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GENOTYPE DIFFERENCE OF –572 G>C AND -174 G>C IL-6 GENE POLYMORPHISM BETWEEN BALINESE POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS AND WITHOUT OSTEOPOROSIS

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Background: Osteoporosis is a silent metabolic disease characterized by diminished bone mass and change in bone microstructure which cause increment of fracture risk. Until now, osteoporosis still becomes one of major health problems around the world. In Indonesia, the incidence of osteoporosis is 25%. Previous study have shown the relation between osteoporosis and IL-6 gene polymorphism at -572G>C and -174 G>C. There are some controversies about the correlation between these polymorphism and osteoporosis because of different result between each study. Genotype G polymorphism at -572 G>C of IL-6 gene has been correlated with lower Bone mineral density (BMD) and Genotype G polymorphism at -174G>C of IL-6 gene has been correlated with higher BMD value. In Indonesia, there are still no study about the association between IL-6 gene polymorphism and osteoporosis. In the future this IL-6 gene polymorphism could be used as a genetic marker for osteoporosis in postmenopausal woman. The objective of this study is to determine the difference of genotype of -572G>C and -174G>C polymorphism of IL-6 gene and osteoporosis in Balinese postmenopausal women. **Method:** This research design is a case control study. Sample was obtained at orthopedic outpatient clinic of Sanglah General Hospital, Bali-Indonesia from June 2012 until November 2012. The diagnosis of osteoporosis is described as BMD value with T score ≤ -2.5 SD using DEXA. All sample's peripheral blood are taken to be isolated for DNA and analyzed for IL-6 gene polymorphism at -572G>C and -174G>C using Real Time PCR. Data obtained was analyzed with chi square test using SPSS. **Results:** This research found 11 osteoporosis sample from total 52 with no difference sample characteristic between case and control ($p > 0.05$). Using Chi square test, There was a significant differences between genotype -572 G>C; IL-6 gene polymorphism in Balinese postmenopausal woman with osteoporosis and in Balinese postmenopausal woman without osteoporosis ($c^2 = 41.85$ and $p = 0.001$) and no significant differences between genotype -174 G>C;IL-6 polymorphism in Balinese postmenopausal woman with osteoporosis and in Balinese postmenopausal woman without osteoporosis($c^2 = 0.283$ and $p = 0.518$). This study discovered significant differences between genotype -572 G>C; IL-6 gene polymorphism in Balinese postmenopausal woman with osteoporosis and in Balinese postmenopausal woman without osteoporosis. **Conclusion:** We concluded that ethnic genetic profile variations affect the role of IL-6 gene polymorphism. In the future, this IL-6 gene polymorphism could be used as risk factor for osteoporosis in Balinese premenopausal woman

Keywords: postmenopausal, osteoporosis, BMD, IL-6 gene, polymorphism

INTRODUCTION

Osteoporosis is a silent metabolic disease characterized by decreased Bone Mineral Density (BMD) and diminished bone fragility and bone fractures.[1] Until now osteoporosis is still becoming a major public health problem worldwide. Osteoporosis is known as a multifactorial disease. Environment has influenced in BMD such as diet, calcium intake and exercise. Age and gender have correlation with BMD value and the risk of bone fractures.[2,3] Genetic factor hold a strong determination of BMD.[4-6] Previous study has shown the association between BMD and gene polymorphism especially hormone, vitamin and cytokines.[7-10] IL-6 is one of the cytokines that involved in bone differentiation particularly in osteoclast differentiation.[11] *IL-6 gene* polymorphism has been connected with BMD in postmenopausal women, proposing that genotype variants of IL-6 may influence osteoporosis susceptibility.[12,13] The promoter region of -572 G>C and -174 G>C of *IL-6 gene* enclose a common polymorphism, where GG genotype of -572 G>C and CC genotype of -174 G>C has been correlated with decreased promoter activity and plasma IL-6 level [14,15] Bone metabolism and BMD have associated with these promoter variants.[16]The purpose of this research is to determine the

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difference genotype -572 G>C and -174 G>C polymorphism of IL-6 gene between Balinese postmenopausal women with osteoporosis and without osteoporosis.

MATERIAL AND METHODS

Subjects

Fifty two participants were postmenopausal women attending the outpatient clinic of Orthopaedic and Traumatology Department, Sanglah General Hospital, Bali-Indonesia. Written informed consent was obtained from all the participants and proven ethical clearance was issued by local ethical committee medic. The ethnicity of all women was Balinese. The life style factors including postmenopausal period, dietary calcium intake, smooking history, alcohol consumption, tea and coffee consumption questionnaires completed at baseline. Exclusions criteria from this study were women who had undergone ovariectomy, rheumatoid arthritis or ostroarthritis. None of the participants had received any medication known to affect bone metabolism such as biphosphonate. This population has been divided into two groups according to their lumbar spine T score of BMD. The first group (n=11) was *osteoporosis* women whose lumbar spine T score was lower than -2.5 SD and the second group (n=41) was non *osteoporosis* women whose lumbar spine T score was greater than -2.5 SD.

BMD measurement

Area BMD (g/cm²) at the lumbar spine L2-L4 and femoral neck was measured by dual energy X-ray absorptiometry (DEXA). The coefficient of variation for BMD was 0.52%.

