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RESEARCH ARTICLE

ASSOCIATION OF VITAMIN D LEVEL AND VITAMIN D RECEPTOR A-1012G POLYMORPHISM WITH PSORIASIS – CASE CONTROL STUDY

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ABSTRACT

Background: Psoriasis is a chronic inflammation of the skin caused by combination of genetic, immune and environmental factors. The Vitamin D receptors alongside with plasma vitamin D (25(OH)D) level have known to be related with psoriasis. Vitamin D receptor polymorphism is one of the multiple polymorphisms that predispose individuals to several diseases. It is possible that this polymorphism is different among psoriatic patients and healthy one particularly in Medan, Indonesia population.

Objective: We aimed to investigate associations between plasma level of 25(OH)D and one VDR gene polymorphism (A-1012G) with psoriasis.

Methods: Fourty four psoriatic patients and 44 healthy control subjects' DNA samples were obtained in this case control study and genotyped for A-1012G polymorphism by Polymerase Chain Reaction (PCR). The plasma vitamin D (25(OH)D) level of case and control subjects were examined using Enzyme Linked Immunosorbent Assay (ELISA).

Results: Significant lower plasma 25(OH)D levels were found in control group ($p<0.001$) which consist of mostly young adult female. There is no significant relationship between AA, AG and GG genotype variances of A-1012G polymorphism with psoriasis ($p=0.124$).

Conclusion: No significant association found between A-1012G polymorphism and psoriasis but there was a significant difference found between vitamin D level and psoriasis ($p<0.001$). From this study, vitamin D deficiency were more common in young adult female.

Keywords : Polymorphism, Psoriasis, Vitamin D, Vitamin D Receptor

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INTRODUCTION

Psoriasis is a skin condition which characterized by chronic inflammation caused by infiltration of inflammatory cells into the epidermis and alters the differentiation and proliferation of keratinocytes.[1] About 2-3% of the world's population is suffered from the disease [2], while the prevalence of psoriasis in Indonesia reaches 0.3% - 2.6%.[3, 4]

The etiology of this disease is still not clearly understood, but a combination of genetic, immune and environmental factors is known to be related with the incidence of psoriasis.[5] Several studies have found loci that are susceptible to psoriasis [6, 7] and one locus that is known to be very related to the incidence of psoriasis is PSOR1 in the major histocompatibility complex (MHC) class 1 region on 6p21 chromosome.[8] However, other studies have also found loci in non-human leukocyte antigen (non-HLA) regions that are susceptible to psoriasis, one of which is the vitamin D receptor (VDR) gene.[9]

The VDR is an intranuclear receptor. It regulates the transcription of vitamin D responsive gene and the active hormone 1,25-dihydroxyvitamin D3. VDR is also known as one of regulators of vitamin D levels (25(OH)D). Many suggest that low level of vitamin D correlated with VDR gene polymorphisms. These VDR polymorphisms have been known to be related with certain diseases, such as Cancer[10], Asthma[11], Atopic Dermatitis[12], Psoriasis[13] and many more. In the last few years, researchers have found that there was a correlation of low vitamin D levels (25 (OH) D) with the risk of occurrence and severity of psoriasis.[14, 15] As a result, topical vitamin D and its analogs have now been effective alternatives to treat psoriasis[16] in addition to other optional medicines.[17]

There are nearly 300 VDR gene polymorphisms have been analyzed, some are in the regulatory region.[18] In 2004, a new polymorphism was found in VDR promoter region of the start codon at exon 1a / Dp5 (-1163 to -818 bp). Band variant sequences showed the presence of A-G polymorphisms in the promoter region of exon 1a at -1012 base pair (bp) (A-1012G) [10]. Later, studies are conducted to find a correlation between this polymorphism with several diseases, one of them is psoriasis.[19, 20]

There are conflicting results regarding A-1012G polymorphism and its relation with psoriasis. Halsall failed to find correlation between this polymorphism with psoriasis[19] but in 2014, Richetta conducted the same study and found that A-1012G polymorphism is correlated with the disease. He found that A-1012G polymorphism may cause lower expression of VDR mRNA and increased the risk of psoriasis.[20] Although the functional role of this promoter variant is still largely unknown, this VDR (A-1012G) polymorphism is important to investigate because it may cause a decrease in the amount of VDR mRNA expression.[20] As is well known vitamin D needs to bind to its receptors to work in cells by forming VDRE complex, low amount of VDR will lead to less effective vitamin D treatment for psoriasis.

The aims of this study are to analyze the relationship between plasma vitamin D level with psoriasis and also to find a correlation between VDR polymorphism A-1012G (rs4516035:A>G) with psoriasis.

