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Prevalence of Vitamin D receptor gene polymorphism among Chronic Kidney Disease patients in Puducherry – A Cross Sectional study

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Abstract

Background: The vitamin D receptor (VDR) axis plays a vital role in activation of vitamin D and responsible for normal physiological renal functions. Any polymorphism of VDR gene lead to the drastic changes in the renal tissues and causes enormous damage to the glomerular function and renal failure. This study was aimed to find the allelic and genotypic frequency of ApaI gene in CKD patients.

Methods: This was a cross sectional study involving 50 CKD patients. ApaI gene polymorphism were genotyped using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) method. All the statistical analysis was carried out using the SPSS software version 24.

Results: Among 50 CKD study participants, AA (wild-type) genotype of ApaI gene was present in 15 patients (30%), Aa (heterozygous) genotype was present in 30 patients (60%) and aa (mutant) genotype was present in 5 patients (10%). CKD patients with Aa and aa genotypes were found to have significantly elevated serum urea, creatinine, uric acid, random blood sugar, potassium and chloride when compared to the AA genotype. Additionally patients with Aa and aa genotypes were found to have decreased haemoglobin levels and sodium when compared to AA genotype.

Interpretation & Conclusion: 'a' allele of ApaI gene, 'Aa' and 'aa' genotypes of ApaI gene were prevalent in chronic kidney disease patients in Puducherry.

Keywords: Chronic kidney disease, Single nucleotide polymorphism, Vitamin D receptor gene.

Introduction

Chronic Kidney Disease (CKD) is defined based on the two clinical criteria, first criteria includes glomerular filtration rate (GFR) < 60ml/min/1.73m^2

for ≥ 3 months with or without evidence of kidney damage and the second criteria includes the evidence of kidney damage with or without decreased GFR for ≥ 3 months.[1] CKD is a global threat for developing countries because of the expensive and lifelong therapy.[2,3]

CKD was considered as a multi-factorial chronic inflammatory condition which involves glomerular part of the nephrons. The chronic inflammation of the glomerulus, affects the normal filtration process of the kidney finally resulting in reduced GFR.[4] Decreased GFR leads to oliguria. Thus one of the major clinical presentation of CKD patients were elevated serum creatinine and urea along with oliguria.[5]

CKD has vast spectrum of presentation, previously CKD was developed in elderly patients but presently many studies demonstrated that young age group people were developing CKD.[6] These young age group population were not associated with any family history of CKD and proper etiology, so currently researchers provoked the etiology of CKD and found that many genetic polymorphisms plays an important role in CKD.

Several gene studies revealed that CKD was linked with the single nucleotide polymorphism (SNP) of Vitamin D receptor gene, Angiotensin receptor gene, Renin gene and Interleukin – 6 gene. Among these, VDR gene was frequently associated with CKD.[7,8]

VDR is a member of nuclear receptor family of transcription factor, it is also called as calcitriol receptor or nuclear receptor subfamily (group I & member 1).[9]

Human VDR gene located on chromosome 12q, consist of 11 exons along with introns and made up of 75kb.[10] The four common SNP of VDR gene includes BsmI, ApaI, FokI and TaqI.[11] ApaI gene is located on intron 8 of chromosome 12q, it consists of two different alleles (A,a) and its polymorphism is adenine/cytosine (A/C) variation in intron 8 of 5' promoter region. Among these four SNP, BsmI and ApaI have been identified as risk factors in the progression of CKD.

This is the first study conducted in Union Territory of Puducherry to evaluate the prevalence of ApaI gene polymorphism among CKD patients. Thus the purpose of this study was to reveal the allelic and genotypic frequency among CKD patients in Puducherry.

Materials and Methods

This was a cross-sectional study done at Sri Manakula Vinayagar Medical College & Hospital (SMVMCH), after obtaining permission from Institutional Ethics committee.

A total of 50 CKD patients were recruited for the study from Nephrology Department of SMVMCH, Puducherry. Informed written consent was obtained from all the CKD study participants.

