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Association Between Some Candidate Gene Variants And The Development Of Osteoporosis

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ABSTRACT

Osteoporosis is a polygenic condition that has been linked to more than 30 candidate genes, the majority of which are also involved in bone mineral density regulation (BMD). Because the receptor of vitamin D (VDR) governs Vit-D action, VDR gene polymorphisms may be linked to a variety of disorders. The COL1A2 (collagen type I alpha 2) gene encodes collagen type I, which is a component of the bone matrix and may be a risk factor for osteoporotic fracture. There were no significant variations in ApaI genotype (rs7975232) frequencies between the patients and controls, according to the current findings. heterozygous C/A with homozygous C/C (OR=0.1365, 95 percent CI=0.0072 - 2.5895, P= 0.1847), and the homozygous variation A/A genotypic frequency compared to homozygous C/C (OR=0. 1127, 95 percent CI= 0.0060 - 2.1070, P= 0.1440). In the VDR ApaI (rs7975232) polymorphism, a difference in significancy appears in allele Afrequenciesofpatients compared to groups of control (OR= 0.9252, 95 percent CI =0.4556 - 1.8784, P=0.8295). Between the patients and controls, there were no significant variations in BsmI genotype (rs15444410) frequencies. heterozygous A/G vs. homozygous G/G genotypic frequency (OR=0.3652, 95 percent CI=0.0402 - 3.3174, P= 0.3709) and the homozygous variation A/genotypic A's frequency compared to homozygous G (OR=0.2833, G's 95 percent CI= 0.0293 - 2.7444, P= 0.2763). In the CAT C-262T polymorphism, difference in significancy appears infrequency of A allele between control and patients' groups (OR= 0.7463, 95 percent CI = 0.4075 - 1.3669, P=0.3433). Between the patients and controls, there were substantial variations in COL1A2 genotype (rs412777) frequencies. heterozygous A/C vs. homozygous A/A genotypic frequency (OR=0.2298, 95 percent CI= 0.0613 -0.8614, P= 0. 0292), and the homozygous variation C/C genotypic frequency compared to homozygous A/A (OR=0.9375, 95 percent CI=0.1326 -6.6285, P= 0.9484). In the COL1A2 gene (rs412777) polymorphism, there were no significant changes in C allele frequencies between patients and controls (OR= 0.8759, 95 percent CI =0.4911 -1.5621, P=0.6536).

Key words: Osteoporosis, COL1A2 polymorphism, VDR polymorphism, PCR-RFLP.

1. Introduction

Osteoporosis is a systemic bone disease that primarily affects the elderly. Bone remodeling (bone resorption and creation) is disrupted in this condition, as a result, bone mass is lost, bone fragility develops, and eventually fracture occurs. Osteoporotic fractures can cause disability, decreased quality of life, and, in the end, death – they affect every aspect of a patient's life (Ichikawa et al. 2006). There have also been studies that have revealed the prevalence of this illness in a particular geographic location; for example, in 2009, an Iranian multi-center study indicated that 70% of women and 50% of men aged 50 had osteoporosis or osteopenia (Thakkinstian et al. 2004).As a major predictor of bone strength in early adulthood, peak bone mineral density (BMD) is critical in the prediction of osteoporotic fracture later in

life. Numerous studies show that genetic factors, in addition to well-known characteristics like race, sex, age, diet, hormonal state, menopausal status, smoking, alcohol use, and physical activity, have a significant impact on bone strength. Genetic factors are thought to account for up to 80% of BMD variance, according to research (Thakkinstian et al. 2004; Xiao et al. 2012).

The nuclear receptor superfamily includes the VDR gene, which is found on the long arm of chromosome 12 (12q13.11), and several studies have demonstrated that VDR gene polymorphisms have a role in the etiology of osteoporosis (Thakkinstian et al. 2004). VDR TaqI (rs17880019), VDR BsmI (rs1544410), VDR FokI (rs17881966), and VDR ApaI polymorphisms are among the VDR polymorphisms (rs17879735)(Ozel et al. 2011; Zhang et al. 2003).

Other studies have discovered a connection between VDR gene variants and bone health problems including osteoporosis (Mencej-Bedrač et al. 2009). A meta-analysis of BsmI, TaqI, ApaI, and FokI VDR polymorphisms in females showed no clear relationship to OP (Zintzaras, Rodopoulou, and Koukoulis 2006).

Collagen changes throughout time as a result of bone disease and has a role in osteoporosis etiology (Saito and Marumo 2010). Bone hardness and age-related changes in bone quality are both influenced by the collagen network (Xiaodu Wang et al. 2001). It has been discovered that age-related changes in bone tissue result in decreased fracture resistance, decreased bone strength and flexibility, and altered collagen fiber network function (X. Wang et al. 2002). COL1A1 and COL1A2 candidate genes have been demonstrated to be essential determinants in the development of osteopenia and osteoporosis, as well as influencing bone metabolism.The COL1A2 polymorphism might be a genetic risk factor for the development of osteoporosis (Majchrzycki et al. 2015). Although procollagen type I gene mutations (COL1A1 or COL1A2) have been related to roughly 85% of cases with osteogenesis imperfecta (OI), no hot spots have been linked to particular clinical features (Augusciak-Duma et al. 2018).

