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Assessment of Vitamin D Level in Patients with Recurrent Renal Calcium Stones

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ABSTRACT

Background: a higher concentration of the active vitamin D metabolite colic calciferol 1,25-dihydroxyvitamin D3 is associated with increased urinary calcium excretion, which could lead to increase the risk of stone formation.
Objectives: To assess vitamin D level in patients with recurrent renal calcium (Ca) stones.
Patients and methods: This study was conducted in Urology Department at Menoufia University Hospital during the period from March 2018 to December 2019. 96 patients were included in this study divided into two groups. The first group (group 1) consisted of 76 patients with renal Ca stone and the second group (group 2) consisted of 20 cases without renal stone. Patients who were included in this study seeking for treatment of recurrent renal stone that proved to be Ca stone by CT KUB or stone analysis.
Results: there was no statistically significant difference between the two groups regarding vitamin D level, there was no statistically significant difference between different types of calcium stone in relation to vitamin D levels (P= 0.981). There was no statistically significant correlation between vitamin D levels with age (P=0.300), Hounsfield unites of stone (P=0.650) or serum creatinine (P=0.690).
Conclusion: There was no statistically significant difference between studied groups regarding vitamin D level. Further studies are needed to determine whether there is any untoward consequence of a concomitant rise in intestinal absorption and urinary excretion of calcium associated with vit D supplementation in idiopathic stone formers.
Keywords: Calcium stone, Vitamin D, Recurrent, Renal stone, Urinary excretion.

INTRODUCTION

Urinary stone disease is a common and recurrent disorder with an estimated lifetime risk in the United States with incidence of 6–12%, and a recurrence rate of up to 50%. The estimated prevalence of kidney stone disease is about 3–5 % in females and 10–15 % in males [(1)]. The most common type (more than 80 %) is the calcium-based renal stone, and high urine calcium excretion is a strong risk factor for stone formation [(2)]. Both dietary pattern and genetic factors can influence urinary calcium (Ca) excretion and take a role in the pathogenesis of renal stones [(3)]. Prior studies showed that a higher concentration of the active vitamin D metabolite colic calciferol 1, 25-dihydroxyvitamin D3 (1.25 $(OH)_2$ D3), is associated with increased urinary calcium excretion, which could lead to increase the risk of stone formation [(4)]. Despite vitamin D played an important role in maintaining bone health, as well as a variety of other physiologic functions, many clinicians are not going to treat vitamin D deficiency or insufficiency in renal stone formers because of the risk of increasing urinary calcium excretion. This likely derives from the fact that vitamin D is often cited as a risk factor for renal stones [(5)]. The vitamin D endocrine cycle helps to maintain extracellular Ca^{++} level through its action in kidneys, bones, parathyroid (PTH) and intestine. 1,25 (OH)2D3 produced in the kidney (proximal tubules) enhances intestinal calcium absorption, controls bone remodeling and suppresses of PTH function (hormone production and cell growth). These effects of vitamin D help in calcium homeostasis [(6)]. Epidemiological studies of patients who developed renal stones showed that they have osteopenia, osteoporosis and hypovitaminosis D [(7)]. The aim of the present study was to assess vitamin D level in patients with recurrent renal calcium stones.

PATIENTS AND METHODS

This study was conducted in Urology Department at Menoufia University Hospital during the period from March 2018 to December 2019. 96 patients were included in this study divided into two groups.

76 patients with recurrent Ca renal stone and 20 cases without renal stone (control group). Patients included in this study were seeking for treatment of recurrent renal stone that proved to be Ca stone by CT or stone analysis.

Ethical consideration:

All participants were volunteers. All of them signed a written informed consent with explaining the aim of study before the study initiation. **Protocol of the study was approved by Ethical Scientific Committee of Menoufia University Hospital.**
All of them were selected according to inclusion and exclusion criteria.

Inclusion criteria: recurrent renal stones adult cases (20-50) years and Ca stone proved by CT or stone analysis.