Inclusion criteria

1. CKD patients with age group 45 – 70 years was included in this study.
2. CKD patients with blood urea value > 40mg/dl
3. CKD patients with serum creatinine> 1.2mg/dl
4. CKD patients with estimated glomerular filteration rate (e GFR)

< 60ml/min/1.73m^2

5.CKD patients with estimated creatinine for males : < 97ml/min and

for females : < 88ml/min was included in the study.

Where e GFR was calculated using the modified of diet in rural disease formula (MDRD).

Where estimated creatinine clearance was calculated using Cockcroft-Gault formula.[12]

6. CKD study participants were staged based on the KDIGO (Kidney Disease Improving Global Outcomes) CKD staging by using GFR.[13,14]

Exclusion criteria

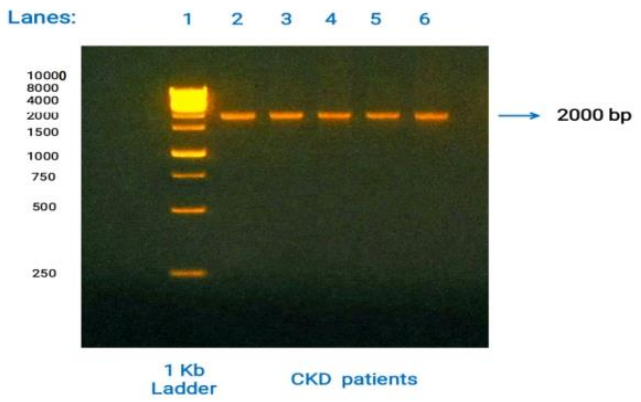
- Patients with acute renal failure
- Patients with renal cell carcinoma

After getting proper informed consent 2 ml of Ethylene Diamine Tetra-acetic Acid (EDTA) blood sample was collected from patients for Deoxyribo Nucleic Acid (DNA) extraction.[15] CKD patients from Nephrology OPD were selected using random sampling method.

Genetic Analysis

The extracted DNA was subjected to PCR. Gradient PCR amplification was done to detect ApaI gene polymorphism using VDR forward primer with 5'-CAACCAAGACTACAAGTACCGCGTCAGTGA- 3' (GC content = 50%, Tm = 68.1˚C) and ApaI reverse primer with 5'-GCAACTCCTCATGGCTGA GGTCTCA- 3' (GC content = 60%, Tm = 67.9˚C) to produce ApaI gene amplicon with 2000bp length.[16] Figure – 1 shows the amplification of ApaI gene with 2000bp.

Figure 1: Analysis of amplified ApaI gene by 2% agarose gel electrophoresis



Determination of ApaI genotype

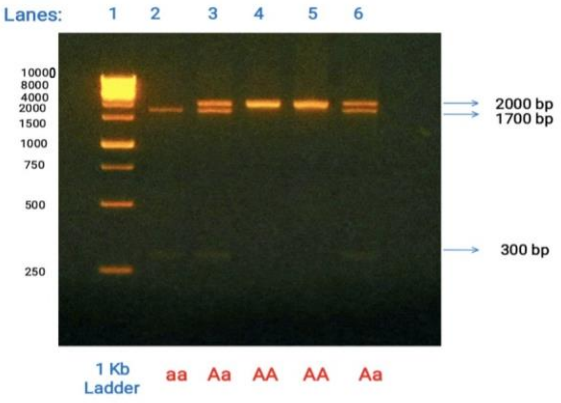
Thermo cycling consisted of denaturation at 95˚C for 120 seconds, annealing at 65˚C for 60 seconds and extension at 72˚C for 180 seconds, for 30 cycles followed by final extension at 72˚C for 5 minutes.

PCR products were detected by 3 % agarose gel electrophoresis with ethidium bromide stained and visualized by UV transilluminator.

Then ApaI gene was subjected to ApaI restriction enzyme (Brand name: Fermentas) digestion for 30 minutes at 25˚C with ApaI restriction enzyme and the digested products represents the RFLP. This RFLP were detected by 3% agarose gel electrophoresis stained with ethidium bromide and visualized under UV transilluminator.

