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DIALYSIS – TRANSPLANTATION

Pretreatment serum FGF-23 levels predict the efficacy of calcitriol therapy in dialysis patients

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Pretreatment serum FGF-23 levels predict the efficacy of calcitriol therapy in dialysis patients.

Background. The predictor for the result of calcitriol therapy would be useful in the clinical practice of secondary hyperparathyroidism. Fibroblast growth factor-23 (FGF-23) is a newly found circulating phosphaturic factor. Its circulating level is elevated in uremia.

Methods. Dialysis patients with plasma intact parathyroid hormone (iPTH) levels greater than 300 pg/mL were included in the study. Calcitriol was intravenously injected three times a week. The patients whose plasma iPTH levels dropped below 300 pg/mL within 24 weeks were defined as those who had been successfully treated. A sandwich enzyme-linked immunosorbent assay (ELISA) system that detects human FGF-23 was applied.

Results. Sixty-two patients were analyzed. The pretreatment FGF-23 levels were related to the iPTH levels, calcium × phosphate product levels, and history of active vitamin D therapy. The pretreatment FGF-23, iPTH, and calcium levels were lower in the patients who would be successfully treated with calcitriol. A logistic regression study revealed that the pretreatment iPTH and FGF-23 levels significantly affected the therapy results. Analyses using a receiver-operated curve revealed that FGF-23 was the best screening test for identifying patients with future refractory response to calcitriol therapy. The treatment would be successful in 88.2% of those with FGF-23 ≤9860 ng/L and iPTH ≤591 pg/mL, while it would be successful in only 4.2% of those with FGF-23 >9860 ng/L and iPTH >591 pg/mL.

Conclusion. Pretreatment serum FGF-23 levels were a good indicator in predicting the response to calcitriol therapy. The measurement of serum FGF-23 levels, especially in combination with iPTH levels, is a promising laboratory examination for the clinical practice of secondary hyperparathyroidism.

Key words: fibroblast growth factor-23 (FGF-23), intact parathyroid hormone (iPTH), calcitriol, secondary hyperparathyroidism.

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Secondary hyperparathyroidism is a common complication among long-term dialysis patients, and in many cases, intermittent intravenous calcitriol administration is an effective therapy [1]. However, refractoriness to medical therapy occurs in cases with developed hyperparathyroidism, presumably due to changes in the biological properties of the parathyroid cells [2–4]. Continuing medication with active vitamin D metabolites in patients with refractory secondary hyperparathyroidism is not only ineffective, but is also likely to worsen their prognosis because of the medication's unfavorable effects on calcium and phosphate metabolism [5, 6].

It would therefore be useful to predict this refractoriness before initiation of the calcitriol therapy. In this regard, pretreament plasma intact parathyroid hormone (iPTH) levels and serum calcium (Ca) levels have been found to influence the effectiveness of active vitamin D therapy, and ultrasonographic parathyroid morphometry may help to predict this effectiveness [7, 8]. Serum inorganic phosphate (Pi) may also be implicated in the refractoriness of secondary hyperparathyroidism [9]. The above factors are currently used as predicting factors in clinical practice; their reliability, however, remains unsatisfactory. As such, the identification of other indicators that can predict the effectiveness of calcitriol therapy would certainly improve the quality of therapy of secondary hyperparathyroidism.

Fibroblast growth factor-23 (FGF-23) [10] is a natural circulating phosphaturic factor that was discovered in samples obtained from patients with autosomal-dominant hereditary rickets [11] and tumor-related osteomalacia [12]. Circulating FGF-23 seems to play a role in the regulations of phosphaturisis and vitamin D 1-α hydroxylase activity [13–17].

Circulating FGF-23 levels are elevated in patients with chronic renal failure [18–20], and these levels may play an important role in the development of secondary

hyperparathyroidism in predialysis patients [21]. Circulating FGF-23 levels affect the future development of secondary hyperparathyroidism in dialysis patients with mild secondary hyperparathyroidism. As such, it is possible that pretreatment circulating FGF-23 levels may allow us to predict the refractoriness to calcitriol therapy.

We have therefore carried out a prospective clinical study. The aim of this study was to verify the impact of pretreatment serum FGF-23 levels in predicting the refractoriness to calcitriol therapy in dialysis patients with moderate to severe secondary hyperparathyroidism.

