Vitamin D Receptor Gene Polymorphism rs1544410 (BsmI) Associated with Essential Hypertension in Iraqi Patients

Aseel R. Jabir*, Bassim I Mohammad*, Wisam J Mohammed*, Hussein A Saheb**, Ahmed M Sultan**, Asma A Swadi*, Sinaa Abdul Amir Kadhim***

***Department of Pharmacology and Therapeutics, College of Medicine, University of Al-Qadisiyah ,**

****Department of Pharmacology and Therapeutics, College of pharmacy, University of Al-Qadisiyah ,**

*****ALKindy Teaching Hospital , Baghdad - Iraq**

Correspondence: aseelghufran9@gmail.com

(Ann Coll Med Mosul 2023; 45 (2):146-151).

Received: 18th Augu. 2023; Accepted: 1st Octo. 2023.

ABSTRACT

Background: It is suggested that polymorphisms in the Vitamin D receptors gene (rs1544410 BsmI) may affect blood pressure in Iraqi patients with essential hypertension (EH). Essential hypertension accounts for *95%* of all cases of hypertension. EH seems to be considerably affected by genetic factors and environmental factors.

Aim: To understand how vitamin D receptor gene polymorphism (VDR rs1544410 BsmI) 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. PCR-TETRA ARM technique was used, and blood samples were genotyped and examined for the polymorphisms BsmI (rs15444410) genes.

Results: The most frequent allele was C (95,53%) while the most frequent genotype was TC (57, 63%). There was no statistical difference between the actual and expected frequency distribution, according to Hardy-Weinberg equilibrium. The effect of VDR polymorphism rs1544410 on blood pressure indicates (the mean systolic blood pressure in homozygous TT, heterozygous TC, and homozygous CC carrier patients was 147, 151, and 147 respectively p=0.4. On the other hand, mean diastolic blood pressure in homozygous TT, heterozygous TC, and homozygous CC carrier patients was 87, 90, and 88 respectively p=0.3) there was no statistically significant effect on systolic and diastolic blood pressure.

Conclusion: Our study revealed that Vit D receptor gene polymorphism rs1544410 was not related to Vit D level, there was no statistically significant effect of rs1544410 on systolic and diastolic blood pressure.

Keywords: Essential Hypertension, Vit.D receptor, Polymorphism, rs1544410, JNC8.

تعدد الأشكال الجيني لمستقبلات فيتامين د (rs1544410 (BsmI المرتبط بارتفاع ضغط الدم الأساسي لدى المرضى العراقيين

أصيل رياض جابر* ، باسم أرحيم محمد* ، وسام جاسم محمد*** ، حسين علي صاحب** ،
أحمد محمود سلطان** ، أسماء عبدالجليل سوادي* ، سيناء عبد الامير كاظم*
*فرع الادوية والعلاجيات ، كلية الطب ، جامعة القادسية ، **فرع الادوية والعلاجيات ، كلية الصيدلة ، جامعة القادسية ، ***مستشفى الكندي التعليمي ، بغداد ، العراق

الخلاصة

الخلفية: يقترح أن تعدد الأشكال في جين مستقبلات فيتامين د (rs1544410. BsmI) قد يؤثر على ضغط الدم لدى المرضى العراقيين الذين يعانون من ارتفاع ضغط الدم الأساسي (EH). يمثل ارتفاع ضغط الدم الأساسي ٩٥٪ من جميع حالات ارتفاع ضغط الدم . يبدو أن EH يتأثر بشكل كبير بالعوامل الوراثية والعوامل البيئية.

الهدف: فهم كيفية تأثير تعدد الأشكال الجيني لمستقبلات فيتامين د (VDR rs1544410 BsmI) على ضغط الدم لدى المرضى العراقيين الذين يعانون من ارتفاع ضغط الدم الأساسي في محافظة الديوانية.

المواد والطرق: هذه دراسة وصفية مقطعية رصدية أحادية المركز لـ ٩٠ مريضًا يعانون من ارتفاع ضغط الدم الأساسي. تم استخدام تقنية PCR-TETRA ARM وتم تنميط عينات الدم وفحصها للتأكد من تعدد أشكال جينات (rs15444410) BsmI.

