Correlation between 25-Hydroxyvitamin D Serum Levels with Telomere Length in Premenopausal Minangkabau Ethnicity Women

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Abstract

Background: Aging is associated with an increased prevalence of non-communicable diseases. Premenopause is a phase in aging characterized by reducing the biological functions of cells. Aging can be measured by cell biomarker, namely telomeres. Telomere length can be influenced by various factors including vitamin D. Vitamin D acts as an anti-proliferation and anti-inflammatory cell. This study aimed to examine the relationship between 25-hydroxy vitamin D serum levels with telomere length of Minangkabau premenopausal women in Padang city.

Method: This cross-sectional study was recruited ninety-three Minangkabau premenopausal women in Padang city. The recruitment subject was using a multistage random sampling technique. 25-hydroxy vitamin D serum levels were measured by the ELISA method. Telomeres length was measured by qPCR using O'Challagan & Fennech method. The analysis was carried out by univariate and bivariate with Pearson correlation.

Results: The average serum 25-hydroxy vitamin D levels were 27.79±1.38 ng/ml. The average telomere length was 474.13 ± 2.02 bp. There was a correlation between 25-hydroxy vitamin D serum levels with telomere length ($r = 0.267$, $p = 0.01$).

Conclusion: This study concluded that there was a correlation between 25-hydroxy vitamin D serum levels with telomere length of Minangkabau premenopausal women in Padang city. An increase of 1 ng/ml 25-hydroxy vitamin D serum levels slowed down telomere shortening 0,583 bp.

Keywords: *Vitamin D, Telomere, Premenopause, Minangkabau.*

Introduction

Non-communicable diseases are a global and national problem faced today. Data from the World Health Organization (WHO) Global Report on Non-Communicable Disease in 2017 states that non-communicable diseases cause 40 million deaths per year or around 70% of deaths worldwide. This figure is expected to increase to around 52 million deaths per year in 2030. More than 9 million of all deaths from non-communicable diseases occur at the age of 40 years and over[1].

Data Riskesdas in 2013 showed an increase in the prevalence of non-communicable diseases from the previous year. Non-communicable diseases suffered

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by many people aged 40 years and over. Around 47% of the death rates due to noncommunicable disease in Indonesia are women[2].

Premenopause is a phase in the aging process, which is a transition from reproductive to non-reproductive periods that occur at the age of 40-55 years. During this time biological and endocrine changes such as the occurrence of estrogen hormone fluctuations that affect the menstrual cycle[3]. The hormone estrogen has a role as an antioxidant and increases telomerase activity. Low estrogen levels at the end of premenopause will increase the risk of non-communicable diseases that will be encountered when entering menopause in the next 5 until 10 years[4].

Aging at the cellular level can be measured by examining telomeres, which are cell aging biomarkers. Telomeres are structures that play a role in protecting and preventing the fusion and degradation of chromosomes located at the ends of chromosomes[5]. Telomer experiences a shortening of around 24.8-27.7 base pairs per year. This shortening process is in line with increasing age. Progressive shortening of telomeres causes aging, apoptosis or oncogenetic transformation of somatic cells[6].

Telomere length can be influenced by various factors, including body mass index, hormone therapy, antioxidant intake, chronic diseases, multivitamins, minerals and sex. One vitamin that has a role in maintaining telomere length is vitamin D. Vitamin D is a fat-soluble vitamin that acts as a steroid hormone that has many vital roles in mineral metabolism, bone health, proliferation, cell differentiation and apoptosis as well as anti-inflammatory properties. Research shows that vitamin D plays a role in maintaining genomic and telomere stability. Vitamin D levels can affect telomere length through anti-inflammatory mechanisms and controlling the rate of cell proliferation[7,8].

Indonesia is a tropical country that receives sun exposure throughout the year. Even though they are exposed to sunlight throughout the year the prevalence of vitamin D deficiency in Indonesia tends to be high at 50% in women aged 45-55 years[9], and 35.5% in women aged 60-75 years[10].