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Exclusion criteria: children, congenital anomalies (ex: PUJO, ….), renal impairment (serum creatinine >2) and diabetes mellites.

All patients were assessed preoperatively by careful history taking. Sheet was performed for all patients included in this study, including personal history **(**name, age, sex, address, residence, occupation, marital status and special habits) and past history of any medical disease like diabetes, hypertension, chronic kidney diseases and its nature.

Duration, treatment and any drug intake and its regimen and duration of intake. Present history **(**onset, course, duration, site, number, recurrence and previous treatment) and history of any previous surgical interventions.

Inclusion criteria for control group: all healthy subjects aged 20-50 years old of both sexes as well as subjects who had no previous renal stones. General and physical examination was done for all patients participating in this study.

All patients and control were subjected to routine laboratory investigation complete blood picture**,** kidney function tests, liver function tests. All patients and control underwent systematic measurement of 25-OH VitD concentration in serum.

Human 25-Dihydroxy vitamin D (25-OH-D) ELISA Kit:

Test principle: The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human 25-dihydroxy vitamin D（25-OH-D) in samples. Add 25-Dihydroxy vitamin D（25-OH-D) to monoclonal antibody enzyme well which is pre-coated with human 25-Dihydroxy vitamin D（25-OH-D) monoclonal antibody. Then, add 25-Dihydroxy vitamin D（25-OH-D) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex.

Then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the human substance 25-Dihydroxy vitamin（25-OH-D) of sample were positively correlated.

Statistical Analysis

Results were tabulated and statistically analyzed by using a personal computer using MICROSOFT EXCEL 2016 and SPSS v. 21 (SPSS Inc., Chicago, IL, USA**.** Statistical analysis was done using descriptive that include percentage (%), mean and standard deviation. Analytical that includes Chi-Squared (χ^2), t test, Pearson correlation, and ROC curve. A value of P less than 0.05 was considered statistically significant.

RESULTS

There was no statistically significant difference between the studied groups regarding age (p=0.031) and sex (p= 0.063). There was no statistically significant difference between studied groups regarding vitamin D level (p=0.904) **(**Table 1**).**

Table (1): Comparison between the studied groups regarding age, sex, and vitamin D level.

	Studied subjects				**t test**	**P value**
	with renal stone (n=76)		**without renal stone (n=20)**			
Age: - Mean ± SD	42.68 ± 6.98		40.05 ± 11.47		0.84	0.31
	No	**%**	**No**	**%**	**X^2**	**P value**
Sex: - Male - Female	50 26	65.79 34.21	12 8	60.0 40.0	1.40	0.063
Vitamin D (IU) Mean ± SD	32.79 ± 2.44		33.14 ±11.20		0.121	0.904^{NS}

SD: standard deviation ***:** significant. **T:** independent t test **X^2:** chi-square

Concerning distribution of previous operation type, most of patients with renal Ca stone had ESWL (50%) followed by PCNL (27.63%) and Pyelolithotomy by 19.74%, (Fig 1). Concerning distribution of Ca stone type, most of patients had Ca oxalate (69.74%), followed by mixed Ca oxalate and Ca phosphate (15.79%), then Ca phosphate presented by (14.47%), (data not showed in table).

There was no statistically significant difference between different types of calcium stone in relation to Vitamin D levels (P= 0.981) (Table 2).

Table (2): Several studied variables and vitamin (D) levels in relation to types of calcium stone.

