Original Article

*APA*I VDR GENE POLYMORPHISM: CROSS-SECTIONAL STUDY IN THAI POSTMENOPAUSAL WOMEN

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Abstract

Osteoporosis is most common bone disease in elders, which is defined by loss of bone density with higher risk of bone fracture. Many studies have focused on relationship of vitamin D receptor (VDR) gene variation and risk of osteoporosis in postmenopausal women. However, *Apa*I VDR gene polymorphism is sparely reported among Thai population. This study was proposed to evaluate genotypic polymorphism of *Apa*I (T>G; rs7975232) VDR gene polymorphism between risk and control groups in postmenopausal women. In addition, bone mineral density (BMD) screening and other bone related markers were also measured. Results was shown that BMI, BMD, calcium and ALP of risk and control groups were significantly different. Mean of BMI in control group was trended to overweight and mean of BMD in risk group was osteopenia. Therefore, calcium and ALP in both groups were within reference range. The genotypic frequencies of risk group were 15.2% (n = 7) wild type (TT); 63.1% (n = 29) heterozygous variant (TG); and 21.7% (n = 10) homozygous variant (GG). Controls were 16.7% (n = 4) wild type (TT); 70.8% (n = 17) heterozygous variant (TG); and 12.5% (n = 3) homozygous variant (GG). Odd ratio of osteoporosis in *Apa*I heterozygous (TG) and homozygous (GG) variants were significantly increased in risked postmenopausal women ($p<0.02$ and <0.001, respectively). Homozygous variant (GG) was highest OR value (OR = 1.90, 1.04-4.32). Genotypic of *Apa*I VDR gene is useful to evaluate occurrence of osteoporosis especially in Thai postmenopausal women who are carried homozygous variant.

Keywords: *Apa*I polymorphism, osteoporosis, postmenopausal women, VDR gene.

INTRODUCTION

Osteoporosis is most common bone disease in elders, which is defined by reduction of bone mass and strength, structure disrupts and consequence in bone fractures [1]. Low bone mineral density (BMD) is common indicator of osteoporosis and risk of bone fracture especially in postmenopausal women [2, 3]. Vitamin D is function on calcium and phosphate distribution between in bone composition and blood circulation, which is mediated through vitamin D receptor [4, 5, 6]. Vitamin D receptor (VDR) is play role on vitamin D functions i.e., cell maturation and divisions. Polymorphisms of VDR gene including *Apa*I, *Bsm*I, *Cdx*2, *Fok*I and *Taq*I are significantly important and associated with various diseases [5, 7].

Many studies have focused on relationship of vitamin D receptor (VDR) gene variation and risk of osteoporosis in postmenopausal women; however, the results are still controversial [5, 8, 9]. In addition, relationships of VDR gene polymorphisms and BMD in postmenopausal women are also conflicted [10, 11, 12]. According on meta-analysis study, postmenopausal women who carried VDR *Apa*I and VDR *Fok*I gene variances are susceptible to osteoporosis rather than wild type.

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In addition, VDR *Bsm*I and *Fok*I gene polymorphisms are increase risk of osteoporosis in Asian women [13]. "T" allele of *Fok*I VDR gene is risk factor of osteoporosis in Indian and Iranian women [14, 15]. In addition, TT genotype of *Fok*I VDR gene is more susceptible to osteoporosis in Thai postmenopausal women including osteoporosis and osteopenia [16, 17]. Aa and aa genotypes of VDR *Apa*I gene (T>G; rs7975232) are significantly increase risk in Saudi osteoporotic women and "a" allele is higher frequent in osteoporotic patients [18]. VDR *Apa*I is located in the 3′-regulatory region of intron 8 and genomic variation can affect io physiological functions of vitamin D [19]. Postmenopausal women who carried VDR *Apa*I variants are lower risk compared with wide type. However, controversial studies had reported with inconsistent finding [8, 19, 20-23]. There is sparely information of VDR *Apa*I polymorphism in Thai postmenopausal women. This study was proposed to evaluate genotypic polymorphism of *Apa*I VDR gene between risk and control groups in postmenopausal women. In addition, BMD screening and other bone markers in risk and normal groups were also measured.

Materials and methods

Study Population

Study was cross-sectional designed and conducted with 1,500 participants who joined in program of osteoporotic prevention, Sumut Songkhram, Thailand during 2020-2021. Participants who met to inclusion and exclusion criteria as described in previous studies [17, 24], were randomly selected (N = 70). All selected subjects had informed and signed consent before they were joined in the study. Biodata of each subject was collected by interviewing with questionnaire. Additionally, measurement of body weight and height was used to calculate body mass index (BMI) and represented as kg/m2. Normal BMI was defined as normal weight (18.5–24.9) in Asian population. Research protocol had reviewed by Ethics Committee of Suan Sunandha Rajabhat University (COA.1-050-2020). This study was also approved from director of osteoporosis prevention program.