The aim of this study was to utilize an RFLP to identify the VDR gene's distribution (ApaI &BsmI) and COL1A2 gene (rs412777) polymorphism in Iraqi osteoporosis patients.

2. Material and Methods

2.1. Ethical Statement

Every volunteer has informed written consent. The ethics committee of the MOH and MOHSER in Iraq's ethical approval for scientific research has accepted this research.

2.2. Study Population

The study subjects comprised from 64 patients selected from Imam Al-Hussein Medical-City all were female as patients' group with age range (20-82 years). The control group study included 36 people

apparently healthy that also were females with age range (20–71) years. All subjects in this study were taken written consent before participation in this study.

Osteoporosis Patients were diagnosed with DEXA Scan device, A DEXA scan is an imaging test that measures bone density (strength). DEXA scan results can provide helpful details about your risk for osteoporosis (bone loss) and fractures (bone breaks). This test can also measure your body composition, such as body fat and muscle mass, by measuring the T-score and Z-score.

2.3. DNA Extraction and Genotyping

Blood DNA was extracted and purified using a Geneaid extraction and purification kit (UK). After extracting DNA from blood samples, the research groups were genotyped using the PCR-RFLP technique. The targeted DNA locations were amplified using design specific primers bought from Macrogen firm in South Korea, which were used to detect VDR (rs7975232). 5'- CAGAGCATGGACAGGGAGCAA-3' (forward primer)and 5'-GCAACTCCTCATGGCTGAGGTCTC-3' (revers primer). Volume of reaction was 20 µl, 1 µl forboth forward and reverse primer, 12.5 µl frommaster mix, 3 µl of Genomic DNA, and 2.5 µl of nuclease-free water were added to the reaction volume. A thermocycler was used for amplification (Biometra, Germany) device setting was: 5 min for pre-denaturation at 95°C; 30 cycles for latedenaturationfor 20 sec. at 95°C, annealing for 20 seconds at 62°C, extending for 20 seconds at 72°C; thenextention step which was 5 min (SZ, HF, and AR 2017). Specific primers for VDR (rs1544410) were purchased from the Macrogen firm in South Korea. 5'-AGTGTGCAGGCGATTCGTAG -3' and 5'- ATAGGCAGAACCATCTCTCAG -3' are the forward and reverse primers, respectively. The following settings were used in a thermocycler (Biometra, Germany) for amplification: 5 minutes of pre-denaturation at 95°C; 30 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 62°C, and extending for 30 seconds at 72°C; and a 5-minute final extension (Marozik et al. 2013). Design specific primers were used to identify COL1A2 (rs412777), which were bought from Macrogen firm in South Korea.5'- GTTTCATCCGTGGCAGCATC -3' (forward primer) and 5'-GACTGGACTGATTCGCAGGA -3' (revers primer).Volume of reaction was 20 µl, 1 µl of each forward and reverse primer, 12.5 µl of Green Master Mix, 3 µl of Genomic DNA, and 2.5 µl of nuclease-free water were added to the reaction volume. The following settings were used in a thermocycler (Biometra, Germany) for amplification: Pre-denaturation for 5 min at 95°C; 30 cycles for late denaturation for 20 sec. at 95°C, annealing for 20 seconds at 62°C, extending for 20 seconds at 72°C; then extension step which was 5 min.

Gel electrophoresis (cleaver science – UK) was used to electrophorese PCR products in 1 percent agarose at 75 V and observe them using ethidium bromide. To take photographs, a gel documentation system (Cleaver Scientific –UK) was employed.

2.4. Polymerase Chain Reaction Method for Restriction Fragment Length Polymorphism (PCR RFLP).

The PCR product was digested with 2 units of one of the particular endonucleases (ApaI) for 14 hours at 37°C for VDR (rs7975232), as directed by the manufacturer (Promega company). The PCR product was

digested with 2 units of one of the particular endonucleases (BsmI) for 5-15 minutes at 65°C for VDR (rs1544410), as directed by the manufacturer (Biolabs company). Electrophoresis in 1 percent agarose at 75 V using gel electrophoresis (cleaver science – UK) was used to examine the PCR and digestion products, which were observed with ethidium bromide.

2.5. Analytical Statistics

SPSS statistical software (version 23; SPSS Inc., Chicago, IL) was used for all statistical analyses, and p0.05 was considered statistically significant.

3. Results and Discussion

As a first step, genomic DNA (Fig. 1) was isolated from blood samples in order to amplify the VDR genes (rs15444410 andrs7975232)area.

