International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 8 No. 2 Sep. 2014, pp. 556-566

http://www.ijias.issr-journals.org/

# Relationship with Vitamin D Receptor (RVD) Gene and Essential Arterial Hypertension in Moroccan Population

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**ABSTRACT:** Vitamin D receptor (VDR) gene is recognized as candidate gene for susceptibility to several disease such cardiovascular disease, or arterial hypertension (AHT). The main objective of this study was to investigate a possible association between polymorphisms of the VDR gene and AHT among Moroccan patients. *Materials and Methods*: 177 clinically diagnosed AHT patients and 222 healthy controls from the Moroccans population were recruited. ***Bsm*-**I(rs1544410), ***Fok*-**I(rs10735810) and ***Apa*-**I (rs7975232) single nucleotide polymorphisms(SNPS) of VDR gene were analyzed using PCR-RFLP reaction. A Vitamin D level was determined using ELISA. *Results*: Fok-I polymorphism had a strong association with AHT in all genetics models. It was found that FF carriers tend to have higher HDL-c levels than the ff genotypes (55.30±17.15versus 40.24±10.59, p<0.001), and lover trigltyceride levels (109.02 ± 59.33 versus 122.95 ± 21.61, p=0.0103) in comparison to ff genotypes. There were 7 haplotypes with a frequency higher than 5*%,* constructed from VDR polymorphisms, with the following order: ***Fok*-**I, ***Bsm*-**I, and ***Apa*-**I . Haplotype H4 (f B A) and H6 (f b A) was associated with increased risk of AHT. *Conclusions:* The Fok-I VDR polymorphism seems to influence blood pressure, and while appears to affect the plasma lipid profile, but no significant association with the ***Bsm*-**I, and ***Apa*-**I .

**KEYWORDS:** VDR gene, Polymorphisms, Haplotypes, Arterial hypertension, Morocco.

## 1 INTRODUCTION

Arterial hypertension (AHT) is a real global public health problem; it affects more than 1.56 billion adults worldwide in 2025 [1]. Individuals with HTA have a higher risk of developing Coronary Artery Disease (CAD), Cerebro-Vasular Disease and Heart Failure than those without HTA [2]. Indeed, according to the American Heart Association, one of the main risk factors for the development of CAD is the HTA and dyslipidemia [3], [4]. The vast majority of these studies show that low levels of vitamin D are associated with higher blood pressures and a higher prevalence of HTA in large samples of the U.S. population [5], Germany [6] and the United Kingdom [7]. Regulation of phospho-calcium homeostasis and robustness of the human skeleton are the effects of vitamin D classic, but the discovery of the vitamin D receptor (VDR) in several human cells suggests a role for vitamin D in diseases as extra skeletal. The vitamin D is involved at renal cells in the regulation of the rennin-angiotensin-Aldosterone System (RAAS) and its interaction with the RAS to determine the intracellular medium of calcium into vascular smooth muscle [8]. The genetic studies involved in AHT can not only understand the psychopathological

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mechanisms of the disease, but may also elucidate the biochemical and physiological pathways involving several risk factors causing susceptibility to disease. Recent advances in molecular genetics and statistical methodologies allow better interpretation of genetic in AHT disease. Vitamin D receptor (VDR) gene is considered a candidate gene for susceptibility to several diseases such as cardiovascular disease and AHT.

The aims of this study, is to investigate the association of VDR gene polymorphisms and haplotypes with AHT among Moroccan patients, and evaluate its interaction with anthropometrical and biochemical parameters.

## 2 PATIENTS AND METHODS

### 2.1 STUDY POPULATION

This study concerned 399 Moroccan adult volunteers, 177 patients diagnosed with AHT by medical corps according to International recommendation (Systolic Blood Pressure >140 mmHg and/or Diastolic Blood Pressure> 90 mmHg, or treated by antihypertensive drugs) and 176 sex-matched healthy controls. The volunteers were recruited in the Pasteur Institute of Morocco in Casablanca and informed about the aim and method of the study. A questionnaire was performed including lifestyle, social demographics information's, past medical history, geographical origin, family history, the presence of hypertension, diabetes, and hyper-cholesterolemia. The subjects with co-morbidities were excluded from this study. The anthropometrics measurements age, gender, weight, height, waist and hip-circumference, body mass index and diastolic and systolic blood pressure were taken for all volunteers. All participants in this study were unrelated individuals of similar ethnicity from different geographic and ethnic backgrounds in Morocco.