Figure – 2 shows the three different patterns of ApaI gene restriction fragments were obtained in agarose gel electrophoresis. ApaI gene without restriction sites will produce single fragment of 2000bp (Homozygous wild – AA genotype), ApaI gene with restriction sites will produce either 2 fragments with 1700bp & 300bp (Homozygous mutant - aa genotype) or produce 3 fragments with 2000bp, 1700bp & 300bp (Heterozygous - Aa genotype) respectively.[17]

Figure 2: Analysis of ApaI gene restricted products on 3% agarose gel electrophoresis



Statistical Analysis

Statistical analysis was done using SPSS software version 24.0. Allele and genotypic frequencies were done by direct gene counting method. Comparison of ApaI genotypes with the study variables were expressed as mean ± SD and were done using student t test. The association between genotypes of ApaI polymorphism in CKD patients and type 2 diabetes mellitus was calculated using Odds ratio (OR) and 95% confidence intervals (CI). p values < 0.05 were considered significant.

Results

Table 1: Demographic characteristics of study participants (n = 50)

Study Parameters	Value(Mean ± SD)
1. Age (years)	57.54 ± 7.33
2. Gender (%) Male Female	 30 (60%) 20 (40%)
3. Blood Urea (mg/dl)	63.86 ± 15.53
4. Serum Creatinine (mg/dl)	3.40 ± 1.69
5. Serum Uric acid (mg/dl)	10.45 ± 2.76
6. Random Blood sugar (mg/dl)	182.06 ± 67.77
7. Serum Sodium (mEq/L)	130.43 ± 0.89
8. Serum Potassium (mEq/L)	6.62 ± 0.66
9. Serum Chloride (mEq/L)	112 ± 1.02
10. Serum Calcium (mg/dl)	8.12 ± 0.04
11. Serum Phosphorus (mg/dl)	6.68 ± 0.09
12. Haemoglobin (g/dl)	10.08 ± 1.77

n: Number of subjects, SD: Standard deviation, %: Percentage

Table 1 shows the demographic characteristics of the study participants. All the 50 CKD patients involved in the study showed elevated urea, creatinine, uric acid, potassium & Chloride level. And further decreased sodium & haemoglobin level are noted in the CKD patients. Thus abnormally elevated renal profile along with dyselectrolytemia was seen in the CKD study participants.

Table 2: Comparison of ApaI genotypes with study parameters.

Study variables	AA genotype (n=15)	Aa+ aa genotypes (n=35)	p value
1. Age (Years)	57.66 ± 6.77	57.57 ± 7.81	0.97
2. Urea (mg/dl)	53.11 ± 8.44	69.84 ± 15.47	< 0.001*
3. Creatinine (mg/dl)	2.39 ± 1.26	3.93 ±1.66	< 0.001*
4. Uric acid (mg/dl)	8.79 ± 2.34	12.47 ± 2.88	< 0.001*
5.Random Blood Sugar(mg/dl)	104.94 ±10.80	225.44 ± 42.44	< 0.001*

6. Serum Sodium (mEq/L)	132.11 ± 0.79	129.89 ± 0.87	< 0.001*
7. Serum Potassium (mEq/L)	3.93 ±0.33	5.01 ± 0.43	< 0.001*
8. Serum Chloride (mEq/L)	107.23 ± 0.09	112.89 ± 1.09	< 0.001*
9. Serum Calcium (mg/dl)	9.78 ± 0.18	8.10 ± 0.03	< 0.001*
10. Serum Phosphorus (mg/dl)	4.89 ± 0.05	6.28 ± 0.07	< 0.001*
11. Haemoglobin (g/dl)	11.89 ± 0.28	9.16 ± 1.26	< 0.001*

Data are represented in mean±SD. CKD: Chronic kidney disease, n: Number of subjects

* considered as significant.