النتائج: كان الأليل الأكثر شيوعا هو C (٩٥,٥٣%) بينما النمط الجيني الأكثر شيوعا كان TC (٥٧,٦٣%). لا يوجد فرق إحصائي بين التوزيع التكراري الفعلي والمتوقع حسب توازن هاردي-واينبرج. يشير تأثير تعدد الأشكال VDR rs1544410 على ضغط الدم (متوسط ضغط الدم الانقباضي في مرضى TT متماثل الزيجوت، TC متغاير الزيجوت، ومرضى حاملي CC متماثلين كان ١٤٧، ١٥١، و ١٤٧ على التوالي = ٠.٤. من ناحية أخرى، متوسط ضغط الدم الانبساطي في متماثل الزيجوت كان مرضى TT و TC متغاير الزيجوت ومرضى حاملي CC متماثلين ٨٧ و ٩٠ و ٨٨ على التوالي (p = 0.3) ولم يكن هناك تأثير ذو دلالة إحصائية على ضغط الدم الانقباضي والانبساطي.
الاستنتاج: كشفت دراستنا أن تعدد أشكال جينات مستقبل فيتامين د rs1544410 لم يكن مرتبطًا بمستوى فيتامين د، كذلك لم يكن هناك تأثير ذو دلالة إحصائية لـ rs1544410 على ضغط الدم الانقباضي والانبساطي.

الكلمات المفتاحية: ارتفاع ضغط الدم الأساسي، مستقبلات فيتامين د، تعدد الأشكال، rs1544410، JNC8 .

INTRODUCTION

Hypertension is one of the most significant risk factors for dementia, chronic renal disease, ischemic heart disease, stroke, and other CVDs. High blood pressure is the leading avoidable cause of CVD death and disease burden in most locations and all over the world [1]. HTN affects up to 40% of Iraqi persons over the age of 25, with women having a higher prevalence [2]. It is common to state that essential hypertension is idiopathic, although this is only partially true because little is known about genetic variations, genes that are overexpressed or under-expressed, and the intermediary phenotypes that these genes regulate to produce BP [3]. The 8th report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 8) categorized blood pressure values as shown in the Table.1 [4].

Table 1 Classification of blood pressure.

Category	systolic		diastolic
Normal	<120	and	<80
Pre-hypertension	120-139	or	80-90
Stage 1 hypertension	140-159	or	90-99
Stage 2 hypertension	>160	or	≥100

According to the Iraqi Ministry of Health, 30% of Iraqis have high blood pressure in 2019 [5]. According to a survey, 35.6% of people in Iraq had excessive blood pressure. Only 7.9% of people were on medication and under control, with women benefiting more than males (9.3% female, 6.6% male) [6]. Cardiovascular disease alone is thought to be responsible for 27% of all fatalities, according to the Iraqi Ministry of Health's 2019 study [5]. The most recent WHO statistics, which were published in 2020, showed that hypertension was responsible for 2,451 fatalities in Iraq. Many risk factors can have an impact on cardiac output and peripheral resistance, which include: genetics, overactive sympathetic nervous system, renal factors, vascular factors, hormonal factors, obesity, obstructive sleep apnea, and environmental factors and Vitamin D deficiency. A 25-hydroxyvitamin D (25 [OH] D) level below 30 ng/ml, indicates vitamin D deficiency, which has been linked to an increased risk of hypertension. Vitamin D may change calcium concentrations in vascular smooth muscle cells, which may have an impact on vascular tone. As intracellular calcium accumulates, renin secretion is inhibited in juxtaglomerular cells. Both vitamin D and RAS, which regulate calcium and salt, may be related to the emergence of hypertension [7]. 1,25(OH)2D3, is a hormone that negatively regulates endocrine function and suppresses the expression of the renin messenger RNA (mRNA) in the RAAS. Vitamin D's biological effects are mediated by its ability for binding to VDR, where SNPs can alter arterial blood pressure and hasten the development of hypertension. The findings of the studies support the hypothesis that VDR Bsm I polymorphisms may be associated with susceptibility to essential hypertension [8].rs1544410 was chosen among many VDR gene SNPs because it was more frequent in the samples of this study and blood pressure can be influenced by this SNP. The goal of the current investigation was to understand how vitamin D receptor gene polymorphism (VDR rs1544410 BsmI) affects blood pressure in Iraqi patients with essential hypertension in Al Diwaniya province.

MATERIAL AND METHODS

Subjects

In this study, 90 persons (20-70 years old, 50 men, and 40 women) were enrolled. 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 Al-Qadisiya University's Department of Pharmacology and Therapeutics in the province of Diwaniyah.

Genotyping

DNA Extraction

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

PCR – TETRA ARM Technique

PCR-TETRA ARM technique was used, and blood samples were genotyped and examined for the polymorphisms of the Bsm I (rs1544410). Genotyping was performed using the following primers sequences; (table 2, figure 1 and 2)

Table 2 The PCR primers with their sequence, amplicon size, and annealing temperature.