### 2.2 ETHICS STATEMENT

All participants provided informed consent before participation in this study. The institutional committee of ethical research of Pasteur Institute of Morocco approved this study.

### 2.3 BIOCHEMICAL'S ANALYSIS

The blood was collected in EDTA tubes and storedin a – 20 °C freezer prior to analysis. Fasting glucose plasma (FGP), Cholesterol Total (TC), triglyceride (TG) and High-Density Cholesterol (HDL-C) levels were performed using the Vitros 5.1 (F.S CHEMISTERY SYSTEM). Low-density Cholesterol (LDL-C) was determined using the Friewalds formula. was calculated according to the Friedwald's formula. 25(OH)D level was measured byenzyme linked immunosorbent assays (ELISA) (EUROIMMUM, Lubeck, Seekamp). The intra-assay variation was 2.4- 4.4% and inter assay was < 21 % Vitamin D status was classified as recommendations. A 25(OH)D level of < 20 ng/mL as deficiency, a level of 20–30 ng/mL as insufficiency, and a level of > 30 ng/mL as normal.

### 2.4 ISOLATION OF DNA

Genomic DNA was extracted from peripheral blood leucocytes using the salting-out method. DNA quality was determined using 1 % agarose gel electrophoresis followed by staining with ethidium bromide. Purity of DNA was determined by taking the optical density of the samples at 260 nm and 280 nm using the Nanodrop Analyzer spectrophotometer.

### 2.5 VDR GENOTYPING

PCR and RFLP were performed for genotyping of SNPs: *Fok*-I (rs10735810), *Bsm*I (rs1544410), *Apa*-I (rs7975232) of VDR gene. Biometra thermal cycler and Taq Polymerase were used to perform all reactions PCR.

#### 2.5.1 *FOK-I* POLYMORPHISM

Genomic DNA was amplified in a total volume of 10 µL containing approximately 50 ng of genomic DNA, 200 µMOL/L of DNTPs, 10 Pmol of each Primers, 1,5 MMOL/L of mgcl2, o,5 U Taq Polymerase and 1 µl of 10× PCR Buffer. A fragment of 270 bp including the *Fok-I* (rs10735810) polymorphism was amplified using two oligonucleotides:

Forward: 5'- AGCTGGCCCTGGCACTGACTCTGGCTCT-3',

Reverse: 5'- ATGGAAACACCTTGCTTCTTCTCCCTC -3'.

The conditions of the PCR starts an initial denaturing at 94°C for 5 minutes, followed by 35 cycles of 94°C for 40 second, 61°C for 40 second, 72°C for 50 second, and a final extension of 72°C for 7 minutes. The PCR products were digested for 1 hour at 37°C with 3Unit of *Fok-I* restriction enzyme (*Biolabs NEW ENGLAND, R0109S*), then the PCR products of digestion were elecrophoresed on a 3% of Agarose (UltraPure Agarose, invitrogen) with bromide ethidium and visualized under UV Illumination and photographed. The wild type homozygote (FF), heterozygote (Ff) and mutant homozygote (ff) showed one band (270BP), three banbs (270, 210, 60) and tow bands (210 and 60), respectively, because the substitution create a *Fok-I* Recognition Sequence which digest the 270 bp into 210 and 60 bp.

#### *2.5.2 BSM-I* POLYMORPHISM

Genotyping for *Bsm*I (rs1544410) was performed with the following primers:

Forward: 5′- CAACCAAGACTACAAGTACCGCGTCAGTGA-3′,

Reverse 5′-AACCAGCGGGAAGAGGTCAAGGG -3′.

A 820 bp fragment VDR gene was amplified using the PCR under reactions conditions identical to those used for the *Fok-I* polymorphism. The PCR products were digested with BsmI restriction enzyme (*Biolabs NEW ENGLAND, R0134S*), for one hour at 65°C. The digest fragment were seprated in a 3% of Agarose (UltraPure Agarose, invitrogen) with bromide ethidium and visualized under UV Illumination and photographed. The wild type homozygote (BB), heterozygote (Bb) and mutant homozygote (bb) showd one band (820bp), three banbs (820, 650, 170) and tow bands (650 and 170), respectively, because the substitution create a *Bsm-I* Recognition Sequence wich digest the 820 bp into 650 and 170 bp.

