ORIGINAL ARTICLE

CONTENTS

Vitamin D receptor gene rs2228570 (FOKI) polymorphism associated with essential hypertension in Iraqi patients

Aseel R. Jabir[1], Hussein A Saheb[2], Bassim I Mohammad[1], Ahmed M Sultan[2], Sinaa Abdul Amir Kadhim[1], Asma A Swadi[1]
[1]DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS, COLLEGE OF MEDICINE, UNIVERSITY OF AL-QADISIYAH, IRAQ
[2]DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS, COLLEGE OF PHARMACY, UNIVERSITY OF AL-QADISIYAH, IRAQ

ABSTRACT

Aim: To understand how vitamin D receptor gene polymorphism (VDR rs2228570) affects blood pressure in Iraqi patients with essential hypertension in Al Diwaniya province.

Materials and Methods: This is a single-center observational cross-sectional descriptive study of 90 patients with essential hypertension. Using the PCR-TETRA ARM technique, blood samples were genotyped and examined for the polymorphisms of FOKI (rs2228570) gene.

Results: The most frequent allele was A (121, 67%) while the most frequent genotype was AG (55, 61%). There was no statistical difference between the actual and expected frequency distribution, according to Hardy-Weinberg equilibrium. The effect of VDR polymorphism rs 2228570 on blood pressure indicates (the mean systolic blood pressure in AA, AG, and GG carrier patients was 149, 150 and 166 respectively, P=0.29. On the other hand, the mean diastolic blood pressure in AA, AG, and GG carrier patients was 89, 89, and 94 respectively P=0.6) there was no statistically significant effect on systolic and diastolic blood pressure.

Conclusions: there is no statistically significant effect of VDR rs2228570 on SBP and DBP (p = 0.6), vitamin D receptor gene polymorphism rs2228570 was related to vitamin D level.

KEY WORDS: single nucleotide polymorphism, rs2228570, essential hypertension, vitamin D receptor gene

Wiad Lek. 2024;77(7):1387-1393. doi: 10.36740/WLek202407112 DOI

INTRODUCTION

Blood pressure is commonly expressed as the ratio of systolic to diastolic pressure (the pressure the blood exerts on the artery walls while the heart contracts and relaxes) [1]. A systolic blood pressure (SBP) of 140 mm Hg or higher, or diastolic blood pressure (DBP) of 90 mm Hg or higher, is considered as hypertension [2]. Primary and idiopathic hypertension is other names for essential hypertension. This type of high blood pressure is the most common in 90 to 95 percent of people. Essential hypertension appears to be largely influenced by genetic factors [3]. Hypertension is the greatest cause of death worldwide. Because the primary symptoms of hypertension are difficult to detect, many cases (40-50%) commonly go untreated, gaining it the term "silent killer" [4]. It is estimated that genes that govern vitamin D levels are causative of 30-50% of the BP fluctuations [5]. Identifying the genes that regulate vitamin D levels in essential hypertension may provide a more accurate factor in understanding the disease's molecular pathophysiology. The RAAS plays an important role in the physiologic control of sodium and potassium balance, intravascular volume, and blood pressure [6]. Excessive RAAS activity is now well documented to raise the cardiovascular risk that is minimized by reducing or blocking the RAAS [7]. Low vitamin D levels have been linked to clinical outcomes previously associated with increased RAAS function, such as hypertension, inflammation, and CVD [8]. Animal investigations showed that 1,25(OH)2D3-VDR complex adversely regulates renin expression and that vitamin D-induced RAAS activity can prevent poor vascular outcomes to the same extent as pharmaceutical angiotensin receptor antagonism [9]. This theory has been supported by human studies, which showed that reduced circulating vitamin D concentrations are associated with higher plasma renin activity and angiotensin II concentrations [10, 11] and that vitamin D deficiency is associated with higher RAAS activity, which can be reduced with vitamin D3 therapy intervention [9, 12]. Fok I (rs2228570) is a VDR gene SNP that has received a lot of attention. This polymorphism was discovered in the early 1990s, which is comprised of T to C mutation in exon-2. Because the mutation occurs in a start codon (ATG), the C variation causes an alternate start site to be employed, resulting in a larger protein [13]. The Fok I polymorphism can result in shortened proteins and is connected with an increased risk of hypertension. The

Table 1. PCR primers with their sequence, amplicon size, and annealing temperature

