Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 10218 - 10227
Received 25 April 2021; Accepted 08 May 2021.

Association of Vitamin D Receptor Gene Polymorphism and Calcium-Containing Renal Stone Disease in the Babylon Province Population

Salah Hashim[1a], Rabab Omran[2b], Ammad Hassan Mahmood[3]

1Biology Department, College of Science, University of Karbala. 2Department of Biotechnology, University of Babylon's College of Science. 3Department of Urology, University of Babylon's College of Medicine.

[a)]Corresponding author: salahhashem5@gmail.com
[b)]anotherauthor: omranaljelawi@gmail.com

ABSTRACT. The main reason for kidney stones is unknown, but it is thought to be linked to the vitamin D receptor gene (*VDR*). Attempted to evaluate the association between calcium stone disease and the *VDR* start-codon (T/Crs2228570) polymorphism in the Babylon Province population to determine the polymorphism's eventual role in calcium stone formation.Method: From January to December 2020, blood samples were obtained from 60 patients admitted to the Ibn Al-Nafees laboratory and Hillah Hospitals in Babylon Province, Iraq. Furthermore, normal people were used as a control group (40 samples). A polymerase chain reaction technique was used to genotype *VDR* single-nucleotide polymorphisms (SNPs), followed by single-strand conformation polymorphism. As a result, DNA sequencing was used to verify these DNA polymorphisms.**Results**:Due to the existence of SNPs within the studied area, the conformational haplotypes of *VDR*, exon4, and intron 3 were got in three patterns, including two, three, and four bands. These SNPs in exon 4 causing three amino acid substitutions in VDR, includingMet 1→ Thr1, Arg30 → Leu30, and Arg49 → Ser 49.These changes in amino acids were thought to affect the VDR protein's expression and/or function. also, there are significant differences ($P \leq 0.05$) in the serum levels of calcium, Phosphorus, creatinine as well as eGFR level among 2-bands, 3-band, and 4-bands for the *VDR* gene in the calcium-containing renal stones diseases group.**Conclusion**: These findings indicate that the VDR SNP rs2228570, as well as other VDR variants, maybe play a role in kidney stone disease susceptibility.

Keywords: Vitamin D receptor,Renal calcium-containing stones, Single nucleotide polymorphism.

INTRODUCTION

Kidney stone disease is the formation of a crystal concretion in the kidneys. Stone formation is a complex process involving several physicochemical events, including supersaturation, nucleation, growth, aggregation, and retention of urinary stone constituents within tubular cells [1]. The global prevalence and recurrence rates of kidney stone disease are increasing [2], and there are few successful therapies available. Urolithiasis affects approximately 12percent of the global population at some stage in their lives [3]. It affects people of all ages, genders, and races, but men between the ages of 20 and 49 are more affected than women [4].

The most common form of kidney stone is a calcium stone, which appears to be the most common, accounting for around 80% of all urinary calculi. CaOx stone formation is influenced by hypercalciuria (resorptive, kidney leak, absorptive, and metabolic diseases), hyperuricosuria, hyperoxaluria, hypocitraturia, hypomagnesuria, and hypercystinuria [5]. Calcium-based calculi cause about 75% of kidney stone disease, and their prevalence is growing, meaning that environmental and dietary factors are acting on a preexisting genetic history. Recent genetic studies have successfully identified genes that may be involved in the development of kidney stones, indicating that the disease has a known familial nature and important heritability[6].

Vitamin D is important for calcium metabolism. Via an increase in serum calcium levels, it may increase the chance of urinary stone development. The vitamin D receptor regulates the effects of vitamin D. There is evidence that a pathologic rise in VDR in the intestine, kidney [7], and bone [8] can trigger changes in

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 10218 - 10227
Received 25 April 2021; Accepted 08 May 2021.

calcium transport, so any change in the VDR may affect calcium metabolism and, as a result, be related to an increased risk of urolithiasis. More research is needed to determine whether *VDR* gene polymorphisms are linked to nephrolithiasis. Polymorphisms in the VDR gene have been related to the production of kidney stones in a variety of populations, according to the majority of these studies [9],[10].
There is no evidence of a connection between the *VDR* gene polymorphism and the renal calcium-containing stone disease in Iraq's Babylon Province population. As a result, we conducted a study to look into the risk factor for *VDR* rs2228570 patients suffering from the renal calcium-containing stone disease.

