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The Relationship Between FokI Vitamin D Receptor Polymorphisms on The Incidence Of Diabetic Retinopathy In Type 2 Diabetes Mellitus Patients At Sanglah Hospital Denpasar Bali

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ABSTRACT

Introduction: Complications of T2DM result in many organs of the body including visual disturbances (diabetic retinopathy). Polymorphisms in vitamin D receptor gene FokI, increase the risk of complications of T2DM including DR. This study aims to determine the FokI polymorphism in vitamin D receptor gene in DMT2 patients at Sanglah Hospital Denpasar Bali and to determine whether there is a relationship between vitamin D receptor polymorphisms FokI on the incidence of DR in DMT2 patients at Sanglah Hospital Denpasar Bali.

Methods: Design of the research was a cross-sectional analytical study. To detect the status of the FokI polymorphism, DNA segment amplification was performed using the PCR method using a primer specific for the FokI polymorphism. Furthermore, sequencing was performed to detect the base pattern at the location of the FokI SNP. Statistical analysis was performed by using SPSS 25.sav. P-value <0.05 was considered statistically significant.

Results: There were 38 samples that were eligible to be analysed and successfully sequenced. The mean age of the sample was 50.68 years with the proportion of DR and non-DR of 73.7% and 26.3%, with the majority of DR patients being male (65.8%). In our multivariate analysis, we found a significant relationship between VDR FokI polymorphism with hypertension among diabetic retinopathy patient (p=0.036; adjusted OR=18; 95% CI=1.694-191.233).

Conclusion: Heterozygous (C/T) VDR Fok I polymorphism was the most frequent in this finding. There is no significant relationship between FokI polymorphisms in vitamin D receptor gene and the incidence of RD in T2DM patients at Sanglah Hospital Denpasar.

Keywords: Diabetic retinopathy, polymorphism FokI, receptor vitamin D.
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INTRODUCTION

Diabetic is one of the aging signs. Complications of DM are many and dangerous, that's the reason DM is called the silent killer. DM can affect various organs of the body and cause visual disturbances in the eyes (retinopathy), cataracts, kidney disease, stroke, heart disease, sexual function disorders, impaired wound healing, blood vessel disease, lung infections and various other diseases(Bhatt *et al.*, 2016). Diabetic retinopathy (DR) is one of the DM complications, and leading cause of blindness worldwide. It is characterized by retinal macrovascular abnormalities caused by long-term hyperglycemia. The risk factors for DR are multi-factorial, one of which is genetic factors.[2]

In 2017 the prevalence of Diabetes Mellitus (DM) in the world reached 424.9 million people and is estimated to reach 628.6 million people in 2045 and makes DM the seventh leading cause of death in the world according to the International Diabetes Federation (IDF). Around 95% of the world's population who suffers from diabetes are type 2 DM. The increase in the prevalence of diabetic in Indonesia is quite significant, from 6.9% in 2013 to 8.5% in 2018. Bali province also had an increase of DM prevalence based on doctor's diagnosis from residents aged 15 years in 5 years significantly as much as 20%, which in 2013 was 1.5%, and in 2018 it was 1.8%.[3]

Wihandani's research in 2018 linked genetic polymorphisms to the incidence of RD in Bali. This study is a case-control study that includes 35 patients with type 2 DM (DMT2) with RD with a control of 35 patients with non-DR. PCR analysis and DNA sequencing were performed to detect polymorphisms C (-106) T and C (-12) G in the regulatory region of the Aldose Reductase (ALR2) gene. ALR2 functions

as an enzyme that limits the speed of the polyol pathway. This study concluded that the C (-104) T polymorphism in the regulatory region of the Aldose Reductase (ALR2) gene is a risk factor for RD in T2DM patients in Bali, Indonesia.[4] Understanding the clinical course of diabetes in this population is critical for establishing evidence-based clinical practice recommendations, identifying research priorities, allocating resources, and establishing health care policies.[5]

One of the factors for the incidence of DR is vitamin D deficiency. In the 1980s vitamin D deficiency was limited to the effects of rickets, osteoporosis and osteomalacia. Research in the last 10 years has proven the role of vitamin D in various non-skeletal conditions such as cancer/malignancy, high blood pressure, DM and cardiovascular disease. Vitamin D supplementation has shown a reduction in the incidence and disorders mentioned above.[6] The number of people with vitamin D deficiency is estimated to be relatively low in Indonesia and also countries with year-round sun exposure.[7] This estimate turned out to be not entirely correct. Vitamin D deficiency was found in more than a third (35.1%) of the total elderly female population in Jakarta and Bekasi.[8]

Vitamin D deficiency is generally defined as a serum 25(OH)D concentration of less than 20-25 nmol/L, whereas insufficiency is defined as a serum 25(OH)D concentration between 25 and 75 nmol/L. A 25(OH)D level of 75 nmol/L or higher is required for all physiological functions of vitamin D and should therefore be considered the optimal threshold. Without adequate sun exposure, it is almost impossible to achieve adequate levels of vitamin D from nutritional sources.[9]

The relationship between diabetes mellitus and vitamin D deficiency has been widely studied. Several studies have shown the relationship between polymorphisms and the presence of vitamin D receptors in pancreatic cells with a person's genetic susceptibility to developing type 1 diabetes. Other studies have shown that hypovitaminosis D as an environmental factor can cause diabetes. Meta-analytical studies have shown an association between vitamin D deficiency and type 2 diabetes. The possible mechanism underlying this role is that vitamin D stimulates beta cells directly and through its role in calcium levels in pancreatic beta cells causes an increase in insulin secretion. It is suspected that vitamin D also increases glucose absorption in the periphery and increases insulin sensitivity (Khan, *et al.*, 2011).