Primer	Sequence	Amplicon	Annealing
rs2228570 (FOKI)	Inner forward 70 AAGTGCTGGCCGCCATTGCCTACA (24)	A-allele 201 bp. G-allele 257 bp. Two outer primers 404 bp.	69°C
	Inner reverse 70 CCGTGGCCTGCTTGCTGTTCTTACAGGTAC (30)		
	Outer forward 70 AAAATGCAAGGGCTCCCTTCATGGAAACA (29)		
	Outer reverse 70 CGAAGGCACTGTGCTCAGGCCTGG (24)		

Table 2. Substances used in study, their producer, and their place of origin

No.	Chemical	Company and Origin
1	TBE buffer	Intron (Korea)
2	Agarose	MarLiJu (Korea)
3	Ethidium bromide	BioBasic (Canada)
4	Ladder	Bioneer (Korea)
5	Primers	Macrogen (Korea)

Table 3. JNC8 classification of blood pressure [19]

Category	Systolic		Diastolic
Normal	<120	and	<80
Pre-hypertension	120-139	or	80-90
Hypertension (Stage 1)	140-159	or	90-99
Hypertension (Stage 2)	>160	or	≥100

rs2228570 polymorphism is induced by thymine to cytosine transfer, which causes a translational frameshift defined by an expansion of the open reading frame to the next initiation codon (ATG), leading to the synthesis of a shortened 424 amino acid protein. ATG-encoded methionine (M1 form) was found in the f allele, whereas ACG-encoded methionine (M4 form) was present in the F allele [14]. The shortened protein in people with the FF genotype is hypothesized to increase the development of essential hypertension by boosting renin and angiotensin II production [15, 16]. It is suggested that the shortened protein's transcription activity is more than that of the full-length protein. Furthermore, the shortened protein's enhanced reactivity to 1,25(OH)2D3 may change VDR function as well as vitamin D in cells and tissues [17]. This shows that 1.25(OH)2D3 can suppress renin expression in humans and raise the risk of cardiovascular and metabolic disorders [18].

AIM

The aim of this research is to understand how vitamin D receptor gene polymorphism (VDR rs2228570) affects blood pressure in Iraqi patients with essential hypertension in Al Diwaniya province.

MATERIALS AND METHODS

STUDY DESIGN, PATIENTS RECRUITMENT, SETTING AND TIMING

The study involved 90 people (50 men and 40 women). This is a single-center observational cross-sectional descriptive study for hypertension patients of Iraqi nationality who have been diagnosed using the Eighth Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. All candidate patients were evaluated and diagnosed by a cardiologist or other professional caregiver. The study, which lasted from July 2022 to July 2023, was carried out at the Al-Diwaniyah teaching hospital and the Department of Pharmacology and Therapeutics, College of Medicine, University of Al-Qadisiya, Iraq. The laboratory work was done in the Al-Qadisiyah University's Department of Pharmacology and Therapeutics in the province of Diwaniyah.

QUESTIONNAIRE FORMULA

The following data were collected from the patients: name, age, sex, race, senior status, patient identification number, comorbidities, diagnosis, serum lipid level, uric acid, glucose, blood urea, and serum creatinine.

ETHICAL CONSIDERATIONS

The College of Medicine at Al-Qadisiyah University's Ethics Committee approved the study, and all patients received an explanation of the procedures before providing their informed permission.

PRIMERS USED IN THE CURRENT STUDY

Using the PCR-TETRA ARM technique, blood samples were genotyped and examined for polymorphisms in the FOKI (rs2228570). The Integrated DNA Technologies (IDT) website and data from the National Center for Biotechnology Information (NCBI) were used to create polymerase chain reaction (PCR) primers for the VDR