	Type of calcium stone				
	Ca oxalate	**Ca Phosphate**	**Ca oxalate + Ca phosphate**	**F Test**	**P value**
Age (year): -Mean ± SD	43.13 ± 6.40	41.09 ± 8.36	42.17 ± 8.42	0.42	0.56 NS
H. U: - Mean ± SD	943.40 ± 559.16	916.64 ± 587.10	957.75 ± 424.28	0.78	0.47 NS
S. creatinine (µmol/L) - Mean ± SD	1.38 ± 0.26	1.33 ± 0.26	1.43 ± 0.26	0.40	0.68 NS
Hb (g/dL) - Mean ± SD	12.07 ± 1.30	12.05 ± 1.30	12.46 ± 1.10	0.48	0.62 NS
Size of stone (cm): - Mean ± SD	1.15 ± 1.34	1.50 ± 1.54	1.55 ± 1.09	0.64	0.53 NS
Vitamin D levels (IU): - Mean ± SD	32.77 ± 2.99	32.85 ± 3.55	32.8 ± 2.3	0.23	0.981

H.U: Hounsfield unites of stone **S. creatinine**: serum creatinine **Hb:** hemoglobin
SD: standard deviation **NS:** non-significant.

There was no statistically significant correlation between vitamin D levels with age (P=0.300), Hounsfield unites of stone (P=0.650) and serum creatinine (P=0.690) (Table 3).

Table (3): correlation between Vitamin (D) levels with several studied variables.

	Vitamin (D) levels	
	r	**P value**
Age (year)	-0.11	0.300
H. U	-0.05	0.650
S. creatinine (µmol/L)	-0.04	0.690
Hb (g/dL)	0.01	0.970
Types of Calcium stone	0.002	0.988
Site of Stone (cm)	-0.183	0.114

r: correlation coefficient

ROC curve analysis showed that area under curve (AUC) of vitamin D was 0.494 with sensitivity of 0.829% and specificity of 1.00% (Table 4, Fig 1).

Table (4): Determination of cutoff value of type of calcium stone and vitamin D

Test Result Variable (s)	**Sens %**	**Spec %**	**PPV**	**AUC**	**Std. Error[a]**	**Asymptotic Sig.[b]**	**95%CI**
Vitamin D	0.829	1.00	21.7500	0.494	0.068	0.935	0.360-0.628