Several previous studies have shown that there are variations in vitamin D receptor polymorphisms in diabetes and the metabolic syndrome. Vitamin D receptor polymorphisms, especially FokI, increase the risk of complications of DM including DR.[11] A systematic review and meta-analysis of 15 observational studies with 17,664 study subjects showed that vitamin D deficiency increases the risk of DR. The results of the analysis showed that serum 25(OH)D < 20 ng/ml increased the risk of developing DR with OR + 2.03 (95% CI: 1.07-3.86) (Luo *et al.*, 2017).

One study suggests that there is a role for vitamin D and DR receptor polymorphisms. A Korean study conducted on 537 patients with type 2 diabetes showed that patients with the B allele (BB or Bb) tested with the BsmI polymorphism (rs1544410; BB, Bb, bb) had a lower risk of developing RD than subjects without the B allele. (bb) with a ratio of 7.4% VS 17.3%, p = 0.035.[13] A study in Lebanon in 136 subjects with DM showed that the mean 25(OH)D levels in subjects with DR were significantly lower than in patients without diabetic retinopathy (12.3 ± 5.5 versus 21.8 ± 13.7, p < 0.001). Multivariate analysis showed that low 25(OH) levels were predictors of diabetic retinopathy (OR 2.8, 95% CI 2.1–8.0, p < 0.05).[14] In a study that looked at the genotypic expression of the vitamin D receptor, TaqI, BsmI, and FokI showed that the vitamin D receptor polymorphism, TaqI was an independent protective factor against diabetes after considering the factors of age, sex, and body mass index (OR: 0.35, 95% CI= 0.144–0.831, p = 0.018).[15]

A study in Brazil showed that there was no significant difference in the incidence of type 2 diabetes in terms of genotype and allele frequencies of vitamin D receptor polymorphisms between the two groups.[16] A large cross-sectional study in Korea to look at 25(OH)D levels and the incidence of RD involved 18363 patients with DM. The results showed that the mean levels of 25(OH)D were low in DM patients. Vitamin D levels in men are 19.2 ng/ml, and in women are 17.9 ng/ml. There was a relationship between vitamin D levels and the degree of retinopathy. Severity of DR increased at low vitamin D levels (OR, 0.15; 95% CI, 0.03–0.83; p =0.043) (Jee *et al.*, 2014). Research that discusses the vitamin D receptor FokI and DR is important to see biomarkers of the prognosis of DR. Prevention of DR is very important to improve the quality of life of T2DM patients. Research linking vitamin D polymorphisms FokI and RD is still rare. Thus, this study aims to determine the FokI polymorphism in vitamin D receptor gene in DMT2 patients at Sanglah Hospital Denpasar Bali and to determine whether there is a relationship between vitamin D receptor polymorphisms FokI on the incidence of DR in DMT2 patients at Sanglah Hospital Denpasar Bali.

METHODS

Study Design and Sample Preparation

A cross-sectional analytical study to determine the relationship between *FokI* polymorphisms in vitamin D receptor and the incidence of DR in DMT2 patients. This research was conducted in Integrated Biomedical Laboratory Unit, faculty of medicine, universitas Udayana, and the DNA samples and related data were obtained from biological materials stored in the Department of Biochemistry, Faculty of Medicine, Udayana University. The eligible criteria of this sample were type 2 DM patients, ethnic Balinese, aged 40-60 years, have suffered from DM for 5-15 years and are willing to participate in the study. Diabetic retinopathy is diagnosed by means of slit-lamp bio-microscopic observation. The sample size is calculated with an error rate of 5% with 80% research power.

Sample Assessment

To detect the status of the *FokI* polymorphism, DNA segment amplification was performed using the PCR method using a primer specific for the *FokI* polymorphism. Furthermore, sequencing was performed to detect the

base pattern at the location of the *FokI* SNP (rs:2228570). We used 5'-GCC TGC TTG CTG TTC TTA CA-3' as forward primer, and 5'-ACC TTG CTT CTT CTC CCT CC-3' as reverse primer.