METHODS

Sampling

A case-control study was conducted between January and December 2020. Patients at the Ibn Al-Nafees and the Hillah Hospitals in Babylon, Iraq were taken with blood samples.In this study, a total of 100 blood samples were used. A total of 60 blood samples were collected from patients with Calcium Kidney Stones Disease. There were 40 samples of apparently healthy people used as a control group. A venous blood sample of about five milliliters was taken from each study participant. Each blood sample was divided into two sections: The first (2 ml) was collected into EDTA-containing tubes for genetic analysis, and the second (2 ml) was centrifuged at 3000 rpm for 15 minutes to extract the serum, which was then stored in Eppendorf tubes at -20 oC until required. Both of the participants were tested by a specialist physician. Hyperparathyroidism patients were excluded.

PhysiologicalParameters

Using a reflotron plus analyzer, calcium, phosphorous, and creatinine were directly measured in serum (Roche company- Germany). The glomerular filtration rate was calculated using the online site WWW. National Kidney Foundation (eGFR calculate).

SSCP-PCR technique to identify genotypes

After extracting DNA from blood samples, the study groups were genotyped using the SSCP-PCR technique. A Geneaid extraction and purification kit was used to extract and purify DNA from blood.The targeted DNA sites were amplified using design-specific primers obtained from Macrogen company in South Korea, which were used to identify VDR (rs2228570). 5'- ATGTATGAGGGCTCCGAAGG-3' and 5'- CCTTCATGGAAACACCTTGC-3' are the forward and reverse primers, respectively.

To get the reaction volume up to 20 µl, 1 µl of each forward and reverse primer, 12.5 µl of Green Master Mix, 3 µl of Genomic DNA, and 2.5 µl of nuclease-free water were added to the reaction volume of PCR.Amplification was conducted in a thermocycler (Biometra, Germany) with the following settings: 3 min pre-denaturation at 94°C; 30 cycles with denaturation for 30 seconds at 94°C, annealing for 30 seconds at 60°C, extending for 30 seconds at 72°C; and a final extension of 3 min.

PCR products were electrophoresed in 1 percent agarose at 75 V using gel electrophoresis (cleaver science – UK) and visualized with ethidium bromide. A gel documentation system (Cleaver Scientific –UK) was used to take photos. After electrophoresis of exon 4 and part of intron 3 of the VDR PCR fragment, the clear and bright bands were found. These amplified fragments are then suitable for SSCP experiments[11].Followed by 30 cycles of PCR amplification, an equivalent volume of SSCP denaturing-loading buffer was applied to 10 of each PCR amplicon (95 percent formamide, 0.05 percent bromophenol blue, 0.05 percent xylene cyanol, and 20 mM EDTA, pH 8). After being denatured for 8 minutes, PCR amplicons were directly put on ice and kept freezing for at least 10 minutes. The samples were then loaded

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 10218 - 10227
Received 25 April 2021; Accepted 08 May 2021.

onto polyacrylamide gels that were neutral. Afterward, gels were stained with a silver staining procedure that was highly sensitive[12],[13].

The single-stranded (ssDNA) and double-stranded (dsDNA) DNA bands were observed in the higher portion of the gel and the lower portion of the gel, respectively. The genetic pattern of each amplicon is determined by the variation of ssDNA in SSCP gels, and the SSCP-PCR conditions were 8 percent polyacrylamide gel electrophoresis power applied: 200 V, 90 mA for 160 minutes. After that, silver staining was used to visualize the gels.

Sequencing

According to Macrogen sequencing laboratory instructions, each PCR amplicon representing a specific PCR-SSCP banding pattern was submitted for Sanger sequencing reactions from both the forward and reverse termini (South Korea). All observed PCR-SSCP banding pattern sequences have been aligned with the program DNA Bio Edit 7.2.5 and edited.

Statistical analysis

SPSS statistical software (version 23; SPSS Inc., Chicago, IL) was used for all statistical analyses, and $p \leq 0.05$ was deemed statistically significant.