METHODS

Patients

Chronic hemodialysis patients whose plasma iPTH levels were greater than 300 pg/mL were included in the study. Patients with malignancy or chronic inflammatory diseases were excluded. The study was designed according to the Helsinki Declaration, and was approved by the ethics committees of the medical facilities involved. The patients were duly informed about the study, and written consent was obtained before enrollment.

Methods

The washout period consisted of ceasing the use of active vitamin D agents four weeks prior to initiation of the study. After completion of the washout period, 1 μg of calcitriol was intravenously injected three times per week at the end of every dialysis session as an initial dosage. Thereafter, the amount of calcitriol per injection varied between 0.5 and 1.5 μg so that the serum Ca concentrations would not exceed 11.0 mg/dL, or so the Ca × inorganic phosphate (Pi) product would not exceed 75 $(mg/dL)^2$ on the third day after the last dialysis session. Hemodialysis conditions, including dialyzers, dialysate compositions, and anticoagulants, remained unchanged throughout the study period. The Ca concentration of the dialysate was 3.0 mEq/L. The goal of the treatment was to reduce the iPTH levels to less than 300 pg/mL. The treatment was continued for the 24 weeks of the study period unless the goal of the treatment was achieved, or unless one of the following events occurred: (*1*) the use of other active vitamin D analogs, glucocorticoid agents, bisphosphonates, or any other antiosteoporotic agents; (*2*) hypercalcemia with the serum Ca levels exceeding 11.5 mg/dL; and (*3*) hyperphosphatemia with the serum Pi levels exceeding 10.0 mg/dL on the third day after the last dialysis session, despite the increased dose of oral phosphate binders. Those patients with hypercalcemia, hyperphosphatemia, or any other harmful events associated with the calcitriol therapy were subjected for surgical parathyroidectomy after withdrawing from the study. The measured serum Ca levels were adjusted with the albumin levels as follows when they were lower than 4.0 g/dL: Ca = measured Ca levels + [4.0− albumin levels (g/dL) × 0.8] mg/dL. The corrected serum Ca is given throughout the paper. The treatment was also discontinued if any other intolerable events occurred.

The serum Ca, Pi, albumin, and plasma iPTH levels were monitored at least once every four weeks in each dialysis unit. The plasma iPTH levels were measured with a two-site immunoradiometric method (Nichols Institute, San Juan Capistrano, CA, USA). Serum FGF-23 levels were determined with a sandwich enzyme-linked immunosorbent assay (ELISA) system using two kinds of monoclonal antibodies requiring the simultaneous presence of both the N-terminal and C-terminal portions of FGF-23 (Kainos Laboratories, Inc., Tokyo, Japan) [22]. The interassay variation in the normal and elevated concentration range was found to be <6%. All of the blood samples mentioned above were collected at 9:00 a.m. on the third day after the last dialysis session.

The effect of treatment was judged 24 weeks after initiation of the calcitriol therapy. The patients whose plasma iPTH levels dropped below 300 pg/mL within the 24 weeks without harmful events were defined as those for whom treatment had been successful.

Statistical analysis

All values are expressed as mean ± SD, and $P < 0.05$ was considered statistically significant. Paired and unpaired *t* tests were applied to compare the results between groups. A logistic regression method was applied to sort out the factors that significantly affected the results of calcitriol therapy. For the logistic analysis, the measured values were divided into five levels according to the ranks in order to compare the obtained odds ratios impartially. An analysis using a receiver-operated curve (ROC) [23, 24] was performed to verify the screening abilities of each factor to discriminate patients who would be successfully treated with calcitriol. Statistical computations were performed using an Apple Macintosh G4 computer with the Stat View 5.0 software (SAS Institute, Inc., Cary, NC, USA).

RESULTS

The clinical courses

Sixty-two patients were included in the study. Their general clinical features are listed in Table 1.

None of the patients began to use glucocorticoid agents, bisphosphonates, or antiosteoporotic agents during the study. Calcium bicarbonate was the only oral phosphate binder used during the observation period, and the daily dosage was changed in none of the patients. The treatment was discontinued in two patients because of hypercalcemia that exceeded 11.5 mg/dL. The treatment

Table 1. Summary of the clinical features of all 62 patients included in the study

Age *years*	Gender	Duration of hemodialysis	Primary disease
58.9 ± 12.8	M40:F22	120.8 ± 98.5 (M)	CGN 44 DMGS 8 NSc 3 Others 4 Unknown 3

Abbreviations are: CGN, Chronic glomerulonephritis; DMGS, diabetic glomerulosclerosis; Nsc, hypertensive nephrosclerosis.