MATERIAL AND METHODS

Study Subjects

Samples were consisted of 88 blood plasma and isolated DNA, 44 psoriasis patients and 44 healthy control subjects. Samples were obtained from the previous study at the Laboratorium Terpadu Faculty of Medicine Universitas Sumatera Utara. The sample size was determined from the sample size calculation for case control studies. The study was approved by the Ethical Committee of the Faculty of Medicine Universitas Sumatera Utara and Haji Adam Malik Hospital, Medan, No: 365/TGL/KEPK FK USU-RSUP HAM/2019.

The case subjects were psoriatic patients from Haji Adam Malik Hospital and Universitas Sumatera Utara Hospital. The inclusion criteria for case subjects were psoriatic patients and over 18 years old. People who were fit in inclusion criteria but refused to cooperate in this study were excluded. Among the case group subjects, there were 19 men (mean age ± standard deviation (SD) = 45.0 ± 12.4) and 25 were women (mean age ± SD = 41.0 ± 15.5). The control subjects consist of students and staff of Universitas Sumatera Utara and Medistra Health Institute who were not suffer from psoriasis, have no history of psoriasis in the family, over 18 years old and were willing to participate in the study. Among the control subjects, there were 12 men (mean age ± SD = 29.0 ± 11.5) and 32 were women (mean age ± SD = 22.3 ± 2.6). The study was done at Laboratorium Terpadu Faculty of Medicine Universitas Sumatera Utara from May until September 2019.

Plasma Level of Vitamin D (25-hydroxy-cholecalciferol (25(OH)D))

Collected blood samples from previous study enabled measurement of the plasma level of a vitamin D3 derivative (25-hydroxy-cholecalciferol) using an immunoenzyme assessment technique (DBC 25(OH)D ELISA kit, Canada). The results were referred to normal serum ranges of 25(OH)D based on American Society for Bone and Mineral Research in 2011: deficient: 0-20 ng/ml; insufficient: 21-29 ng/ml; sufficient: 30-100 ng/ml.

Vitamin D Receptor Genotyping

Genotyping of A1012G (rs4516035) was done by PCR-based restriction enzyme digestion [10]. The polymorphism was amplified in a 25-µL PCR with 2 µL DNA, 12,5 µL PCR Master Mix (Thermo Scientific TM Dream Taq Green PCR Master Mix; Catalog number: K1081), 4,5 µL Nuclease Free Water and Forward Reverse Primers 1 µL each. The primer sequences were Forward: 5′-CCT CCT CTG TAA GAG GCG AAT AGC GAT-3′ and Reverse: 5′-GGA CAG GTG AAA AAG ATG GGG TTC-3′. The PCR conditions were described as followed: Denaturation step with 94 °C temperature for 5 minutes followed by three cycles of annealing step with 55 °C and 37 cycles with 65 °C temperature for 45 seconds. Every cycle underwent a denaturation step with 94 °C for 45 seconds and 45 seconds of extension at 72 °C followed by 2 minutes final extension at 72 °C.

The forward primer of A-1012G was adjacent to the polymorphism and introduced to EcoRV restriction site which occur in allele A, but not in allele G. The PCR product was imposed to restriction enzyme digestion with 5 Units of EcoRV enzyme (Thermo Scientific Eco32I

(EcoRV); Catalog number: ER0301) at 37°C for 2 hours followed by electrophoresis with 4% agarose gel. The restricted A allele showed two bands of 150 base pair (bp) and 27 bp, while the rare G allele that has no restriction site remained uncut and showed a single band of 177 bp (Figure 1).

Statistical Analysis

Age and sex of subjects were expressed as mean ± SD. Mann-Whitney U test was used to compare the plasma vitamin D between case and control group. Genotype and allele frequencies of subjects A-1012G gene polymorphisms were determined by direct counting. Associations between polymorphisms and psoriasis were assessed by using the Chi-Square statistics. In this study, a p-value of < 0.05 considered to be statistically significant.

RESULTS

The age and sex frequencies data among subjects were obtained from a previous study from a collaborative research with the same samples set.[21] The case group has a higher mean age (47.13 years) than control group (24.09 years). Also, there were more female (56.8%) who suffered from psoriasis than male (43.2%).

