**Genetic investigation of osteoporosis genes in postmenopausal and association of vitamin D receptor gene TaqIrs (731236) with type 2 diabetes**

**Abstract**

This study aims to investigate how the body interacts with medications that genetically influence osteoporosis, which helps in diagnosing, treating, and preventing fractures and reducing their negative effects. The study was conducted on (100 women) with osteoporosis, 80 women with postmenopausal osteoporosis, and 20 healthy women who were randomly selected to study the relationship between osteoporosis and the Taq1 (rs731236) single nucleotide polymorphism (SNP) in the VDR gene using PCR. Among 60 patients, 15 (25%) TT, 24 (40%) GG, and 21 (35%) TC heterozygotes were found. TT, CC and TC (31, 25.4; 10, 4.3; 9, 20.4) respectively, are the proportion and distribution of people with (rs 731236) that deviate from those predicted under Hardy–Weinberg equilibrium. (p < 0.001}), 15.843, and rs 731236), making it statistically significant. The (rs 731236) SNP did not differ statistically significantly from the other mean of the biomarkers (D3, ALP, PTH, Progesterone, and E2). Mean age, BMI, T-score, using one-way analysis of variance, the study groups were compared. The mean T. score of the controls did not differ significantly. The mean BMI differed significantly (p = 0.02). Patients' bone mineral density )BMD( (p = 0.026) and mean T. score (-3.27 ± 0.53) for the TT allele are substantially greater than those for the CC allele (-2.66 ± 0.80), According to post hoc analysis using (LSD) amendment, the results showed that the mean BMD of CC alleles (0.75 ± 0.04) was significantly lower than the mean BMD of the TT allele (0.80 ± 0.06). The T-score and BMD showed a significant difference, however the demographic characteristics of PMO patients with DM type 2 and PMO patients without DM type 2 did not differ significantly in the (rs 731236) SNP.

**Keywords:** Molecular, Osteoporosis, Genes, Postmenopausal Women, Type 2 Diabetes.

**Introduction**

Osteoporosis is a common condition that causes fragility fractures by causing a systemic decrease of bone mass and microarchitecture. The medical and societal consequences of osteoporosis, especially postmenopausal osteoporosis, will increase as the population ages [1]. A number of environmental factors influence pathogenesis. The fact that several aspects of bone strength are heritable shows that genes also have a significant impact [2]. Since osteoporosis is mostly genetically determined, it is impossible to fully and publicly demonstrate the etiology of osteoporosis until the processes of gene activity that underlie the condition are understood. Some of this issue has been addressed by osteoporosis gene expression investigations. Instead of looking at the DNA level, gene expression studies look at the relationships between particular genes and diseases at the mRNA level. Many studies have shown that the vitamin D receptor (VDR) gene, which belongs to the group of nuclear receptors and is located on the long arm of the chromosome, plays a major and pivotal role in determining the causes of osteoporosis. This method can provide a deeper understanding to determine how genes work and to identify some of the metabolic processes that lead to this disease [3].

One of the potential genes controlling bone strength and metabolism is thought to be the one encoding the vitamin D receptor [4]. The nuclear transcription factor VDR mediates (1,25(OH)2D3) activity to affect bone remodeling, calcium absorption, and mineralization rate. 1α,25(OH)2D3/VDR Osteoporosis, cancer, type 1 and type 2 diabetes, arteriosclerosis, vascular disease, and infections are among the chronic illnesses of aging that can be avoided by managing gene expression and acting promptly [5]. Studies have shown that postmenopausal women with type 2 diabetes mellitus (T2DM) had a higher prevalence of osteoporosis and related fractures. A research found that 25% of postmenopausal women with type 2 diabetes developed osteoporosis, and that traits like less physical activity were strongly correlated with lower (BMD) and osteocalcin levels [6]. According to a different study, T2DM patients generally have higher BMD than non-diabetic controls, which may be due to body mass index (BMI) and fasting insulin levels [7]. Numerous research has looked at genetic differences linked to osteoporosis in postmenopausal women.