Table 2. The changes in biochemical data throughout the study

	0W ($N = 62$)	8W ($N = 48$)	24W ($N = 45$)
A			
iPTH *pg/mL*	702 ± 294	614 ± 321	564 ± 329[a]
Ca *mg/dL*	9.1 ± 1.0	9.7 ± 9[b]	9.7 ± 9[c]
Pi *mg/dL*	6.0 ± 1.3	6.3 ± 1.1	6.8 ± 1.7[b]
FGF-23 *pg/mL*	19771 ± 20780	25354 ± 24063	28823 ± 23128[a]
	0W	8W	24W
B	($N = 45$)	($N = 45$)	($N = 45$)
iPTH *pg/mL*	758 ± 306	613 ± 310[c]	564 ± 329[c]
Ca *mg/dL*	9.2 ± 1.0	9.6 ± 9[c]	9.7 ± 9[c]
Pi *mg/dL*	6.0 ± 1.3	6.3 ± 1.2[a]	6.8 ± 1.7[c]
FGF-23 *pg/mL*	23192 ± 22171	26640 ± 24583	28823 ± 23128[a]

Calcitriol therapy reduced the plasma iPTH levels and increased the serum Ca, Pi, and FGF-23 levels. Samples were obtained three days after the last dialysis session. All the patients in group A participated (unpaired ± test). Group B comparised 45 patients who received the calcitriol therapy throughout the 24 weeks (paired ± test). The normal FGF-23 range among healthy volunteers is 26.3 ± 8.4 pg/mL.

[a] $P < 0.05$ vs. 0W.

[b] $P < 0.01$ vs. 0W.

[c] $P < 0.001$ vs. 0W.

goal was achieved in 15 patients before the 24th week. The remaining 45 patients received calcitriol therapy three times per week for 24 weeks. Of those 45 patients, the plasma iPTH levels were below 300 pg/mL in 11 patients, and above 300 pg/mL in 34 patients at the 24th week.

Table 2 shows the changes in biochemical data throughout the study. Calcitriol therapy reduced the plasma iPTH levels, and increased the serum Ca, Pi, and FGF-23 levels.

The pretreatment serum levels of serum FGF-23

The pretreatment Ca × Pi product levels, months after initiation of the chronic hemodialysis therapy, as well as previous active vitamin D therapy within one-year period before the washout period were found to be significantly related to the pretreatment serum FGF-23 levels (Figs. 1 and 2). The pretreatment iPTH levels also showed a weak correlation with FGF-23.

Clinical profiles of the successfully treated patients

The pretreatment plasma iPTH, serum Ca, and serum FGF-23 levels were significantly lower in patients who would be successfully treated (Fig. 3).

Prediction of successful treatment

The overall successful treatment rate was 26/62 (41.9%). A logistic regression study revealed that the pretreatment serum FGF-23 and plasma iPTH levels significantly affected the results of calcitriol therapy (Table 3).

An analysis using an ROC was performed to verify the screening abilities of iPTH, Ca, FGF-23, and Pi levels. The area under the curve (AUC) obtained by FGF-23 (8429.5) was more than those of iPTH (7970.1, $P < 0.05$), Ca (7959.4, $P < 0.05$), and Pi (5170.9, $P < 0.0001$). The maximum value of sensitivity (%) × specificity (%) product was 76.9 × 83.3 = 6410.3 by FGF-23, 76.9 × 75.0 = 5769.2 by iPTH, 84.6 × 63.9 = 5406.0 by Ca, and 65.4 × 44.4 = 2906.0 by Pi, respectively. When 84.6% of sensitivity was obtained by FGF-23, the corresponding specificity was 75.0% (Fig. 4, Table 4).

The treatment would be successful in 88.2% of those with FGF-23 ≤9860 ng/L and iPTH ≤591 pg/mL, while it would be successful in only 4.2% of those who with FGF-23 >9860 ng/L and iPTH >591 pg/mL (Table 4).

