

Serum iFGF-23, sKlotho, and 1,25 (OH)₂ D₃ Vitamin Levels in Kidney Transplant

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Abstract: Aim of the study: Chronic kidney disease is characterized by elevated iFGF-23 level, which is known to regulate phosphate, little is known about the relationship between iFGF-23 and phosphate homeostasis in posttransplant patients, however. We will look at the iFGF-23 level and correlate it with sKlotho, and 1,25 (OH)₂ D₃ Vitamin in posttransplant patients. Material and methods: This study was conducted using 60 kidney transplant patients. 34 healthy subjects enrolled as a control group. Blood samples were withdrawn for measuring the levels of serum iFGF-23, sKlotho, 1,25 (OH)₂ D₃ Vitamin, Calcium, Phosphorus, iPTH, 25 (OH) D vitamin. CKD-EPI is used to calculate GFR. Results: iFGF-23 levels were elevated in the posttransplant period compared with healthy subjects. iFGF-23 levels were measured as 263.64±153.08 pg/ml in transplant patients and 155.05±73.40 pg/ml within the control group. sKlotho levels were measured as 2.82±1.76 ng/ml and 3.72±3.59 ng/ml in transplant patients and control groups respectively. 1,25 (OH)₂ D₃ Vitamin levels were measured as 49.56±13.73 pg/ml and 48.42±12.13 pg/ml in transplant patients and control group respectively. The results of this study revealed a significant correlation between iFGF-23 and sKlotho both in transplant patients and in the control group. Conclusions: Significantly elevated iFGF-23 and iPTH level accompanied by decreased GFR activity suggests a progressive deficiency in phosphate homeostasis.

Keywords: iFGF-23, sKlotho, 1, 25 (OH)₂ D₃, Kidney Transplantation

1. Introduction

Calcium homeostasis has been extensively studied over the past several decades. We know relatively little about the regulation of phosphate homeostasis [1]. Fibroblast growth factor-23 (FGF-23) is known to regulate calcium and phosphate homeostasis. Increased serum phosphate concentration increases FGF-23 expression by osteocytes and osteoblasts in bone. Reduction in renal function increases serum phosphate level contributing to the secretion of FGF-23 further enhancing the excretion of phosphate per nephron. As the progression of chronic CKD circulating FGF-23 increases gradually. Elevated serum FGF-23 level inhibits 1,25 (OH)₂ D₃ synthesis in the kidney. Decreased 1,25 (OH)₂ D₃ level induces PTH secretion by the parathyroid gland [2,

3].

Klotho is produced mostly in the kidney and the parathyroid gland. Klotho is secreted by the cell. It is also known that it is expressed from cleavage of the intracellular domain of the full-length protein by secretases. Both ways result in soluble Klotho [4]. FGF receptors (FGF-Rs) activates the biological effects of FGF-23. Compared to FGF-R alone, Klotho/FGF-R complex binds to FGF-23 with higher affinity [5]. It is suggested that sKlotho deficiency causes FGF-23 resistance to further causes CKD [6, 7].

Kidney transplantation results in normalization of renal functions further improves mineral and hormone homeostasis. However, elevated FGF-23 level increases renal phosphate wasting which causes hypophosphatemia is known to be a common problem in the early posttransplantation period [8, 9]. Decreased 1,25 (OH)₂ D₃ level is associated with elevated

FGF-23 level as FGF-23 inhibits 1,25 (OH)₂ D₃ secretion [10].

The recent study showed that the FGF-23 level decreases rapidly in 3 months after transplantation but 90% of receivers with functioning graft still have a higher FGF-23 level compared to healthy subjects [11]. It is shown that iPTH and iFGF-23 levels are persistently elevated in the long term (10 > years posttransplantation) transplant patients with well-functioning graft. It is suggested that FGF-23 resistance has evolved in the parathyroid gland before transplantation and persists long after the transplantation [12]. There is not enough study for homeostasis of minerals, iFGF-23, sKlotho, 1,25 (OH)₂ D₃ and iPTH (intact PTH) in the posttransplantation period. We aimed at finding an association of iFGF-23 with sKlotho, and 1,25 (OH)₂ D₃ in posttransplantation patients.

2. Materials and Methods

60 kidney transplant patients (18 females, 42 males) who were followed up in the Transplantation Polyclinic of Gazi University, Department of Nephrology were included in this study. Patients were 20 years old or older. 34 healthy subjects (16 females, 18 males) who were in the same age group and sex with the patient groups were enrolled in this study as a control group. People who have any chronic or acute disease were excluded. Written informed consent was obtained from all participants.