SNP genotyping

DNA was extracted from peripheral venous blood using High Pure DNA extraction kit from Roche. Real Time PCR was performed to identify the differentiation of genotype of -572 G>C and -174 G>C polymorphism of *IL-6 gene*. The reference of primer and probe came from Tip Mol Biol. Modified Real Time PCR procedure was conducted by Medical Genetic Laboratory UWKS.

Statistical analysis

Statistical analysis was performed by using The SPSS program. Results were expressed as mean and standard deviation. The subject characteristic was analyzed by *Kolmogorov-Smirnov* to ensure data normality. Differences between means were analyzed by *t-independent*. The significance of differences between two groups was assessed using χ^2 test. All examination of this study considered that *P* value of < 0.05 was statistically significant.

RESULTS

The Characteristics differences of subjects can be seen in Table 1. No characteristic difference of subjects including age, height, weight, BMI, menarche age and postmenopausal age between case and control have been explained in Table 1.

Table 1
Characteristics differences of subjects

Variable	Case (n=11)	Control (n=41)	*p*
Age (year)	59.7(5.1)	57.4(5.2)	0.216
Weight (kg)	57.7(5.6)	62.9(6.8)	0.103
Height (cm)	151.8(4.9)	154. 2(5.5)	0.191
BMI	25.0(2.55)	26.3(3.4)	0.256
Menarche age (year)	14.0(1.4)	14.1(1.4)	0.720
Postmenopausal age (year)	50.5(4.1)	50.4(3.7)	0.942

The profile of -572 G>C and -174 G>C SNP genotype are presented in Table 2 and Table 3.

Table 2
Genotype differentiation of -572 G>C polymorphism Gene IL-6 in Balinese women with osteoporosis and without osteoporosis

Variable		Groups		c^2	*p*
		Case	Control		
Gene	GG (%)	11(100)	2(4.87)	41.85	0.001
	CCGC (%)	0 (0)	39(93.13)		
Total		11	41		

Remarks: case; Balinese women with osteoporosis, control; Balinese women without osteoporosis,
gene; -572G>C polymorphism of IL-6 gene

Table 3
Genotype differentiation of -174 G>C Gene IL-6 in Balinese women with osteoporosis and without osteoporosis

Variable		Groups		c^2	*p*
		Case	Control		
Gene	GG (%)	1(9)	2(4.87)	0.283	0.518
	CCGC (%)	10 (91)	39(93.13)		
Total		11	41		

Remarks: case; Balinese women with osteoporosis, control; Balinese women without osteoporosis,
gene; -174G>C polymorphism of IL-6 gene

The difference of frequencies of genotype between these two groups (case and control) of -572 G>C IL-6 gene polymorphism were significant ($p<0.05$). The frequencies of GG genotype -572 G>C polymorphism of IL-6 gene has been confirmed as 100% in case (osteoporosis). No significant difference in the frequencies of GG/CC/GC genotypes of -572 G>C polymorphism of IL-6 gene in the control group. -174 G>C polymorphism of IL-6 gene confirmed no significant difference of genotype frequencies between case and control ($p>0.05$). GG/GC/CC genotypes of -174 G>C polymorphism of IL-6 gene distributed almost the same frequencies between case and control.

DISCUSSION

In this research we investigated the genotype differentiation between case (osteoporosis) and control (non osteoporosis) in promoter region of

IL-6 gene -572 G>C and -174 G>C IL-6 gene polymorphism have been suspected to have a role in bone mechanism and differentiation especially in osteoclast differentiation. The important finding in this study discovered that GG genotype of -572 G>C polymorphism IL-6 gene in all the case (osteoporosis) but no significant genotype difference of -174 G>C polymorphism found in case and control.

In previous study explained that two polymorphism (-572 G>C and -174 G>C) in promoter region IL-6 gene correlated with BMD. Ferrari et al, 2003 told that GG genotype have association with the lower BMD in postmenopausal women (16) and Feng et al, 2003 published that CC genotype increased the bone fracture in postmenopausal woman in Japan.[17]

This research proved that GG genotype of -572 G>C polymorphism IL-6 gene associated with lower value of BMD (osteoporosis). The results same with the publication of Feng et al, 2003 and different with Magan˜a et al, 2008.[1]

This study concluded that GG genotype of -572 G>C polymorphism IL-6 gene could be used as genetic factor to look at the risk of osteoporosis in Balinese postmenopausal woman. The bigger population still needed to ensure that GG genotypes of -572 G>C polymorphism IL-6 gene as genetics tool to diagnose osteoporosis in postmenopausal woman.

The limitation of our study was small size for six month research period. The groups could be expanded as osteoporosis, osteopeni and normal would give stronger statistical results to look at the association between BMD and IL-6 polymorphism. However, the genotype difference between BMB and IL-6 polymorphism in Balinese postmenopausal women was the first in Indonesia populations.

The different results between other study explained that might be the ethinicity has the strong relation with the IL-6 polymorphism. New and different studied are needed to establish the new insight into this topic and to give informative results for understanding the genetic determination of postmenopausal osteoporosis

CONFLICT OF INTEREST

The author declare that they have no conflict of interest

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