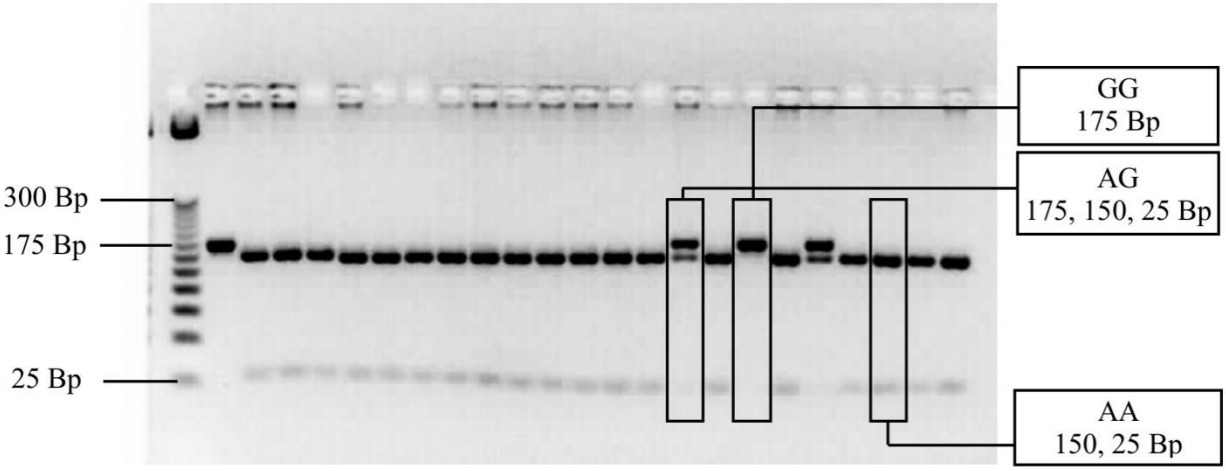

Figure 1. Restriction Fragment Length Polymorphism of A-1012G

Table 1. Association between plasma vitamin D level with Psoriasis

Plasma Vitamin D Level	Case (%)	Control (%)	*p*
Mean	25.92 ± 11.86	12.96 ± 7.27	<0.001*
Deficient	16(30.8)	36(69.2)	
Insufficient	14(66.7)	7(33.3)	<0.001**
Sufficient	14(93.3)	1(6.7)	

*data measured using Mann-Whitney U test for mean plasma vitamin D level in each group
** data measured using Chi Square for Vitamin D status in each group

Table 2. Association between A-1012G polymorphism with psoriasis based on allele and genotype

A-1012G polymorphism	Allele		Genotype		
	A (%)	G (%)	AA (%)	AG (%)	GG (%)
Case	72 (81.8)	16 (18.2)	29 (65.9)	14 (31.8)	1 (2.3)
Control	80 (90.9)	8 (9.1)	37 (84.1)	6 (13.6)	1 (2.3)
p-value	0.08		0.124		

p-value <0.05 considered as statistically significant

Table 3. Hardy-Weinberg Equilibrium (HWE) of A-1012G VDR gene polymorphism

Groups	Allele		HWE X^2(p)
	A	G	
Case	0.82	0.18	0.21
Control	0.91	0.09	1.35

p-value <0.05 considered as statistically significant

The relationship of plasma vitamin D levels with the incidence of psoriasis was analyzed and shown in Table 1. Categorical data were assessed using Chi Square and Mann-Whitney test for numerical data. The proportion of vitamin D deficiency was found more common in the control group compared to the case group (p <0.001).

The association of the A-1012G polymorphism with the incidence of psoriasis was shown in Table 2. The AA variant were more common in the control group (84.1%) rather than in the case group (65.9%) while the AG variance in the case group (31.8%) was greater than the control group (13.6%) and the GG variance was the same percentage between the two groups at 2.3%. There was no relationship between genotype variants of AA, AG and GG with the incidence of psoriasis (p = 0.124).

From the type of allele, allele A in the control group was more abundant than the case group that is equal to 90.9% in the control group and 81.8% in the case group. Allele G was found more in the case group at 18.2% compared to the control group at 9.1%. There was also no relationship found between alleles A and G with the incidence of psoriasis (p = 0.08).

Based on the type of polymorphism obtained in the study sample, analysis was made to assess the Hardy-Weinberg equilibrium (Table 3). The value of p = 0.21 in case groups and p = 1.35 in control group showed no significant results (p> 0.05). These results indicated that the genotypes polymorphism of the VDR A-1012G gene were in accordance with Hardy-Weinberg equilibrium.