This study was approved by the ethics committee of Zekai Tahir Burak Women's Health Education and Research Hospital under code number 12/2017 on January 17th, 2017.

The blood samples which were collected after 8-10 hours of fasting from individuals in the patient group and control group were put into tubes that were protective-free and anticoagulant-free. The blood samples centrifuged 10 minutes at the speed of 3600 RPM and serums were collected. Serum Ca and P levels were measured by using the Beckman Coulter AU5800 auto-analyzer. iPTH, and 25 OH Vitamin D levels were measured by using Beckman Coulter DXI 800 Access Immunoassay System auto-analyzer. To calculate the eGFR levels CKD-EPI formula was used. A well-functioning transplant was defined as an eGFR ≥ 45 ml/min/1.73m². The remaining serum was stored at -80°C until other measurements were performed.

Serum sKlotho levels were measured by using Human soluble alpha klotho (SaKL) ELISA Kit, catalog number 201-12-5446 supplied from SunRed Biotechnology.

Serum 1,25 (OH)₂ D₃ levels were measured by using Immucrom kits on Shimadzu LC. MS/MS device.

Serum iFGF-23 concentrations were measured using Human intact fibroblast growth factor-23, iFGF-23 ELISA Kit, catalog number 201-12-8625 supplied from SunRed Biotechnology. The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the

level of Human iFGF-23 in samples. iFGF-23 was added to monoclonal antibody enzyme well which was pre-coated with the human iFGF-23 monoclonal antibody, waited for incubation then added iFGF-23 antibodies labeled with biotin, and combined with Streptavidin-HRP to form an immune complex, then carried out incubation and washed again to remove the uncombined enzyme. Then added Chromogen Solution A, B, the color of the liquid changed into blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the Human Substance iFGF-23 of the sample were positively correlated.

Statistical Methods

Data that was gathered as a result of the study is analyzed by using SPSS Statistics 20. Findings in tables are given as mean \pm standard deviation. To determine significant differences of a parameter between different groups Student t-test is used if the test statistic follows a normal distribution, Mann-Whitney U test is used if the test statistic does not follow a normal distribution. For the correlation analysis of parameters, Pearson test is used if test statistics follow a normal distribution, the Spearman test is used if test statistics do not follow a normal distribution. $p < 0.05$ is taken as statistically significant.

3. Results

The mean levels of iFGF-23, sKlotho and 1,25 (OH)₂ D₃ Vitamin in the control group were 155.05 \pm 73.40 pg/ml, 3.72 \pm 3.59 ng/ml, and 48.42 \pm 12.13 pg/ml respectively. On the other hand, the mean levels of iFGF-23, sKlotho and 1,25 (OH)₂ D₃ Vitamin in the transplant patients were 263.64 \pm 153.08 pg/ml, 2.82 \pm 1.76 ng/ml, and 49.56 \pm 13.73 pg/ml respectively.

Means and standard deviations of all parameters for transplant patients and the control group are given in Table 1.

This study revealed a significant increase in iFGF-23 and iPTH in transplant patients compared with the control group ($p < 0.05$, $p < 0.05$ respectively). We observed that sKlotho was decreased in transplant patients compared to the control group, but the decrease was not significant ($p > 0.05$). It was seen that 1,25 (OH)₂ D₃ Vitamin increased in transplant patients compared to the control group, but it is not significant.

We observed that the eGFR level was significantly decreased in transplant patients compared with the control group ($p < 0.05$). We observed that the Phosphorus level was significantly declined in transplant patients group compared with the control group ($p < 0.05$). We didn't observe a significant change in 1,25 (OH)₂ D₃ Vitamin levels in transplant patients compared to healthy subjects.

We found that there is a significant correlation between iFGF-23 and sKlotho ($p < 0.05$) in the control group on the other hand there is no correlation between these parameters ($p > 0.05$) in transplant patients.

Table 1. Biochemical parameters.

	Trans Patients (n=60)	Control Group (n=34)	p
Age	40.83±13.76	36.53±7.19	
Gender, n (%)			
Male	42 (70)	16 (47)	
Female	18 (30)	18 (53)	
Median Trans Year (Min - Max)	6 (1 - 21)		
Ca (mg/dl) *	9.57±0.81	9.42±0.37	0.041
Phosphorus (mg/dl) *	3.21±0.73	3.54±0.49	0.007
25 (OH) D Vitamin (µg/l)	14.76±10.88	16.40±10.00	0.259
eGFR (ml/dk/1.73m ²) *	55.39±19.79	112.87±14.32	0.000
iPTH (pg/ml) *	116.62±74.43	45.75±25.60	0.000
iFGF-23 (pg/ml) *	263.64±153.08	155.05±73.40	0.000
sKlotho (ng/ml)	2.82±1.76	3.72±3.59	0.934
1,25 (OH) ₂ D ₃ Vitamin (pg/ml)	49.56±13.73	48.42±12.13	0.816

* There is a significant change in the transplant group compared with that of the control group. Values are presented as mean±SD unless stated otherwise.