Primer	Sequence	Amplicon	Annealing
VDR rs1544410 (BsmI)	Inner forward 10 GCAGAGCCTGAGTATTGGGAAGGC 24	C allele: 161 bp. T allele: 230 bp. Two outer primers 347 bp.	65℃
	Inner reverse 10 GGGCCACAGACAGGCCTTCA 20		
	Outer forward 10 TCCTCTTCGGCCTTTTCTCCCTCT 24		
	Outer reverse 10 AGAGCCCCTGTGGTGTGTGGAC 22		

PCR Product Analysis

The PCR products were analyzed by agarose gel electrophoresis using a UV transilluminator, the PCR products were seen

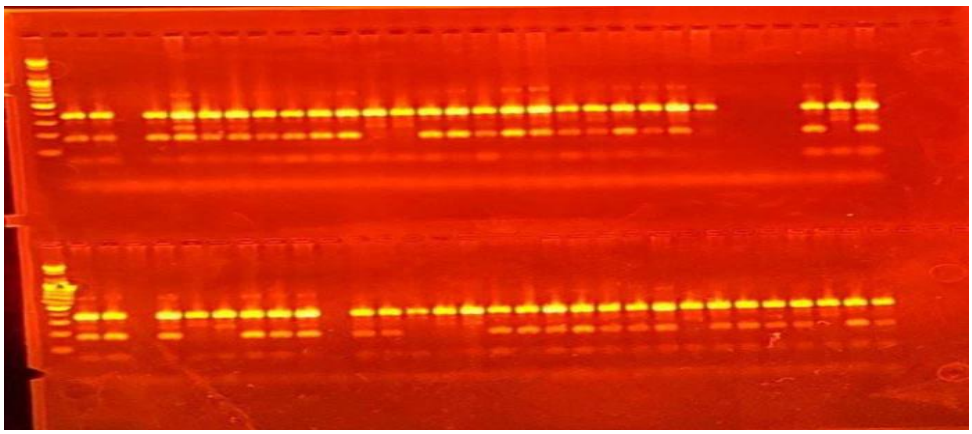

Figure 1 Image of electrophoresis of Agarose gel that demonstrates the analysis of PCR product of Bsm I (rs1544410) gene from other blood samples of patients.

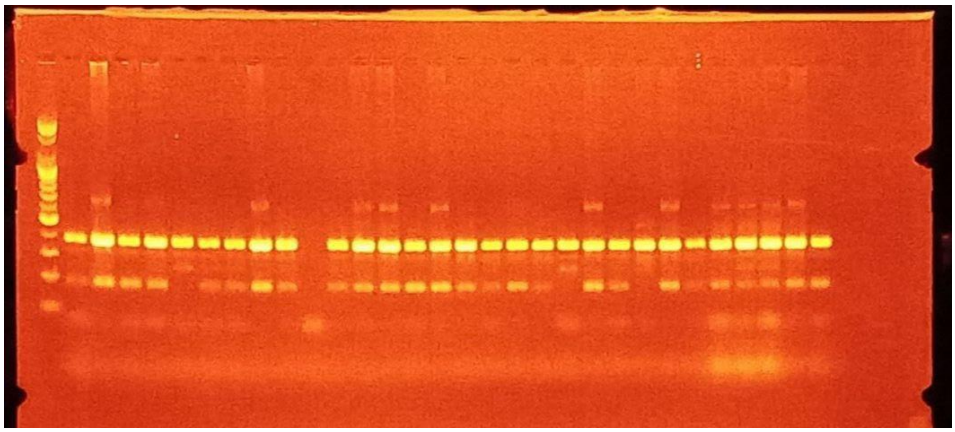

Figure 2 Image of agarose gel electrophoresis that demonstrates the analysis of PCR product of BsmI (rs1544410) gene from blood samples of other patients.

RESULTS

Alleles and Genotype Frequency of VDR gene rs1544410

The most frequent allele was C (95,53%) while the most frequent genotype was TC (57, 63%). There was no statistical difference between the actual and expected frequency distribution, according to Hardy-Weinberg equilibrium (P>0.05) (Table 3).

Table 3 The genotype and frequency of VDR alleles in each patient included in this investigation.

	Genotype	Actual		Expected by Hardy-Weinberg law		P value
		Number	Frequency	Number	Frequency	
VDR rs1544410	CC	14	0.16	20	0.22	0.19 (NS)
	TC	57	0.63	45	0.5	
	TT	19	0.21	25	0.28	
	Total	90	1	90	1	
	Allel					
	T	85	0.47	NA	NA	
	C	95	0.53	NA	NA	
	Total	180	1			

Effect of VDR Polymorphism rs1544410 on Vit D Level

As shown in Table 4, the mean plasma level of Vit.D was higher in homozygous TT carriers (7.7), and lower in heterozygous TC carriers (5.8). There was no statically significant relationship in the plasma level of Vit.D between TT carriers and CC carrier P value was >0.05 (Figure 3).