AUC: area under curve PPV: positive predictive value

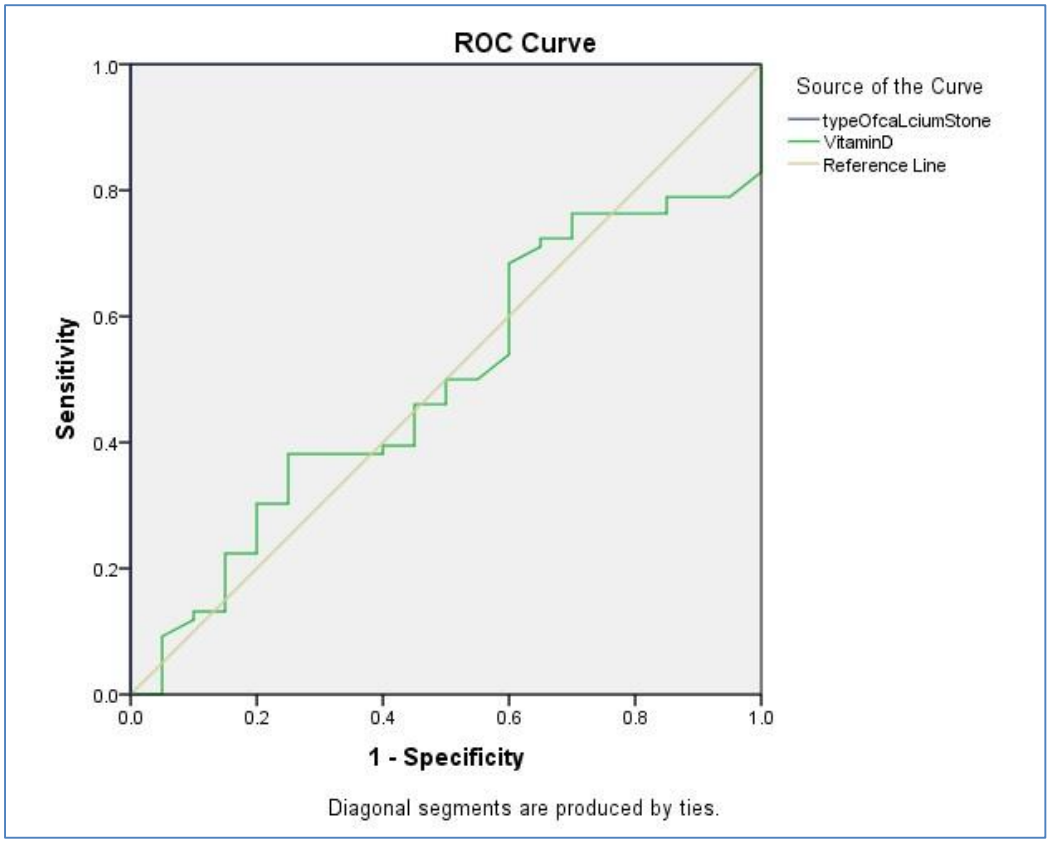

Fig. (1): ROC curve for the validity and predictivity of vitamin D.

DISCUSSION

The present study showed that, there was no statistically significant difference between the studied group regarding age (p=0.031) and sex (p= 0.063). Our results agree with the study done by **Mohammad Jafari *et al.*** [10] who showed that the prevalence of urinary stones in male patients was (64%), which was almost the same of our results. Our results are in the same line with a study by **Taheri *et al.*** [8] on 26 kidney stone patients who were treated for vitamin D deficiency, the mean age of the studied patients was 47.5 ± 12.31 years, and 67.9% of them were males.

The current study showed that, there was no statistically significant difference between studied groups regarding vitamin D level. Our results agree with the study of **Tang *et al.*** [11] who presented serum levels of vitamin D_3 in individuals with urinary tract stone and did not show significant difference compared to control group. On contrary to our results, **Beigi *et al.*** [12] showed that serum levels of vitamin D_3 were significantly higher in the group with recurrent stone than those who had a urinary stone for the first time or the control group. In addition, they found no significant difference in serum levels of vitamin D_3 in the group with urinary stone for the first time in comparison with control group.

Regarding Ca level in urine, **Penniston *et al.*** [13] concluded that consuming vitamin D_3 supplements did not increase the levels of calcium in the urine of vitamin D_3 deficient post-menopausal women without urinary tract stones [13]**.** In contrast, **Leaf *et al.*** [9] described that the consumption of vitamin D_3 supplements in patients with vitamin D_3 deficiency and urinary tract stone resulted in increasing of levels of calcium in urine. Different explanations for vitamin D deficiency and kidney stone formation could be provided. Secondary hyperparathyroidism in vitamin D-deficient group has been suggested as a reason for the higher risk of kidney stone in this group [14]**.** Another probable association may be the similar risk factors. Some major risk factors for vitamin D deficiency are living at higher altitudes, older age, female sex, low socioeconomic status, malnutrition, and obesity, which is the only common risk factor [15, 16, 17].

Our current study showed that there was no statistically significant different between different types of calcium stone in relation to age (p=0.56) and Hounsfield unites of renal stone (p=0.047). Our study agrees with the study done by **Shahnani *et al.*** [18] where they found that there was no significant difference between HU of calcium oxalate and calcium phosphate stones. Thus, they were analyzed as "calcium stones" collectively. Calcium stones had the distinct range of HU without any overlap in comparison with other types of renal stones. They had HU > 448 in all of the CT radio-densities. Another study was conducted by **Lieske *et al.*** [19] where they

demonstrated that Ca Ox stones are the most common stones across the age and sex spectra, but they are particularly common in middle-aged men. This study is consistent with a historical series from other large referral laboratories that reported that about 80% of stones are composed of Ca Ox and/or hydroxyapatite (HA) [20, 21, 22]. The most recent large series of a central laboratory for Veterans Administration facilities across the United States found several important trends including an increasing likelihood of calcium phosphate stones in a given individual as the number of stone events increased [23].