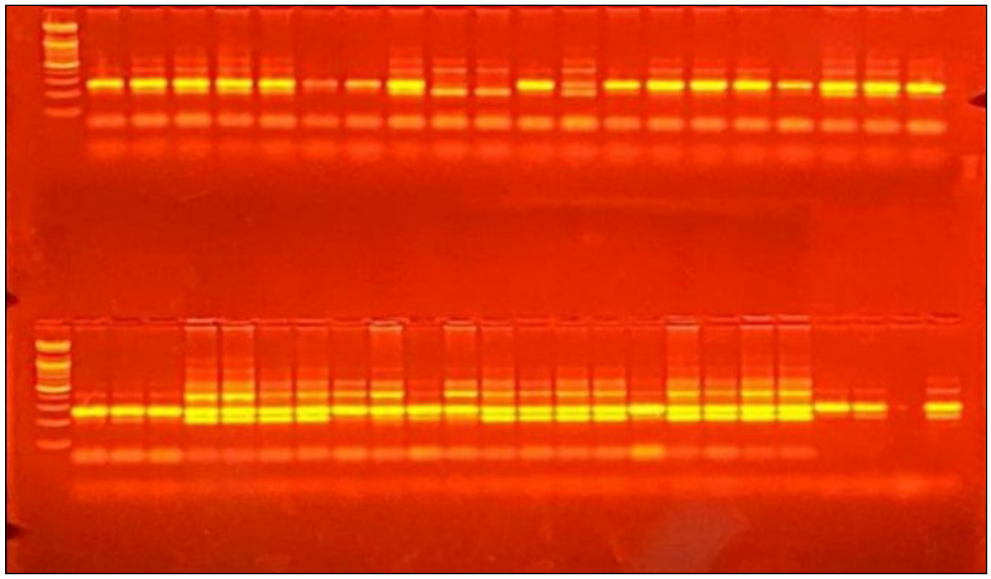

Fig. 1. Image of electrophoresis of agarose gel that demonstrates the analysis of PCR product of FOKI (rs2228570) gene from certain blood samples of patients.

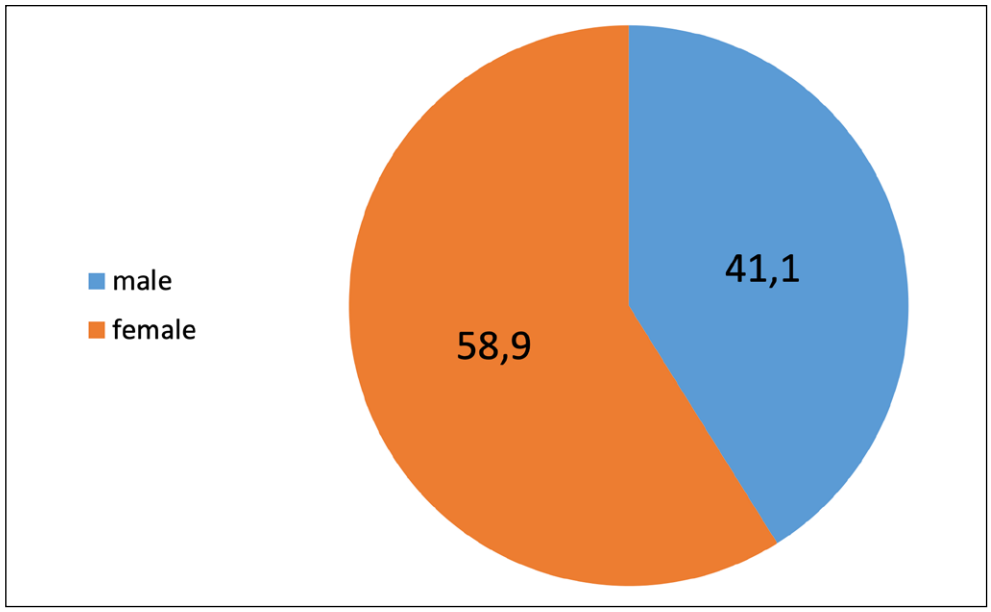

Fig. 2. Sex distribution of participant hypertensive patients in the study (n=90).

FOKI (rs2228570) genes. The primers were designed especially for this study using information for SNP sequence available online (https://www.ncbi.nlm.nih.gov/), SNP data and online tool for tetra arms primer design (http://primer1.soton.ac.uk/primer1.html), and for sequence SNP primer design (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/). The complementary nature of the given primers was further verified using the BLAST gene bank tool to ensure that they were not complementing the non-targeted gene. Deionized distilled water (DDH2O) was used to dissolve the lyophilized primers in the master tube to obtain a concentration of 100 pmol/l. Then, 10 pmol/µl was created as the working solution by transferring 10 µl from the master tube to another tube and bringing the total volume to 100 µl with DDH2O (Table 1).These primers were provided by Bioneer Company, Korea.

CHEMICALS THAT WERE USED IN THE CURRENT STUDY

Table 2 shows the chemical compounds employed in this investigation, along with their place of origin and Production Company.

BLOOD SAMPLING

4ml of blood was taken from each patient, divided into two parts, and aspirated from the antecubital vein.

- 1ml of the patient's whole blood was taken in an EDTA-containing tube for DNA extraction and kept at -20 C until DNA extraction.
- 3ml of the patient's whole blood was collected in a gel tube, spun for five minutes at 5,000 revolutions per minute, and the serum was collected for use in biochemical assays.