We found that iFGF-23 is significantly correlated with 25 (OH) D Vitamin and iPTH ($p<0.05$, $p<0.05$ respectively) whereas there is no significant correlation between iFGF-23 and sKlotho, 1,25 (OH)₂ D₃ Vitamin, Ca, and P ($p>0.05$ for all) in transplant patients.

This study revealed that there is a significant correlation between iFGF-23 and 25 (OH) D Vitamin ($p<0.05$) in the

control group. iFGF-23 was not correlated with Ca, P, and 1,25 (OH)₂ D₃ Vitamin in the control group.

The results of the correlation analysis of iFGF-23, sKlotho, and 1,25 (OH)₂ D₃ Vitamin with each other and with other parameters in transplant patients and in the control group are given in Table 2.

Table 2. Correlation analysis.

		Transplant Patients			Control Group		
		iFGF-23	sKlotho	1,25 (OH) ₂ D ₃ Vitamin	iFGF-23	sKlotho	1,25 (OH) ₂ D ₃ Vitamin
Calcium	r	-.112	.075	.309*	-.083	-.044	-.136
	p	.394	.571	.016	.641	.804	.445
Phosphorus	r	.021	-.157	-.077	-.109	-.012	-.145
	p	.872	.231	.561	.539	.947	.413
iPTH	r	.619*	.031	.031	.173	.336	.075
	p	.000	.814	.812	.327	.052	.674
25 (OH) D Vitamin	r	-.345*	-.034	.152	.467*	.222	-.096
	p	.007	.798	.247	.005	.208	.588
eGFR	r	-.206	.059	.020	-.186	-.124	-.020
	p	.113	.655	.881	.292	.484	.910
iFGF-23	r		.030	.208		.720*	.081
	p		.823	.110		.000	.651
sKlotho	r	.030		.041	.720*		.112
	p	.823		.756	.000		.527
1,25 (OH) ₂ D ₃ Vitamin	r	.208	.041		.081	.112	
	p	.110	.756		.651	.527	

* Significant correlation

4. Discussion

In a study conducted by Economidou et al., on 18 transplant subjects, it was found that hypophosphatemia occurs early following renal transplantation and resolves almost completely at 1 year after transplantation [13]. In a study which is conducted on 247 kidney recipients who had received a graft more than one year prior to the study, FGF-23 was elevated in the recipients across all CKD stages [14]. In a study conducted on 40 long-term kidney transplant patients with well-functioning kidney transplants who received a first kidney transplant more than 10 years ago, it was revealed inappropriately high levels of iPTH and iFGF23 consistent with a state of persistent hyperparathyroidism [12]. Many studies showed that renal Phosphate wasting is

persistent in the posttransplantation period even with normophosphatemia [14-16]. Our study revealed that the iFGF-23 level was elevated significantly in long-term kidney transplant patients with well-functioning transplants compared with those in the control group. A well-functioning transplant was defined as an eGFR ≥ 45 ml/min/1.73m².

Although, kidney transplantation results in significant improvement in mineral metabolisms especially on phosphorus and calcium metabolisms, the eGFR level is suboptimal in the posttransplant period [17]. We found a significant deficiency in eGFR levels in transplant patients compared with those in healthy subjects.

Elevated iFGF-23 level implies a decline in 1,25 (OH)₂ D₃ Vitamin level. This effect occurs in the presence of sKlotho [18]. In many studies it is shown that there is no association

between the iFGF-23 level and 1,25 (OH)₂ D₃ Vitamin level in the posttransplantation period. It is also shown that 1,25 (OH)₂ D₃ Vitamin level tends to be higher in posttransplant patients [13, 17]. In our study, although the iFGF-23 level was significantly increased, 1,25 (OH)₂ D₃ Vitamin was not changed significantly in the transplant group compared to the control group. Moreover, correlation analysis between iFGF-23 and 1,25 (OH)₂ D₃ Vitamin didn't show a significant correlation between these parameters in transplant patients.