#### 2.5.3 *APA-I* POLYMORPHISM

Genotyping for *Apa-I (rs7975232)* was performed with the following primers:

Forward: 5′- CAGAGCATGGACAGG GAGCAA-3′,

Reverse 5′- GCAACTCCTCATGGCTGAGGTCTC -3′.

A 2000 bp fragment VDR gene was amplified using the PCR under reactions conditions identical to those used for the *Fok-I* polymorphism. The PCR products were digested with *ApaI* restriction enzyme *(Promega, REF R6361,USA)* for one hour at 65°C. The digest fragment was separated in a 3% of Agarose (UltraPure Agarose, invitrogen) with bromide ethidium and visualized under UV Illumination and photographed. The wild type homozygote (AA), heterozygote (Aa) and mutant homozygote (aa) showed one band (2000bp), three banbs (2000, 1700, 300) and tow bands (1700 and 300), respectively, because the substitution create a *Apa-I* Recognition Sequence which digest the 2000 bp into 1700 and 300 bp. All genetics and molecular analyses were performed in the Molecular and genetic laboratory in Pasteur institute of Moroccoo.

### 2.6 STATISTICAL ANALYSIS

The Biochemical and Anthropometrical parameters were given as means and standard deviations in the tables. The student test was applied to compare quantitative values that follow a Normal Distribution. Otherwise, the Manne-whithney test-test is used. Chi-square and logistic regression analysis were performed to evaluate the association between T2DM and genotypes and haplotypes VDR. Analysis of the regression logistic was adjusted by gender and age. The P-Value of less than o.o5 was considered statistically significantly. All analysis statistics were performed using STATA software, version 11.00. A P-Value was automatically corrected using the bonfferoni correction by multiplying with the comparisons number. For all Haplotypes frequencies, estimation and comparison, we used P-LINK SOFTWARE, VERSION 1-07. The frequencies less than 5% of all haplotypes were not considered in this analysis. Linkage Disequilibrium between each pair of VDR gene polymorphism was estimated using HAPLO-VIEW Software Version 4-2.

## 3 RESULTS

### 3.1 PATIENTS AND CONTROLS CHARACTERISTICS

The clinical characteristics of the study subjects are shown in Table 1.

*Table 1. Characteristics of the studied groups.*

| Characteristics | Controls (n=177) | Patients (n= 222) | P-value |
|---|---|---|---|
| **Age (years)** | 56.94±11.47 | 49.63±14.96 | **<0.001** |
| **BMI (kg/m2)** | 28.51±4.25 | 27.41±3.77 | 0.0055 |
| **Sbp (mmgHg)** | 127.92±6.65 | 147.83±17.78 | **<0.001** |
| **Dbp (mmgHg)** | 73.21±4.14 | 84.47±10.10 | **<0.001** |
| **FGP (mg/dl)** | 87.11±8.28 | 125.98±43.94 | **<0.001** |
| **TC (mg/dl)** | 157.38±70.48 | 174.96±13.12 | 0.0745 |
| **TG (mg/dl)** | 95.07±35.92 | 122.49±62.64 | **<0.001** |
| **LDL-C (mg/dl)** | 107.72±78.85 | 112.64±10.62 | 0.0657 |
| **HDL-C (mg/dl)** | 58.91±18.73 | 44.99±7.30 | **<0.001** |
| **Vitamin D level(ng/ml)** | 30.28±13.05 | 25.20±9.47 | **0.0002** |
| **Vitamin D normal(>30 ng/ml).** | 50% (n=88) | 28% (n=62) | <0.001 |
| **Vitamin D insufisancy(20-29 ng/ml)** | 30% (n=53) | 30% (n=67) | 0.907 |
| **Vitamin D deficiency (<20 ng/ml)** | 20% (n=36) | 42% (n=93) | <0.001 |

*BMI: bodymass index, TC: Serum total cholesterol, TG: Triglycerides, FGP: Fasting plasma glucose, HDL-C: High-Density Lipoprotein Cholesterol, LDL-C: Low-density Lipoprotein Cholesterol, Sbp systolic blood pressure, Dbp diastolic blood pressure.*

The mean age of the control group was 56.94 years. The mean age of the patients group was 49.63 years. Compared with control subjects, patients with AHT had a lower vitamin D level (25.20±9,47 ng/ml vs. 30.28±13.05 ng/ml, p<0.05). 30% of AHT patients were Vitamin D insufficient (20-29 ng/ml) and 42% % of the patients were Vitamin D deficient (<20 ng/ml). Among control subjects, 30% of the subjects were Vitamin D insufficient and 20% of the subjects were Vitamin D deficient. 30% of the patients with AHT had normal vitamin D levels (> 30 ng/ml), and 50% of control subjects had normal vitamin D levels (> 30 ng/ml). The prevalence of Vitamin D deficiency is significantly higher in patients with AHT than in the control subjects. In addition, Triglyceridemia (TG), Cholesterol LDL, Total Cholerestrol (TC), Fasting Glucose Plasma (FGP) and blood pressure and were significantly different between AHT patients and controls.