In osteoporotic postmenopausal women, variations in bone mineral density (BMD) have been linked to the LRP5 gene polymorphism, notably the A1330V variant, although it is not significantly associated with type 2 diabetes [7][8]. Additionally, the lactose intolerance variant of the MCM6 gene (rs4988235) was associated with lower BMD and a higher risk of DM type 2 in postmenopausal women [9]. Variants in the DKK1 gene have been connected to abnormal bone metabolism in postmenopausal women with DM type 2, indicating a potential hereditary component to the risk of osteoporosis [10].

Furthermore, the population's genetically complicated osteoporosis has been demonstrated by The association between TS and MTHFR gene polymorphisms with a tendency to osteoporotic spinal compression fractures [11]. Genetic variables, including as polymorphisms in genes like VDR, OPG, ESR1, Col1A1, eNOS, WNT16, and LRP5, significantly impact women's risk of osteoporosis. These hereditary traits impact fracture risk and bone mineral density, highlighting the need of genetic testing in assessing an individual's susceptibility to osteoporosis. The goal of this study is to better understand the genetic consequences of the VDR gene in order to create targeted treatments and preventative measures for osteoporosis in Iraqi women.

**Material and method**

The study was conducted on 100 specimens; they were chosen at random from the teaching hospital of Al-Hussein Medical City from the Karbala City. this cross-sectional study was to investigate the connection between patients with osteoporosis and the Taq1(rs731236) SNP in the VDR gene polymorphism. eighty postmenopausal women with osteoporosis were included in the population survey. These patients, who vary in age from 55 to 70, have 40 T2DM diagnoses and 40 non-T2DM diagnoses. and 20 people who seemed to be healthy postmenopausal women were chosen at random from the general population with comparable age and location to act as controls. for the extraction of DNA. Peripheral blood samples from patients and controls were obtained using EDTA tubes. Next,a ReliaPrepTM Blood DNA Miniprep System (Promega) was used to extract DNA from entire blood samples. Following the determination of the DNA's content and purity [12], we carried out a polymerase chain reaction (PCR).

Using a particular primer, allele specific PCR was used to amplify the target gene (VDR gene) [13]. Under sterile circumstances, the ALLEL SPECIFIC PCR reactions were carried out in PCR tubes with 25μl volumes. DDH2O and the master mix, which comprised the ideal quantities of the reaction needs (MgCl2 1.5 mM), (polymerase Taq1 U) and (dNTP 200 μM), were used to finish the reaction mixture to 25μl. in a microcentrifuge to fully combine the solutions at room temperature the PCR tube is Centrifuged at (2000 rm) for 30 seconds. A number of primer quantities and template DNA volumes (0.5, 1, 1.5; 1, 2,3, 4, 5, 6μl) respectively, were examined to enhance the reaction conditions Reaction programs for the Taq1 VDR gene polymorphism (rs731236) using the PCR technique. Based on the NCBI database, to retrieve all gene information and SNP details and to perform PCR, Genius software and a primer pair specifically designed for the VDR gene were used. Following gene amplification, agar gel electrophoresis was carried out using Robinson and La Fleche's methodology [14].

**Statistical analyses**

Using t-test were evaluated utilizing the patients, the mean values for the two groups patient (women with postmenopausal osteoporosis) and control group (healthy women), which provides variables that are continuous as mean standard deviation. Mean levels of continuous characteristics between genotypes were assessed using SPSS 28.0, which included Student's t -test and ANOVA. Alleles and genotypes were categorical data published as frequencies. Every statistical analysis has a significance level of 0.05 [15]. A mathematical formula known as the Hardy-Weinberg equilibrium (HWE) connected allele frequencies to genotypes [16] [17]. The following formula display, p2+2pq+q2 = 1Since (p and q) denote the minor and major distributions of Allele respectively. allelic frequencies will stay remain throughout generations in the absence of mutation, gene migration, selection, or genetic drift, which provides a quantitative explanation, ensures that in a sizable population that reproduces at random.

Using the previously described technique, distributions of Allele (frequencies) were calculate in order to evaluate HWE. By comparing observed genotypes to expected values, the chi-squared test can be used to assess how far a population deviates from HWE. Next, the anticipated genotype frequencies were ascertained. The P value indicates the number of population where it appears less than (0.05), In terms of indicating the small population size based on the mean standard deviation from HWE. The online program web-Assotest (www.ekstoem.com) was used to calculate HWE. Using multiple inheritance patterns, In Windows system and using SPSS program, multinomial logistic regression was read and was examined the associated with osteoporosis and the association between allele frequencies and genotype. the reference wild type, TT. T was the dominant allele, and C was the minor allele.