In processing sample, we mix the reaction consisting of one of primer reverse and one of primer forward with each concentration was 9.5 M, nuclease free water, 1 DNA template and 12.5 Go Taq green master mix twice with a total reaction of 25 l. Go Taq green master mix composition is reaction buffer pH 8.5, each 400 M dATP, dGTP, dCTP, dTTP, 3mM MgCl2, Taq DNA polymerase and loading dye (Promega, USA). Then we optimizing the annealing temperature of 57°C-62°C, the optimal temperature is 61°C. Amplification of DNA samples using the following cyclical parameters, as follows initial denaturation at 94°C for 5 minutes followed by 35 cycles at 94°C for 30 seconds, 61°C for 30 seconds, followed by 72°C for 1 minute, and the final extension at 72°C for 7 minutes. Electrophoresis of PCR amplification results using 2% agar. Examination of Fok1 polymorphism by sequencing technique was sent to Genetics Science. The sequencing results were then read using the Snapgene application to localize the FokI SNPs.

Statistical Analysis

Statistical analysis was performed with the SPSS 25.sav. Descriptive study was performed in frequency and mean data. Analytic study was assessed by using Mann-Whitney, fisher's-exact, and unpaired t-test. Furthermore to assessed variable independency and get rid the confounding factors, we use multivariate analysis (logistic regression) for all categorical study with p-value ≤0.25. P-value <0.05 was considered statistically significant.

RESULTS

The total sample of patients was 38 people, with an average age of 50.68±6.9 years, the majority of DR patients was male (65.8%). Diabetic retinopathy patients have suffered from type 2 DM an average of 7.09 ± 3.7 years, with some of them having a family history of DM (65.8%). Blood sugar levels in the sample were taken two hours after eating (post-prandial) and randomized blood sugar. Based on these examinations, it was found that the average random blood sugar level was 78.76±64.4 mg/dL, and the post-prandial blood sugar average was 258.10±78., mg/dL, with an average HbA1C. of 7.39±1.51%. In addition, several patients also had a history of hypertension ranging from pre-hypertension to grade II hypertension, with the majority of patients having a history of grade I hypertension (47.4%). Based on the condition of the patient samples, there were a total of 38 DMT2 patients, 28 samples had DR, and 10 samples were without DR Initially, it was planned to have 23 samples each, but the biological samples of the non-DR

Table 1. Baseline Characteristic of The Research.

Variables	N (38)
Age, (years)	50,68±6,9
Gender, n (%)	
Female	13(34,2)
Male	25(65,8)
Duration of diabetes, (years)	7,09±3,7
Family history, n (%)	
Yes	25(65,8)
No	13(34,2)
Blood pressure, (mmHg)	
Systolic	139,34±14
Diastolic	88,68±7,4
Blood pressure classification n (%)	
Normal (<130/<85 mmHg)	5(13,2)
Pre-hypertension (130-139/85-89 mmHg)	8(21,1)
Hypertension *grade* I (140-159/90-99 mmHg)	18(47,4)
Hypertension *grade* II (≥160/≥10 mmHg)	7(18,4)
BMI, (kg/m²)	23,74±3,15
Belly circumference, (Cm)	93,07±6,05
Blood sugar, (mg/dL)	
Random blood sugar	178,76±64,14
Post prandial blood sugar	258,10±78,5
HbA1C, (%)	7,39±1,51
Patient status, n (%)	
DR	28(73,7)
NDR	10(26,3)
Polymorphism (rs2228570), n (%)	
Wildtype (TT)	3(7,9)
Homozygous (CC)	8(21,1)
Heterozygous (CT)	27(71,1)

[a]Classification of blood pressure based on the 2020 International Society of Hypertension Global Hypertension Practice Guidelines; [b]Allele ancestor = T

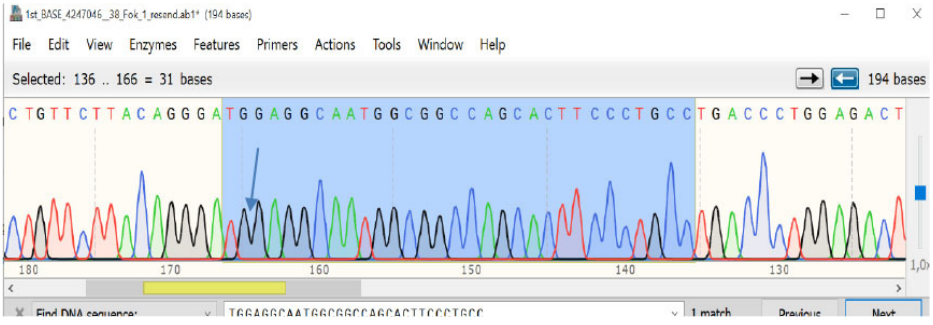

Figure 1. Visualization Of Sanger Sequencing Results from TT Genotype PCR Products (Wildtype).

Figure 2. Visualization Of Sanger Sequencing Results from PCR Products Of Homozygous CC Genotypes.