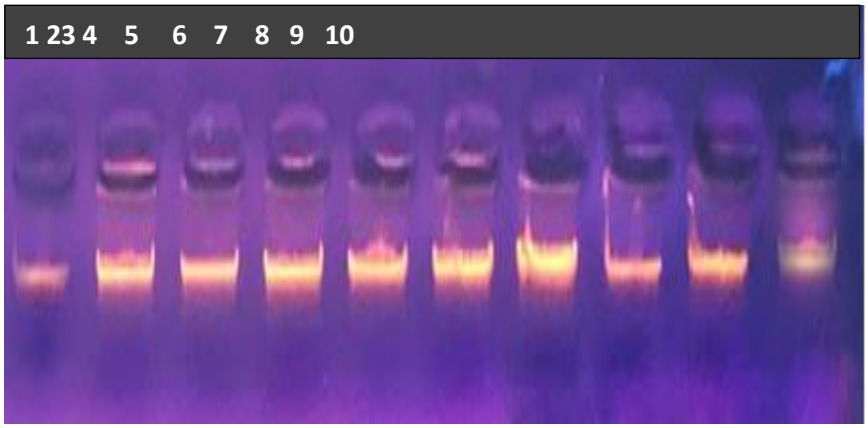

Figure 1: The electrophoresis pattern of gnomic DNA isolated from osteoporosis patients' blood samples and healthy control groups. (Genomic DNA from blood samples is represented by lanes 1 through 10; electrophoresis settings were 1 percent agarose, 75 V, 20 mA for 1 hour, stained with ethidium bromide).

3.1. Genotyping of VDR (rs7975232) Gene Polymorphisms

Genomic DNA was amplified using specified primers and completed by the Thermo-cycler apparatus under ideal conditions for VDR (rs7975232) genotyping. The results confirmed the existence of a single band (745bp) in an agarose gel containing the VDR (rs7975232) gene's target sequence (Fig. 2).

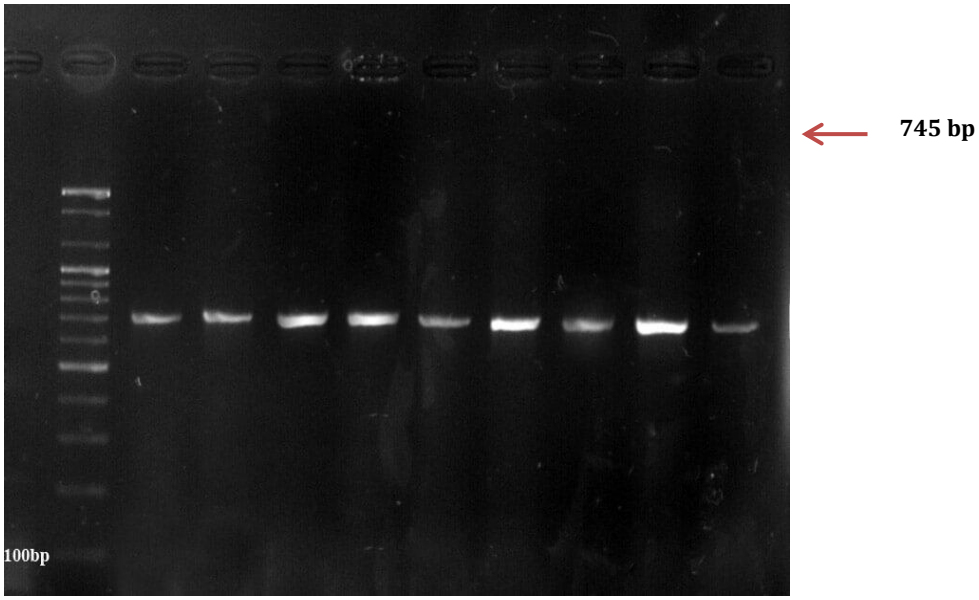

Figure 2 :With a particular primer, gel electrophoresis (agarose) amplified product patterns of vitamin D receptor (rs7975232). (Lans 1–9: PCR products of vitamin D receptor (rs7975232) (745bp) from osteoporosis patients and healthy controls; M: DNA size marker.) 1 percent agarose concentration; 75 V, 20 mA for 120 minutes; precast ethidium bromide staining technique). Electrophoresis conditions: 1 percent agarose concentration; 75 V, 20 mA for 120 minutes; precast ethidium bromide staining technique).

To detect the rs7975232SNP in the VDR gene, the PCR products of the VDR (rs7975232) target sequences were digested with ApaI (5' GGGCCC3') restriction enzyme (Fig. 3). The PCR-RFLP findings revealed the presence of three distinct genotypes, as shown in Figure(3). The first homozygous A1/A1 (AA) shows the anticipated 745bp fragment, but the second homozygous A1/A2 (AG) showed 745, 528, and 217 fragments.The third A2/A2 (CC) segment is 528 and 217 bp long.