The high prevalence of vitamin D deficiency is associated with lifestyle and environmental factors, including the lifestyle of Indonesian women who tend to avoid sunlight, spend more time indoors, use of sunscreen and low intake of food sources of vitamin D[11]. Besides, older women are more at risk of vitamin D deficiency because of the reduced ability of the skin to synthesize vitamin D[12].

Vitamin D deficiency is associated with telomere shortening. This deficiency is related to the function of vitamin D in maintaining genomic stability[13]. However the effect of vitamin D on telomeres is still controversial. The purpose of this study was to examine the relationship between serum 25 (OH) D levels and telomere length of the Minangkabau premenopausal women.

Material and Method

This research is a cross-sectional study of 93 ethnic Minangkabau premenopausal women aged 40-55 years. Subjects were selected by multistage random sampling. The inclusion criteria in this study were willing to enter the study by signing an informed consent, aged 40-55 years, Minangkabau ethnic, having menstrual disorders and not using hormonal contraception. The exclusion criteria in this study were not coming and not being found when collecting research data, suffering from chronic diseases such as diabetes mellitus, hypertension, cancer (obtained from anamnesis). This research has obtained approval from the Andalas University Ethics Committee.

Telomere length is measured using the O'Callaghan and Fenech method. Venous blood was taken in 5cc cubital fossa then stored in a vacutainer. Blood samples were carried out by DNA isolation in the Biomedical Laboratory of the Faculty of Medicine, Andalas University, Padang. Following procedures in the Pure Link genomic DNA isolation kit. The isolation process consists of blood lysis, DNA binding, washing and eluting. The DNA concentration was measured using nano drops. Examination of 25-hydroxy vitamin D serum levels was carried out according to the procedure using an ELISA kit from DBC Canada. Data were analyzed using Pearson correlation parametric analysis. Abnormal data were transformed into Log 10. Significant correlation if $p < 0.01$. Data analysis using SPSS 20.

Results

The results of this study are shown in table 1. The average age of respondents was 46.52 ± 1.08 years, with a minimum age of 40 years and a maximum age of 54 years. The mean serum 25 (OH) D level was 27.79 ± 1.38 ng/ml with a minimum value of 10.96 ng/ml and a

maximum value of 58.88 ng/ml. The average telomere length was 474.13 ± 2.02 bp with a minimum telomere length of 102.32 bp and a maximum of 2041 bp.

Table 1. Average of Age, 25-Hydroxyvitamin D Serum and Telomere Length

Variables	Mean ± SD	Minimal value	Maximal Value
Age (year)	46,52 ± 1,08	40	54
25(OH)D Serum (ng/ml)	27,79 ± 1,38	10,96	58,88
Telomere Length (bp)	474,13 ± 2,02	102,32	2041

Table 2. Regression Model for 25-Hydroxyvitamin D Serum and Telomere Length

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1,833	,320		5,736	,000
	25(OH)D Serum	,583	,220	,267	2,648	,010