RESULTS

In contrast to the control group, the patient groups showed no significant differences ($P \leq 0.05$) in serum Phosphorus and Calcium levels (Table1). In addition, the findings showed that the patient groups had significant differences in creatinine concentrations and eGFR levels as compared to the control group, as shown in table 1.

As a first step, DNA was extracted from a blood sample to separate the genomic DNA of patients and healthy controls. Figure 1 depicts the electrophoresis of genomic DNA on a gel. (1% agarose, 75 V, 20 mA for 1 hour).

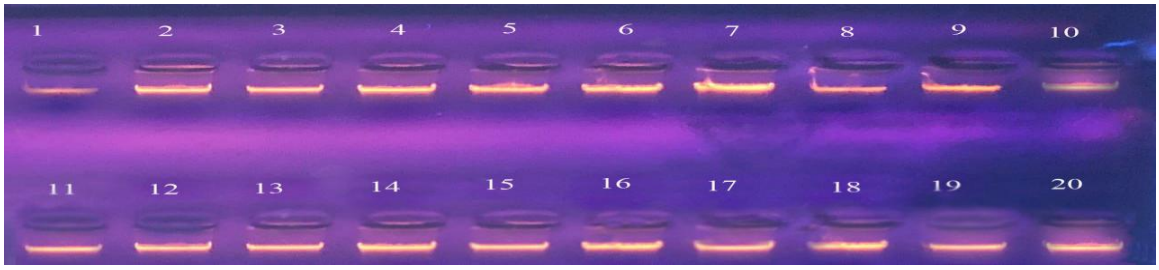

Figure 1: Genomic DNA extracted from blood samples of renal calcium-containing stone disease patients and healthy control groups on an agarose gel electrophoresis pattern. 1 percent agarose, 75 V, 20 mA for 1 hour, stained with ethidium bromide electrophoresis conditions.

Tables 1: Some physiological parameters of patients with renal diseases andhealthy control groups

Groups	**Mean ± SD**		***P*-value**
Parameters	**Patients**	**Control**	

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 10218 - 10227
Received 25 April 2021; Accepted 08 May 2021.

Calcium (mmol/l)	**2.26 ± 0.17**	**2.30 ± 0.09**	**0.113**
Phosphours (mmol/l)	**1.19 ±0.23**	**1.140 ± 0.16**	**0.241**
Creatinine (µmol/l)	**113.19± 79.41**	**80.12 ± 11.01**	**0.01***
eGFR (ml/min /1.73 m^2)	**79.76± 34.3**	**99.6 ± 14. 1**	**0.001***

*P ≤ 0.05; SD: Standard Deviation

For VDR genotyping using the RCR-SSCP technique, the genomic DNA of renal calcium-containing stone disease patients and controls was amplified using specific primers constructed according to the exon 4 region of Homo sapiens VDR reference ID: NG 008731.1. Under optimal conditions, the thermocycler apparatus was used to conduct genomic DNA amplification. The findings revealed the presence of a single band (403bp) of the target sequence of an exon 4 region of VDR, which includes a danger region, on an agarose gel (Fig. 2) (rs2228570).