Table 4. Effect of VDR polymorphism rs 2228570 on blood pressure

Genotype rs2228570	Systolic BP means	SE	P value	Diastolic BP mean	SE	P value
AA	149	2.7		89	1.2	
AG	150	1.9	0.29 NS	89	0.9	0.6 NS
GG	166	1.5		94	6	

Table 5. Effect of VDR polymorphism rs 2228570 on blood pressure

Genotype rs2228570	Numbers	Mean Vid D level	S.E	P value
AA	33	4.4	0.44	
AG	55	7.5	1.05	0.028
GG	2	9.8	4.3	

BLOOD PRESSURE MEASUREMENT

A mercury sphygmomanometer was used to take blood pressure (BP) measurements. Before getting the measurements, the patient was instructed to sit comfortably and rest for five minutes with her right arm unclad and her legs uncrossed. The palm of the right arm was then raised and put on the table. The proper cuff size was selected. The cuff was kept at the same level as the heart during measurements. Patients are classified in the Eighth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC8) as shown in table 3.

GENOTYPING

Genomic DNA from blood samples was extracted by using a DNA isolation kit (Frozen Blood) Geneaid, USA.

PCR–TETRA ARM TECHNIQUE

Using the PCR-TETRA ARM technique, blood samples were genotyped and examined for polymorphisms in the FOKI (rs2228570) (Fig.1).

STATISTICAL PROCESSING

SPSS version 25 was used to make the statistical analysis. The data were shown as mean ± SE. Allelic and genotypic frequencies for each SNP were calculated. P value <0.05 was considered statistically significant.

RESULTS

DEMOGRAPHIC DATA

This study included 90 Iraqi hypertensive patients, 58.9% (n=53) were females and 41.1% (n=37) were males (Fig.2), with the mean ± SD age (years) of the study group 53.2±13.8, and mean ± SD BMI of 29±5 kg/m^2.

EFFECT OF VDR POLYMORPHISM RS 2228570 ON BLOOD PRESSURE

As shown in Table 4, the mean systolic blood pressure in homozygous AA, heterozygous AG, and homozygous GG carrier patients was 149, 150, and 166 respectively. On the other hand, mean diastolic blood pressure in homozygous AA, heterozygous AG, and homozygous GG carrier patients was 89, 89, and 94 respectively there was no statistically significant effect of rs 2228570 on systolic and diastolic blood pressure.

EFFECT OF POLYMORPHISM RS2228570 ON VIT D LEVEL

As shown in table 5, the mean plasma level of Vit D was higher in homozygous GG carriers (9.8), and lower in homozygous AA carriers (4.4). The difference was statistically significant in the plasma level of Vit D between AA carriers and AG carriers (P value was <0.028), (Fig.2). Although the mean Vit D level in GG carrier was higher than that of AG and AA carriers but it is difficult to decide whether the difference is statistically significant or not because we have only two patients carry GG allele.

DISCUSSION

The most frequent allele was A (67%) while the most frequent genotype was AG (61%). G allele has minor frequency 33% and GG genotype was 2% (p=0.8). There have been numerous studies looking at correlations between the rs2228570 variant and EH [20]. However, the findings have been contentious and inconclusive. In a study in Morocco, Errouagui and coauthors [21] studied 177 patients with hypertension and 222 normotensive persons of both genders and discovered that rs 2228570 polymorphism was related to AH in genotypes that are codominant, dominant, or recessive. The

