Urol Res (2000) 28:155–158

ORIGINAL PAPER

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Loss of regulation of circulating 1,25-dihydroxyvitamin D with paradoxically decreased serum phosphate levels in individuals with recurrent kidney stones

Received: 27 January 1999 / Accepted: 25 June 1999

Abstract A low serum phosphate concentration is characteristic in individuals in whom kidney stones form, this being related to serum 1,25-dihydroxyvitamin D, parathyroid hormone and urinary phosphate excretion. In order to determine whether these parameters are related to recurrence of stone formation, they were analyzed in single and recurrent stone formers as well as controls. An inverse correlation between serum levels of phosphate and 1,25-dihydroxyvitamin D was observed in control subjects, indicating that a drop in serum phosphate results in upregulated circulating 1,25-dihydroxyvitamin D level in controls. While the circulating low phosphate level upregulated the 1,25-dihydroxyvitamin D level in single stone formers, the elevation was less than expected from the drop in serum phosphate in recurrent stone formers. The results thus suggest that loss of upregulation of 1,25-dihydroxyvitamin D by serum levels of phosphate might be important for stone formation. The possibility of deregulation of 1,25-dihydroxyvitamin D to maintain physiological requirements in stone formers and prevent further nephrolithiasis therefore warrants attention.

Key words 1,25-Dihydroxyvitamin D · Recurrence of kidney stone formation · Phosphate · Stone formers · Hypercalciuria · Normocalciuria

Introduction

Urolithiasis is a common disease with an annual incidence (number of patients per 100,000 general population) of 92.5 in Japan [1], 164 in the United States [2], 300 in Norway [3], and 200 in Sweden [4]. Recurrence of stone formation occurs in more than 50% of patients if no medical adjustment is made after primary treatment [5]. Although no data are available for Japan, medical care costs for urolithiasis were estimated to be more than two billion dollars spent in the United States in 1986 [6]. Therefore, clarification of the pathogenesis of nephrolithiasis is a high priority for development of prevention approaches.

A low serum concentration of phosphate, as compared with healthy subjects, is evident in normocalciuric stone formers [7]. This is due to renal leakage of phosphate which therefore is a major risk factor for stone formation [7, 8]. Serum phosphate levels are regulated by circulating 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D) levels with reverse correlation evident in healthy subjects [9].

Therefore, it is logical that low serum phosphate levels might be linked to abnormal regulation of serum 1,25-$(OH)_2$D levels. Since this has not been clarified, the present study was conducted with the aim of establishing the relationship between serum 1,25-$(OH)_2$D, phosphate levels and the risk of stone formation.

Materials and methods

Twenty-eight single stone formers, 29 recurrent stone formers, and 21 non-stone formers as controls participated in the present study approved by the ethics committee. The stone formers suffered from calcium-containing kidney stones. The diagnosis of kidney stones was mainly based on roentgenograph findings and the analysis of stone constitution. Informed consent was obtained from all subjects. Non-stone formers were admitted to Mie University Hospital to determine whether they suffered from urological disease, malignancies, or renal dysfunction. Clinical examinations proved that all were negative. Stone formers with renal tubular acidosis, hyperparathyroidism, renal hypercalciuria, aminoaciduria, or infectious stones were excluded from this study. Also, stone formers with elevated serum parathyroid hormone (PTH) levels did not participate. Blood and urine samples were collected at least twice after 12-h fasting and serum 1,25-$(OH)_2$D, parathyroid hormone, calcium and phosphate levels were measured. Urine samples

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were analyzed for assessment of calcium and phosphate excretion and the results were expressed as the ratios to urine creatinine. The mid-region of parathyroid hormone-(44–68) was determined, using the method described previously [10]. The inter- and intra-assay coefficients of variation (CVs) were 4.3 and 5.2%, respectively, and the sensitivity was 100 pg/ml. For assays of serum 1,25-$(OH)_2D$ levels, Watanabe's method was used [11]. The inter- and intra-assay coefficients of variation were 5.1 and 6.7%, respectively, and the sensitivity was 4 pg/ml. Unpaired *t*-tests were used to analyze the statistical significance. *P*-values less than 0.05 were defined as statistically significant. The data are represented as mean ± SD.