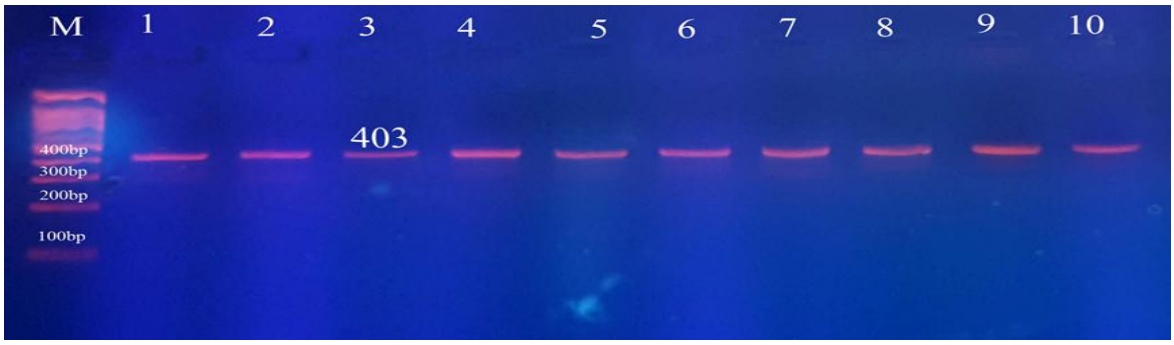

Figure 2: Electrophoresis of VDR amplified product patterns on an agarose gel in patients and stable controls. M: refers to DNA scale marker (403bp); Lanes 1 - 10 represent PCR products of the VDR gene (403bp) from patients and healthy control groups. Electrophoresis conditions were as follows: 1 percent agarose concentration; 75 V, 20 mA for 120 minutes. Ethidium bromide was used as a staining tool.

As shown in Fig. 3 of PCR-SSCP gel electrophoresis, the existence of various conformational DNA polymorphisms was found according to the number of bands: 2-bands, 3-bands, and 4-bands.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 10218 - 10227
Received 25 April 2021; Accepted 08 May 2021.

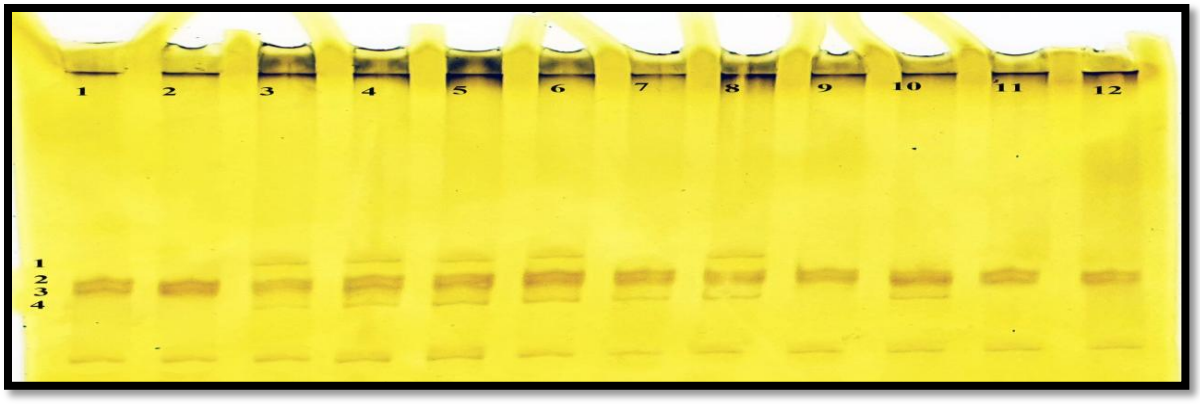

Figure 3:*VDR* gene polymorphisms of renal calcium-containing stones patients and healthy control subjects according to the number of the bands using the PCR-SSCP method. Numbers (1-4) refers to band location. Lanes 1 –10 refer to *VDR* gene PCR-SSCP fragment patterns. Lanes 1 & 2 represent 2-bands haplotype of control; Lanes 3, 4, 5, 6 & 8 represent 4-bands haplotype of patients; Lanes 7 and 10 represent 3-bands haplotype of patients; Lanes 9,11& 12 represent 2-bands haplotype of patients.

The results also revealed that conformational polymorphism distributions among haplotypes in the patient community were 2-bands (31.66%), 3-bands (45%), and 4-band (23.33%), respectively, compared to 2-bands (85%), 3-bands (15%), and 4-band (0%) in the healthy group. There is a connection among DNA polymorphisms and the number of bands in patients as compared to controls, as shown in table 2.

Table 2:The number of bands in the PCR-SSCP haplotype distribution of the VDR gene, as well as their relationship with patient and control groups.

Conformational haplotypes	**Patient group No. (%)**	**Control group No. (%)**	***P*-value**	**OR**	**95% CI**
2-bands [a]	19 (31.66%)	34(85%)	0.0001*	8.025	2.824-22.959
3-bands	27 (45%)	6 (15%)			
4-bands	14 (23.33)	0 (0%)	0.007*	51.3	2.899-908
3-bands [a]	27(45%)	6 (15%)	0.2	6.85	0.36-130.438
4-bands	14 (23.33)	0 (0%)			
Total number	60	40			

*P ≤ 0.05; OR= (95%CI);

The current results lead to the existing significant differences (P≤ 0.05) in serum calcium, phosphorus, creatinine, and eGFR levels for the VDR gene in the renal calcium-containing stones diseases group among 2-bands, 3-bands, and 4-bands in the renal calcium-containing stones diseases category as shown intable 3.