frequency of the GG genotype was significantly lower in hypertension patients than in controls (P=0.002). The average vitamin D concentrations of patients with the AA, AG, and GG genotypes were "28.06±10.57, 29.04±11.97", and "26.40±19.15 ng/mL", respectively. Nevertheless, there were no statistically significant variations in vitamin D levels between AA and AG genotype patients (P=0.6463) or those with AA and GG (P=0.0767) [21]. In a prospective study by Wang et al. [22] recruited 1,211 Caucasian American men. Hypertension affected 695 of the patients. The vast majority of hypertensive people with a VDR gene polymorphism and the prevalence of Fok I polymorphism was found in 885 subjects. Only the recessive model revealed a link between the VDR rs2228570 and the risk for hypertension. In model 2, the (HR) multivariate hazard ratio for the occurrence of hypertension was associated with the GG genotype. Patients with the GG genotype were more likely than those with the AG and AA genotypes to have an increased risk of hypertension in relation to 25 (OH) D concentration [22]. In an Italian investigation, Cottone and colleagues recruited 72 control volunteers of both sexes and 71 patients with essential hypertension. AA, AG, and GG genotype frequencies were 50.7%, 42.3%, and 7.0%, in patients with hypertension, respectively, and 40.3%, 40.3%, and 40.0%, respectively, in participants in good health 50.0% and 9.7%, respectively. Patients with hypertension had frequencies of alleles of A and G of 71.8% and 28.2%, respectively, while normotensive people had allelic frequencies of 65.3% and 34.7%, respectively. All three Fok I polymorphism genotypes had distinct diastolic blood pressures (P=0.018). Diastolic blood pressure was greater in GG genotype patients compared to AG genotype patients (P=0.002) 25 (OH) D levels were shown to be negatively correlated with heartbeat blood pressure, and this association was significant statistically. Among patients with the AG genotype (P=0.035). No particular genotype or allele was associated with hypertension. Additionally, no correlation between the Fok I polymorphism and RP activity was found [23]. Our results indicate the mean plasma level of Vit.D was higher in homozygous GG carriers (9.8), and lower in homozygous AA carriers (4.4). There was a statistically significant difference in the plasma level of Vit D between AA carriers and AG carriers (P value was <0.028) Although the mean Vit D level in GG carrier was higher than that of AG and AA carriers but it is difficult to decide whether the difference is statistically significant or not because we have only two patients carry GG allele and thereby there is no statistically significant effect of VDR rs2228570 on SBP and DBP (p=0.6) and responsiveness to valsartan with 45.5% were considered as responders and 54.4% as non-responders. This may be either because of the small size of the samples in this study or a potent indication of the absence of association between this variant and EH in the Iraqi population. There is no clear explanation for why the rs2228570 variation is linked to hypertension. However, it has been proposed that the rs2228570 polymorphism is brought on by T to C change that causes a frameshift in translation marked by the creation of a truncated 424-amino-acid protein by extending the open reading frame to the next start codon (ATG) "ATG encoded methionine (M1 form)" was found in 427 amino acid protein's G allele, whereas "ACG encoded methionine (M4 form)" was found in the A allele. It is believed that the shortened protein in people with the AA genotype enhances the production of renin and angiotensin II, which in turn helps to develop essential hypertension [16].

CONCLUSIONS

Our research is the first of its kind in Iraq that investigates allele and genotype frequency in Iraqi patients with essential hypertension. Our result showed the most common allele for rs2228570 was the A allele 67% while the most frequent genotype was AG (61%), frequency of another genotype AA and GG were 37% and 2% respectively. Vit D receptor gene polymorphism rs2228570 was related to vit D level and there was no statistically significant effect on systolic and diastolic blood pressure.

REFERENCES

1. Oparil S, Acelajado MC, Bakris GL et al. Hypertension. Nat Rev Dis Primers. 2018;4:18014. doi: 10.1038/nrdp.2018.14. DOI
2. Zhou B, Carrillo-Larco RM, Danaei G et al. Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. The Lancet. 2021;398(10304):957-980. doi:10.1016/S0140-6736(21)01330-1. DOI
3. Winters WL. Hypertension (Pathology). Encyclopedia Britannica. 2024. https://www.britannica.com/science/hypertension. [Accessed 12 March 2024]