Table 2 shows the comparison of ApaI genotypes with study parameters. Out of the 50 CKD study participants, 15 patients were having AA genotype (wild type without SNP), 30 patients were with Aa genotype (Heterozygous type with SNP) and 7 patients were with aa genotype (Homozygous mutant type with SNP).

We divided the CKD patients according to their ApaI genotypes and compared the study parameters in terms of indices of renal involvement. There were significant increase in serum creatinine, urea, random blood sugar, potassium and chloride in CKD patients with ApaI polymorphism than compared to the CKD patients without ApaI polymorphism. And further sodium and haemoglobin level were significantly decreased in CKD patients with ApaI SNP when compared to the CKD patients without ApaI SNP. Among 50 CKD study participants, 30 (60%) were males and 20 (40%) were females.

Table 3: Distribution of ApaI genotype and allele frequency among CKD patients

Study participants	Genotypic frequency			Allele frequency	
	AA	Aa	aa	A	a
CKD patients (n = 50)	15 (30 %)	30 (60 %)	5 (10 %)	0.60 (60%)	0.40 (40%)

CKD: Chronic Kidney Disease, n: Number of patients, % : Percentage

Table 3 shows the distribution of ApaI polymorphism among CKD patients, Out of 50 CKD patients, 15 patients were having AA (wild) genotype, that is 30%, 30 patients were having Aa (heterozygous) genotype, that is 60%, followed by 5 patients were having aa (mutant) genotype, that is 10%. And among 50 patients, 60% of patients were having 'A' allele and 40% of patients were having 'a' allele of ApaI gene.

Discussion

The primary objective of this study was to find the allele and genotypic frequency of VDR ApaI gene polymorphism among CKD patients and to study the relation between ApaI genotypes and the severity of CKD patients.

In this study, we demonstrate that CKD patients with 'a' allele at intron 8 of chromosome 12q had higher serum creatinine, urea, uric acid, random blood sugar, potassium, chloride and presence of

anaemia and hyponatremia than those with 'A' allele.

This is the first study to document an association between ApaI gene polymorphism among CKD patients in South Indian population.

Hussain et al. from Saudi Arabia, did a detailed meta-analysis and found that vitamin D receptor TaqI and ApaI genetic polymorphisms were associated with end stage renal disease (ESRD).[18]

Wang et al. from china, found a strong association of VDR gene polymorphisms with ESRD. And further ESRD patients with ApaI gene polymorphism had increased risk of high-turnover renal osteodystrophy.[19]

Nagamani.S et al. found a highly significant association between ApaI gene spolymorphism and CKD disease progression.

Many studies demonstrated that CKD patients were associated with dyselectrolytemia which includes hyponatremia, hyperkalemia and hyperchloridemia.[20,21]

CKD patients usually presents with anaemia, and further CKD patients on haemodialysis were strongly associated with very severe anaemia.[22]

In contrast to our result, few studies revealed that ApaI gene polymorphism was not associated with CKD in Turkey and Caucasian individuals.[23,24]

Based on the evidence from both animal and human studies, recently researchers demonstrated that the VDR activation protects against renal injury. Further, it was found that VDR could be a novel therapeutic target for kidney diseases in near future. And attempt has been made to develop new classes of pharmacological activators of VDR, which was aimed to design with better therapeutic efficacy and reduced side effects for the treatment of kidney diseases.[25]

In our study, a statistically significant relationship was observed between a allele, Aa and aa genotypes of ApaI polymorphism.

Limitations of the study were small sample size, the study excluded CKD patients on peritoneal dialysis, our study does not included control group and there is no long-term follow-up of CKD patients.

Conclusion

Our results demonstrated that ApaI polymorphism of VDR gene influence the risk of CKD development. ApaI SNP could be added to the list of potential markers to Nephrologists in determining the CKD risk profile. Our findings suggest that genetic variation in BsmI gene has an impact on the development of CKD in the Puducherry. Thus, molecular genetic assessment possess vital role in progression of CKD. This study needs to be extended in a larger sample size to establish this association more accurately.

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