Even so, using only the gel visualization, it may be tough to establish the pattern of all resolved SSCP bands. As a result, sequencing must be used to validate these DNA polymorphisms. Many SNPs were found between the one resolved haplotypes and between the VDR, Intron 3, and exon4 for reference sequences, according to the sequencing results. According to the reference sequence alignment of the human VDR gene (NCBI Reference Sequence: NG 008731.1), the findings revealed the existence of several SNPs as shown in Fig. 4 and Table 4.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 10218 - 10227
Received 25 April 2021; Accepted 08 May 2021.

Table 3: The relationship between DNA polymorphisms and physiological parameters in patient gro ups based on the number of bands for the VDR gene.

PCR-SSCP haplotypes Band No. (n)	Mean ± SD			
	Phosphuros (mmol/l)	Creatinine (µmol/L)	Calcium (mmol/L)	eGFR
Patient				
2-bands (n=19)	1.31±0.3 **c**	79.18±10.9 **c**	2.26±0.12 **b**	98.05 ±12.55 **a**
3-bands (n=27)	1.13±0.16 b	169.02±117.3 **b**	2.13±0.33 **a**	59.41±39.02 **b**
4-bands (n=14)	1.11±0.15 **a**	153.29±145.5 **a**	2.29±0.07 **c**	82.16 ±40.46 **c**

Means having the different letters in the same column differed significantly(*P ≤ 0.05); SD :(Standard Deviation); eGFR: estimated Glomerular Filtration Rate.