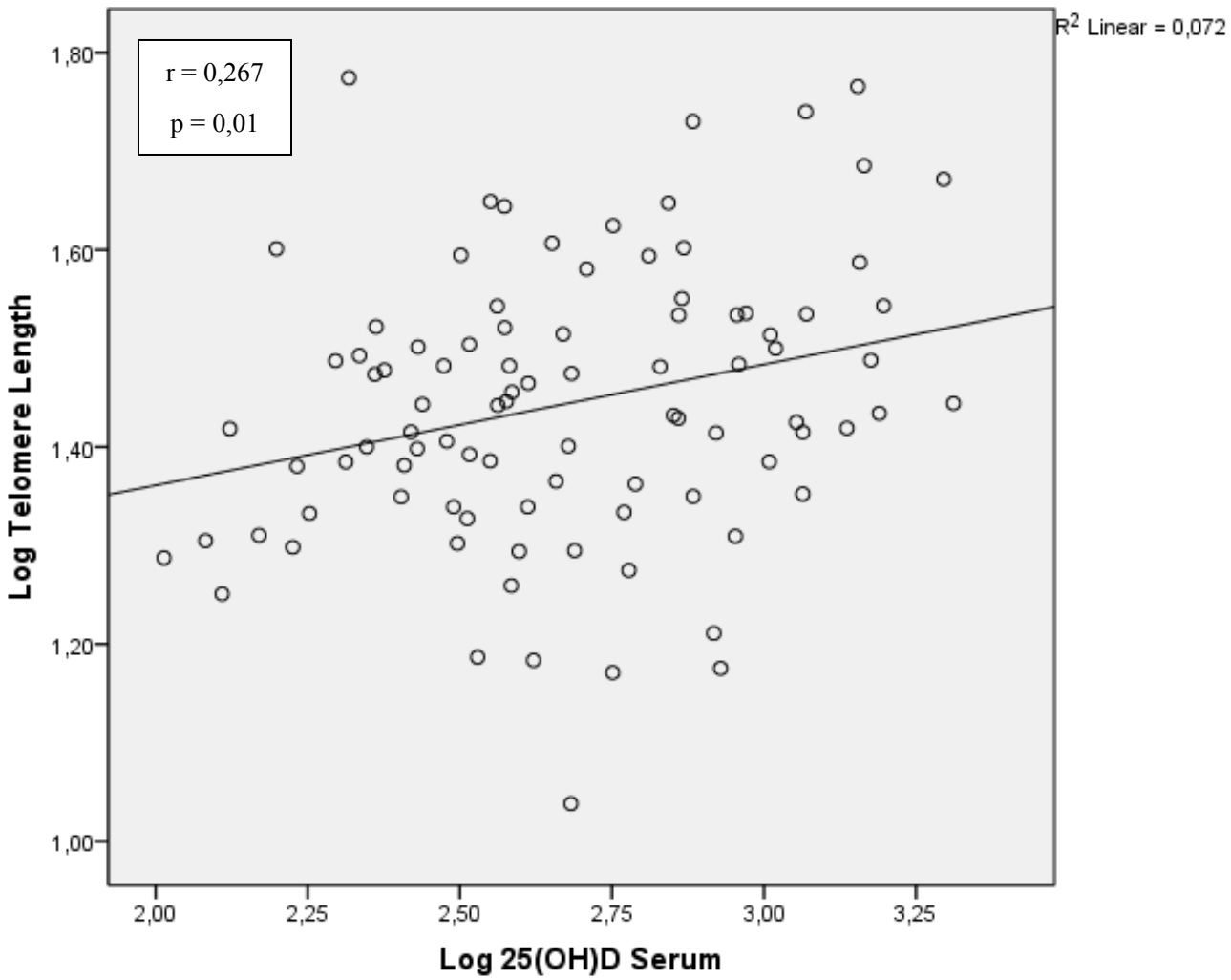

Figure 1. Correlation between 25-Hydroxyvitamin D Serum levels and Telomer Length

Figure 1 showed a significant correlation between serum 25 (OH) D levels and telomere length (p = 0.01, r = 0.267). R2 = 0.072 indicates that serum 25 (OH) D levels affect telomere length by 7%. Regression prediction equation in this study obtained Y = 1.833 + 0.583 X or telomere length = 1.833 + 0.583 levels 25

(OH) D (ng/ml). This means that every 1 ng/ml increase in serum 25 (OH) D levels slows shortening of telomere 0.583 bp (table 2).

Discussion

Telomeres are nucleoprotein structures at the ends of chromosomes that play a role in maintaining genomic stability and preventing fusion and degradation of chromosomes during the process of cell division. Telomeres consist of a sequence of 6 TTAGGG hexanucleotide base pairs that repeat hundreds or even thousands of times. Telomeres will experience a shortening with age. The length of human telomeres ranges from 4-15 kbp. Telomeres will experience a shortening of around 24.8-27.7 base pairs per year. Telomere length can be influenced by various factors, including body mass index, antioxidant intake, chronic diseases, physical activity and gender. Progressive shortening of telomeres will result in cell aging and an increased risk of degenerative diseases[6,14].

