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ALTERATIONS OF VITAMIN D RECEPTOR GENE POLYMORPHISM AND HYPERPARATHYROIDISM IN END STAGE RENAL DISEASE

Nashwa Abdelelah Mohamed[1]*, Mohamed Abd-El Moniem Mahmoud[1], Ayman El-Baz El-Agroudy[2] and Abdel-Aziz Fotouh Abdel-Aziz[3]

[1]Department of Chemistry, Faculty of Science, Port Said University, Port Said, Egypt.
[2]Department of Chemistry, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
[3]Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt.

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***Corresponding Author**
Nashwa Abdelelah Mohamed
Department of Chemistry, Faculty of Science, Port Said University, Port Said, Egypt.

ABSTRACT

This study included 65 chronic renal failure patients and 25 healthy volunteers as normal control group. Deficiency of vitamin D is associated with high level of iPTH leads to development of secondary hyperparathyroidism (sHPT).This study estimated that the levels of 25(OH) D and calcium were significantly lower; on the other hand, the levels of serum phosphorus and iPTH were significantly higher in comparison with those in the control group. It was observed that there were significant negative correlations between serum iPTH and 25(OH) D levels, Phosphorus and 25(OH) D levels and serum calcium and phosphorus levels in chronic renal failure patients. On the other hand, there were significant positive correlations between serum calcium and 25(OH) D levels also, between serum iPTH and phosphorus levels. Vitamin D receptor (VDR) gene FOKI polymorphism plays an effect role on iPTH level in chronic renal failure patients. In this study there were significant differences of iPTH levels in genotype groups of FOKI gene polymorphism in CRF patients as FF genotype patients had a higher level of iPTH than Ff and ff genotype patients.

KEYWORDS: Chronic renal failure, intact parathyroid hormone, Secondary hyperparathyroidism, Vitamin D.

INTRODUCTION

Chronic kidney disease (CKD) is recognized as a major health problem and economic issue with an increasing incidence and prevalence. Chronic renal failure (CRF) is defined as the presence of kidney damage, manifested by abnormal albumin excretion or decreased kidney function, quantified by measured or estimated glomerular filtration rate (GFR).[1] Chronic renal failure is associated with multiple metabolic disturbances of calcium, phosphorus and vitamin D which play a key role in the development of secondary hyperparathyroidism (sHPT).[2]

Vitamin D is a pro-hormone essential for life which present in few types of foods and is produced endogenously in the skin by a photochemical reaction. The final step of vitamin D activation occurs in the kidneys involving a second hydroxylation reaction to generate the biologically active metabolite 1, 25(OH) 2-Vit D.[3]

Vitamin D receptor is a ligand-dependent transcription factor, belonging to the steroid nuclear receptor gene family, which binds to specific DNA sites to modify the expression of target genes.[4] Polymorphisms of vitamin D receptor (VDR) gene have been studied as genetic markers of many diseases as cancer, diabetes and kidney diseases.[5] Genotype of the TaqI VDR gene polymorphism was reported in Iranian patients with renal failure, also genotype of FokI VDR gene polymorphism was detected in hemodialysis patients.[6]

Secondary hyperparathyroidism is one of the main complications in patients with chronic renal failure.[7] Vitamin D regulates calcium level, bone homeostasis and regulation of intact parathyroid hormone (iPTH) secretion[8] as decreased serum levels of 1,25-dihydroxy vitamin D (calcitriol) cause an increase in parathyroid hormone secretion and the development of secondary hyperparathyroidism.[9] Secondary hyperparathyroidism may be influenced by vitamin D receptor (VDR) gene polymorphism FokI which may be risk factors in chronic renal failure patients.[6] This study investigated vitamin D receptor gene Fokl polymorphism and the level of intact parathyroid hormone also, estimated the potential link between them in chronic renal failure patients in order to know the role of this gene as predisposing risk factors and morbidity indicator in these patients.

SUBJECTS AND METHODS

This study included 90 subjects; 65 patients with mean age 45.16 ± 14.01 years they were 39 (60%) males and 26 (40%) females. All patients were undergoing dialysis treatment

following diagnosis of chronic renal failure disease (CRF) by nephrologists in the Urology and Nephrology Center, Mansoura University; each patient was dialyzed 3 times per week. Twenty five healthy volunteers as normal control group were collected from donner blood bank, Mansoura University, with mean age 39.30 ±10.20 years; they were 13(52%) males and 12 (48%) females. Serum creatinine, uric acid, blood urea nitrogen (BUN) and liver function parameters as serum albumin, total bilirubin, liver enzymes ALT, AST and ALP were tested. The control subjects had normal kidney function, normal concentration of vitamin D and normal level of intact parathyroid hormone (iPTH), they were free from any kidney diseases. The studied subjects (patients and controls) were in the same socioeconomic class and had similar nutritional habits.