**Results**

Through the results that appeared, the study obtained association rates between osteoporosis and the gene associated with it. The P value, 95% confidence interval, and odds ratio (OR) were used. Also, adjustments were made to the data based on age and body mass index to display the output data. The P values, 95% confidence interval, and odds ratio were recalculated.

**Table .1** Genetic paradigms of inheritance.

|  |  |
| --- | --- |
| **VDR gene** | **Genetic model** |
| T vs. C | Allelic model |
| CC+ TC | Dominant model| |
| TT+ TC | Recessive model |
| TT,TC,CC | Co-dominant model |
| 2CC\*+TC | Additive model |

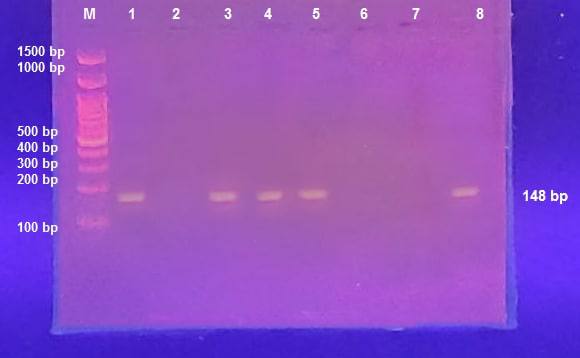
\*mutant type

**Table .2** DNA purity and concentration of patient groups (postmenopausal osteoporosis, and healthy women).

|  |  |
| --- | --- |
| DNA | Mean ± SD |
| Purity of (DNA) | 1.90 ± 0.10 |
| Con. of DNA(µg/ml) | 39.12 ± 18.41 |

## **reactions of amplification**

The VDR gene's rs731236 SNP genotypes were assayed using allele-specific PCR to quickly screen for polymorphism in the patients. For rs731236, amplification products with a size of 148 bp were produced. Following electrophoresis, the PCR result was directly visible on an agarose gel that was stained with ethidium bromide when exposed to ultraviolet light. Three genotypes for each SNP were determined by analyzing the amplification results: for rs 731236, the genotypes are CC (mutant type), TT (homozygous wild type), and TC (heterozygous type) in the (fig.1).



**Fig.1.** VDR (rs 731236) gene Genotyping.

**Frequency of the rs731236 T/C allele in the study populations and genotype distribution.**

For the VDR gene (T>C) (rs731236), the study participants were split into three genotypes: one heterozygous (TC), One homozygous for the mutation type C (CC) allele. There were 15 (TT) genotypes (25%), 24 (CC) genotypes (40%), and 21 heterozygous (TC) genotypes (35%), among 60 patients. For SNP rs731236 in the VDR gene, Using Fisher's exact test, the genotype results of the current study are presented in Table 3. A summary of the genotypes of the study participants, based on the VDR gene (rs731236) polymorphisms in the patient group, the C and T alleles (57% and 42%), is presented for the 80 women who participated in our study. There were (TC, CC and TT) genotypes 9(18%), 10(20%), and 31(62%) respectively, in the twenty-control group. Both the T allele was (75%) and the C allele was (25%) from the size of the samples (80 patients) of the VDR gene (rs731236) SNP were found in the control group.

**Table 3.** Frequency of the rs731236 T/C allele in the study populations and genotype distribution.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Alleles Genotype** | | | | | | |  |
| **T/C** | **TT** | **TC** | **CC** | **Total** | **T** | **C** | **Frequencies** |
| **Control**  **No. (%)** | 15 (62) | 1 (18) | 4(20) | 20(100%) | 0.75 | 0.25 | 2.68 |
| **patient No. (%)** | 19(25) | 26 (35) | 35 (40) | 80(100%) | 0.423 | 0.577 | 0.984 |
| **95% CI** |  |  |  |  |  |  | 0.159 |
| **Odd**  **ratios** | Reference | 0.433 | 0.366 |  |  |  |  |
| ***P value*** | **-** | **0.007** | **<0.001** |  |  |  |  |

**Assessment of Hardy-Weinberg equilibrium and genetic power estimation for gene polymorphism (VDR-Taq1).**

The results of comparing the tested population's observed and expected SNIP values with (rs 731236) are displayed in Table 4 and Fig. 2. Since both the distribution and the proportion of individuals with rs731236 differed from what would be expected under the equilibrium of Hardy-Weinberg, the results showed statistically significant, the most important of which were these results: TT, CC and TC (31, 25.3; 10, 4.6; 9, 20.5) respectively, {X2=15.842, P < 0.001}.