Bone Densitometry

BMD was determined on calcaneal bone by quantitative ultrasound bone densitometer (SONOST-2000, OsteoSys, Korea) and measurement was performed and reported under manufactural instruction. Level of BMD and osteoporosis risk in postmenopausal women were defined according to WHO classification (WHO Europe, 1994) including osteoporotic (T-score at or below -2.5), osteopenia (T-score between -1.0 and -2.5), and normal (T-score at above -1.0). After BMD screening, normal BMD level was defined as normal group (N = 24), and poor BMD level, osteoporotic and osteopenia was defined as risk group (N = 46). Low BMD women were received health consults and supplementation after provided research information.

Biochemical Measurement

Whole blood sample (5 ml) was collected from median cubital vein on early morning. Each collecting blood was drawn in clotting blood tube (3 ml) and ethylenediaminetetraacetic acid (EDTA) tube (2 ml). Clotting blood was centrifuged and serum was separated from clotting blood tube within 2 h. Serum alkaline phosphatase (ALP) and Ca were measured by automatic analyzer, COBAS c501 (Roche-diagnostics, Switzerland).

DNA Extraction and Genotyping

Genomic DNA was extracted from EDTA blood by the QIAamp blood DNA mini kit (QIAGEN Thailand, Bangkok, Thailand) and DNA was storage at -20 °C. Genomic DNA was amplified and genotyped on VDR (rs7975232) gene by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Specific primers were forward primer (5′-GGATCCTAAATGCACGGAGA-3′) and reverse primer (5′-ACGTCTGCAGTGTGTTGGAC-3′). PCR mixture was performed by OnePCR Ultra Supermix with fluorescent dye (Bio-Helix, GibThai, Thailand). The PCR program was as follows: Initial denaturation at 95 °C for 5 min, cycle denaturation at 95 °C for 1 min, cycle annealing at 55 °C for 1 min, cycle extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. PCR steps were repeated in 35 cycles, and product was maintained at 8 °C until it was removed [18, 25, 26]. Amplified PCR product (265 bp) was determined by 2.0% agarose gel electrophoresis. RFLP technique was genotyped by digestion of PCR product with *Apa*I restriction endonuclease enzyme (R0109S, NEB). Reaction mixture (30 μl) as follows: PCR product (10 μl), *Apa*I restriction endonuclease enzyme (1 μl), reaction buffer (2 μl) and sterile water (17 μl). Reaction mixture was incubated at 37 °C in water bath for 20 min, and DNA fragments were produced. DNA fragments and DNA ladder (OmniMARK, Bio-Helix, Taiwan) were separated on 2.0% agarose gel electrophoresis (BIO-RAD, Thailand). DNA fragment pattern was developed under gel doc system (Universal Hood II, BIO-RAD Laboratories-Segrate, Italy). Result interpretation was including TT genotype was one band of 265 bp (unable cut); GG genotype was represented two fragments of 146 and 119 bp (two allele cut); TG was generated three fragments of 265, 146 and 119 bp and defined as heterozygous or one allele cut [25, 26]. Quality control was done by 15% randomized sequencing.

Statistical analysis

Personal data and biochemical levels of each group were reported by descriptive statistics. Comparison of personal data and biochemical parameters between normal and risk groups was calculated by using independent *t*-test. *Apa*I VDR allele was evaluated for Hardy-Weinberg equilibrium. Chi-square was used to calculate odds ratios (ORs) on different of *Apa*I genotypes as risk factor for osteoporosis and 95% confidence intervals (CIs). Statistically significant difference

was judged at $p<0.05$.

Results and Discussion

Bone-Related Parameters in Postmenopausal Women

Physical and biochemical characteristics of risk and control groups was shown in Table 1. Age, BMI, BMD, calcium and ALP were described as mean ± standard error of the mean. Age was not significantly different between two groups. BMI, BMD, calcium and ALP of risk and control groups were significantly different. Mean of BMI in control group was trended to overweight and mean of BMD in risk group was osteopenia. Therefore, calcium and ALP in both groups were within reference range. Risk of osteoporosis is trend to increase in aging and women, especially in postmenopausal period [27, 28]. Multifactorial factors of osteoporosis risk are including low dietary calcium intake, vitamin D deficiency, low BMI, reduction of physical activity and reduction of estrogen levels in postmenopausal women [29]. Thai people are become proportionally aging and risk of osteoporosis is trend to increase [17, 30]. Serum calcium and ALP are significantly increased in postmenopausal women with osteoporosis, which had been reported in previous studies. There is implied that serum calcium and ALP are converse correlate to BMD value [17, 31, 32].