Blood sample collection and examination

Six milliliters venous blood was collected using a disposable plastic syringe from each patient and healthy individuals. All samples of patients were collected before the dialysis. Two milliliters from 6 ml were collected on ethylene diamine tetraacetic acid (EDTA) for measuring hemoglobin level and extraction of DNA using (G-spin$^{TM)}$, total DNA extraction Kit supplied by intron biotechnology, IBT-QMS-GT1704 (R01-2012-01) for DNA isolation and purification from whole blood samples. DNA stored at 20°C for amplifying by polymerase chain reaction (PCR) then, restriction fragment length polymorphism (RFLP) was performed for detection VDR gene polymorphism using FOKI (rs10735810) restriction enzyme within forward primer 5'- AGC TGG CCC TGG CAC TGA CTC TGC TCT-3'and reverse primer 5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3'. The rest of blood (4ml) were collected on free tubes without addition anticoagulants, were centrifuged at 3000 rpm for 5 minutes to obtain serum and measured 25(OH) D by using enzyme- linked immunosorbent assay (ELISA) using DRG 25-OH Vitamin D (total).[10] Serum intact parathyroid hormone (iPTH) was measured by Electrochemilumenecence immunoassay; Elecsys 2010[11], also serum calcium and phosphorus levels were measured by colorimetric end point method according to the method of.[12]

Statistical analysis

Data were obtained using Statistical package for social Sciences (SPSS) version 19.0 software. Data were expressed as mean ± standard deviation (M±SD). Results of CRF patients and control subjects were performed using chi- square analysis, independent t-test. Multiple comparisons were performed using Kuskalwallis test followed by Mann-whitney.

Correlation between parameters was determined by pearson's correlation coefficient (r). Chi square and odds ratio were calculated with 95% confidence interval. A probability value less than 0.05 was considered statistically significant.

RESULTS

As observed in table (1), there was no significant difference of age and body mass index (BMI) in CRF patients when compared with control subjects. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly higher in CRF patients than in control subjects, ($p < 0.001$). On the other hand, the level of hemoglobin was significantly lower in CRF patients as compared to control subjects ($P<0.001$).

Table 1: Demographic and Clinical Data of Studied Subjects.

Parameters	Control Subjects (n=25)	CRF patients (n=65)
Age(years) (Mean ± SD) *P*	39.30 ±10.20	45.16 ± 14.01 >0.05
BMI(kg/cm²) (Mean ± SD) *P*	31.01 ± 5.20	28.60 ± 4.80 >0.05
SBP(mmHg)/ 24h (Mean ± SD) *P*	122.10 ± 6.80	158.80 ± 13.10 < 0.001**
DBP(mmHg)/ 24h (Mean ± SD) *P*	78.01 ± 4.20	96.20 ± 5.10 < 0.001**
Hemoglobin (mg /dl) (Mean ± SD) *P*	13.20 ± 1.10	8.90± 2.30 < 0.001**

*Significant value ($p<0.05$). Data were expressed as mean ± SD. Results were obtained using independent t-test. Body mass index (BMI). Systolic blood pressure (SBP). Diastolic blood pressure (DBP).

In the present study, clinical parameters such as creatinine, blood urea nitrogen (BUN) and uric acid were tested for chronic renal failure (CRF) patients, (n=65), as well as control subjects (n=25). table (2) shows the levels of creatinine, BUN, uric acid and alkaline phosphatase were significantly increased in CRF patients when compared with control subjects ($p<0.001$). On the other hand, there was lower significant in the level of albumin in chronic renal failure patients than in control subjects, also as observed there were no

significant in the levels of alanine aminotransferase (ALT) enzyme, aspartate aminotransferase (AST) enzyme, and total bilirubin (TB).

Table 2: Renal and liver function parameters in chronic renal failure patients and control subjects.