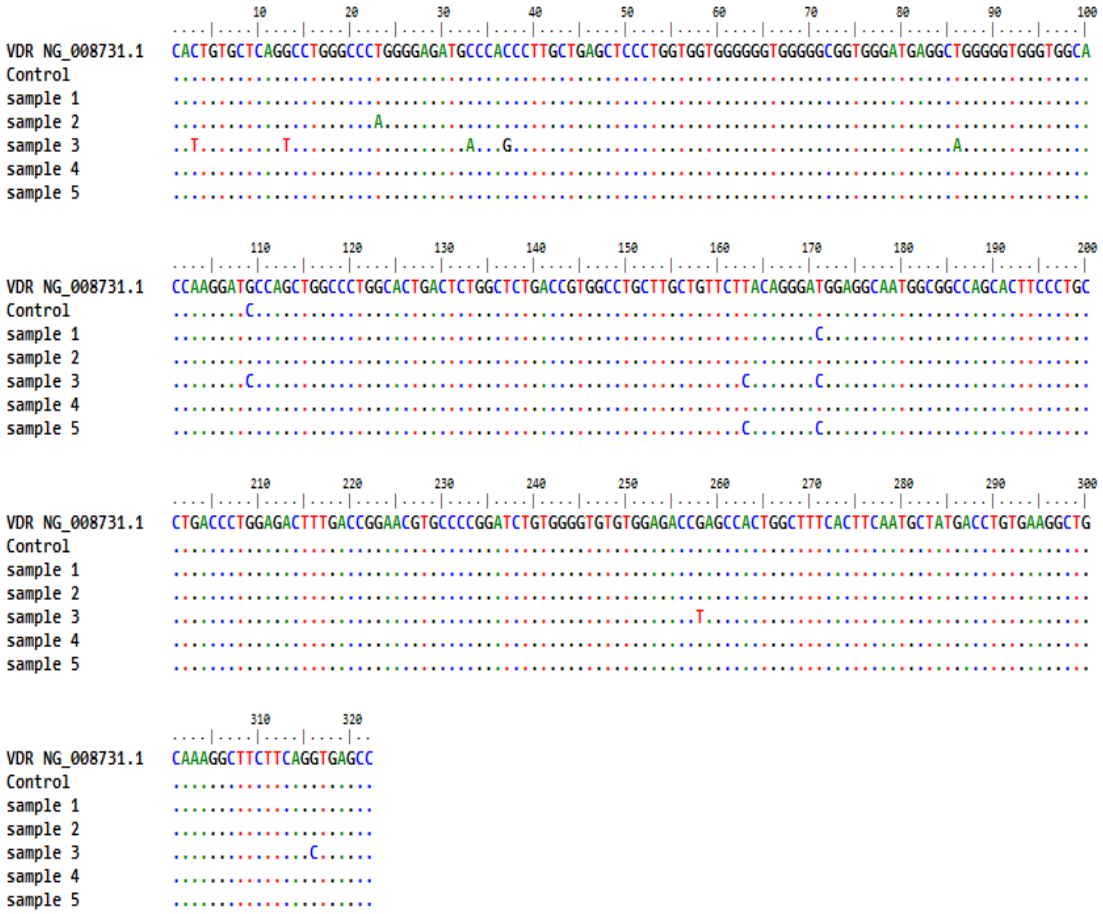

Figure 4: Sequences alignment fragment results of *Homo sapiensVDR* gene, Exon-4 and part of intron- 3 region reference ID: NG_008731.1 by Bio Edit software version7.2.5. Numbers 1 to 5 representa patient sample.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 10218 - 10227
Received 25 April 2021; Accepted 08 May 2021.

The sequencing results revealed there were three SNPs in the exon-4 region. most of these SNPs are previously recorded in NCBI such as rs2228570 (exon 4 regions), rs1238820289, and rs1034172632 (intron region) as showed in the table (4). Whereas, other SNPs are novel and not recorded previously like that located at the chromosomal site 47879280 (Intron), 47879260 (Intron), 47879174 (Intron), 47879025 (Exon), and 47878967 (Exon) (Table 4) . The non-coding variant may be linked to increased or decreased transcriptional activity as a result of altered trans-acting protein factor binding behavior to particular DNA sequences in the promoter region.

When using the Bio Edit software version 7.2.5 to translate the DNA sequence according to the reference sequence alignment of the human VDR gene ID: NG 008731.1. As shown in fig.4, the findings appeared to alter three amino acid residues in the Vitamin D receptor protein.These amino acid substitutions were Met 1→ Thr 1 in Sample 1, 3 and 5 (Fig.4) , Arg30 → Leu30 in sample 3 and Arg49 → Ser 49 also in sample 3 . These changes were thought to have an effect on the VDR protein's expression and/or function .

Table 4: All SNPs in exon 4 and intron 3 region of *VDR* gene.

Location (Intron or Exon)	**SNP**	**Code**	**Amino acid (AA)**	**Code**	**Amino acid**	**Seq.Base/AA**	**Previosly recorded SNP**
47879280 (Intron)	C/T	-	-	-	-	3	-
47879270 (Intron)	G/T	-	-	-	-	13	**rs1238820289**
47879260 (Intron)	T/A	-	-	-	-	23	-
47879250 (Intron)	C/A	-	-	-	-	33	**rs1034172632**
47879246 (Intron)	C/G	-	-	-	-	37	**rs540879004**
47879197 (Intron)	T/A	-	-	-	-	71	**rs1385324339**
47879174 (Intron)	G/C	-	-	-	-	109	-
47879120 (Intron)	T/C	-	-	-	-	163	**rs1333385322**
47879112 (Exon)	T/C	ATG	M	ACG	T	171/1	**rs2228570**
47879025 (Exon)	G/T	CGA	R	CTA	L	258/30	-

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 10218 - 10227
Received 25 April 2021; Accepted 08 May 2021.