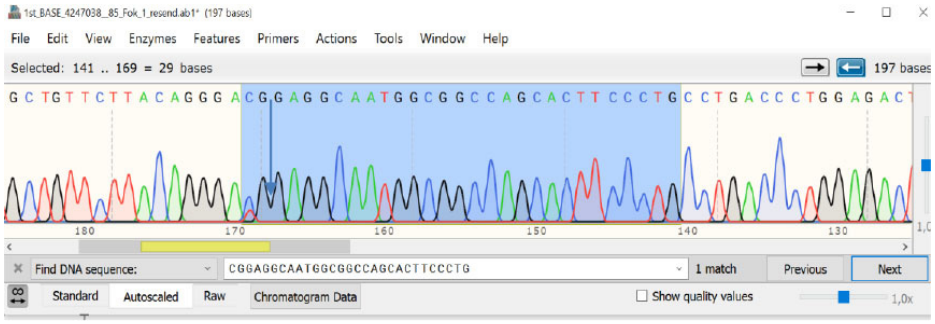

Figure 3. Visualization Of Sanger Sequencing Results from CT. Heterozygous Genotype PCR Products.

Table 2. Normality Test By Using Saphiro-Wilk Test.

Numeric variables	P-value[a]
Ages, (Years)	0,55
Duration diabetes, (Years)	0,04*
Systolic, (mmHg)	0,02*
Diastolic, (mmHg)	0,00*
BMI, (kg/m^2)	0,45
Random blood sugar, (mg/dL)	0,005*
Post prandial blood sugar, (mg/dL)	0,15
HbA1C, (%)	0,08
Waist circumference, (Cm)	0,04*

[a]Shapiro-Wilk; *p-value <0,05 (data distribution is not normal)

group were already dry so that their DNA could not be detected. For this reason, the available DR samples were added up to 28 samples. Eligible samples were processed by PCR and then electrophoresed. The PCR product was then sent to PT Genetics Science for sequencing. The Snapgene application is used to read the sequencing results (figures 1, 2 and 3). In the analysis of the polymorphism of the vitamin D receptor FokI at rs2228570, 7.9% of DM patients did not have this polymorphism (wildtype), while 71.1% of homozygous (CC) and heterozygous (CT) polymorphisms were found. More detailed basic characteristics can be seen in table 1.

Furthermore, the numerical variables were tested for normality to determine the distribution of the data using the Saphir Wilk test because the number of samples was <50 (Table 2). The distribution of the data is considered normal if the p value> 0.05, while the distribution was considered abnormal if the p value <0.05. Based on the results of the analysis, it was found that the variables of age, BMI, HbA1C and post-prandial blood sugar had normal data distributions (p>0.05) so that the bivariate analysis used was unpaired t-test, while other variables were distributed abnormal will be analyzed using Mann-Whitney.

Relationship Between the Baseline Characteristics Of The Patient Sample And The Incidence Of Diabetic Retinopathy

Furthermore, bivariate analysis was carried out to determine the relationship between the incidence of diabetic retinopathy (DR vs. Non-DR) with FokI polymorphism and confounding factors (age, BMI, blood sugar level, HbA1c, family history, hypertension, duration of diabetes and abdominal circumference). From the results of the analysis, it was found that there was no significant relationship between FokI vitamin D receptor polymorphisms and the incidence of retinopathy status. Re-classification of polymorphic genotypes to TT vs. Non-TT (TC+CC) and CC vs. Non-CC (TC+TT) also did not show a significant relationship. However, the results of the bivariate test showed that the proportion of hypertension in patients with DR (27 people; 71.1%) was higher than patients without DR (NDR) (6 people; 15.8%). Fisher-Exact test confirmed that the difference was significant (p= 0.012). However, there were no other variables that were significantly associated with retinopathy status in the study sample (Table 3).

Multivariate Analysis Of The Relationship Between Hypertension And The Incidence Of DR

Because there was no significant relationship between FokI vitamin D receptor polymorphisms and the incidence of DR in the bivariate test, multivariate analysis was continued to assess the only variable that was significantly associated with the incidence of DR. From the results of logistic regression analysis, it was found that hypertension status was a factor that increased the risk of developing DR in the study sample with an adjusted odds ratio of 18.00 (95% CI: 1.69-191.23; p=0.017) (Table 3).

DISCUSSION

Diabetes Mellitus is one of the symptoms of ageing in the human body. T2DM is a metabolic disorder characterized by increased blood sugar due to decreased insulin secretion by pancreatic β cells or impaired insulin function (insulin resistance). Insulin deficiency can occur

Table 3. Relationship Of Sample Characteristics With DR.

Variables	DR status		P-value
	DR	NDR	
Ages, (years)	50,86	50,20	0,800[a]
BMI, (kg/m²)	24,02	22,98	0,503[a]
Blood sugar, (mg/dL)			
Random blood sugar	188,43	151,7	0,151[c]
Post prandial blood sugar	265,04	238,70	0,370[a]
HbA1C, (%)	7,32	7,59	0,638[a]
Family History, n(%)			
Yes	20(52,6)	5(13,2)	0,263[b]
No	8(21,1)	5(13,2)	
Blood pressure, n(%)			
Normal	1(2,6)	4(10,5)	
Hypertension	27(71,1)	6(15,8)	0,016*[b]
Duration diabetes (years)	7,04	7,25	0,961[c]
Belly circumference, (Cm)	93,25	92,57	0,909[c]
Polymorphism (rs2228570), n (%)			
Wildtype (TT)	2(7,1)	1(10)	
Homozygous (CC)	6(21,4)	2(20)	0,784[b]
Heterozygous (CT)	20(71,4)	7(70)	0,783[b]
Regroup TT vs Non-TT			1,000[b]
TT	2 (5.3%)	1 (2.6%)	
TC+CC	26 (68.4%)	9 (23.7%)	
Regroup CC vs Non-CC			1,000[b]
CC	6 (15.8%)	2 (5.3%)	
TC+TT	22(57.9%)	8 (21.1%)	