Table 4 The effect of VDR polymorphism rs1544410 on Vit.D level.

Genotype rs1544410	Numbers	Mean vit D level	S.E	P value
TT	14	7.7	2.7	0.5 (NS)
TC	57	5.8	0.49	
CC	19	7.2	2.18	

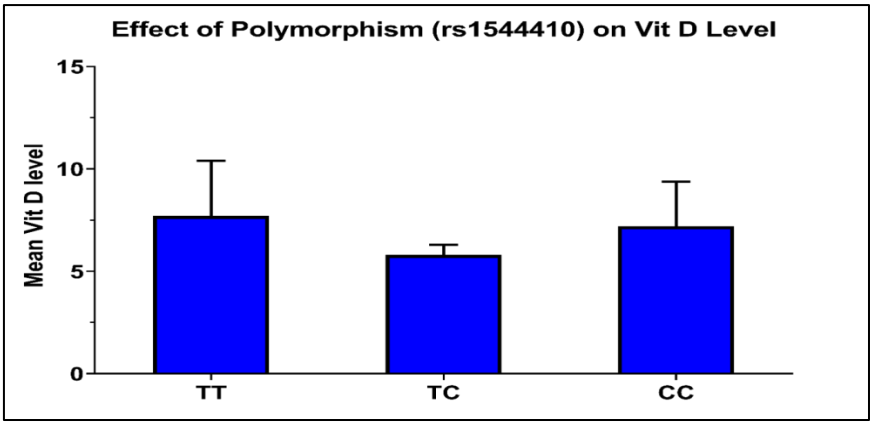

Figure 3 Effect of VDR polymorphism rs1544410 on vit D level.

Effect of VDR Polymorphism rs1544410 on Blood Pressure

As shown in Table 5, the mean systolic blood pressure in homozygous TT, heterozygous TC, and homozygous CC carrier patients was 147, 151, and 147 respectively. On the other hand, mean diastolic blood pressure in homozygous TT, heterozygous TC, and homozygous CC carrier patients was 87, 90, and 88 respectively there was no statistically significant effect of rs1544410 on systolic and diastolic blood pressure.

Table 5 shows the effect of VDR polymorphism rs1544410 on blood pressure.

Genotype rs1544410	Systolic BP means	SE	P value	Diastolic BP mean	SE	P value
TT	147	3.5	0.4 (NS)	87	2.2	0.3 (NS)
TC	151	1.9		90	0.9	
CC	147	3.7		88	1.2	

DISCUSSION

The hormone 1,25(OH)2D3 elicits genomic and nongenomic responses, the biological spectrum of 1,25(OH)2D includes regulation of gene expression in particular organs, which is controlled by the nuclear receptor of vitamin D (a DNA binding protein) that directly engages regulatory sequences close to the target genes [9,10]. BP and incident HT are negatively correlated with vitamin D levels. Studies on animals and human species have revealed that a 1,25(OH)2D3 deficiency may increase RAAS activity both systemically and in the kidney, which may explain why HT develops in people with lower levels of the vitamin in these individuals. An elevated plasma level of renin in the presence of low 1,25-dihydroxyvitaminD3 may increase sympathetic activity and intraglomerular pressure, increasing the risk of EH, decreasing GFR, and consequent cardiovascular injury [11,12]. VDR Bsm I is a nucleotide substitution from A to G in intron 8 that affects transcript stability. It is in linkage disequilibrium with other polymorphisms, and its association with certain diseases is most likely due to this phenomenon. The findings of the studies support the hypothesis that VDR Bsm I polymorphisms may be associated with susceptibility to essential hypertension [8]. The aim of the study was to understand how vitamin D receptor gene polymorphism (VDR rs1544410 BsmI) affects blood pressure in Iraqi patients with essential hypertension in Al Diwaniya province. In our cross-sectional study that did not need a control group, it was found that the most frequent allele was C (53%). The patients had a higher frequency of TC genotypes (63%). While T allele frequency was (47%) and CC genotype frequency was (16%) ($p = 0.19$). there was no statistically significant effect of rs1544410 on systolic blood pressure ($p=0.4$) and diastolic blood pressure ($p=0.3$). 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 rs1544410 variation is linked to hypertension. There have been numerous studies looking at correlations between the rs1544410 variant and EH. However, the findings have been controversial and inconclusive. No consistent studies could be found but a meta-analysis study inconsistently showed that the risk of hypertension was correlated with the VDR rs1544410 (BsmI) variant [13]. In contrast to healthy controls, hypertension patients had a lower prevalence of the VDR BsmI CC genotype. Those who had the VDR BsmI CC genotype were less likely to develop hypertension than those who had the TC or TT genotype ($p=0.005$). In the meantime,

the case group's C allele frequency was more than the control group's (p = 0.04).