### 3.2 GENOTYPIC MODEL DISTRIBUTIONS OF VDR POLYMORPHISMS BETWEEN DT2 SUBJECTS AND CONTROLS AND LINKAGE DISEQUILIBRIUM

The genotype frequencies of the VDR *Bsm*-I, *Fok*-I and *Apa*-I were in agreement with Hardy–Weinberg equilibrium in all groups ( Table 2). In AHT patients, the frequencies of ff genotypes of VDR *Fok*-I is significantly decreased compared to control group (OR = 0.24, 95% CI = 0.10–0.58, *P* = 0.002). In all genetic models, a strong association was observed between Fok-I polymorphism and patients with AHT. For other polymorphisms VDR Bsm-I and Apa-I, we did not observe significant differences in all genetic models between cases and controls (P> 0.05).

Standardized Linkage-Disequilibrium Coefficient D' was calculated for all pairs of polymorphism to determine the extent of Linkage Disequilibrium (LD) among the 3 polymorphisms. Figure.1 shows that with the exception of Bsm-I and A pa-I Polymorphisms which were in strong linkage Disequilibrium (D'=50), others polymorphism were not in Linkage Disequilibrium.

***Table 2 VDR Gene Polymorphisms and susceptibility to arterial hypertension patients***

| *Model/SNP* | *Controls(n= 177)* | *Patients (n= 176)* | *OR (95% CI)* | *P-Value* |
|---|---|---|---|---|
| **Fok-I** | | | | |
| Codominant | | | | |
| F/F | 79 (45.4%) | 120 (54.5%) | 1.00 | |
| F/f | 74 (42.5%) | 91 (41.4%) | 0.6 (0.42-1.03) | 0.068 |
| f/f | 21 (12.1%) | 9 (4.1%) | **0.24(0.10-0.58)** | **0.002** |
| Dominant | | | | |
| F/F | 79 (45.4%) | 120 (54.5%) | 1.00 | |
| F/f-f/f | 95 (54.6%) | 100 (45.5%) | **0.57(0.37-0.87)** | **0.009** |
| Recessive | | | | |
| F/F-F/f | 153 (87.9%) | 211 (95.9%) | 1.00 | |
| f/f | 21 (12.1%) | 9 (4.1%) | **0.29(0.12-0.69)** | **0.005** |
| HWE | 0.569 | 0.103 | | |
| **Bsm-I** | | | | |
| Codominant | | | | |
| B/B | 18 (13.2%) | 19 (11.4%) | 1.00 | |
| B/b | 57 (41.9%) | 71 (42.8%) | 0.98(0.45-2.13) | 0.965 |
| b/b | 61 (44.9%) | 76 (45.8%) | 0.99(0.46-2.12) | 0.974 |
| Dominant | | | | |
| B/B | 18 (13.2%) | 19 (11.4%) | 1.00 | |
| B/b-b/b | 118 (86.8%) | 147 (88.6%) | 0.99(0.48-2.03) | 0.978 |
| Recessive | | | | |
| B/B-B/b | 75 (55.1%) | 90 (54.2%) | 1.00 | |
| b/b | 61 (44.9%) | 76 (45.8%) | 0.9 (0.60-1.57) | 0.907 |
| HWE | 0.421 | 0.696 | | |
| **Apa-I** | | | | |
| Codominant | | | | |
| A/A | 36 (22.8%) | 44 (22.1%) | 1.00 | |
| A/a | 89 (56.3%) | 113 (56.8%) | 1.05(0.61-1.82) | 0.851 |
| a/a | 33 (20.9%) | 42 (21.1%) | 1.22(0.63-2.41) | 0.554 |
| Dominant | | | | |
| A/A | 36 (22.8%) | 44 (22.1%) | 1.00 | |
| A/a-a/a | 122 (77.2%) | 155 (77.9%) | 1.1 (0.65-1.85) | 0.731 |
| Recessive | | | | |
| A/A-A/a | 125 (79.1%) | 157 (78.9%) | 1.00 | |
| a/a | 33 (20.9%) | 42 (21.1%) | 1.18(0.68-2.03) | 0.56 |
| HWE | 0.110 | 0.055 | | |