[a]unpaired t-test; [b]Fisher's-exact; [c]Mann-whitney

Table 3. Logistic Regression Between Hypertension Variables and DR

Variable	Unstandardized Coefficients		Wald	Adjusted OR	95% CI		P-value
	B	SE			Upper	Lower	
Constant	-1,504	0,451	11,106	0,222			0,001
Hypertension (Yes vs No)	2,890	1,206	5,747	18,000	1,694	191,233	**0,036**

due to damage to pancreatic β cells due to external influences (chemicals, viruses, and bacteria), desensitization or a decrease in the number of glucose receptors in the pancreas gland due to the ageing process and insulin receptor damage in peripheral tissues. Insulin resistance mainly occurs due to obesity, lack of physical activity, and ageing (Chia *et al*., 2018).

T2DM can cause complications in various organs of the body, one of which is in the eyes, so that it causes vision problems. This condition is often referred to as diabetic retinopathy (DR). Several previous studies have shown variations in vitamin D receptor polymorphisms in T2DM and metabolic syndrome. Vitamin D receptor polymorphisms, especially FokI, increase the risk of complications of DM, including DR. For this reason, this section will discuss further the results obtained and their comparison with other studies. Vitamin D plays a role in immunoregulation, which indicates that vitamin D participates in developing several chronic diseases.[19,20] Vitamin D deficiency is common, especially in the elderly, where the ability to synthesize vitamin D is reduced.[21] Whereas low levels of vitamin D contribute to diseases due to ageing, one of which is DM.[22] Previous studies have found that vitamin D levels are positively correlated with insulin sensitivity in T2DM patients; this shows that vitamin D deficiency can affect glucose intolerance, altered insulin secretion, either through direct activation of vitamin D receptors or indirectly through calcaemic hormones and also through inflammatory processes.[21]

Vitamin D and its receptor complex play a role in regulating insulin secretion from the β cells.[21] It has been demonstrated in several studies that several vitamin D receptor polymorphisms are associated with DM and insulin secretion[23,24], as well as with obesity-related metabolic changes.[25] Variations in the FokI vitamin D receptor gene have been shown to correlate with the development and complications of diabetes, one of which is diabetic retinopathy.[26,27]

Baseline Of Diabetic Retinopathy Patients

Based on the characteristics, DR patients aged 50.68 ± 6.95, and most of them were male (Table 1). Not much different from a study conducted in Sweden in DR patients with a mean diagnosed age of 53.1

± 11.3.[27] However, research conducted at Sanglah Hospital in 2016-2017 found that DR patients were diagnosed at various ages ranging from < 30 years to 70 years. However, the majority of patients were aged between 50-69 years (70.4%). In addition, based on gender, most patients are male (66.7%); of course, these findings are inversely proportional to this study (Wibawa *et.al,* 2018). Based on the National Institute for Health and Care Excellence (NICE), the target HbA1C level of DM patients is 6.5%[29], while in this study, the HbA1C level of patients was still above the target (7.39±1.51%). This HbA1C level is not much different from the study conducted by Henricsson et al. in DR patients, 7.90 ± 1.61% for age 30 years or more.[27] In this study, the proportion of FokI vitamin D receptor polymorphisms in T2DM samples that did not have this polymorphism (wildtype/TT) was 7.9%, while 21.1% was homozygous (CC), and heterozygous (CT) polymorphisms were found to be 71.1%.

Another study divided the sample based on Non-Proliferative Diabetic Retinopathy (NPDR) and Proliferative Diabetic Retinopathy (PDR) in DR patients. Research conducted at Sanglah Hospital Denpasar was 77.8% of the PDR (Wibawa, *et. al.*, 2018). Similar studies found that PDR had a higher proportion than NPDR (>51%).[27] In PDR, new blood vessels mostly grow along the posterior hyaloid, and a sudden contraction of the vitreous can cause rupture of these fragile vessels.[30] However, because it uses stored biological material and its related data, it is not easy to assess the proportion of PDR and NPDR events in Sanglah Hospital through this study.