In an inconsistent Spanish Transversal study on a healthy population showed that males with the TT genotype had greater SBP than men with the other genotypes (P = 0.006). Furthermore, among men with the genotype CC, there was a statistically significant correlation among 25hydroxyvitaminD3, SBP (r: 0.53, P 0.002), and DBP (r: 0.48, P 0.005). A recent GWAS found that the VDR rs1544410 gene is related to hypertension. Systolic blood pressure was greater in males with the rs1544410 CC genotype than in men with the TC or TT genotype, but not in women [14].

CONCLUSION

In this research, we discovered that homozygous TT genotypes had greater plasma levels of vitamin D whereas homozygous TC genotypes had lower levels (p = 0.5). However, no statistically significant effect was seen of VDR rs1544410 on systolic and diastolic blood pressure and Vit D receptor gene polymorphism rs1544410 was not related to Vit D level.

RECOMMENDATIONS

More research with bigger sample numbers and family-based analysis is needed to confirm this association. Future studies should also focus on gene-gene, gene-environment interaction, as well as haplotype patterns.

REFERENCES

1. Hypertension World Health Organization: WHO; 2023 [updated 03/16/2023; cited 2023 06/26]. Available from: https://www.who.int/news-room/fact-sheets/detail/hypertension.
2. Amen S, Rasool B, Muhammad H. Hypertension in Iraq. Medical Journal of Babylon. 2021;18(3):275-. (http://doi.org/10.4103/mjbl.Mjbl_24_21)
3. Carretero OA, Oparil S. Essential hypertension: part I: definition and etiology. Circulation. 2000;101(3):329-35.
4. Bell K, Twiggs J, Olin BR, Date IR. Hypertension: the silent killer: updated JNC-8 guideline recommendations. Alabama pharmacy association. 2015;334:4222.
5. Noncommunicable Diseases World Health Organization: WHO in Iraq; [cited 2023 06/26]. Available from: https://www.emro.who.int/iraq/priority-areas/noncommunicable-diseases.html.
6. Muna A, Al-Badri HJ, Mousa NA. Hypertension Control among Adult Iraqis. Journal of the Faculty of Medicine Baghdad. 2022;64(3):145-52.
7. Saxena T, Ali AO, Saxena M. Pathophysiology of essential hypertension: an update. Expert Review of Cardiovascular Therapy. 2018;16(12):879-87. (http://doi.org/10.1080/14779072.2018.1540301)
8. Nunes IFOC, Cavalcante AACM, Alencar MVOB, Carvalho MDF, Sarmento JLR, Teixeira NSCCA, 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):1211-20. (http://doi.org/https://doi.org/10.1093/advances/nmaa076)
9. Haussler MR, Jurutka PW, Mizwicki M, Norman AW. Vitamin D receptor (VDR)-mediated actions of 1α, 25 (OH) 2vitamin D3: genomic and non-genomic mechanisms. Best practice & research Clinical endocrinology & metabolism. 2011;25(4):543-59.
10. Kurokawa R, Yu V, Näär A, Kyakumoto S, Han Z, Silverman S, et al. Differential orientations of the DNA-binding domain and carboxy-terminal dimerization interface regulate binding site selection by nuclear receptor heterodimers. Genes & development. 1993;7(7b):1423-35.
11. Karadeniz Y, Özpamuk-Karadeniz F, Ahbab S, Ataoğlu E, Can G. Vitamin D deficiency is a potential risk for blood pressure elevation and the development of hypertension. Medicina. 2021;57(12):1297.
12. Tomaschitz A, Pilz S, Ritz E, Grammer T, Drechsler C, Boehm BO, et al. Independent association between 1, 25-dihydroxyvitamin D, 25-hydroxyvitamin D and the renin–angiotensin system: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Clinica chimica acta. 2010;411(17-18):1354-60.
13. Zhu Y, Li Z, Ding N, Yi H. The association between vitamin D receptor gene polymorphism and susceptibility to hypertension: a meta-analysis. European Review for Medical & Pharmacological Sciences. 2019;23(20).
14. Muray S, Parisi E. Influence of vitamin D receptor gene polymorphisms and 25-hydroxyvitamin D on blood pressure in apparently healthy subjects. Journal Of Hypertension.21(11):2069-75.