DISCUSSION

Recent findings demonstrated that FGF-23 plays an important role in the regulation of phosphate and vitamin D metabolism [25, 26]. Several lines of evidence indicating that chronic renal failure accompanies significant increase in serum FGF-23 levels; however, the measurement of serum FGF-23 levels has not been applied as a clinical laboratory examination. In this study, serum FGF-23 levels were found to be a good indicator for predicting the refractoriness to calcitriol therapy. It is naturally understandable that pretreatment iPTH levels affect the result of calcitriol therapy. To our astonishment, the predictability of FGF-23 was at least comparable to that of iPTH itself. Today, the reliability of iPTH and Ca levels remains unsatisfactory in estimating the refractoriness to calcitriol therapy, and therefore, the aimless continuation of the therapy is often performed. Although Pi is recognized as an activating factor for parathyroid function, this study revealed that pretreatment serum Pi levels could not predict the result of calcitriol therapy [27]. Nevertheless, we do not intend to neglect the need for Pi control in uremic patients.

Recently, vascular calcification associated with uremia has drawn attention of many researchers [28, 29]. The vascular calcification is caused at least in part by the overtreatment with active vitamin D therapy [30]. The estimation of the refractoriness to medical therapy would help avoiding the overtreatment with calcitriol and reduce the risk of vascular calcification. Thus, the measurement of circulating FGF-23 levels is a promising laboratory examination that may bring an improvement in the clinical treatment of secondary hyperparathyroidism. The usefulness was even emphasized when it was applied

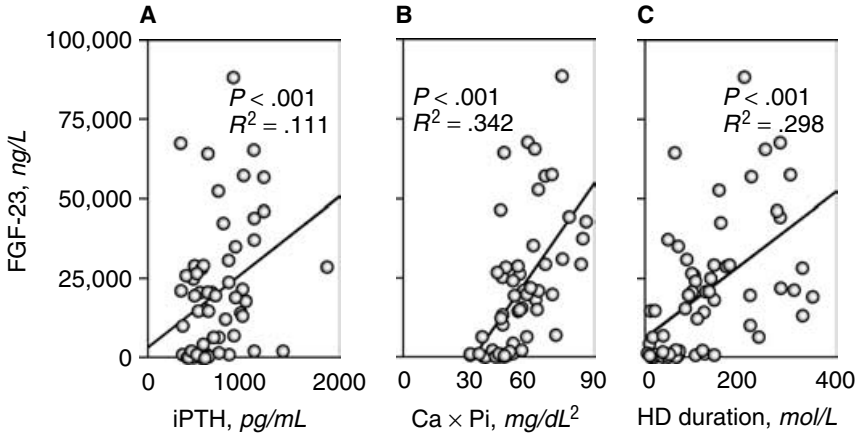

Fig. 1. The pretreatment iPTH levels (*A*), Ca × Pi product levels (*B*), and months after the initiation of chronic hemodialysis therapy (*C*) showed weak correlations with the pretreatment serum FGF-23 levels.

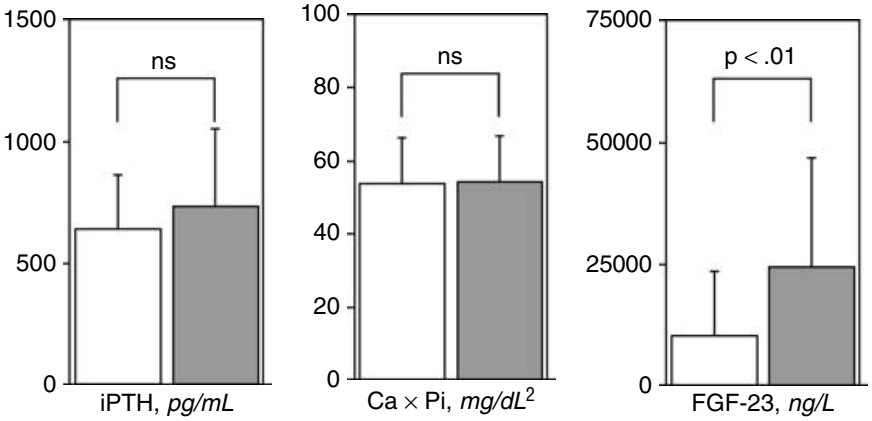

Fig. 2. The previous active vitamin D therapy within the one-year before the washout period independently related to the pretreatment serum FGF-23 levels from iPTH levels or Ca × Pi product levels. Open bars signify patients who had not been treated with calcitriol for at least one year before the washout period. Closed bars signify patients who had been treated with calcitriol within one year before the washout period.