The present study showed that, there was no statistically significant difference between different types of calcium stone (calcium oxalate, calcium phosphate and mixed Ca oxalate + phosphate) in relation to vitamin D levels (P= 0.981). In another study by **Abbaszadeh *et al.*** [24] they demonstrated that there was a direct significant correlation between the level of vitamin D and calcium serum level. In addition, the study that was done by **Nguyen *et al.*** [25] demonstrated no relationship between vitamin D levels and urinary stones. In contrast to our results, **Fallahzadeh *et al.*** [26] showed significant relationship between vitamin D and calcium levels.

The current study showed that there was no statistically significant correlation between vitamin D levels with age (P=0.300), Hounsfield unites of stone (P=0.650), serum creatinine (P=0.690), hemoglobin (P=0.970), types of calcium stone (P=0.988) and site of stone (P= 0.114). In study by **Johri *et al.*** [27], they estimated the prevalence of vit D deficiency (serum 25-OH vit D $\leq$ 30 nmol/L) in a large cohort of renal stone formers [28]. It was found that thirty-one percent of these patients were vit D deficient (serum 25-OH vit D 20.4 ± 5.6 nmol/L; 8.2 ± 2.2 ng/mL), while 57% were vit D insufficient (48.9 ± 12.3 nmol/L; 19.6 ± 4.9 ng/mL), and the rest (12%) classified as vit D replete (97.4 ± 22.4 nmol/L; 39.0 ± 9.0 ng/mL). The groups were not significantly different in age. In addition, multiple correlation studies revealed no association between 24-h urine excretion rates of calcium, oxalate, citrate, phosphate or urinary pH, respectively, and serum 25-OH vit D.

CONCLUSION

There was no statistically significant difference between studied groups regarding vitamin d level, Also, there was no statistically significant difference between different types of calcium stone (calcium oxalate, calcium phosphate and mixed Ca oxalate + phosphate) in relation to vitamin d levels. We would recommend monitoring urinary calcium excretion in vit D-supplemented stone formers. Further studies are needed to determine whether there is any untoward consequence of a concomitant rise in intestinal absorption and urinary excretion of calcium associated with vit D supplementation in idiopathic stone formers.

REFERENCES

1. **O'Kell A, Grant D, Khan S (2017):** Pathogenesis of calcium oxalate urinary stone disease: species comparison of humans, dogs, and cats. Urolithiasis, 45 (4): 329-36.
2. **Dornbier R, Bajic P, Van Kuiken M *et al.* (2019):** The microbiome of calcium-based urinary stones. Urolithiasis, 3: 1-9.
3. **Asai M, Sowmiya K, Anbu N (2019):** Investigation of factors influencing urolithiasis in patients from northern metro zone a cross sectional observation study. Int J Trans Res Ind Med., 1 (2): 41-7.
4. **Melo T, Esper P, Zambrano L *et al.* (2019):** Expression of vitamin D receptor, CYP27B1 and CYP24A1 hydroxylases and 1, 25-dihydroxyvitamin D 3 levels in stone formers. Urolithiasis, 3: 1-8.
5. **Vitale C, Bermond F, Guiotto C *et al.* (2019):** FP006 metabolic effects of cholecalciferol supplementation in kidney stone formers with vitamin d deficiency. Nephrology Dialysis Transplantation, 34 (1): 106-111.
6. **Fleet J (2017):** The role of vitamin D in the endocrinology controlling calcium homeostasis. Molecular and Cellular Endocrinology, 453: 36-45.
7. **Verdelli C, Corbetta S (2017):** Kidney involvement in patients with primary hyperparathyroidism: an update on clinical and molecular aspects. European Journal of Endocrinology, 176 (1): 39-52.
8. **Taheri M, Tavasoli S, Shokrzadeh F *et al.* (2019):** Effect of vitamin D supplementation on 24-hour urine calcium in patients with calcium Urolithiasis and vitamin D deficiency. International Braz J Urol., 45 (2): 340-6.
9. **Leaf D, Korets R, Taylor E *et al.* (2012):** Effect of vitamin D repletion on urinary calcium excretion among kidney stone formers. Clinical Journal of the American Society of Nephrology, 7 (5): 829-34.
10. **Mohammad Jafari H, Barzin M, Salehifar E *et al.* (2014):** Etiologic and epidemiologic pattern of urolithiasis in north Iran; review of 10-year findings. Iran J Pediatric, 24 (1): 69–74.
11. **Tang J, McFann K, Chonchol M (2012):** Association between serum 25-hydroxyvitamin D and nephrolithiasis: The National Health and Nutrition Examination Survey III, 1988–94. Nephrology Dialysis Transplantation, 27 (12): 4385-9.
12. **Beigi M, Shirani M, Motamedi M (2018):** The effect of serum levels of vitamin D in stone recurrence in patients with urinary tract stone. Journal of Renal Injury Prevention, 7 (2): 167-173.
13. **Penniston K, Jones A, Nakada S *et al.* (2009):** Vitamin D repletion does not alter urinary calcium excretion in healthy postmenopausal women. BJU International, 104 (10): 1512-6.
14. **Tavasoli S, Taheri M (2019):** Vitamin D and calcium kidney stones: a review and a proposal. International Urology and Nephrology, 51 (1): 101-11.
15. **Beveridge L, Struthers A, Khan F *et al.* (2015):** Effect of vitamin D supplementation on blood pressure: a systematic review and meta-analysis incorporating individual patient data. JAMA Internal Medicine, 175 (5): 745-54.