47878967 (Exon)	G/C	AGG	R	AGC	S	316/49	-

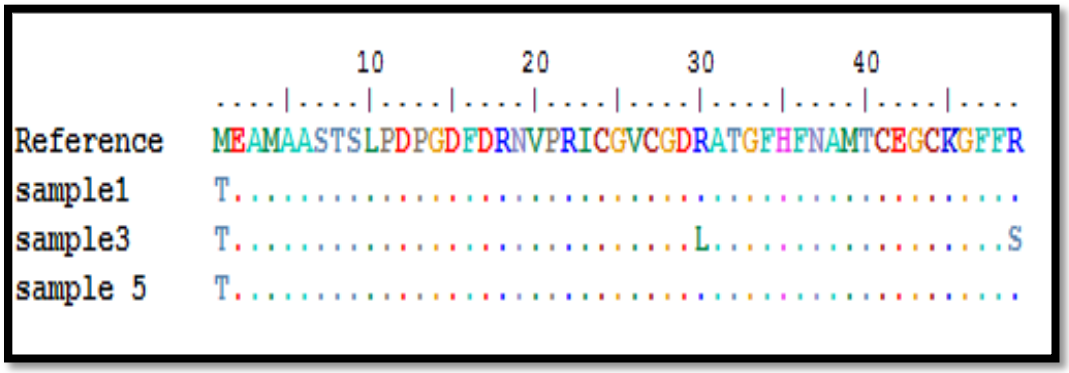

Figure 4: Pair sequence alignment of VDR amino acids based on Bio Edit software version 7.2.5 alignment. 1,3 and 4 refers to the patient's sample

DISCUSSION

Cell development, calcium absorption from the gut, cell differentiation, and androgen and estrogen activation are all regulated by the vitamin D receptor. Vitamin D, protein kinase A, parathyroid hormone, and growth factors all help to increase VDR levels. Minor changes in receptor structure, such as protein composition changes caused by DNA sequence variants, may have a minor effect on the cellular response to 1,25 (OH)2D3. Human VDR polymorphisms are being studied as potential prognostic markers for a variety of illnesses. Any VDR alteration will interfere with calcium metabolism, putting you at risk for kidney stones[14] .

The VDR helps to preserve calcium homeostasis by influencing bone resorption and increasing calcium absorption. The VDR's stone-forming mechanism is still a mystery. Allelic variations in the 3` - UTR region can affect messenger RNA stability and/or translation, lowering vitamin D activity and thus increasing the risk of stone disease [15]. Polymorphic variations in the VDR gene have been related to stone disease in some studies, but not all [16]..

The findings of our current study agree in part with Miyamoto & Yamamoto, who found that the VDR polymorphism C/T (rs2228570) at the translation initiation codon appears to result in the production of a small (by three amino acids) protein with increased biological activity and is related to increased bone mineral density in premenopausal Japanese women [17]..

Bid *et al*., found significant differences ($P< 0.001$) between the healthy control and stone patient groups in their study. When compared to the CC/TT genotype, the CT genotype was linked to a 3.234-fold increased incidence of renal stone (OR 3.234; 95 % CI 1.964, 5.325). In normal health subjects, the allelic distribution of the C/T polymorphism of VDR (rs2228570) was 238/94, while in stone patients, it was 166/110[18].

The results of this study indicate that the *VDR* (rs2228570) polymorphism may play a role in the development of renal calcium stones.The detection of genetic markers could lead to early intervention in patients at risk of calcium stone disease and, in the long run, to the prevention of urolithiasis. Besides, determining the risk of calcium stone disease in a patient's family member or the risk of recurrence in a patient can provide a tool for medical therapy and follow-up pace.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 10218 - 10227
Received 25 April 2021; Accepted 08 May 2021.

CONCLUSION

These findings indicate that the rs2228570 in the start-codon of *VDR* gene can play a role in susceptibility to renal calcium-containing stone disease.

REFERENCES

[1] Alelign, T., & Petros, B. (2018). Kidney Stone Disease: An Update on Current Concepts. *Advances in Urology*, *2018*.

[2] Yasui, T., Okada, A., Hamamoto, S., Ando, R., Taguchi, K., Tozawa, K., & Kohri, K. (2017). Pathophysiology-based treatment of urolithiasis. *International Journal of Urology*, *24*(1), 32-38.3.

[3] Chauhan, C. K., Joshi, M. J., & Vaidya, A. D. B. (2009). Growth inhibition of struvite crystals in the presence of herbal extract Commiphora wightii. *Journal of Materials Science: Materials in Medicine*, *20*(1), 85.

[4] Sorokin, I., Mamoulakis, C., Miyazawa, K., Rodgers, A., Talati, J., &Lotan, Y. (2017). Epidemiology of stone disease across the world. *World Journal of Urology*, *35*(9), 1301-1320.