**Table 4.** Hardy-Weinberg equilibrium based on the genotype (rs 731236) in the control group.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Genotypes** | | | **Alleles** | | **The equilibrium of Hardy–Weinberg**  **( X2 test)** |
| T | C |
| **Genotype N=50** | **Frequencies** | **%** | 0.71 | 0.29 | X2= 15.842  P < **0.001** \* |
| TT (Wild) | 31 | 62 |
| CC(homozygous mutant) | 10 | 20 |
| TC( heterozygous mutant ) | 9 | 18 |
| \* statistically significant | | | | | |

**Fig 2**. Frequencies % of the VDR observed versus expected genotypes of rs 731236 for the sample of people.

**Demographic parameters evaluation in the VDR rs731236 genotype.**

For demonstrating the difference between demographic data and the (rs 731236) SNP in table 5 (in control) and table 6 (in patients), a one-way ANOVA test was used to compare BMI, age, T. score and BMD in study groups. The mean T. score of the controls did not differ significantly. Patients' mean BMIs varied significantly (p = 0.02), as shown in Table 6. Patients' BMD (p = 0.026) and mean T. score (-3.27 ± 0.53) for the TT allele are substantially greater than those for the CC allele (-2.67 ± 0.80), according to (post-hoc testing) with LSD modification. The results were display the mean BMD for the TT (0.80 ± 0.06) is substantially greater than that of the CC (0.75 ± 0.04), according to post-hoc analysis with LSD adjustment.

**Table 5**. The characteristic associated with control (rs 731236) SNP.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Demographic parameters** | **The** **SNP (rs 731236) (n= 50)** | | | **P-Value** |
| **TC (n=9)** | **CC (n=10)** | **TT(n=31)** |  |
| **BMI** | 29.87 ± 1.79 | 30.69 ± 5.76 | 29.79 ± 5.26 | 0.882 |
| **BMD** | 0.87 ± 0.05 | 0.85 ± 0.05 | 0.79 ± 0.05 | 0.478 |
| **Age** | 52.78 ± 3.15 | 54.80 ± 3.77 | 58.10 ± 9.11 | 0.143 |
| **T. Score** | -1.61 ± 0.40 | -1.66 ± 0.48 | -1.68 ± 0.39 | 0.899 |

n= number of subjects, percentage% and mean ± SD. This is how the result was displayed

**Table 6.** Demographic characteristic in (rs 731236) SNP in patients with PMO (with and without DM type 2).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Demographic parameters** | | **rs 731236 (n= 60)** | | | | | **P-Value** | |
|  | | | | |
| **TT (n=15)** | **CC (n=24)** | | **TC (n=21)** | |
| **BMI** | | 29.36 ± 4.96 | 30.54 ± 5.09 | | 28.66 ± 3.46 | | 0.174 | |
| **Age** | | 65.07 ± 9.25 | 61.92 ± 6.93 | | 65.38 ± 7.16 | | 0.260 | |
| **T.Score** | | -3.27 ± 0.53 | -2.67± 0.80 | | -2.63 ± 0.78 | | **0.020** \* | |
| **BMD** | | 0.80 ± 0.06 | 0.75 ± 0.04 | | 0.76 ± 0.09 | | **0.026\*** | |
| **PMO** | **With T2DM** | 7 | | 14 | | 9 | | 0.559 | |
| **Without T2DM** | 8 | | 10 | | 12 | |

n= number of patients, p<0.05 and \* is indicating a significant and mean ± SD.