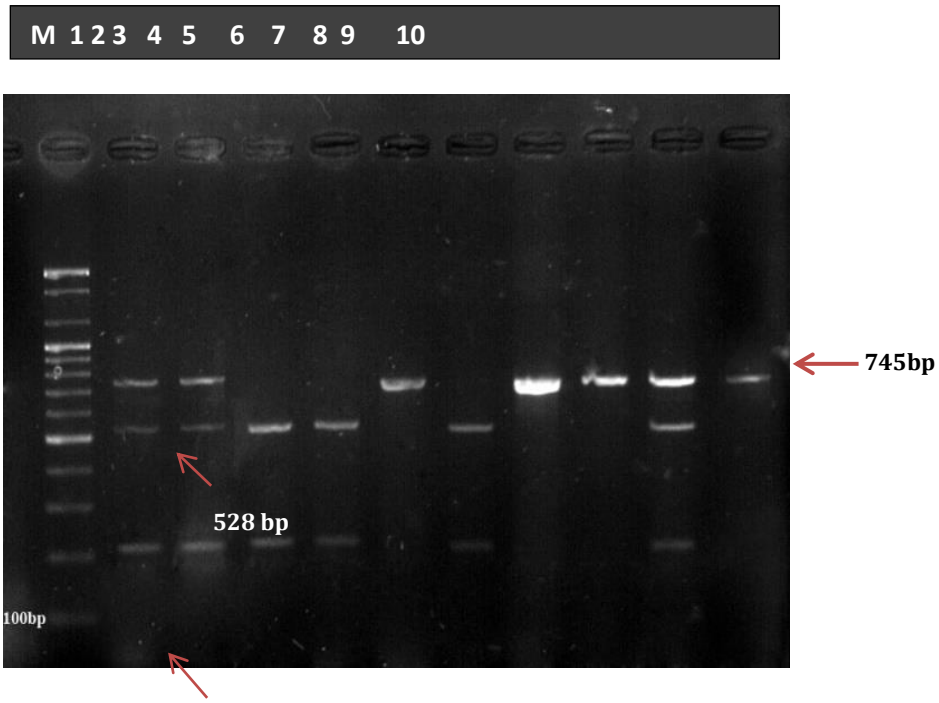

Figure 3 : Electrophoresis patterns of allelotyping of VDR (rs7975232) gene of osteoporosis patients and healthy control groups using ApaI enzyme by PCR-RFLP method. (M: DNA ladder (100 bp); Lanes 5,7,8&10 refer to a homozygous allele (AA) had a single band with 745 bp molecular size; Lanes 1,2&9 refer to a heterozygous allele (CA) had 3 bands with 745, 528 &217 bp; Lanes 3,4&6 refer to a homozygous allele (CC) had a two bands with 528 &217 bp molecular size).

3.2. The Genotypes Distribution of (rs7975232) Polymorphisms with Allele Frequency in Control and Case Groups.

Table 1 shows the distribution of VDR (rs7975232) gene polymorphism in the patients and controls groups (1). In the control group, mutant homozygote AA had the greatest genotype (58%) followed by CA heterozygote genotype (42%) and homozygote genotype CC (0 percent). In cases of osteoporosis, the most common genotype mutant homozygote AA (48.4%), CA heterozygote genotype (42.1%), and homozygote genotype CC homozygote genotype CC homozygote genotype CC homozygote genotype CC homozygote genotype CC homozygote genotype CC homozygote genotype CC homozygote genotype CC homozygote genotype CC homozygote (9.5 percent), The VDR ApaI polymorphism demonstrated significant differences between patients and controls (CA vs CC: OR=0.1365, 95 percent CI =0.0072 - 2.5895, P= 0.1847). In the VDR ApaI polymorphism, there were no significant differences between control and patient's groups (AA vs. CC: OR=0.1127, 95 percent CI =0.0060 - 2.1070, P= 0.1440).

Table 1: Genotype distribution and odd ratio of rs7975232 polymorphisms between the patient's vs healthy control.

Genotype of rs7975232	PatientsNo.(%)	ControlNo.(%)	Significance level	O.R	CI (95%)
CC[a]	6 (9.5%)	0 (0%)	-	-	-
CA	27 (42.1%)	15 (42%)	0.1847	0.1365	0.007-2.589
AA	31 (48.4%)	21 (58%)	0.1440	0.1127	0.006 - 2.107
Total No.	64	36	-	-	-
Allele	Frequency	Frequency			
C	0.31	0. 21	-		
A	0.69	0.79	0.8295	0.9252	0.455-1.878

P ≤ 0.05; OR=(95%CI); a [reference]

Zhang et al.(Zhang et al. 2003)found a link between the ApaI (AA) genotype and the risk of osteoporosis in Chinese postmenopausal women in 2003(Dundar et al. 2009). In 2009, researchers looked into the ApaI genotype distribution in Turkish menopausal women, finding that those with the (aa) genotype had poorer bone density and higher blood calcium levels than those with the (AA) genotype. ApaI rs7975232 SNPs were shown to be substantially related with the incidence of postmenopausal osteoporosis in Caucasians (dominant comparison: OR = 0.77, P = 0.007; allele comparison: OR = 0.81, P = 0.04), according to another study. ApaI rs7975232 polymorphisms may alter the risk of postmenopausal osteoporosis in Caucasians,

according to this meta-analysis(Chen et al. 2020). Furthermore, comparing osteoporotic patients and controls, there was no significant change in the frequency distribution of genotype and allele for VDR ApaI(Ahmad et al. 2018). When compared to controls, osteoporotic patients had a significant increase in the ApaI (Aa) and (aa) genotype frequencies (P = 0.002 and P 0.0001, respectively). ApaI's minor "a" allele was found to be substantially more prevalent in patients than in controls (P 0.0001) (Mohammadi et al. 2014). There are many polymorphic sequence variations in the VDR gene. Multiple sclerosis, osteoporosis, vitamin D-dependent rickets type II, and other complicated disorders have been related to VDR genotypes (Cantorna and Mahon 2004; Valdivielso and Fernandez 2006).