DISCUSSION

In this study there were more female than male in psoriasis group. This contradicts with the result of WHO report

from different studies that showed more men were diagnosed with psoriasis.[2] This could be due to founder effect or women tend to be more concerned about their skin condition than men. The mean age in the case group was 47.13 years. The same result was also found in several studies which showed that the average psoriasis case was found at the age of 46 to 49 years (4th decade).[15, 22] There was a wide difference of mean age between case and control groups in this study. However, the level of vitamin D in adult could be measured from 18 until 70 years old with not much difference in terms of vitamin D needs and levels as long as there was no liver and kidney disfunction involved.[23]

In the examination of vitamin D levels, it was observed that insufficiency and deficiency were found more common in control group than case group. This interesting finding contradicts the results of various studies which state that vitamin D deficiency levels are more common in psoriatic patient compared with the healthy control group (not psoriasis) .[15, 24] There are several factors that can cause vitamin D deficiency such as decreased vitamin D synthesis due to lack of exposure to UVB rays, use of drugs, impaired liver and kidney function and malabsorption.[25] Moreover, subjects from case group could not recall the history of their previous psoriasis treatment. As is well known that vitamin D and its analogues may have been used as psoriasis treatment and they can alter patients vitamin D level. In addition, the control group is dominated by female students where the tight schedule keeps them away from sunlight exposure, physically become less active and in general female students use closed clothing and sunscreen. Studies showed that the most common predisposing factors for vitamin D deficiency in Southeast Asian countries were young people, women, living in urban areas and lack of outdoor physical activity.[26, 27] Nimitphong and Holick also stated that the prevalence of vitamin D deficiency in Asian countries is more common in young populations than older populations.[28]

Several studies have suggested that the role of genetics cause differences between the VDR gene polymorphisms in the psoriasis group and the normal population (not psoriasis).[1, 29] In this study the AA genotype was the most common variant followed by AG and GG in both the case and control groups, but the highest number of AA genotypes was found in the control group. These results are different from the other studies in Europe which found that AG is the most common genotype found in A-1012G polymorphisms followed by AA and GG.[19, 20] These results indicate that different gene polymorphism can be found in different races and ethnicities.

The relationship between A-1012G polymorphism with the incidence of psoriasis in this study was not statistically significant ($p= 0.124$), this is consistent with a previous study by Halsall that found no correlation between this polymorphism and psoriasis.[19] In other diseases the polymorphism of A-1012G was also not found to be related, such as in multiple sclerosis[30], polycystic ovary syndrome[31] and breast cancer[32], but the study in 2004 managed to find an association between the polymorphism of the VDR A-1012G gene with the incidence of malignant melanoma.[10] In contrast to the results of research conducted by Richetta in 2014 which states there is a relationship between the polymorphism of the VDR A-1012G gene with the occurrence of psoriasis. The study is the only study that found an association of AA

genotype polymorphism in the A-1012G gene with the risk of psoriasis.[20] Based on his study, the A allele in A-1012G polymorphism may cause a lower VDR mRNA expression that led to a higher risk of psoriasis.[20]

In this study, the A allele was more numerous than the G. Allele A was mostly found in the control group. Although the role of this VDR promoter polymorphism is not clearly known, it is possible that this polymorphism modulates binding of a transcription factor. This promoter area has been analyzed through TESS (Trademark Electronic Search System) database and demonstrates the polymorphism lies within the core sequence of GATA-3 binding site in the A allele, while in the G allele this binding site is not present.[33] In 1993, researchers showed that DNA strands containing the core sequence of AGATAT (the reverse orientation of the A allele) bound to human GATA-3, while none of the core sequence of AGACAT (the reverse orientation of the G allele) bound to GATA-3.[34] GATA-3 is a transcription factor that is able to regulate the differentiation of T cells from naive T cells to Th-2 cells[35], since psoriasis is an inflammation mediated by Th-1 cells, it can be concluded that the A allele in A-1012G polymorphism is a protection against psoriasis.

The Hardy-Weinberg deviation results showed that genotypes in both groups were in HWE. This result assumes that all 5 principles of HWE are fulfilled which are a large breeding population, random mating, no allelic mutation, no gene flow and no natural selection.

The limitation of this study was vitamin D levels only examined once, while a person's vitamin D status can change according to conditions and factors such as season, physical activity, food, genetic variation, that can affect a person's vitamin D levels within one year.[36] Multivariate analyses are also needed to find correlation between several other factors with psoriasis.

CONCLUSION

In conclusion, there is no association between A-1012G polymorphism with psoriasis. Vitamin D deficiency was found more numerous in control group which in majority consist of young females. Although there is no association found, allele A has a tendency to have a protective role against psoriasis. Further studies with larger sample size and multivariate analysis are recommended to do in the future to unravel more of association of A-1012G with psoriasis

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