Parameters	Control Subjects (n=25)	CRF patients (n=65)
Creatinine (mg/dl) (Mean ± SD) *P*	0.50 ± 0.10	8.80 ± 0.30 < 0.001**
BUN (mg/dl) (Mean ± SD) *P*	7.40 ± 0.40	86.60 ± 7.90 < 0.001**
Uric acid (mg/dl) (Mean ± SD) *P*	4.20 ± 0.30	6.60± 0.20 < 0.001**
Albumin(g/dl) (Mean ± SD) *P*	4.55 ± 0.14	3.26 ± 0.19 < 0.001**
ALT(Iu/l) (Mean ± SD) *P*	17.10 ± 0.41	18.30± 0.33 >0.05
AST(Iu/l) (Mean ± SD) *P*	19.90 ± 0.26	21.10 ± 0.22 >0.05
ALP(Iu/l) (Mean ± SD) *P*	81.20 ± 4.20	139.50 ± 61.10 < 0.001**
TB(mg/dl) (Mean ± SD) *P*	0.71 ± 0.03	0.74 ± 0.08 >0.05

*Significant value ($p<0.05$).Data were expressed as mean ± SD. Results were obtained using independent t-test. Blood urea nitrogen (BUN). Alanine aminotransferase (ALT) enzyme. Aspartate aminotransferase (AST) enzyme. Alkaline phosphatase (ALP) enzyme. Total bilirubin (TB).

As observed in table (3), there were highly significant in the levels of serum intact parathyroid hormone (iPTH) and phosphorus in CRF patients when compared with control subjects but there were lower significant in the levels of serum 25(OH) D and calcium than control subjects.

Table 3: Biochemical Features of studied subjects with control subjects.

Parameters	Control Subjects (n=25)	CRF patients (n=65)
iPTH pg/ml (Mean ± SD) *P*	39.30 ±10.20	246.11 ± 34.21 < 0.001**
25(OH)D (ng/ml) (Mean ± SD) *P*	46.20 ± 4.60	12.11 ± 0.20 < 0.001**
Calcium (mg/dl) (Mean ± SD) *P*	10.15 ± 0.10	8.01 ± 0.20 < 0.001**
Phosphorus (mg/dl) (Mean ± SD) *P*	3.20± 0.09	5.40 ± 0.18 < 0.001**

*Significant value (p<0.05). Data were expressed as mean ± SD. Results were obtained using independent t-test. Intact parathyroid hormone (iPTH).Vitamin D 25(OH) D.

The genotyping and allelic frequency of vitamin D receptor gene is presented in table (4). The genotype frequency of vitamin D receptor gene in the normal control group is 52% for FF, 36% for Ff and 12% for ff. In contrast to CRF Group, it is presented as follow 24.62% for FF, 43.08% for Ff and 32.30% for ff with statistically significant differences in all genotype in CRF patients when compared to control group(p<0.0001). whereas, the alleles frequency of vitamin D in the normal control is 74.0%, for F, 26.0% for f, while in CRF patients it is presented as follow 41.5% for F allele, 58.5% for f allele with statistically significant difference in all genotype in CRF patients when compared to the control group (P<0.0001).

Table 4: Genotyping and allelic frequency of VDR-FOK1 gene polymorphism in all studied groups.

		Control subjects (n=25)		CRF patients (n=65)		P value	OR(CI95%)
		No	%	No	%		
Genotypes	FF	13	52.00%	16	24.62%	-	1(Ref)
	Ff	9	36.00%	28	43.08%	0.004	2.3 (1.3-4.1)
	ff	3	12.00%	21	32.30%	<0.0001	10.0 (4.08-24.5)
	Ff+ff	12	48.0%	49	75.3%	<0.0001	3.49 (2.03-5.99)
Allels	F	37	74.0 %	54	41.5%	<0.0001	1(Ref)
	f	13	26.0 %	76	58.5%		3.2 (2.18-4.75)

P: Probability OR: odd's ratio CI: confidence interval

As observed in table (5), serum intact parathyroid hormone, vitamin D, calcium and phosphorus levels classified by FOKI polymorphism in CRF patients, there were no significant between genotype of FOKI in CRF patients in all parameters except iPTH level was significance (p=0.001). As observed in figure (1) agarose gel electrophoretic analysis of FOKI F/f polymorphism after Fast Digestion analysis Lane M (100bp) represents the molecular marker. Lane (3, 4, 5) represents F/F showing one band at 265bp, lane (2, 7, 9) represents F/f heterogeneous genotype show the three bands (265, 196 & 69). Lane (6, 8, 10) represents f/f show two bands at (196, 69) bp.