3.3. Genotyping of VDR (rs15444410) Gene Polymorphisms

Genomic DNA was amplified using specified primers and completed by the Thermo-cycler device under ideal conditions for VDR (rs15444410) genotyping. The results confirmed the existence of a single band (191bp) in an agarose gel containing the target sequence of the VDR (rs15444410) gene (Fig. 4).

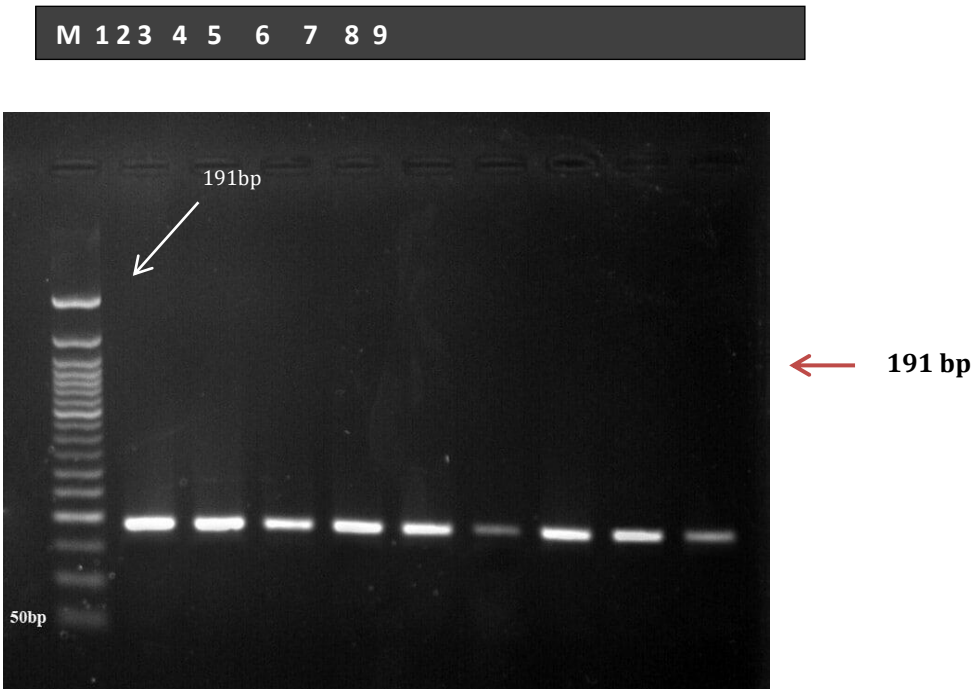

Figure 4: With a particular primer, gel electrophoresis of an amplified product patterns of vitamin D receptor (rs15444410). (M: DNA size marker; lanes 1–9: PCR results of osteoporosis patients and healthy controls for vitamin D receptor (rs15444410) (191bp).) 1 percent agarose concentration; 75 V, 20 mA for 120 minutes; precast ethidium bromide staining technique). Electrophoresis conditions: 1 percent agarose concentration; 75 V, 20 mA for 120 minutes; precast ethidium bromide staining technique).

After that, the PCR products of the VDR (rs15444410) target sequences were digested with BsmI (5' GAATGCN ↓ 3')restriction enzyme (Fig.5) to detect the rs15444410 SNP in VDR gene. The genotypes of the studied subjected has been distributed into three groups based on the presence or absence of the Polymorphisms: A/A homozygous 191 bp, A/G heterozygote demonstrated 191, 115 & 76 bp and G/G homozygous 115 & 76 bp (Fig.5).