Relationship Of FokI Vitamin D Receptor Polymorphism With DR Incidence In T2DM Patients

Diabetic retinopathy is an ocular complication in T2DM patients. Microangiopathy due to hyperglycaemia in diabetic patients results in vascular leakage, leading to diabetic macular oedema and capillary occlusion. Capillary occlusion causes retinal ischemia and increased vascular endothelial growth factor (VEGF), resulting in neovascularization and the proliferative stage of DR. A study in China found a significant association between the duration of DM and HbA1C (p<0.05) in the incidence of RD. Meanwhile, there was no statistically significant relationship between gender, age, BMI, systolic and diastolic blood pressure, triglyceride levels, cholesterol, HDL-c and LDL-c.[31] These results are certainly inversely proportional to this study which only found a significant relationship between blood pressure and the incidence of DR (p=0.012).

The results of this study are in line with the meta-analysis conducted by Jiao et al. in 2018, which found that there was no significant relationship between the incidence of RD and FokI vitamin D receptor polymorphisms (p>0.05).[32] However, many other studies have shown a significant relationship between FokI vitamin D receptor polymorphisms and RD.[31]

FokI vitamin D receptor polymorphisms have even been associated with the incidence of DR in gestational diabetes mellitus (GDM) in Turkish women. In that study, it was found that the proportion of TT and CT genotypes increased in women with GDM with DR complications compared to GDM controls without DR complications.[26]

Several things may cause the differences between the findings of this study and previous studies. The number of samples can cause this difference. Zhong's study evaluated 110 non-RD samples, 94 RD samples, and 116 control groups.[31] The difference between the findings in this study and previous results could be due to differences in sample size and research design (case-control vs cross-sectional). The low proportion of samples with NDR in this study also caused the low representation of the comparison group (control). However, several studies have found that the FokI polymorphism is not associated with any aspect of T2DM.[33–36]

Regarding DM as a causative factor for DR, many studies have reported that vitamin D receptor polymorphisms are associated with important aspects of diabetes mellitus. FokI polymorphisms have been reported to be associated with aspects and risk factors of T2DM. Angel et al. (2018) found that the C allele, especially in the TC genotype, is a risk factor for DM in older Chilean adults, whereas this association is less evident in diabetic patients with vitamin D deficiency.[21] The significance of the C allele was also demonstrated by a meta-analysis which found that the C allele of the FokI polymorphism was an allele associated with DM mainly among Asian populations, but a similar relationship was not found in the BsmI, ApaI, and TaqI polymorphisms.[37] In this study, although the heterozygous genotype in all samples, the proportion of TC was the most found (71.1%), it was not statistically significant.

Associated with the significant relationship between blood pressure and the incidence of DR, high blood pressure also affects the blood vessels in the eye. The inability of the capillaries in the retinal endothelium to withstand pressure due to the increase in blood vessels gradually causes the capillaries in the endothelium to burst; several studies have proved this was in a study conducted in 1919 elderly T2DM patients showed a significant relationship (p<0.05) between systolic blood pressure and DR. However, the condition of hypertension is not associated with blood sugar control.[38] In line with previous studies, our study showed a significant relationship with the patient's blood pressure.

Hypertensive conditions also have a relationship with vitamin D polymorphisms, where the vitamin D receptor gene has an essential role in regulating the renin-angiotensin system (RAS), which affects blood pressure regulation; this has been demonstrated in a study with 280 patients with hypertension, showing a significant relationship between vitamin D receptor polymorphism Fok I genotype FF (CC) and hypertension. Therefore, the truncated vitamin D receptor protein formed in homozygous FF (CC) has a role in increasing renin and angiotensin II production, which increases blood pressure.[39] Although this study used a different population, namely DR patients with hypertension, a significant relationship was found to the FokI vitamin D polymorphism with TT vs non-TT genotypes (p=0.036).

Differences in vitamin D levels in DM patients with or without DR complications cannot be separated from the heterogeneity

of the response to vitamin D levels in each DM patient and the effect of gene variations in each individual. One of the genes that control vitamin D intake is the vitamin D receptor, so that this condition can cause vitamin D deficiency.[40] However, the relationship between allele variations of the FokI vitamin D receptor gene and the development of T2DM is still unclear.[25]

The limitation of this study is that the examination of vitamin D levels in DM patients with RD complications was not carried out. The proportion of RD and non-RD samples that can be PCR done is not balanced to affect the results. The sequencing of the vitamin D receptor gene was not performed on different polymorphisms, so it was impossible to determine which type of vitamin D receptor polymorphism had the most effect on RD patients and whether it was protective or increased risk.

CONCLUSION

The frequency of the *FokI* genotype was as follows: wildtype TT was 7.9%, at 21.1% homozygous CC and 71.1% heterozygous CT. There is no significant relationship between *FokI* polymorphisms in vitamin D receptor gene and the incidence of RD in T2DM patients at Sanglah Hospital Denpasar.

CONFLICT OF INTEREST

All researchers declare that there is no conflict of interest related to this article.

ETHICAL APPROVAL

This research has been approved by the ethics commission of Udayana University with no Ethical Clearance: 1052/UN14.2.2.vVII.14/LT/2021.

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AUTHOR'S CONTRIBUTION

All authors contribute equally in compiling this research article.