Calcium homeostasis is also associated with 1,25 (OH)₂ D₃ Vitamin level [7]. A significant increase in Ca along with 1,25 (OH)₂ D₃ Vitamin levels were noted at the end of 3, 6,

and 12 months after transplantation [13]. In our study, we found that Ca levels were elevated significantly in the transplant group compared with those in the control group. 1,25 (OH)₂ D₃ Vitamin levels were increased compared with those in the control group but the increase was not significant. The correlation analysis in our study showed a significant correlation between Calcium and 1,25 (OH)₂ D₃ Vitamin as shown in Figure 1. It is reported that there is no association between iFGF-23 and 1,25 (OH)₂ D₃ Vitamin in posttransplant patients [13]. In accordance with previous reports, we couldn't find an association between iFGF-23 and 1,25 (OH)₂ D₃ Vitamin in the patient group.

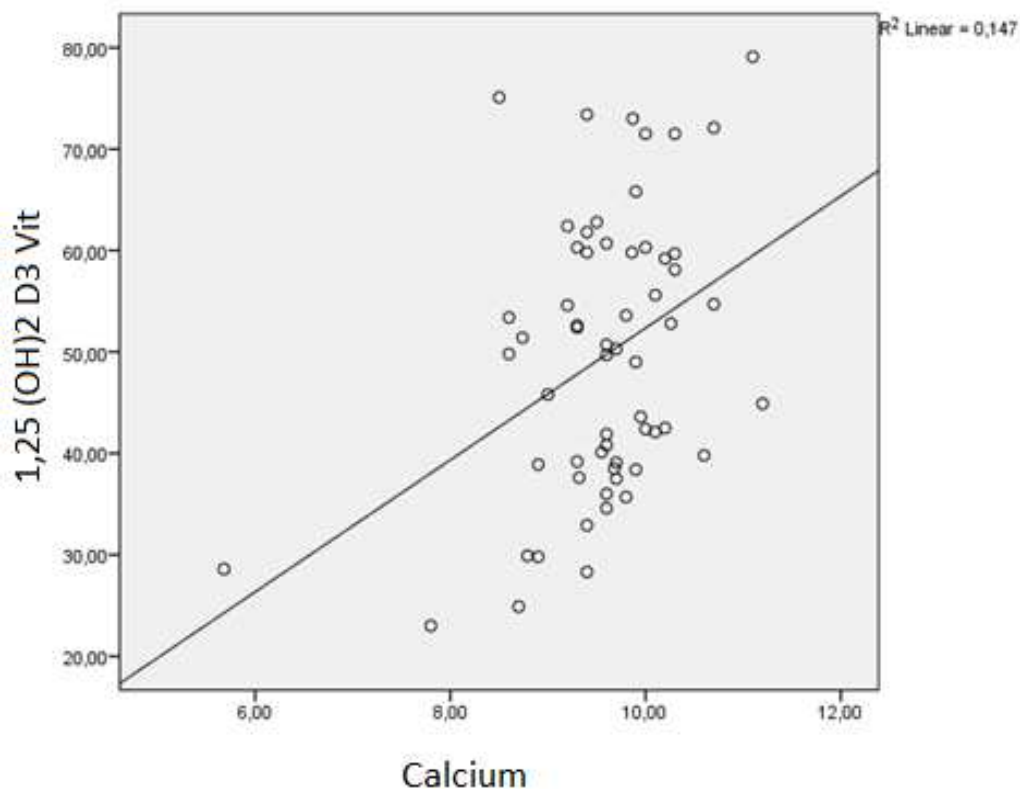


Figure 1. Correlation between Calcium and 1,25 (OH)₂ D₃ Vitamin in the transplant group.

Declining renal function results in decreased sKlotho secretion because of deficiency in mineral metabolisms related with failing kidney [19]. sKlotho level is associated with plasma phosphate in an age-dependent manner, changing from a positive association in young adults gradually to a negative association in the elderly [20]. Although both sKlotho and phosphate levels are declined in the transplant group compared with those in the control group, we couldn't find any correlation between sKlotho and plasma phosphate neither in the transplant group nor the control group.

Compared with FGF-R alone, Klotho/FGF-R complex binds to FGF-23 with higher affinity increasing the effects of FGF-23 significantly [5]. Many studies showed that the sKlotho level is declined in kidney transplant receivers compared with those healthy subjects [19, 21, 22]. In our study we observed a significant decrease in sKlotho level in

transplant patients compared with those in the control group which causes iFGF-23 resistance. On the other hand, in a study covering pretransplantation and posttransplantation periods, it was found that sKlotho levels declined at 1w following transplantation and started increasing gradually afterward. At 12 w it exceeded the pretransplant level and at 52 w it was still increasing [23].

sKlotho is associated with GFR [24]. On the contrary, in a study conducted in a cohort of 312 patients with stage 2-4 chronic kidney disease, it was revealed that there was no significant correlation between sKlotho levels and GFR levels [25]. In our study we couldn't find any significant correlation between sKlotho levels and GFR levels.