Table 5: Serum intact parathyroid hormone, vitamin D, Calcium and phosphorus levels in FOKI polymorphism in CRF patients.

parameters	Genotyping of FOKI polymorphism in CRF patients (n=65)			P-value
	FF	Ff	ff	
N %	16 (24.62%)	28(43.08%)	21(32.30%)	
iPTH pg/ml	326.0 ±122.0	278.0 ±112.0 [a]	252.0 ±103.0 [bc]	0.001*
25(OH)D (ng/ml)	12.14 ±0.30	11.19 ±0.60	12.16±0.40	0.500
Calcium (mg/dl)	8.01 ± 0.40	8.01 ± 0.40	8.01 ± 0.30	0.700
Phosphorus (mg/dl)	5.40 ± 0.20	4.90 ± 0.10	4.90 ± 0.30	0.20

*Significant value (p<0.05). Data were expressed as mean± SD. Results were obtained using Kuskalwallis test followed by Mann-whitney for multiple comparisons. Intact parathyroid hormone (iPTH).Vitamin D 25(OH) D.
a:significance between FF and Ff
b:significance between FF and ff
c:significance between Ff and ff

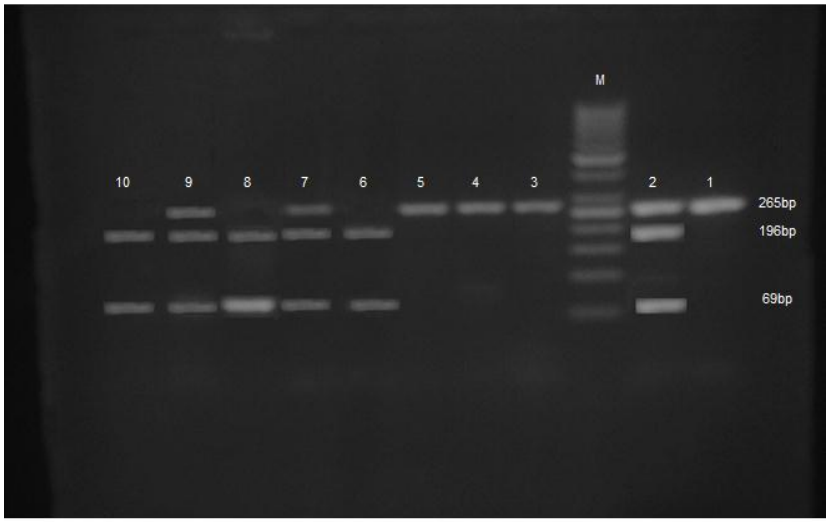

Figure (1): Agarose gel electrophoretic analysis of FOK I F/f polymorphism after Fast Digestion analysis Lane M (100bp) represents the molecular marker. Lane (3, 4, 5) represents F/F showing one band at 265bp, lane (2, 7, 9) represents F/f heterogeneous genotype show the three bands (265, 196 & 69). Lane (6, 8, 10) represents f/f show two bands at (196, 69) bp.

As shown in table (6) there were significant negative correlations between serum (phosphorus and 25(OH) D levels) also, between serum (calcium and phosphorus levels) figures (2 and 3). There was a significant negative correlation between serum iPTH and 25(OH) D level figure (4), on the other hand, there was a significant positive correlation between serum calcium and 25(OH) D figure (5). Also, there was a significant positive correlation between serum (iPTH) and phosphorus levels figure (6).

Table 6: Correlation between studied parameters in chronic renal failure patients.

Parameters	25(OH)D		Calcium		phosphorus		iPTH	
	r	p	r	p	r	p	r	p
25(OH)D	NS		0.6	0.009	-0.7	0.001	-0.7	0.001
Calcium	0.6	0.009	NS		-0.5	0.001	0.090	NS
Phosphorus	-0.7	0.001	-0.5	0.001	NS		0.4	0.001
iPTH	-0.7	0.001	0.090	NS	0.4	0.001	NS	

Not significant (NS), $p< 0.05$; Significant.