REFERENCES

1. Bhatt H, Saklani S, Upadhayay K. Anti-oxidant and anti-diabetic activities of ethanolic extract of Primula Denticulata Flowers. *Indones J Pharm*. 2016;27(2):74-79. doi:10.14499/indonesianjpharm27iss2pp74
2. Mas-Putrawati AA, Bakta M, Suastika K, Habiba-Muhiddin HS, Niti-Susila NK. Polymorphism of Vascular Endothelial Growth Factor Regio Promoter C(-634)G As a Risk Factor of Balinese Type-2 Diabetic Retinopathy. *Bali Med J*. 2015;4(2):49. doi:10.15562/bmj.v4i2.117
3. Kementerian Kesehatan RI. FactSheet Obesitas Kit Informasi Obesitas. *J Kesehat*. Published online 2018:1-8.
4. Wihandani DM, Suastika K, Agus Bagiada IN, Malik SG. Polymorphisms of Aldose Reductase (ALR2) Regulatory Gene are Risk Factors for Diabetic Retinopathy in Type-2 Diabetes Mellitus Patients in Bali, Indonesia. *Open Ophthalmol J*. 2018;12(1):281-288. doi:10.2174/1874364101812010281
5. Huang ES, Laiteerapong N, Liu JY, John PM, Moffet HH, Karter AJ. Rates of complications and mortality in older patients with diabetes mellitus : the diabetes and aging study. *JAMA Intern Med*. 2014;174(2):251-258. doi:10.1001/jamainternmed.2013.12956
6. Leidy. 基因的改变NIH Public Access. *Bone*. 2011;23(1):1-7. doi:10.1001/archinternmed.2008.604.Demographic
7. Pusparini P. DEFISIENSI VITAMIN D TERHADAP PENYAKIT (Vitamin D Deficiency and Diseases). *Indones J Clin Pathol Med Lab*. 2018;21(1):90. doi:10.24293/ijcpml.v21i1.1265
8. Setiati S. Vitamin D status among Indonesian elderly women living in institutionalized care units. *Acta Med Indones*. 2008;40(2):78-83.
9. Pilz S, Pilz S, Zittermann A, et al. Vitamin D testing and treatment : a narrative review of current evidence. *Endocr Connect*. 2019;8:27-43.
10. Khan KA, Akram J, Fazal M. Hormonal actions of vitamin D and its role beyond just being a vitamin: A review article. *Int J Med Med Sci*. 2011;3(3):65-72.
11. Zhang Y, Xia W, Lu P, Yuan H. The Association between VDR Gene Polymorphisms and Diabetic Retinopathy Susceptibility: A Systematic Review and Meta-Analysis. *Biomed Res Int*. 2016;2016. doi:10.1155/2016/5305282
12. Luo B, Gao F, Qin L. The Association between Vitamin D Deficiency and Diabetic Retinopathy in Type 2 Diabetes : Published online 2017:1-12. doi:10.3390/nu9030307
13. Hong YJ, Kang ES, Ji MJ, et al. Association between Bsm1 polymorphism in vitamin D receptor gene and diabetic retinopathy of type 2 diabetes in Korean population. *Endocrinol Metab*. 2015;30(4):469-474. doi:10.3803/EnM.2015.30.4.469
14. Ahmadieh H, Azar ST, Lakkis N, Arabi A. Hypovitaminosis D in Patients with Type 2 Diabetes Mellitus : A Relation to Disease Control and Complications. 2020;2013.
15. Gnanaprakash V, Bodhini D, Kanthimathi S, Ginivenisha K, Shanthirani CS. Association of Vitamin D Receptor (TaqI , BsmI , and FokI) Polymorphisms with Prediabetes and Type 2 Diabetes in Asian Indians. Published online 2021:29-36. doi:10.4103/jod.jod
16. Rodrigues KF, Pietrani NT, Bosco AA, et al. Lower vitamin D levels, but not VDR polymorphisms, influence type 2 diabetes mellitus in Brazilian population independently of obesity. *Med*. 2019;55(5):1-9. doi:10.3390/medicina55050188
17. Jee D, Han K, Kim EC. Inverse Association between High Blood 25-Hydroxyvitamin D Levels and Diabetic Retinopathy in a Representative Korean Population. Published online 2014:1-17. doi:10.1371/journal.pone.0115199
18. Chia CW, Egan JM, Ferrucci L. Age-related changes in glucose metabolism, hyperglycemia, and cardiovascular risk. *Circ Res*. 2018;123(7):886-904. doi:10.1161/CIRCRESAHA.118.312806
19. Chagas CEA, Borges MC, Martini LA, Rogero MM. Focus on Vitamin D, Inflammation and Type 2 Diabetes. *Nutrients*. 2012;4(1):52-67. doi:10.3390/nu4010052
20. Muscogiuri G, Altieri B, Annweiler C, et al. Vitamin D and chronic diseases: the current state of the art. *Arch Toxicol*. 2016;91(1):97-107. doi:10.1007/s00204-016-1804-x
21. Angel B, Lera L, Márquez C, Albala C. The association of VDR polymorphisms and type 2 diabetes in older people living in community in Santiago de Chile. *Nutr Diabetes*. 2018;8(1):31. doi:10.1038/s41387-018-0038-9
22. Meehan M, Penckofer S. The Role of Vitamin D in the Aging Adult. *J aging Gerontol*. 2014;2(2):60-71. doi:10.12974/2309-6128.2014.02.02.1
23. Ortlepp JR, Metrikat J, Albrecht M, von Korff A, Hanrath P, Hoffmann R. The vitamin D receptor gene variant and physical activity predicts fasting glucose levels in healthy young men. *Diabet Med*. 2003;20(6):451-454. doi:10.1046/j.1464-5491.2003.00971.x
24. Angel B, Lera L, Sánchez H, Oyarzún A, Albala C. FokI polymorphism in vitamin D receptor gene: Differential expression of TNFα in peripheral mononuclear cells of type 2 diabetic subjects. *Meta Gene*. 2016;7:1-6. doi:10.1016/j.mgene.2015.10.003
25. Filus A, Trzmiel A, Kuliczkowska-Płaksej J, et al. Relationship between vitamin D receptor BsmI and FokI polymorphisms and anthropometric and biochemical parameters describing metabolic syndrome. *Aging Male*. 2008;11(3):134-139. doi:10.1080/13685530802273426
26. Apayd M, Beysel S, Eyerci N, et al. The VDR gene FokI polymorphism is associated with gestational diabetes mellitus in Turkish women. Published online 2019:1-6.
27. Henricsson M, Nilsson A, Groop L, Heijl A, Janzon L. Prevalence of diabetic retinopathy in relation to age at onset of the diabetes, treatment, duration and glycemic control. *Acta Ophthalmol Scand*. 1996;74(6):523-527. doi:10.1111/j.1600-0420.1996.tb00727.x
28. Wibawa IMS, Budhiastra P, Susila NKN. Karakteristik Pasien Retinopati Diabetik di Rumah Sakit Umum Pusat Sangglah Denpasar Periode April 2016 - April 2017. *E-Jurnal Med*. 2018;7(11):6-11.