It is shown that FGF-23 suppresses both parathyroid hormone secretion and PTH gene expression in a study that was conducted both on rats and in vitro rat parathyroid cultures [26]. On the other hand, it is also shown in a study

conducted both in vitro and in vivo that elevated PTH induced FGF-23 secretion [27]. Thus, PTH and FGF-23 are mutually effective on each other's secretion.

It was shown that iPTH levels decreased around two-third at 3 months posttransplantation compared with the pretransplant period and remained stable at the end of 12 months. Secondary hyperparathyroidism (SHPT) was persistent twelve months after transplantation in about half of the receivers even with well-functioning transplant [3, 13]. In

a study conducted on long-term kidney transplant receivers with median time since transplantation was 18.3 years, it was found that the median levels of iPTH and iFGF-23 were significantly higher than those in the control group [12]. Our study revealed that iPTH levels were elevated significantly in transplant patients compared with those in the control group. Furthermore, the iPTH level was significantly correlated with iFGF-23 levels in the posttransplant period as shown in Figure 2.

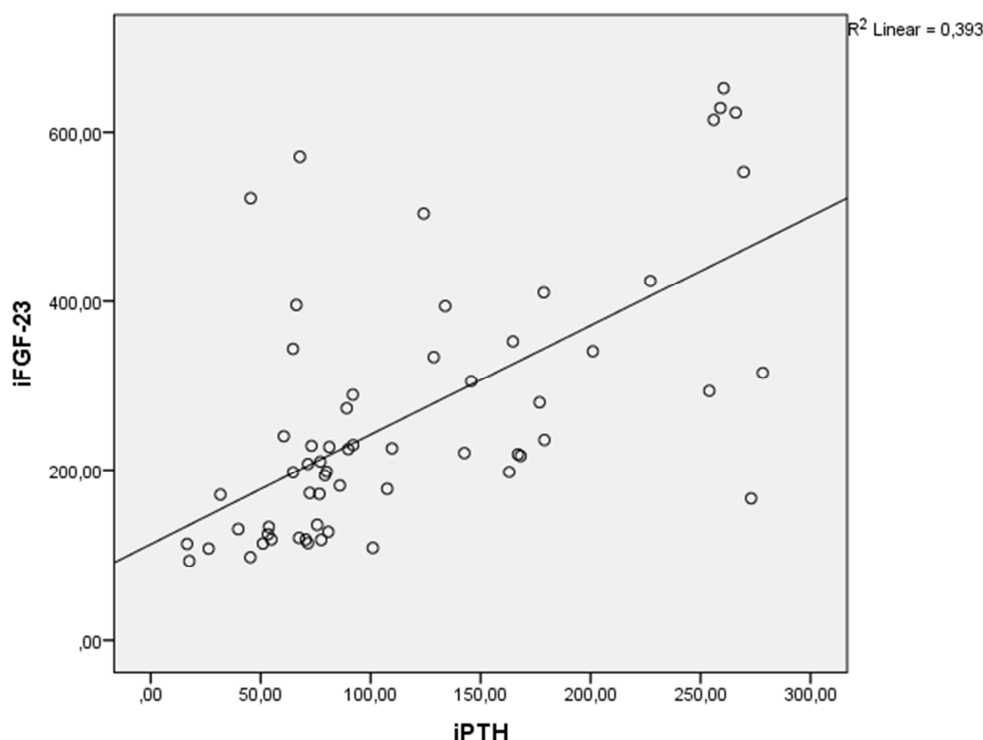


Figure 2. Correlation between iFGF-23 and iPTH in the transplant group.

It was shown that Phosphorous was positively associated with iPTH in the pretransplant period, but there is no correlation in the posttransplant period [3]. In our study, we couldn't find a correlation (data not shown) between Phosphorous and iPTH in the transplant group.

5. Conclusion

Kidney transplantation contributed to the regulation of calcium and phosphate homeostasis. Based on eGFR levels we concluded that kidney transplants are well-functioning in transplant patients. But iFGF-23 and iPTH levels in transplant patients are still significantly higher than healthy individuals.

We found that persistent hyperparathyroidism is associated with significantly increased iFGF-23 and iPTH levels in long-term transplant patients with a well-functioning kidney transplant. sKlotho levels are decreased in transplant patients compared with the control group. We speculate that declined sKlotho levels resulted in iFGF-23 resistance in the parathyroid gland in the pretransplant period and persisted after transplantation.

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