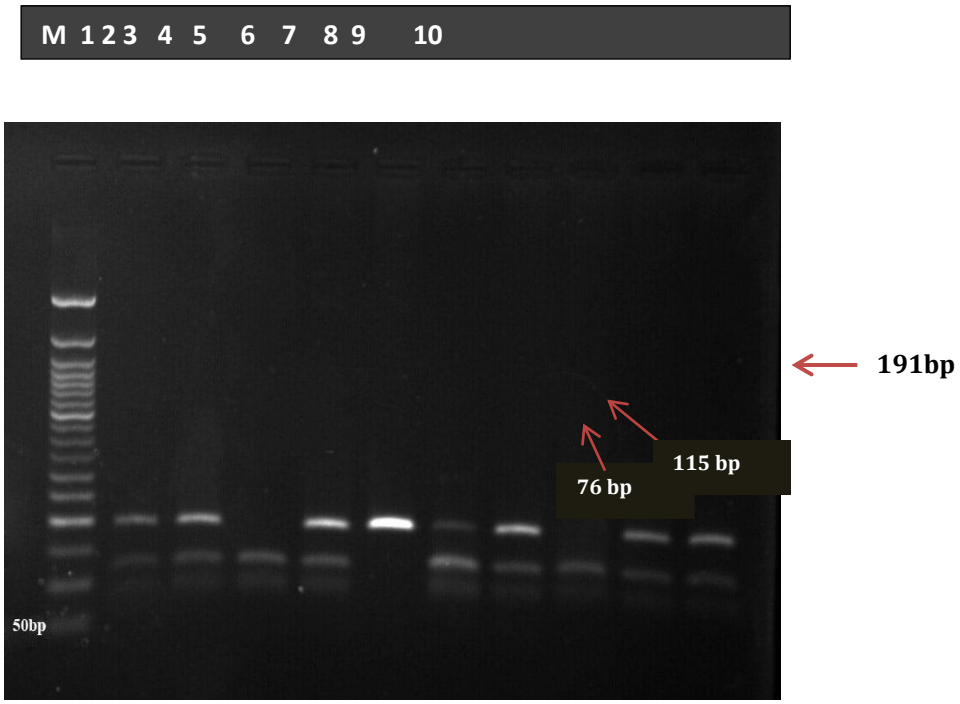

Figure 5: Electrophoresis patterns of allelotyping of VDR (rs15444410) gene of osteoporosis patients and healthy control groups using BsmI enzyme by PCR-RFLP method. (M: DNA ladder (50 bp); Lanes 5 refer to a homozygous allele (AA) had a single band with 191 bp molecular size; Lanes 1,2,4, 6,7,9&10 refer to a heterozygous allele (AG) had 3 bands with 191, 115 &76 bp; Lanes 3&8 refer to a homozygous allele (GG) had two bands with 115 &76 bp molecular size).

3.4. The Genotypes Distribution of VDR (rs15444410) Polymorphisms with Allele Frequency in Control and Case Groups.

Table 1 shows the distribution of VDR (rs15444410) gene polymorphism in the patients and controls groups (2). AG heterozygote genotype (64%) was the most common in the control group, followed by homozygote genotype AA (33.3%) and GG homozygote genotype GG homozygote genotype GG homozygote genotype GG homozygote genotype GG homozygote genotype GG homozygote genotype GG homozygote genotype GG (2.7 percent). AG heterozygote genotype (65.6%) had the highest prevalence of breast cancer disease, followed by homozygote genotype AA (26.5%) and GG homozygote genotype GG homozygote genotype GG homozygote genotype GG homozygote genotype GG homozygote genotype GG homozygote genotype GG

homozygote genotype GG homozygote (7.9 percent),The VDR BsmI polymorphism indicated significant differences between patients and controls (AG vs GG: OR=0.3652, 95 percent CI =0.0402 - 3.3174, P= 0.3709). There were no significant differences between patients and controls in the VDR ApaI polymorphism (AA versus GG: OR=0.2833, 95 percent CI =0.0293 - 2.7444, P= 0.2763).

Table 2: Genotype distribution and odd ratio of rs15444410 polymorphisms between the patient's vs healthy control.

Genotype of rs15444410	PatientsNo.(%)	ControlNo.(%)	Significance level	O.R	CI (95%)
GG[a]	5 (7.9%)	1 (2.7%)			
AG	42 (65.6%)	23 (64%)	0.370	0.365	0.04 - 3.317
AA	17 (26.5%)	12 (33.3%)	0.2763	0.283	0.029 - 2.744
Total No.	64	36			
Allele	Frequency	Frequency			
G	0.41	0. 35			
A	0.59	0.65	0.343	0.7463	0.40 - 1.36

P ≤ 0.05; OR=(95%CI); a [reference]

The VDR gene variation was found to increase the risk of osteoporosis in Indians and women statistically considerably. For the VDR BsmI polymorphism, West Asians had a statistically significant lower risk of osteoporosis. However, after omitting low-quality and Hardy-Weinberg Disequilibrium (HWD) studies, the VDR BsmI polymorphism was revealed to have a substantial reduction in osteoporosis risk only in the entire population. Furthermore, when we assessed the credibility of positive results(Chen et al. 2020). Another study discovered a connection between the VDR BsmI genetic polymorphism and LS BMD levels in paediatric patients: children with the bb genotype were less likely than those with the B allele to have lower BMD levels. There was no discernible change in the levels of FN BMD in children (Bao et al. 2017). In Caucasians, BsmI rs1544410 polymorphisms were found to be significantly associated with the risk of postmenopausal osteoporosis (OR =0.69, P=0.002), while in Asians, FokI rs10735810 polymorphisms were found to be significantly associated with the risk of postmenopausal osteoporosis (OR =0.69, P= 0.002) (comparison of dominant: OR = 0.61, P = 0.0001; comparison of recessive: OR = 2.02, P = 0.001; allele comparison: OR = 0.68, P = 0.002), ApaI rs7975232, BsmI rs1544410, and TaqI rs731236 polymorphisms

may alter the incidence of postmenopausal osteoporosis in Asians, according to this meta-analysis(Fu et al. 2020).

In a meta-analysis of females with the BsmI, TaqI, ApaI, and FokI VDR polymorphisms, no relation to osteoporosis was identified(Zintzaras, Rodopoulou, and Koukoulis 2006). Furthermore, Kow et al. (Kow et al. 2019) discovered that there were no significant differences between the case and control groups in the VDR A/G genotype (p=0. 5, OR [95 percent CI] =1.45 (0.65–3.24), and similarly in the VDR A/A genotype (p=0. 5, OR [95 percent CI] =1.74 (0.66–4.63).

3.5. Genotyping of COL1A2 (rs412777) Gene Polymorphisms

Genomic DNA was amplified using specified primers and completed by the Thermo-cycler apparatus under ideal conditions for COL1A2 (rs412777) genotyping. The results confirmed the presence of a single band (249bp) in an agarose gel containing the COL1A2 (rs412777) gene's target sequence (figure 6).

