of sunlight.

Authors’ Contribution

Jawad, Mazin, and Hayder read, revised, analyzed, and approved the final manuscript, other authors take a part in the data and samples collection.

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Conflicts of Interest Disclosure

The authors declare they have no conflict of interest.

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Ethical Approval

The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration. ■

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