Results

The serum phosphate levels in single or recurrent stone formers were significantly lower than in the controls ($P < 0.05$ and $P < 0.001$, respectively) (Table 1). While urine phosphate excretion in single and recurrent stone formers was significantly elevated compared to controls ($P < 0.0012$, $P < 0.043$, respectively), there was no significant variation in urine phosphate excretion between the two groups ($P = 0.533$). There were no significant differences in serum calcium, PTH levels, and urine calcium-creatinine ratio between stone formers and controls. Moreover, there was no significant variation in values for these parameters between the single and recurrent groups. The serum 1,25-$(OH)_2D$ levels in single stone formers ($n = 28$) and in recurrent stone formers ($n = 29$) were 44.2 ± 13.9 pg/ml, and 35.53 ± 9.05 pg/ml (mean ± SD), respectively, the difference being significant ($P < 0.028$), whereas serum phosphate levels did not differ. The low serum phosphate level did not upregulate the serum level of 1,25-$(OH)_2D$ in recurrent stone formers to the level seen in single stone formers.

As shown in Fig. 1A, the concentration of serum 1,25-$(OH)_2D$ was significantly and inversely related to the concentration of serum phosphate in controls ($r = -0.569$, $P < 0.01$). This correlation was not evident in stone formers (Fig. 1B, C) .

Discussion

The physiological role of $1,25(OH)_2D$ is to maintain bone mineral density and to prevent ectopic calcification. An excess circulating $1,25(OH)_2D$ concentration results in vitamin D toxicity. Deficiency, on the other hand, causes vitamin D-dependent rickets [12]. Most physicians concur that elevated serum $1,25(OH)_2D$ levels produce abnormal calcification in organs and tissues. Recently, Watson and his associates reported that serum $1,25(OH)_2D$ levels were correlated with the degree of calcification in coronary arteries in patients with moderate and high risk for coronary heart disease [13].

Werness and his associates reported that phosphate concentration in urine is related to stone formation [8]. Certainly, single and recurrent stone formers both demonstrated significantly increased phosphate excretion in our study. Renal phosphate leakage exists in stone formers at high frequency [7]. Urinary phosphate excretion discriminates between stone formers and non-stone formers, but does not appear to be a determining factor for recurrent stone formation (Table 1). Circulating calcium and phosphate levels control the rate of 1,25-$(OH)_2D$ production and its circulating levels in healthy subjects [14, 15]. Depletion causes increase of 1,25-$(OH)_2D$ production via PTH. The enzyme, 25-hydroxyvitamin D-1-hydroxylase that is located in proximal tubules, converts 25-hydroxyvitamin D to 1,25-$(OH)_2D$ [16]. The loss of correlation between 1,25-$(OH)_2D$ and serum phosphate in stone formers might suggest dysfunction of renal proximal tubules. However, there is been no direct evidence for such renal tubule dysfunction. Likewise, the findings of the present study cannot authenticate whether low circulating phosphate levels arise from a primary metabolic disorder or are secondary to other factors. Thus to gain an understanding of the cause will require further studies.

The data in this study suggest that recurrent stone formation is not related to urinary phosphate excretion, but rather to circulating 1,25-$(OH)_2D$ levels. However, elevated serum 1,25-$(OH)_2D$ levels have long been known to be associated with absorptive hypercalciuria [16], about 30 to 80% of patients suffering from the latter having high circulating 1,25-$(OH)_2D$ values [17–23]. A recent study using ketaconazole, a p450 inhibitor that impairs proximal tubular production of 1,25-$(OH)_2D$, suggested that in about 60% of patients with idiopathic hypercalciuria, 1,25-(OH)-$_2D$ dependent calcium absorption is reduced [17]. However, abnormal

Table 1 Characterization of stone formers with reference to recurrence. (*SF* stone former). The data presented are means ± SD. Significant differences between values for stone formers and controls were calculated with unpaired *t*-tests and are indicated by $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. A significant difference between serum 1,25-$(OH)_2D$ in single and recurrent stone formers is indicated by $*P < 0.05$

	Controls	Single SF	Recurrent SF
Number of cases	21	28	29
Age	58.7 ± 12.4	54.5 ± 14.9	57.3 ± 11.8
Sex			
Male	16	21	22
Female	5	7	7
Serum phosphate (mg/dl)	3.48 ± 0.52	2.88 ± 0.66*	2.86 ± 0.41**
Serum 1,25-$(OH)_2D$ (pg/ml)	34.2 ± 8.4	44.5 ± 13.4*	35.53 ± 9.05*
Serum calcium (mg/dl)	9.17 ± 0.26	9.07 ± 0.30	9.09 ± 0.32
PTH (pg/ml)	274.3 ± 77.8	284.5 ± 83.9	318.5 ± 103.1
Urinary calcium/creatinine	0.12 ± 0.05	0.13 ± 0.06	0.13 ± 0.05
Urinary phosphate/creatinine	0.49 ± 0.15	0.64 ± 0.17**	0.61 ± 0.19*