4. Bouchard J, Valookaran AF, Aloud BM et al. Impact of oats in the prevention/management of hypertension. Food Chemistry. 2022;381:132198. doi:10.1016/j.foodchem.2022.132198. DOI
5. Prasad M, Rajarajeswari D, Aruna P et al. Status of vitamin D receptor gene polymorphism and 25-hydroxy vitamin D deficiency with essential hypertension. Indian J Clin Biochem. 2022;37(3):335-341. doi:10.1007/s12291-021-00984-z. DOI
6. Laragh JH, Sealey JE. The plasma renin test reveals the contribution of body sodium-volume content (V) and renin-angiotensin (R) vasoconstriction to long-term blood pressure. Am J Hypertens. 2011;24(11):1164-1180. doi:10.1038/ajh.2011.171. DOI
7. Vaidya A, Brown JM, Williams JS. The renin-angiotensin-aldosterone system and calcium-regulatory hormones. J Hum Hypertens. 2015;29(9):515-521. doi:10.1038/jhh.2014.125. DOI
8. Han L, Xu XJ, Zhang JS. Association between vitamin D deficiency and levels of renin and angiotensin in essential hypertension. Int J Clin Pract. 2022;2022:8975396. doi:10.1155/2022/8975396. DOI
9. Vaidya A, Forman JP. Vitamin D and vascular disease: the current and future status of vitamin D therapy in hypertension and kidney disease. Curr Hypertens Rep. 2012;14(2):111-119. doi:10.1007/s11906-012-0248-9. DOI
10. Forman JP, Williams JS, Fisher ND. Plasma 25-hydroxyvitamin D and regulation of the renin-angiotensin system in humans. Hypertension. 2010;55(5):1283-1288. doi:10.1161/hypertensionaha.109.148619. DOI
11. Butler AE, Moin ASM, Sathyapalan T, Atkin SL. Vitamin D association with the renin angiotensin system in polycystic ovary syndrome. J Steroid Biochem Mol Biol. 2021;214:105965. doi:10.1016/j.jsbmb.2021.105965. DOI
12. Jensen NS, Wehland M, Wise PM, Grimm D. Latest Knowledge on the Role of Vitamin D in Hypertension. Int J Mol Sci. 2023;24(5):4679. doi:10.3390/ijms24054679. DOI
13. Carrara D, Bernini M, Bacca A et al. Cholecalciferol administration blunts the systemic renin-angiotensin system in essential hypertensives with hypovitaminosis D. J Renin Angiotensin Aldosterone Syst. 2014;15(1):82-87. doi:10.1177/1470320312471149. DOI
14. Valdivielso JM, Fernandez E. Vitamin D receptor polymorphisms and diseases. Clin Chim Acta. 2006;371(1-2):1-12. doi:10.1016/j.cca.2006.02.016. DOI
15. Nunes I, Pinho F, Cruz M. Influence of polymorphism of vitamin D receptor (Fok I) on hypertension. Brazilian Archives of Biology and Technology. 2020;63:e20190403. doi:10.1590/1678-4324-2020190403. DOI
16. Nunes I, Cavalcante A, Alencar M et al. Meta-Analysis of the association between the rs228570 vitamin D receptor gene polymorphism and arterial hypertension risk. Advances in Nutrition. 2020;11(5):1211-1220. doi:10.1093/advances/nmaa076. DOI
17. Jurutka PW, Remus LS, Whitfield GK et al. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. Mol Endocrinol. 2000;14(3):401-420. doi:10.1210/mend.14.3.0435. DOI
18. Whitfield GK, Remus LS, Jurutka PW et al. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Mol Cell Endocrinol. 2001;177(1-2):145-159. doi:10.1016/s0303-7207(01)00406-3. DOI
19. Li YC, Kong J, Wei M et al. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest. 2002;110(2):229-238. doi:10.1172/JCI15219. DOI
20. Bell K, Twiggs J, Olin BR. Hypertension: the silent killer: updated JNC-8 guideline recommendations. Alabama pharmacy association. 2015;334:4222. https://cdn.ymaws.com/www.aparx.org/resource/resmgr/CEs/CE_Hypertension_The_Silent_K.pdf. [Accessed 12 March 2024]
21. Errouagui A, Charoute H, Ghalim N. Relationship with vitamin D receptor (RVD) gene and essential arterial hypertension in Moroccan population. International Journal of Innovation and Applied Studies. 2014;8(2):556.
22. Wang L, Ma J, Manson JE et al. A prospective study of plasma vitamin D metabolites, vitamin D receptor gene polymorphisms, and risk of hypertension in men. Eur J Nutr. 2013;52(7):1771-1779. doi:10.1007/s00394-012-0480-8. DOI
23. Cottone S, Guarino L, Arsena R et al. Vitamin D receptor gene polymorphisms and plasma renin activity in essential hypertensive individuals. Journal of Human Hypertension.2015;29(8):483-7. doi:10.1038/jhh.2014.113. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Aseel R. Jabir
University of Al-Qadisiyah
University neighborhood, Al-Qadisiyah, Iraq
e-mail: aseelghufran9@gmail.com

ORCID AND CONTRIBUTIONSHIP
Aseel R. Jabir: 0009-0000-4429-0300 A B D
Hussein A. Saheb: 0000-0002-0137-8932 B C
Bassim I. Mohammad: 0000-0001-6732-5940 B C D
Ahmed M. Sultan: 0000-0001-6819-0208 C D
Sinaa Abdul Amir Kadhim: 0000-0001-9375-5581 D E
Asma A. Swadi:0000-0002-7679-1596 D E F

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 12.08.2023
ACCEPTED: 26.06.2024