29. NICE. NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE. Published online 2016:1-3.
30. Singh R, Ramasamy K, Abraham C, Gupta V, Gupta A. Diabetic retinopathy: an update. *Indian J Ophthalmol*. 2008;56(3):178-188.
31. Zhong X, Du Y, Lei Y, Liu N, Guo Y, Pan T. Effects of vitamin D receptor gene polymorphism and clinical characteristics on risk of diabetic retinopathy in Han Chinese type 2 diabetes patients. *Gene*. 2015;566(2):212-216. doi:10.1016/j.gene.2015.04.045
32. Jiao J, Li Y, Xu S, Wu J, Yue S, Liu L. Association of FokI, TaqI, BsmI and ApaI polymorphisms with diabetic retinopathy: a pooled analysis of case-control studies. *Afr Health Sci*. 2018;18(4):891-899. doi:10.4314/ahs.v18i4.7
33. Al-Daghri NM, Al-Attas OS, Alkharfy KM, et al. Association of VDR-gene variants with factors related to the metabolic syndrome, type 2 diabetes and vitamin D deficiency. *Gene*. 2014;542(2):129-133. doi:10.1016/j.gene.2014.03.044
34. Xia Z, Hu Y, Han Z, et al. Association of vitamin D receptor gene polymorphisms with diabetic dyslipidemia in the elderly male population in North China. *Clin Interv Aging*. 2017;Volume 12:1673-1679. doi:10.2147/cia.s145700
35. Zhao Y, Liao S, He J, et al. Association of vitamin D receptor gene polymorphisms with metabolic syndrome: a case–control design of population-based cross-sectional study in North China. *Lipids Health Dis*. 2014;13(1):129. doi:10.1186/1476-511x-13-129
36. Glocke M, Lang F, Schaeffeler E, Lang T, Schwab M, Lang UE. Impact of Vitamin D Receptor VDR rs2228570 Polymorphism in Oldest Old. *Kidney Blood Press Res*. 2013;37(4-5):311-322. doi:10.1159/000350159
37. Li L, Wu B, Liu J-Y, Yang L-B. Vitamin D receptor gene polymorphisms and type 2 diabetes: a meta-analysis. *Arch Med Res*. 2013;44(3):235-241. doi:10.1016/j.arcmed.2013.02.002
38. Klein R, Klein BEK. Blood pressure control and diabetic retinopathy. *Br J Ophthalmol*. 2002;86(4):365-367. doi:10.1136/bjo.86.4.365
39. Swapna N, Vamsi UM, Usha G, Padma T. Risk conferred by FokI polymorphism of vitamin D receptor (VDR) gene for essential hypertension. *Indian J Hum Genet*. 2011;17(3):201-206. doi:10.4103/0971-6866.92104
40. Neyestani TR, Djazayery A, Shab-Bidar S, et al. Vitamin d receptor fok-i polymorphism modulates diabetic host response to Vitamin D Intake. *Diabetes Care*. 2013;36(3):550-556. doi:10.2337/dc12-0919