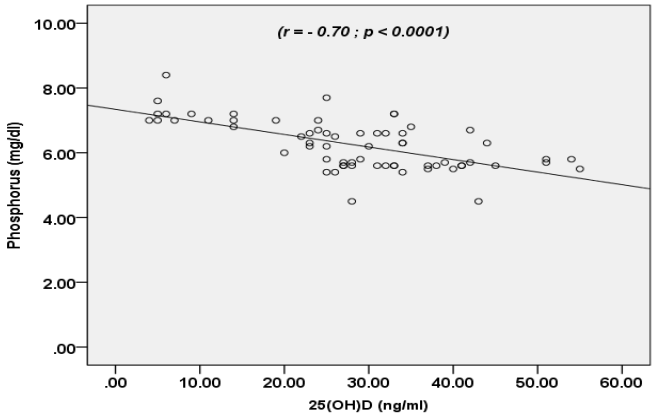

Figure (2): Linear Correlation between phosphorus level and vitamin D level in chronic renal failure patients.

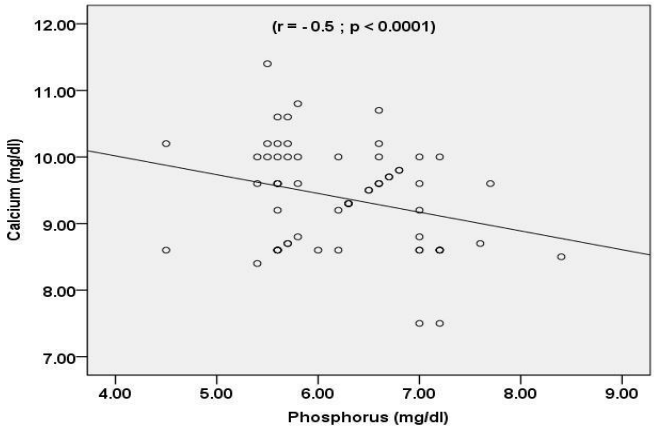

Figure (3): Linear Correlation between calcium level and phosphorus level in chronic renal failure patients.

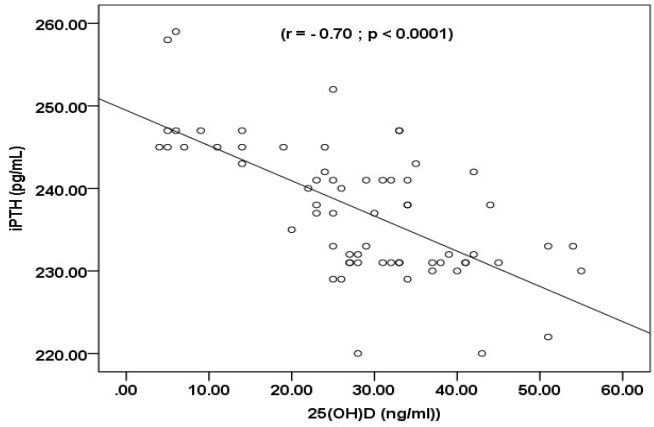

Figure (4): Linear Correlation between iPTH level and vitamin D level in chronic renal failure patients.

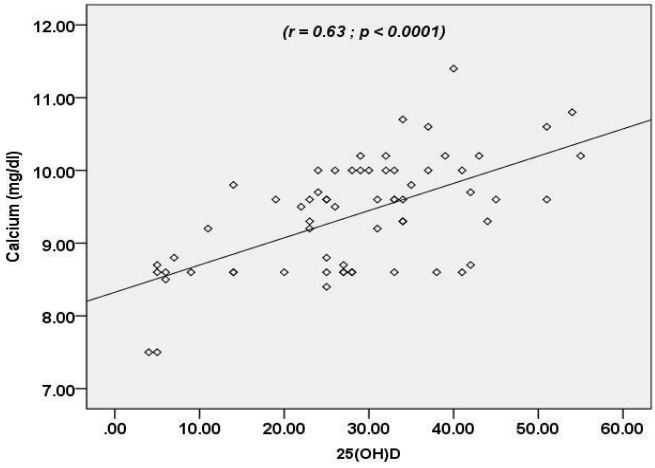

Figure (5): Linear Correlation between calcium level and vitamin D level in chronic renal failure patients.

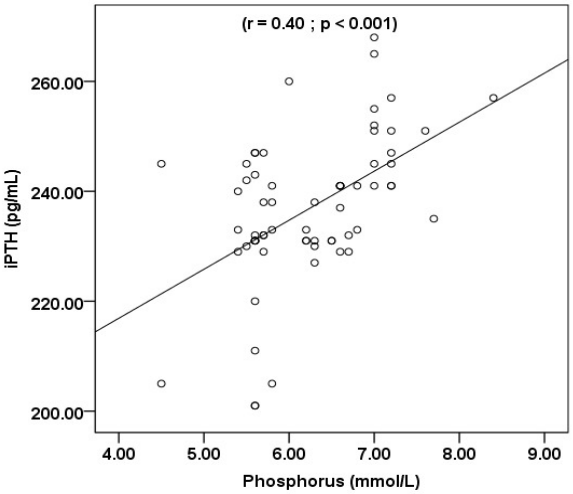

Figure (6): Linear Correlation between iPTH level and phosphorus level in chronic renal failure patients.