16. **Mithal A, Wahl D, Bonjour J *et al.* (2009):** Global vitamin D status and determinants of hypovitaminosis D. Osteoporosis International, 20 (11): 1807-20.
17. **Holick M (2016):** Vitamin D deficiency. New England Journal of Medicine, 357(3):266-81.
18. **Shahnani P, Karami M, Astane B *et al.* (2014):** The comparative survey of Hounsfield units of stone composition in urolithiasis patients. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences, 19 (7): 650-55.
19. **Lieske J, Rule A, Krambeck A *et al.* (2014):** Stone composition as a function of age and sex. Clinical journal of the American Society of Nephrology, 9 (12): 2141-6.
20. **Mandel N, Mandel G (1989):** Urinary tract stone disease in the United States veteran population. II. Geographical analysis of variations in composition. J Urol., 142: 1516–1521.
21. **Herring L (1962):** Observations on the analysis of ten thousand urinary calculi. J Urol., 88: 545–562.
22. **Prien E (1955):** Studies in urolithiasis. III. Physicochemical principles in stone formation and prevention. J Urol., 73: 627–652.
23. **Mandel N, Mandel I, Fryjoff K (2003):** Conversion of calcium oxalate to calcium phosphate with recurrent stone episodes. J Urol., 169: 2026–2029.
24. **Abbaszadeh S, Shahverdi E, Beiraghdar F *et al.* (2018):** Serum Level of Vitamin D3 and Renal Stone in Children. Journal of Comprehensive Pediatrics, 9 (2): 180.
25. **Nguyen S, Baggerly L, French C *et al.* (2014):** 25-Hydroxyvitamin D in the range of 20 to 100 ng/mL and incidence of kidney stones. Am J Public Health, 104 (9): 1783–7.
26. **Fallahzadeh M, Zare J, Al-Hashemi G *et al.* (2012):** Elevated serum levels of Vitamin D in infants with urolithiasis. Iran J Kidney Dis., 6 (3): 186–91.
27. **Johri N, Jaeger P, Ferraro P *et al.* (2017):** Vitamin D deficiency is prevalent among idiopathic stone formers, but does correction pose any risk? Urolithiasis, 45 (6): 535-43.
28. **Looker A, Johnson C, Lacher D *et al.* (2011):** Vitamin D status: USA, 2001–2006. NCHS Data Brief., 59: 1–8.