Genotypic Frequencies and ApaI VDR Gene In Postmenopausal Women

DNA fragments of *Apa*I genotypes was identified as wild type (TT) for 265 bp (uncut), heterozygous variant (TG) for 265, 146 and 119 bp fragments and homozygous variant (GG) for 146 and 119 bp fragments (Fig. 1). Genotypic and allele frequencies of the VDR *Apa*I gene in postmenopausal women were shown in Table 2. The genotypic frequencies of risk group were 15.2% (n = 7) wild type (TT); 63.1% (n = 29) heterozygous variant (TG); and 21.7% (n = 10) homozygous variant (GG). The allelic frequency of "T" versus "G" was 46.7% versus 53.3% in risk group. Controls were 16.7% (n = 4) wild type (TT); 70.8% (n = 17) heterozygous variant (TG); and 12.5% (n = 3) homozygous variant (GG). The allelic frequency of "T" versus "G" was 52.1% versus 47.9% in control group. Odd ratio of osteoporosis in *Apa*I heterozygous (TG) and homozygous (GG) variants were higher in risk group rather than controls ($p<0.02$ and <0.001, respectively). Homozygous variant (GG) was highest OR value (OR = 1.90, 1.04-4.32) and most compromised to osteoporosis. VDR gene polymorphisms are associate to BMD and genotyping of this gene can evaluate risk or predisposition on osteoporosis especially in postmenopausal women [21]. However, relationship of VDR gene variants and loss of BMD reduction are different or contrast due to geographic distribution and ethnical factor [8, 33]. Similar to our finding, heterozygous variant of the *Apa*I VDR genotype is protective factor on osteoporosis while homozygous variant is bone loss factor and related to bone fracture in Tunisian menopausal women with osteoporosis [19]. *Apa*I heterozygous and homozygous variants were significantly increased in osteoporosis patients among Saudi population [18]. Homozygous variant is lower BMD and higher serum calcium than wide type in Turkish menopausal women [34]. In contrast, normal (TT) ApaI genotype is related to osteoporosis incidence in Chinese menopausal women [13]. There is sparely report of ApaI VDR polymorphism in Thai people especially postmenopausal women. *Bsm*I, TaqI and FokI VDR polymorphism in Thai people had been reported and homozygous variant (TT) of *Fok*I VDR is most susceptible to osteoporosis in Thai population [16, 17]]Techapatiphandee et al., 2018; Sudjaroen et al., 2022). Hence, we were provided *Apa*I VDR gene polymorphism in Thai menopausal women as cross-sectional study and significant finding was focused on *Apa*I VDR gene variants and "G" allele may related to risk of osteoporosis or BMD loss. However, sample size of this study is small and may affecting on statistical analysis. Thus, study on larger numbers of Thai menopausal women is needed to conduct.

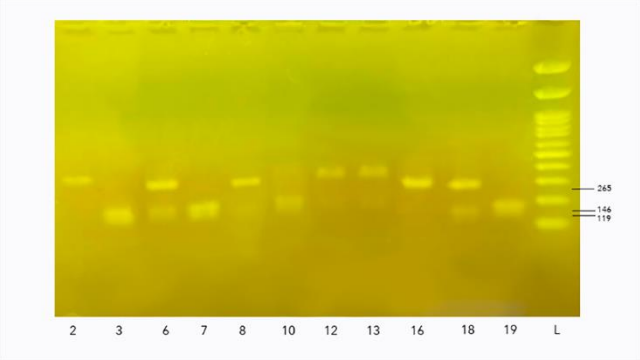

Figure 1 Genotyping of ApaI polymorphism on VDR gene by PCR-RFLP: (a) TT genotype (wild type) was represented as 265 bp fragment (uncut) in Lane 2, 8 and 16; (b) TG genotype was represented as 265, 146 and 119 bp fragments (heterozygous) in Lane 6, 12, 13 and 18; (c) GG genotype was represented as 146 and 119 bp fragment (homozygous) in Lane 3, 7, 10 and 19.

Table 1: Bone-related parameters of risk and control groups in postmenopausal women

Group	Frequency (%)	Age (years)	BMI (kg/m^2)	BMD (T-score)	ALP (U/L)	Ca (mg/dL)
Risk	46 (65.7)	61.2±10.5	21.0±2.4	0.69±0.87	77.4±15.43	9.1±0.40
Control	24 (34.3)	59.6±7.45	23.7±3.2	-1.98±0.86	87.0±20.90	9.9±0.71

***p*-value**	-	0.081	<0.0001**	-	0.035*	<0.0001**
Reference range	-	-	18.5-22.9	> -1.0	30-120	8.2-10.2

*Statistically significant at $p < 0.05$; ** $p < 0.001$

Table 2: Genotypic frequencies and ApaI alleles of risk and control groups in postmenopausal women

***Apa*I polymorphism**	**Frequencies, n (%)**		**Odds Ratio (95% CI)**	***P*-value**
	Risk (n = 46)	**Control (n = 24)**		
TT	7(15.2%)	4(16.7%)	1(Reference)	-
TG	29(63.1%)	17(70.8%)	1.07(0.78-3.57)	0.02
GG	10(21.7%)	3(12.5%)	1.90(1.04-4.32)	<0.001
T	46.7%	52.10%	1(Reference)	-
G	53.3%	47.90%	1.24 (0.92-2.68)	<0.001

Conclusion

We concluded that BMI, BMD, calcium and ALP of risk and control groups were significantly different. Mean of BMI in control group was trended to overweight and mean of BMD in risk group was osteopenia. Genotypic of *Apa*I VDR gene is useful to evaluate susceptibility of osteoporosis in Thai postmenopausal women who are carried homozygous variant (GG).

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Conflict of interest

Authors had declared no conflicts of interest.

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