DISCUSSION

Most patients with chronic renal failure have secondary hyperparathyroidism (sHPT), stimulation of parathyroid function is caused by insufficient production of calcitriol by the kidney, calcium deficiency and increased phosphorus.[13] The progression of renal disease is related to the development of calcium and phosphorus metabolism disorders, it has been well established that reduction of phosphate excretion and production of calcitriol stimulate the activity of the parathyroid gland.[14]

Moderate (controlled) secondary hyperparathyroidism was defined as iPTH between 130 pg/ml and 585 pg/ml (2–9 times the upper limit of normal) and severe (uncontrolled) secondary hyperparathyroidism as PTH > 585 pg/ml (9 times the upper limit of normal). According to recent studies, changes of calcium and phosphorus metabolism in CKD may be due to genetic background and investigation of polymorphisms of vitamin D receptor gene (VDR gene).[15-17]

The incidence of (sHPT) among hemodialysis patients may be caused by genetic heterogeneity, it has been suggested that vitamin D receptor gene can influence the secretion of PTH. The effect of VDR gene polymorphism in CRF patients has been studied due to the role of vitamin D in these patients[18], as vitamin D deficiency has been linked to various disease conditions such as chronic kidney disease.[19] The widespread consequences of vitamin D deficiency have been partly attributed to the distribution of the vitamin D receptor.[20]

Vitamin D receptor (VDR) plays a vital role in mediating the effects of the biologically active form of vitamin D (1, 25, (OH)-D); therefore the variations in these receptors will modulate the consequences associated with vitamin D deficiency.[21] Several researchers have explored this relationship in CKD populations with emphasis on the calcium/ PTH/ calcitriol axis.[22,23]

Many studies have demonstrated that deficiency of vitamin D level is associated with increased chronic kidney disease mortality; lower vitamin D levels are associated with higher mortality and are a useful predictor for early mortality. Vitamin D level reduced because of kidney failure and subsequent absorption of calcium decreases. This study shows Low vitamin D and calcium levels are associated with high levels of iPTH and phosphorus there was a significant positive correlation between phosphorus and iPTH levels (r 0.4 p 0.001) also, there were significant negative correlations between phosphorus and vitamin D levels (r

-0.7 *p* 0.001) and between phosphorus and calcium levels (*r* -0.5 *p* 0.001), also there was a significant negative correlation between iPTH and vitamin D(*r* -0.7 *p* 0.001) levels, these results were in accordance with studies reported by[24,9] that show higher levels of iPTH are correlates of hyperphosphatemia in CRF patients and in contrast with studies reported that lower levels of iPTH and normal levels of calcium which reported by.[19]

Previous studies have reported various different results about the influence of VDR gene FOK1 polymorphism on iPTH in CRF patients. This study investigated relationship between VDR gene FOK1 polymorphism and iPTH levels and observed that no significant difference between genotype of FOKI in CRF patients in all parameters except iPTH level (p=0.001), iPTH levels were different in genotype of FOKI polymorphism as FF genotype patients had higher level of iPTH than Ff and ff genotype patients as in renal failure and predialysis the decrease of vitamin D level stimulate parathyroid gland to increases iPTH levels, FF genotype patients had higher sensitivity to 1,25(OH) D levels so, VDR FOKI polymorphism F allele was shown to have a higher incidence and development of sHPT in end stage renal disease, these results were in accordance with.[24] and contrast with studies reported by.[25]

CONCLUSION

This study shows that deficiency of vitamin D in chronic renal failure patients is associated with high level of intact parathyroid hormone (hyperparathyroidism), also vitamin D receptor gene FOKI polymorphism had effect on the levels of iPTH, so deficiency of vitamin D and high level of iPTH and its association with VDR gene polymorphism may be used as morbidity indicator and risk factors as useful for identification the high risk group in chronic renal failure patients. F allele in VDR FOKI polymorphism was shown to have a higher incidence in the development of sHPT in CRF patients. Finally this study recommends further genetic study about the role of vitamin D and parathyroid hormone in chronic renal failure patients.

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