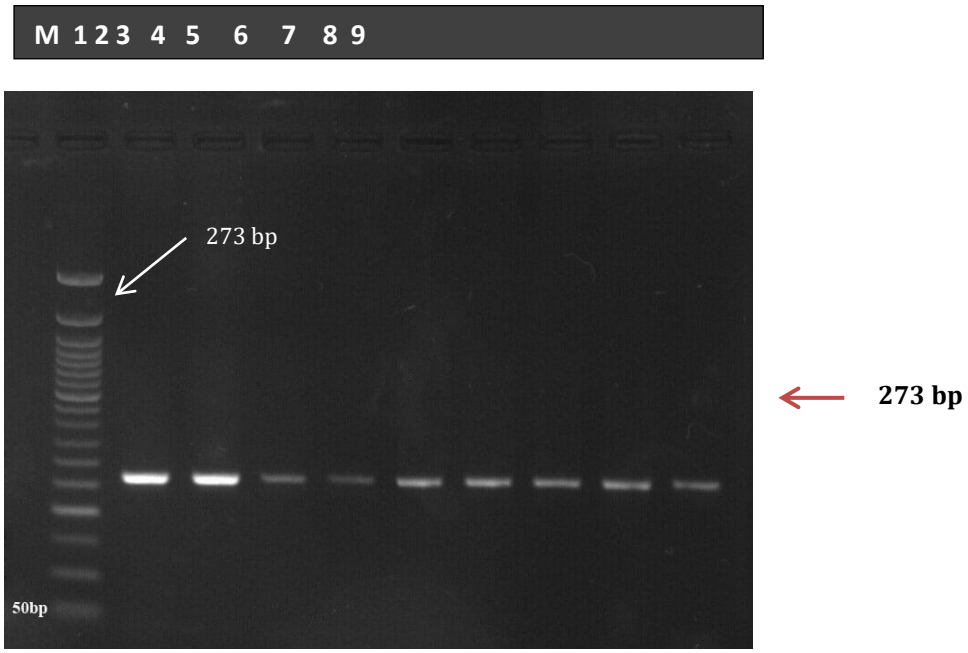

Figure6: COL1A2 (rs412777) amplified product patterns electrophoresed on an agarose gel with a particular primer. Lanes 1 - 9 correspond to COL1A2 (rs412777) (273 bp) PCR results from osteoporosis patients and healthy controls. The following were the electrophoresis conditions: 75 V, 20 mA for 120 minutes; 1% agarose concentration The staining procedure was precast ethidium bromide.

After that, the PCR products of the COL1A2 (rs412777) target sequences were digested with PvuII (5' CAG ↓ CTG 3')restriction enzyme (Fig. 3) to detect the rs412777 SNP in COL1A2 gene (Fig. 3). The results of

PCRRFLP showed that the presence of three different genotypes as in figure (7). the first A1/A1 (CC)homozygous, presents the expected 273bp fragment, the second A1/A2 (AC) demonstrated 273, 148 & 125 bp fragment. While the third A2/A2 (AA) demonstrated 148 & 125 bp fragments.

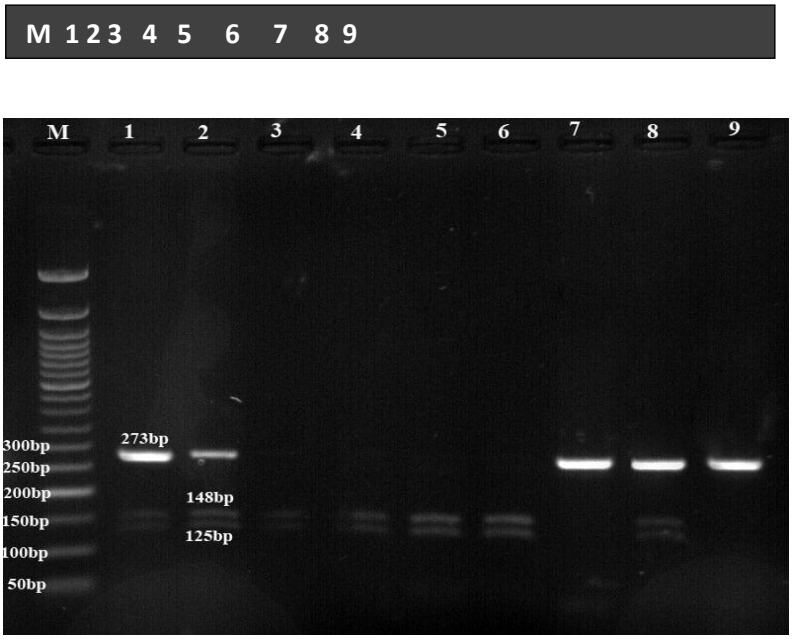

Figure 6: Electrophoresis patterns of allelotyping of COL1A2 (rs412777) gene of osteoporosis patients and healthy control groups using PvuI enzyme by PCR-RFLP methodM: DNA ladder (50 bp); Lanes 3,4, 5&6 refer to a homozygous allele (AA) had two bands with 148&125 bp molecular size; Lanes 1,2&8 refer to a heterozygous allele (AC) had 3 bands with 273,148&125 bp; Lanes 7,9 refer to a homozygous allele (CC) had a single band with 273 bp molecular size.