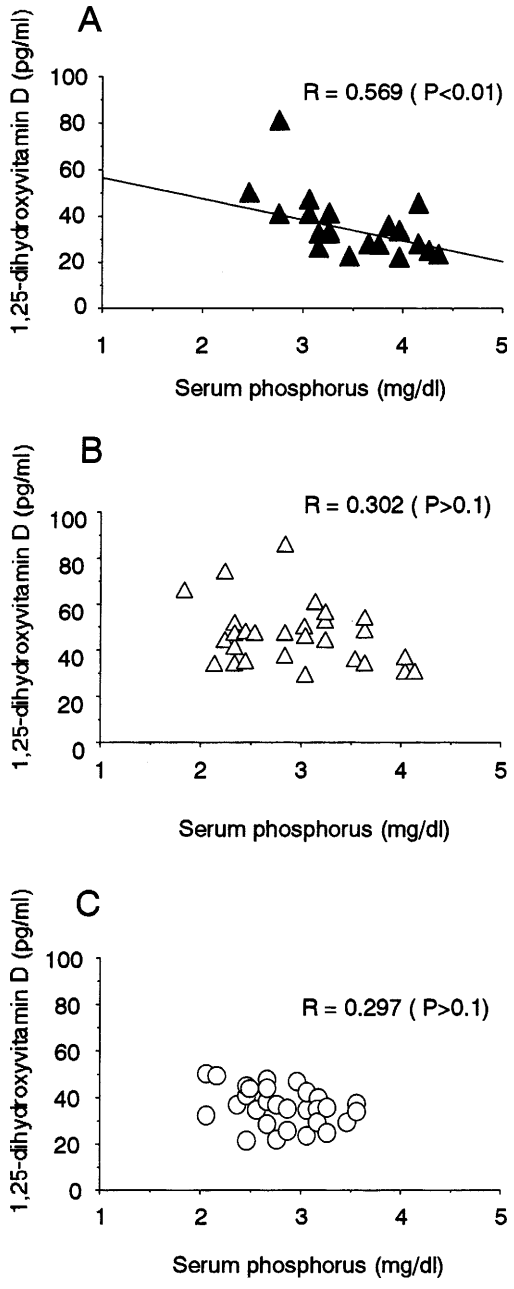

Fig. 1A–C The relationship between morning fasting serum levels of 1,25-$(OH)_2$D and phosphate. **A** shows the results for control patients ($n = 21$). The regression line is expressed by the equation $y = (-9.12) \times (+65.9)$. The standard errors for the slope and intercept are 3.02 and 10.6, respectively. **B** and **C** show the results for single and recurrent stone formers, respectively: no correlation was apparent in these cases

activity of the vitamin D receptor has yet to be proven [19]. Since only two stone formers demonstrated elevated circulating 1,25$(OH)_2$D levels in the present study, we did not focus on urinary calcium excretion, but rather on the relationship between serum 1,25$(OH)_2$D and recurrence of stone formation.

Although patients with some metabolic disorders known to be implicated in calcium-phosphate metabolism and 1,25$(OH)_2$D synthesis were excluded from the present study, type III absorptive hypercalciuria [24] might have been a complicating factor. This might disturb analysis of the relationship between circulating 1,25-$(OH)_2$D and phosphate levels. An increased circulating 1,25-$(OH)_2$D level causes stimulation of the intestinal absorption of calcium, resulting in an increased urinary excretion of calcium. An increased calcium absorption in the intestine might stimulate oxalate absorption. However, statistically, stone formers with hyperoxaluria reveal normal urinary calcium excretion, compared to normal subjects [24]. Rather than urinary oxalate excretion, low circulating phosphate levels were noteworthy. While serum 1,25$(OH)_2$D and phosphate levels were inversely correlated in controls, this was not the case with stone formers. Moreover, circulating 1,25$(OH)_2$D was lower than expected from the observed phosphate decrease in the recurrent stone formers (Table 1). Thus, this loss of regulatory response appeared to be related to stone formation and to be most pronounced in the recurrent stone formers. However, the relationship between the pathogenesis of stone formation and loss of regulatory response of circulating 1,25$(OH)_2$D by serum phosphate levels could not be elucidated in the present study.

In conclusion, our reports indicate that lower than expected serum concentrations of 1,25$(OH)_2$D might be related to the recurrence of stone formation. Thus, the data suggest that stone formers lose the inverse correlation between serum levels of phosphate and 1,25-$(OH)_2$D that is observed in control subjects. The possibility that upregulation of serum 1,25$(OH)_2$D levels to maintain physiological requirements might prevent recurrence of nephrolithiasis if patients have no remarkable hypercalciuria therefore deserves further investigation.

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