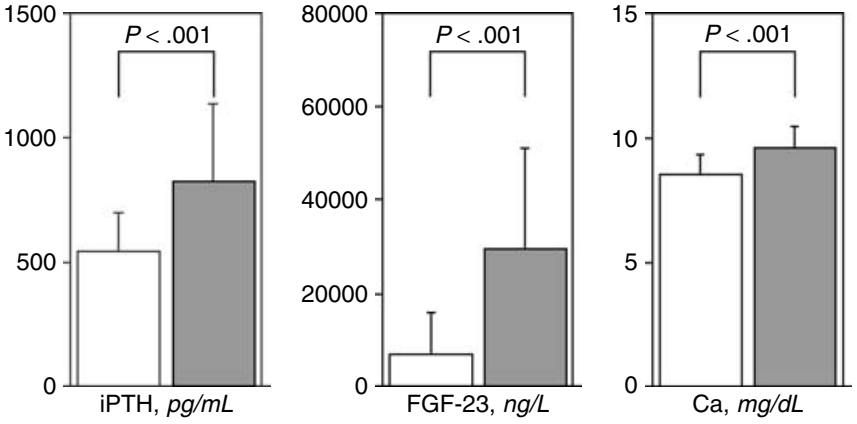

Fig. 3. The pretreatment plasma iPTH levels, serum FGF-23, and Ca levels were significantly lower in the patients for whom treatment had been effective. Open bars signify patients who could be successfully treated with calcitriol. Closed bars signify patients for whom the calcitriol treatment failed.

in combination with iPTH (Table 4). Further prospective clinical studies are necessary to confirm whether the measurement of circulating FGF-23 levels contributes to the improved prognosis of uremic patients with secondary hyperparathyroidism. Moreover, it is of interest whether the pretreatment serum FGF-23 levels predict the result of therapy with not only calcitriol, but also calcitriol analogs such as paricalcitol [31] or maxacalcitol [32].

The mechanisms by which FGF-23 levels are elevated in dialysis patients remain unknown. The levels in dialysis patients are not only generally high, but are distributed across quite a wide range [18–20]. Moreover, the assay system we applied in this study did not detect an inactive metabolite of FGF-23 [22] that would be cleared through urinary excretion. Therefore, the elevated levels of serum FGF-23 in dialysis patients cannot simply be explained by the impairment of glomerular filtration, and suggest that the accelerated production of FGF-23 may coincidently occur. Parathyroid function showed a weak but significant correlation with the serum FGF-23

Table 3. A logistic regression analysis to detect the factors affecting the results of the calcitriol therapy applying five classified levels according to the rank of measured values in FGF-23, iPTH, Ca, and Pi

	P	k^2	Odds ratio	(95% range)
FGF-23	.0055	7.695	3.075	(1.390–6.800)
iPTH	.0066	7.366	2.346	(1.267–4.343)
Ca	.5758	.313	1.234	(.591–2.576)
Pi	.1112	2.537	.578	(.294–1.135)

The study revealed that higher pretreatment circulating FGF-23 and iPTH levels are significantly related to the refractoriness of the calcitriol therapy. FGF-23, −990, 1070–7030, 9860–20500, 20600–29300, 30700− (*ng/L*); iPTH, −470, 472–559, 560–675, 690–890, 900− (*pg/mL*); Ca, −8.0, 8.1–8.8, 8.9–9.3, 9.4–9.9, 10.0− (*mg/dL*) Pi, −4.6, 4.7–5.4, 5.5-6.2, 6.3–7.1, 7.2− (*mg/dL*)

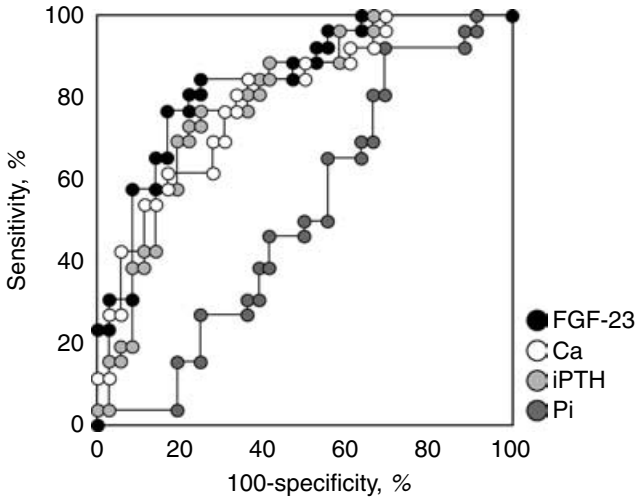

Fig. 4. An analysis using a receiver-operated curve (ROC) to detect the usefulness of screening tests that discriminate patients with secondary hyperparathyroidism who would be refractory to the calcitriol therapy. The ROC analysis revealed that the area under the curve (AUC) obtained by FGF-23 (8429.5) was more than those of iPTH (7970.1, $P < .05$), Ca (7959.4, $P < .05$), and Pi (5170.9, $P < .0001$).