[5]Dal Moro, F., Mancini, M., Tavolini, I. M., De Marco, V., & Bassi, P. (2005). Cellular and molecular gateways to urolithiasis: a new insight. *Urologia internationalis*, *74*(3), 193-197.

[6]Sayer, J. A. (2017). Progress in understanding the genetics of calcium-containing nephrolithiasis. *Journal of the American Society of Nephrology*, *28*(3), 748-759.

[7]Wang, S., Wang, X., Wu, J., Lin, Y., Chen, H., Zheng, X., ... & Xie, L. (2012). Association of vitamin D receptor gene polymorphism and calcium urolithiasis in the Chinese Han population. *Urological research*, *40*(4), 277-284.

[8]Krieger, N. S., Stathopoulos, V. M., & Bushinsky, D. A. (1996). Increased sensitivity to 1, 25 (OH) 2D3 in bone from genetic hypercalciuric rats. *American Journal of Physiology-Cell Physiology*, *271*(1), C130-C135.

[9]Zhou, T. B., Jiang, Z. P., Li, A. H., & Ju, L. (2015). Association of vitamin D receptor BsmI (rs1544410), Fok1 (rs2228570), TaqI (rs731236) and ApaI (rs7975232) gene polymorphism with the nephrolithiasis susceptibility. *Journal of Receptors and Signal Transduction*, *35*(2), 107-114..

[10]Guha, M., Bankura, B., Ghosh, S., Pattanayak, A. K., Ghosh, S., Pal, D. K., ... & Das, M. (2015). Polymorphisms in CaSR and CLDN14 genes associated with increased risk of kidney stone disease in patients from the eastern part of India. *PloS one*, *10*(6), e0130790.

[11] Hashim, H. O., & Al-Shuhaib, M. B. S. (2019). Exploring the potential and limitations of PCR-RFLP and PCR-SSCP for SNP detection: a review. *Journal of Applied Biotechnology Reports*, *6*(4), 137-144.

[12] Al-Shuhaib, M. B. S., Al-Kafajy, F. R., Badi, M. A., AbdulAzeez, S., Marimuthu, K., Al-Juhaishi, H. A. I., &Borgio, J. F. (2018). Highly deleterious variations in COX1, CYTB, SCG5, FK2, PRL and PGF genes are the potential adaptation of the immigrated African ostrich population. *Computers in biology and medicine*, *100*, 17-26.

[13]Byun, S. O., Fang, Q., Zhou, H., &Hickford, J. G. H. (2009). An effective method for silver-staining DNA in large numbers of polyacrylamide gels. *Analytical biochemistry*, *385*(1), 174-175.

[14] Haussler, M. R., Whitfield, G. K., Haussler, C. A., Hsieh, J. C., Thompson, P. D., Selznick, S. H., ... & Jurutka, P. W. (1998). The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *Journal of bone and mineral research*, *13*(3), 325-349.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 10218 - 10227
Received 25 April 2021; Accepted 08 May 2021.

[15] Seyhan, S., Yavascaoglu, I., Kilicarslan, H., Dogan, H. S., & Kordan, Y. (2007). Association of vitamin D receptor gene Taq I polymorphism with recurrent urolithiasis in children. *International journal of urology*, *14*(12), 1060-1062.

[16] Özkaya, O., Söylemezoğlu, O., Mısırlıoğlu, M., Gönen, S., Buyan, N., & Hasanoğlu, E. (2003). Polymorphisms in the vitamin D receptor gene and the risk of calcium nephrolithiasis in children. *European urology*, *44*(1), 150-154.

[17] Arai, H., Miyamoto, K. I., Taketani, Y., Yamamoto, H., Iemori, Y., Morita, K., ... & Takeda, E. (1997). A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. *Journal of Bone and Mineral Research*, *12*(6), 915-921.

[18] Bid, H. K., Kumar, A., Kapoor, R., & Mittal, R. D. (2005). Association of vitamin D receptor-gene (FokI) polymorphism with calcium oxalate nephrolithiasis. *Journal of endourology*, *19*(1), 111-115.