3.6. The Genotypes Distribution of rs412777 Polymorphisms with Allele Frequency in Control and Case Groups.

The distribution observed in COL1A2 (rs412777) gene polymorphism in cases group and control group are showed in Table (3). The highest genotype in control group was heterozygote genotype AC (86.1%) followed by AA homozygote genotype (8.3%), CC homozygote genotype (5.6%). In breast cancer disease, the highest genotype was heterozygote genotype AC (59.3%) followed by AA homozygote genotype (25%), CC homozygote genotype (15.7%). The COL1A2 (PvuI) polymorphism demonstrated significant differences between cases and controls (AC vs AA: OR=0.2298, 95 percent CI= 0.0613 -0.8614, P= 0. 0292). The COL1A2 (PvuI) polymorphism did not differ significantly between patients and controls (CC versus AA: OR=0.9375, 95 percent CI=0.1326 -6.6285, P= 0.9484).

Table 3: Genotype distribution and odd ratio of Rs 412777 polymorphisms between the patient's vs healthy control.

Genotype of rs412777	Patients No.(%)	Control No.(%)	Significance level	O.R	CI (95%)
AA[a]	16 (25%)	3 (8.3%)			
AC	38 (59.3%)	31 (86.1%)	0.029	0.2	0.06 -0.86
CC	10 (15.7%)	2 (5.6%)	0.9484	0.9	0.13 -6.62
Total No.	64	36			
Allele	Frequency	Frequency			
A	0.54	0.51			
C	0.46	0. 49	0.653	0.8	0.491-1.56

$P \leq 0.05$; OR=(95%CI); a [reference]

The relationship between COL1A2 genotypes and osteoporosis is less clear than the relationship between COL1A1 genotypes and the illness. In a number of studies, BMD has been related to GT-repeat polymorphisms, Msp I, Pvu II, and EcoR I of the COL1A2 gene (Lindahl et al. 2009). Lindahl et al. identified links between the COL1A2 gene and BMD in older males from Sweden, the United Kingdom, and Hong Kong (n=2004). According to research of the PvuII polymorphism, the CC genotype had the lowest body weight in women with osteopenia when compared to other genotypes ($p = 0.039$). In a sample of women with osteoporosis, there were no statistically significant links between the PvuII polymorphism and clinical indications. The COL1A2 polymorphism might be a genetic risk factor for osteoporosis development(Zintzaras, Rodopoulou, and Koukoulis 2006). Over 85% of cases of osteogenesis imperfecta

(OI) are connected to mutations in procollagen type I genes (COL1A1 or COL1A2), however no hot spots linked to particular clinical features have been discovered. There is a single mutation in exon 22 of the COL1A2 gene. Collagen type I secretion and intracellular accumulation were unaffected by deletion mutations in COL1A1 that resulted in OI type I. A missense mutation in COL1A2 altered Gly>Cys in the core area of the triple helical domain of the collagen type I molecule, resulting in OI type III. A mutation affected the production of the heterotrimeric form of type I procollagen, while a mutation affected the incorporation of COL1A2 into procollagen type I (Augusciak-Duma et al. 2018). There were no significant changes in allele frequency or genotype distribution between the fracture and control groups for 9 selected SNPs and haplotypes. There was no indication that any of the SNPs or haplotypes examined were linked to fragility fracture. The results were the same after accounting for additional risk variables such as weight, height, and bone mineral density. Our data show no connection between common genetic variations in the COL1A1 and COL1A2 genes and fracture, indicating a complex genetic foundation for osteoporotic fractures (Hu et al. 2011). There was no statistically significant overall effect ($p > 0.06$) in meta-analyses of any genetic model or SNP (Deng et al. 2011). The findings of the two studies that supplied data for the COL1A2 PvuII (rs412777) study were inconclusive, with one stating that the “PP” genotype decreased fracture risk by half (Blades et al. 2010), and the other saying that the P allele (either PP or Pp genotype) increased fracture risk(Lindahl et al. 2009). The combined study described here found no significant overall effect of the COL1A2 PvuII (rs412777) SNP on fracture risk due to the inconsistency of the data. Only Blades et al., (Blades et al. 2010) provide the specific reference SNP number (rs412777) if two separates proximal PvuII sites in the COL1A2 gene have been examined; or if the study intervention on calcium and vitamin D supplementation, the outcomes of the research may change. Significant sub-group heterogeneity was found between sexes when the COL1A1 Sp1 (rs1800012), COL1A2 PvuII (rs412777), and ESR1 PvuII (rs2234693) SNPs were examined, indicating the potential of sex-specific connections. According to epidemiological statistics, males between the ages of 18 and 49 had a greater fracture rate than females in the general population (Curtis et al. 2016).

Conclusion

Based on the present statistical analysis, our study shows no significant association of VDR ApaI (rs7975232) and VDR BsmI (rs15444410) genes with the development of osteoporosis. A significant association of COL1A2 (rs412777) gene with the development of osteoporosis.

Conflict of Interests

The authors have declared no conflict of interests.

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