levels. However, the parathyroid glands themselves were unlikely to produce FGF-23 since fgf-23 mRNA was not detectable in parathyroid glands removed from dialysis patients with severe secondary hyperparathyroidism (data not shown). On the other hand, a recent study suggested that osteoblasts are one of likely candidates that produce circulating FGF-23 [33]. Given the fact that hyperparathyroidism generally accompanies increased activity of bone metabolism, the positive correlation between iPTH and FGF-23 levels may reflect the indirect stimulation of FGF-23 production by PTH via bone cells.

Interestingly, serum FGF-23 levels were rather elevated, while iPTH levels were decreased, in response to the calcitriol therapy in this study. This finding could reasonably explain why previous active vitamin D therapy affected the pretreatment serum FGF-23 levels. The number of months since the initiation of chronic hemodialysis therapy may reflect the total amount of active vitamin D agents used, as these agents had not been used before the initiation of hemodialysis therapy in any of these patients.

Table 4. The discrimination of patients who would be successfully treated with calcitriol by the combination of FGF-23 and iPTH

	FGF-23 ≤ 9860 *ng/L*	FGF-23 >9860 *ng/L*
iPTH ≤ 591 *pg/mL*	15/17	5/12
iPTH >591 *pg/mL*	5/9	1/24

FGF-23 discriminated those patients with sensitivity = (15 + 5)/26 = 76.9% and specificity = (7 + 23)/36 = 83.3%, and so did iPTH with sensitivity = (15 + 5)/26 = 76.9% and specificity = (4 + 23)/36 = 75.0%, respectively. On the other hand, 15/17 = 88.2% of those patients with FGF-23 ≤9860 *ng/L* and iPTH ≤591 *pg/L* would be successfully treated with calcitriol, while the treatment would be failed in 23/24 = 95.8% of those who were treated with FGF-23 >9860 *ng/L* and iPTH >591 *pg/L*.

The longer history of active vitamin D therapy may also be related to the refractoriness to the treatment.

Circulating FGF-23 levels decrease after surgical parathyroidectomy [34]. Therefore, it must not be the decreased action of PTH, but the increased action of $1,25(OH)_2$ vitamin D_3 (1,25D) that caused the elevation of circulating FGF-23 levels in these patients. FGF-23 has a strong suppressive effect on vitamin D-1 α hydroxylase activity in the kidney [25, 35, 36]. On the other hand, administration of 1,25D induced FGF-23 production in mice [25]. FGF-23 production may be promoted by 1,25D administration through decreasing PHEX expression [37], which is a possible regulator of fgf-23 mRNA expression [33]. The cross-talk regulation between FGF-23 and calciotropic hormones in mineral metabolism is suggested, and further studies are needed to elucidate the precise mechanism.

The fact that calcitriol therapy increases FGF-23 levels seems rather convenient from the perspective of predicting the refractoriness of calcitriol therapy based on FGF-23 levels. Although a four-week active drug washout period was set in this study, it would be difficult to have such a period when the measurement of circulating FGF-23 levels is carried out in clinical practice. However, because calcitriol therapy did not decrease the FGF-23 levels, the levels after calcitriol therapy would not underestimate the refractoriness of the parathyroid gland against calcitriol therapy, especially when it is applied in combination with iPTH measurement.

CONCLUSION

Pretreatment serum FGF-23 levels were a good indicator in predicting the efficacy of the calcitriol therapy in dialysis patients with moderate to severe secondary hyperparathyroidism. As such, the measurement of serum FGF-23 levels is a promising laboratory examination in the clinical treatment of secondary hyperparathyroidism, especially when applied in combination with iPTH measurement. Further prospective clinical studies are necessary to confirm whether the measurement of circulating FGF-23 levels contributes to the improved prognosis of the patients.

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