**Biomarkers and (rs 731236) Genotypes.**

The mean D3, ALP, PTH, E2, and progesterone were compared using a one-way ANOVA test in order to illustrate the differences between biomarkers and (rs 731236) SNP table 11. The mean D3 of the patients was found to differ significantly (p < 0.001). The mean of E2 for the TT alleles (28.74 ± 10.28) was higher than that of the CC (26.05 ± 6.87) and TC alleles 25.19 ± 8.30, according to post hoc testing with LSD correction, and that for the TT allele of D3 was higher than that (41.02 ± 7.29) of the CC and TC alleles (15.43±6.97, 23.64±6.98) respectively. The (rs 731236) SNP did not differ statistically significantly from the other biomarker means.

**Table 7.** TheDifference between mean levels of biomarkers with VDR genotype (rs731236) alleles

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Biomarkers** | VDR **(rs 731236) (n= 60 )** | | | **P value** |
|  | | |
|  | | |
| **TT (n=15)** | **CC (n=24)** | **TC (n=21)** |
| **E2** | 28.74 ± 10.27 | 26.05 ± 6.87 | 25.19 ± 8.30 | **0.04 \*** |
| **PTH** | 76.28 ± 24.66 | 83.13 ± 32.96 | 66.23± 26.82 | 0.126 |
| **ALP** | 226.87 ± 50.22 | 217.72 ± 46.23 | 226.42 ± 48.86 | 0.594 |
| **D3** | 41.02 ± 7.29\* | 15.43 ± 6.97 | 23.64 ± 6.98 | **<0.001** \* |
| **Progesterone** | 0.58 ± 0.18 | 0.51 ± 0.14 | 0.58 ± 0.22 | 0.260 |

n= number of patients, p<0.05 and \* is indicating a significant and mean ± SD.

**Discussion**

In line with earlier studies, the current analysis finds a substantial correlation between VDR rs731236 and PMO risk. This study confirms that the VDR polymorphismsrs731236 was significantly associated with increased risk of osteoporosis, independent of age and BMI. It also found that the VDR gene variant rs731236 had a significant effect on bone mineral density and was associated with the risk of osteoporosis in postmenopausal Iraqi women as well as in Saudi Arabia [18]. Strongly correlated VDR SNPs with BMD could be employed as genetic markers for disease identification and screening. Early detection of PMO can help treat the condition and slow its progression. Since polymorphisms in this nuclear receptor gene are crucial to the etiology of the disease, in postmenopausal Iraqi women with osteoporosis, as previously described, the presented results [19] show that the rs731236 polymorphism in the VDR gene is strongly associated with an increased risk of osteoporosis. Therefore, in this study, as the results showed, we evaluated the effect of genetic variations in the gene (VDR rs731236) as a genetic determinant through which bone mineral density and osteoporosis are determined.

The distribution and proportion of people with (rs 731236) in this study are different based on the predictions by (Hardy-Weinberg equilibrium). The noted numbers in the results versus the expected numbers in the statistic were as follows: TT, CC and TC (31, 25.3; 10, 4.6; 9, 20.5) respectively. Another study found that this was statistically significant [20]. Our findings agree with a study in in Belarusian women showing that the activity of various receptors can alter the pattern of vitamin D -mediated gene activation, impacting a range of enzymes involved in the formation and elimination of 25(OH)D. The TaqI 731236 variant showed the strongest correlation with BMD (pFDR = 0.0005 for LS and pFDR = 0.001 for FN) [21]. According to another study, women with gestational diabetes may have reduced vitamin D levels.

Type 2 diabetes was found to be significantly associated with FokI, TaqI, and BsmI polymorphisms in some subgroups, according to a prior review that compiled the findings of 47 case-control studies that were part of that meta-analysis [23]. However, the VDR TaqI polymorphism was found to be unrelated to the incidence of type 2 diabetes in our current investigation. Similar findings were made in a study conducted in the Iraqi community [24]. In the previous Brasilia study [25]@, the genotypic and allelic frequencies of VDR polymorphisms in the control group and the group with type 2 diabetes were not different.

**Conclusion**

Osteoporosis is multifactorial in postmenopausal women, especially those with type 2 diabetes, according to researches. The incidence and prevalence of osteoporosis are influenced by genetic polymorphisms, lifestyle choices, and metabolic disorders, making a thorough approach to risk assessment and therapy necessary. Early PMO diagnosis can help treat the condition and limit its progression. The etiology of the disease is significantly influenced by genetic variants (VDR rs731236) polymorphisms in the nuclear receptor gene.

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