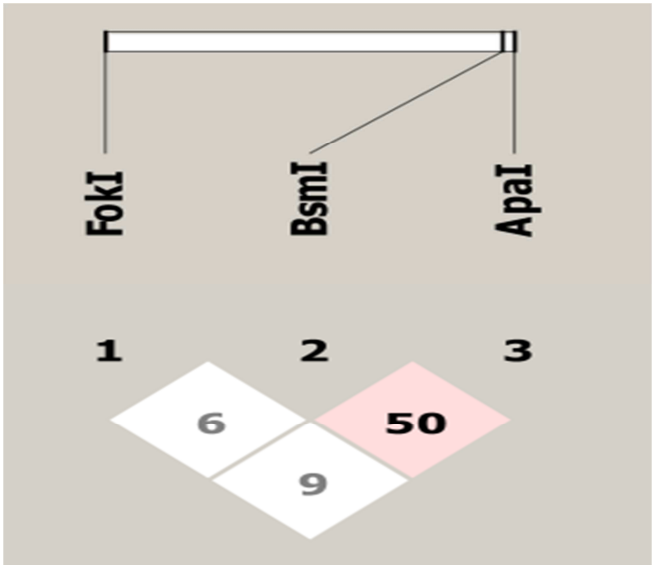


*Fig. 1. Linkage Disequilibrium (LD) between 3 VDR SNPs*

### 3.3 VDR HAPLOTYPES ANALYSIS

The combined effect of three variants (Fok-I, Bsm-I, Apa-I) in the VDR gene was examined. There were 7 haplotypes identified in the VDR gene in our population, with greater than 5% (Table 3). Tow haplotypes confers significant susceptibility to AHT, Haplotypes H4 (f-B-A) (0.4979 (0.255-0.973), p 0.0388) and Haplotypes H6 (f-b-A) ) (0.3985 (0.176-0.901), p 0.0217). No significant association was observed between all other haplotypes. The frequencies of the all haplotypes are listed in Table 3.

*Table 3 Haplotypes frequencies of VDR ( Fok-I, Bsm-I, Apa-I) Gene Polymorphisms in Arterial Hypertension patients and healthy controls*

| Haplotypes | Fok-I (rs10735810) | BsmI (rs1544410) | Apa-I (rs7975232) | Frequency | | OR(95%CI) | P-value |
|---|---|---|---|---|---|---|---|
| | | | | Controls | Cases | | |
| H1 | F | B | a | 0.06555 | 0.06239 | 1.664 (0.681-4.07) | 0.255 |
| H2 | f | b | a | 0.1007 | 0.1184 | 0.6009 (0.318-1.13) | 0.114 |
| H3 | F | b | a | 0.3232 | 0.301 | 1.298 (0.872-1.93) | 0.196 |
| **H4** | **f** | **B** | **A** | **0.0744** | **0.09959** | **0.4979 (0.255-0.973)** | **0.0388** |
| H5 | F | B | A | 0.1738 | 0.1573 | 1.37 (0.801-2.34) | 0.248 |
| **H6** | **f** | **b** | **A** | **0.05791** | **0.08499** | **0.3985 (0.176-0.901)** | **0.0217** |
| H7 | F | b | A | 0.2044 | 0.1763 | 1.289 (0.799-2.08) | 0.296 |

### 3.4 COMPARISONS OF CLINICAL AND BIOCHEMICAL PARAMETERS BETWEEN VDR GENOTYPES

It was found that FF carriers had higher HDL-c levels than the ff genotypes (55.30±17.15versus 40.24±10.59, p<0.001), and had lover triglycerides levels (109.02 ± 59.33 versus 122.95 ± 21.61, p=0.0103) in comparison to ff genotypes.