This study found that the length of telomeres of premenopausal women of Minangkabau ethnicity with an age range of 40-55 years ranged from 102.32 bp to 2041 bp with an average of 474.13 ± 2.02 bp. This study analyzes the telomere length using the O'Challagan & Fenech method, which measures the absolute length of the telomere. This result is lower than the results of a study by Dalgard in the Danish National Twin Registry of 405 women aged 18-64.3 years getting an average telomere length result of 7,010 ± 30 bp[15]. The study of Shin on 54 middle-aged obese women in Korea also obtained longer telomeres with an average of 8,290 ± 1970 bp in premenopausal women[16]. Richards's study obtained a mean telomere length in women in the United Kingdom of 7,000 ± 700 bp[13]. Different telomere lengths can be caused by differences in telomere length at birth, different measurement method used, age, race, diet, physical activity, estrogen levels and body mass index[17,18].

Serum 25 (OH) D levels are the best indicator of vitamin D status[12]. The results showed that serum 25 (OH) D levels of premenopausal women of Minangkabau ethnicity ranged from 10.96 ng/ml to 58.88 ng/ml with a mean of 27, 79 ± 1.38 ng/ml. The results showed that the average Minangkabau premenopausal woman experienced vitamin D insufficiency. Vitamin D insufficiency was based on classification by the Endocrine Society if serum 25 (OH) D levels were obtained between 21-29 ng/ml[12]. The research data showed that respondents who experienced vitamin D deficiency (level 25 (OH) D <20 ng/ml) were 18.3%, vitamin D insufficiency (levels 25 (OH) D 21-29 ng/ml) were 39.8 % and deficiency (25 (OH) D levels 30-100ng/ml) as much as 41.9%.

The results of this study are higher than the results of research conducted by Mazidi in 4347 participants in the National Health and Nutrition Examination Survey (NHANES) with an average level of 25 (OH) D 23.3 ± 9.4 ng/ml on female subjects. This difference in results can be caused by differences in climate and exposure to sunlight, race and different method used in the measurement[19].

25 (OH) D serum levels are related to telomere length. In this study the results obtained were significant and positive patterns with r = 0.267 and p = 0.01. This result is in line with research by Richards of 2160 women in the United States with an average age of 49.4 years. Research by Beilfuss also shows a relationship between serum 25 (OH) D levels and telomere length. This study analyzed data from the National Health and Nutrition Examination Survey (NHANES) 2001-2002 involving 11,039 respondents who found an association of each increase of 10 nmol/L levels of 25 (OH) D increasing telomere length by 0.03 kbp in women aged 45-59 years[20]. Liu conducted a Nurse Health Study study of 1337 white women with an average age of 59 years also found the relationship between serum 25 (OH) D levels with telomere length with a p-value = 0.02[21].

Williams in the Northern Finland Birth Cohort of 5096 respondents with an average age of 31 years, found no relationship between 25 (OH) D levels and telomere length[22]. Cassidy's studied also found no relationship between serum 25 (OH) D levels with telomere length. The difference in the results of this study can be due to differences in respondent's characteristics in terms of age, race, body mass index, intake and lifestyle patterns and climate conditions related to the amount of sun exposure[17].

The mechanism of the relationship between vitamin D levels and telomere length can occur through anti-inflammatory and anti-proliferation mechanisms[19]. Vitamin D in its active form, decreases the number of systemic inflammatory mediators such as interleukin-2 and tumor necrosis factor. Reduced systemic inflammation can result in reduced reactive oxygen

species (ROS) production to prevent telomere erosion[13]. Vitamin D plays a role in reducing the rate of cell proliferation, especially for cells that have the potential for mutations such as cancer cells. Besides, vitamin D can increase telomerase activity which is an enzyme that plays a role in maintaining telomere length[23].

Conclusion and Suggestion

The conclusion from the results of this study there is a relationship between serum 25 (OH) D levels with the telomere length of the Minangkabau ethnic premenopausal women. Every 1 ng/ml increase in serum 25 (OH) D levels slowed the telomere shortening to 0.583 bp. Women should increase their serum 25 (OH) D levels to slowed the telomere shortening.

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Ethical Clearance: This research was approved by ethics committee of Faculty of Medicine, Andalas University No.279/KEPK/FK/2017.

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