*Table 4 Anthropometric and metabolic parameters according to genotypes of VDR polymorphisms.*

| VDR rs10735810 (Fok-I) | | | | | |
|---|---|---|---|---|---|
| | **FF** | **Ff** | **Ff** | **P-value (FF vs Ff)** | **P-value (FF vs ff)** |
| **Age** | 54.22±13.01 | 51.08±14.89 | 53.57±14.59 | 0.1172 | 0.7105 |
| **Systolic blood pressure** | 141.25±18.87 | 137.06±14.46 | 136.31±17.54 | 0.1776 | 0.1450 |
| **Diastolic blood pressure** | 79.88±10.73 | 79.60±8.78 | 77.38±8.61 | 0.9232 | 0.1841 |
| **Fasting plasma glucose** | 110.73±39.24 | 106.82±37.85 | 95.14±25.84 | 0.1644 | 0.0685 |
| **Total cholesterol** | 167.48±55.93 | 164.79±54.20 | 155.19±38.13 | 0.8430 | 0.0862 |
| **Triglycerides** | 109.02±59.33 | 108.26±52.18 | 122.95±21.61 | 0.9531 | **0.0103*** |
| **LDL-cholesterol** | 111.10±59.87 | 108.91±62.79 | 107.96±32.59 | 0.9903 | 0.3948 |
| **HDL-cholesterol** | 55.30±17.15 | 52.51±14.92 | 40.24±10.59 | 0.2275 | **<0.001*** |
| **Vitamin D** | 28.06±10.57 | 29.04±11.97 | 26.40±19.15 | 0.6463 | 0.0767 |
| **BMI** | 27.73±3.98 | 28.00±3.93 | 28.27±4.80 | 0.3996 | 0.5651 |
| **VDR rs1544410 (BsmI)** | | | | | |
| | **BB** | **Bb** | **bb** | **P-value(BB vsBb)** | **P-value (BB vs bb)** |
| **Age** | 56.92±14.36 | 52.99±13.66 | 52.28±14.89 | **0.0528** | 0.1026 |
| **Systolic blood pressure** | 138.56±18.74 | 140.89±18.42 | 138.97±16.64 | 0.2663 | 0.4490 |
| **Diastolic blood pressure** | 77.92±8.53 | 80.14±10.50 | 80.05±9.26 | 0.3505 | 0.2261 |
| **Fasting plasma glucose** | 101.47±27.89 | 106.73±36.26 | 108.25±40.02 | 0.8175 | 0.8238 |
| **Total cholesterol** | 170.72±55.13 | 166.59±53.37 | 160.47±55.17 | 0.5268 | 0.2549 |
| **Triglycerides** | 107.32±42.22 | 106.44±45.75 | 117.92±68.64 | 0.7938 | 0.6677 |
| **LDL-cholesterol** | 114.58±62.72 | 109.40±60.14 | 111.24±58.80 | 0.7422 | 0.8530 |
| **HDL-cholesterol** | 52.18±16.94 | 52.95±16.41 | 51.72±15.04 | 0.5921 | 0.7963 |
| **Vitamin D** | 29.79±11.98 | 27.91±12.18 | 28.28±12.89 | 0.3582 | 0.3875 |
| **BMI** | 26.81±3.88 | 27.91±4.19 | 27.66±4.04 | 0.1780 | 0.2609 |
| **VDR rs7975232 (Apa-I)** | | | | | |
| | **AA** | **Aa** | **aa** | **P-value(AA vs Aa)** | **P-value (AA vsaa)** |
| **Age** | 51.93±13.50 | 52.29±13.34 | 54.31±16.36 | 0.9316 | 0.1081 |
| **Systolic blood pressure** | 138.56±18.74 | 140.89±18.42 | 138.97±16.64 | 0.2663 | 0.4490 |
| **Diastolic blood pressure** | 77.92±8.53 | 80.14±10.50 | 80.05±9.26 | 0.3505 | 0.2261 |
| **Fasting plasma glucose** | 106.47±36.85 | 108.02±38.17 | 110.19±39.41 | 0.7799 | 0.6998 |
| **Total cholesterol** | 169.48±53.15 | 164.59±53.06 | 161.14±55.21 | 0.5369 | 0.5021 |
| **Triglycerides** | 105.72±38.03 | 109.82±48.33 | 111.15±84.52 | 0.7002 | 0.4946 |
| **LDL-cholesterol** | 108.62±55.24 | 109.10±60.00 | 106.53±60.74 | 0.9847 | 0.8900 |
| **HDL-cholesterol** | 52.87±19.61 | 53.03±15.30 | 53.99±16.40 | 0.3904 | 0.3346 |
| **Vitamin D** | 29.42±14.47 | 28.25±11.85 | 28.50±11.63 | 0.5012 | 0.8138 |
| **BMI** | 27.84±4.08 | 27.93±4.05 | 28.03±4.02 | 0.6487 | 0.5599 |

*Data are presented as mean ± standard deviation; statistical significance is shown. *Indicates that the group is significantly different from the first genotype*

## 4 DISCUSSION

Vitamin D is deemed to be involved in several biological processes. Fluctuations in the endocrine system of vitamin D can induce several common chronic diseases such as HTA, bone disease, cardiovascular disease, diabetes, cancer, and

Tuberculosis [9]. Several genetic risk factors and gene-gene and gene-environment have been implicated in the pathogenesis of hypertension. Several studies have indicated that there are many genes involved, and that contribute 30% to 50% of the variation in blood pressure in humans [10]. The VDR gene is one of genes receptor interesting because it is expressed in many tissues [9]. Vascular, pancreatic beta cells and adipocytes is several among cell types that respond to vitamin D [11]. in addition, VDR genotype plays a major role in the homeostasis of glucose and phosphate homeostasis. We conducted this study to clarify the contribution of VDR polymorphisms in susceptibility to AHT among the Moroccan population.

To our knowledge, this is the first study to determined the prevalence of vitamin D deficiency Among Moroccan patients with HAT, and Analyzes the distribution of VDR polymorphisms in relation to anthropometric and biochemical parameters. This study indicates that the prevalence of vitamin D deficiency was significantly higher in AHT (42%) compared to the control (20%). The prevalence of vitamin D deficiency was still in our cohort recent study in subjects Caribbean with type 2 diabetes, which showed that 42.6% [12].

Among the risk factors for vitamin D inadequacy include old age, female sex, dark pigmentation, malnutrition, winter season, lack of sun exposure, covered up the style clothing and obesity [13]. Vitamin D stimulates the intestinal absorption of calcium and phosphorus as well as the improvement of renal reabsorption of calcium, which leads to the elevation of plasma levels of these two important minerals in the human organism [8]. This explains in part, the important biological role of vitamin D in the mineral homeostasis and regulation of bone remodelling [14].

Some interesting results have been reported by Examination Survey National Health and Nutrition Survey (NHANES) on a large cohort of non-hospitalized civilian population in the United States. Martins et al showed in a cohort 15088 subjects increased prevalence of hypertension associated with low serum 25 (OH) vitamin D levels [15]. A recent meta-analysis were performed to quantify the prospective associations of vitamin D status with the risk of hypertension and showed an increased risk of developing hypertension in vitamin D-déficient subjects (RR = 1.76; 95%CI: 1.27-2.44, $P < 0.05$) [16]. Vitamin D is a negative regulator of the renin-angiotensin system. in a study in mice VDR -/-,, the authors observed an elevation in the expression of renin, which is accompanied by an increase circulating levels of angiotensin II, head of the hypertension, cardiac hypertrophy, and increased water consumption [17]. From a molecular point of view, research groups led by Yuan et al have reported the molecular explanation of the direct effect of vitamin D on the transcription of the renin gene. They found that the vitamin D is capable of repressing the transcription of a gene by renin element AMPc response shown on the promoter region of the gene Ren-1c [18] .

The pathogenesis of AHT is considered a multifactorial disease in which genetic and environmental factors play a complex role and is not yet clear defined. Several genes are involved in the molecular mechanisms of AHT and were considered candidates for early disease [19]. Among these genes, the VDR gene is considered particularly good candidate gene for the disease [20].

Our study demonstrated that VDR gene polymorphisms were associated with susceptibility to AHT in the Moroccan population, which can be explained by differences in VDR *Fok-I* genotype distributions between AHT and control Subjects. Carriers of allele f Fok-I SNP could have a protective effect against Vitamin D deficiency, an earlier study indicated that can affect circulating levels of vitamin D and can also affect the cardiovascular risk [21].

In agreement with our results, a recent study has demonstrated Fok-I polymorphism of the VDR gene as a possible risk factor for AHT [22]. The involvement of genetic factors in the pathogenesis of HTA reported in studies by Kulah et al. [23]. They analyzed the degrees of target organ damage and 24 hour ambulatory monitoring of blood pressure with vitamin D receptor gene polymorphism Fok-I of patients with hypertension. Their results indicated a negative correlation between vitamin D level and blood pressure values in the group of patients with the ff/ Ff genotype. In addition, Vaidya A et al. rapport that genetic variation at the Fok-I polymorphism of the VDR gene, in combination with 25(OH)D levels, was associated with HTA. These results support the vitamin D-VDR complex as a potential regulator of renin activity in humans [24].

The molecular explanation for the supposed relationship between Fok-I polymorphism and HTA are only partly understood. The Fok-I polymorphism can be detected by the presence or absence of a Fok-I restriction site within the ATG transcriptional start site of the VDR gene. The gene is transcribed into normal length when the restriction site (f allele) is present and into shortened length when the restriction site (f allele) is absent. The longer VDR protein appears to possess decreased transcriptional activity, leading to lower activation of target cells [25]–[26].

On the other hand, our study revealed that VDR Apa-I and Bsm-I polymorphisms were not correlated with AHT in Moroccan population. The distribution of VDR Apa-I and Bsm-I genotype showed no statistical difference between the control and AHT patient. Few studies have reported the association between this polymorphism and AHT. Muray et al. reported that men with bb of Bsm-I had the highest levels of systolic blood pressure [27].
In contrast, a Previous studies (genome-wide association and candidate gene polymorphism) have focused on the association between VDR gene and development of AHT, but findings have often been inconsistent among different populations worldwide. Generally the discrepancies between studies may be due to false positive finding, replication study lacks power, heterogeneity between studies and heterogeneity across studies. Some studies have reported interactions between VDR polymorphisms and vitamin D in diseases such as Type 1 diabetes mellitus [28], tuberculosis [29] and prostate cancer [30]. Although to our knowledge, this is the first time an interaction between VDR polymorphisms and vitamin D levels has been reported in AHT patients in Moroccan population.

The findings of this study confirm the relationship between biological and clinical traits and VDR genotypes for all patients and controls combined. The Fok-I FF variant was significantly associated with increased levels of HDL cholesterol and decreased levels of triglycerides (all P values <0.05). No Significant association was observed for Bsm-I and Apa-I polymorphism.

It has been suggested that studying haplotypes could be more informative than the study on individual SNPs. The VDR H4 (f B A) and H6 (f b A) haplotypes is significantly associated with increase risk to AHT. These results suggest that each variant in the VDR haplotypes may be associated to some mechanisms that increase blood pressure and cardiovascular disease risk.

The gene coding for the VDR is located on chromosome 12q12-q14. Direct effect of Vitamin D metabolism pathway and cell adiposity differentiation is a possible pathway for such an effect, as VDR is expressed in pre-adipocytes [30]. It is now suggested that the underlying mechanism of the relationship Vitamin D deficiency and chronic diseases is the presence of VDR in several tissues and cells, including vascular and renal cells [31]. Furthermore, an association between polymorphisms of the VDR and body weight and insulin secretion has also been reported [32]- [33].

All data thesis suggest that vitamin D plays an important role in the regulation of many biological processes. Furthermore, pair wise Linkage Disequilibrium comparison performed in this study between 3SNPs: Fok-I (rs10735810), BsmI (rs1544410), Apa-I (rs7975232) of VDR gene, was shown to be related, Suggesting the existence of mechanisms of cooperation for associations with HTA and related traits. The metabolic syndrome (MetS) is a cluster of disorders Including visceral obesity, dyslipidemia, hyperglycemia, and hypertension [34]. The association of the VDR gene polymorphisms with increased risk of MetS has been reported in several studies.

There are a few limitations of our study. Firstly, our sample numbers considered relatively small. Secondly, lack of replication studies of the association of VDR gene polymorphisms and AHT in Moroccan population. Consequently, further studies including larger sample numbers and replication of significant findings are necessary to clarify the role of the VDR gene polymorphism in AHT.

In conclusion, it is evident that vitamin D deficiency has prevailed in Moroccan population with AHT. Alterations in vitamin D action may affect insulin sensitivity, b-cell function or both. Moreover our study documents a correlation between VDR Fok-I gene polymorphisms and susceptibility to AHT in the Moroccan population. The possible role of Vitamin D role in the pathogenesis of AHT is not yet fully elucidated. Knowledge of the molecular and functional implications of VDR polymorphisms is significant for well appreciate their significance and understand their potential clinical implications.

## CONFLICT OF INTEREST

The all authors declare that they have no conflict of interest.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the patients with HTA and their families for their participation in this study.

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