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POLYMORPHISM OF VASCULAR ENDOTHELIAL GROWTH FACTOR REGIO PROMOTER C(-634)G AS A RISK FACTOR OF BALINESE TYPE-2 DIABETIC RETINOPATHY

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Background: Diabetic retinopathy (DR) is one of the complications on diabetic mellitus (DM) patient as a micro vascular retina disorder which caused by a long term of hyperglycemia. This is one of the blindness causes in the world. This research aims to find out Polymorphism of VEGFC(-634)G gene as a risk factor of DR on the Balinese with DM type-2 (DMT2). **Method:** This study is applying two designs, analytical cross sectional and case control. The case is DMT2 patient with DR(+), DMT2 without DR as control. The sequencing technique was performed to evaluate polymorphism and plasma VEGF levels were determined by ELISA. **Results:** Cross sectional study (hospital based) came out with quite high number of DR, 57 people of 97 study samples. This study shows the existence of genetic variation on Gen VEGF C(-634)G, with most often genotype of CG (51.5%). Polymorphism C(-634)G as the risk factor of DR (OR=1.815 CI-95%= 1.077-3.057, *p*=0.025), and high level of VEGF were also significant (QR=3.75 , CI-95% 1.34-10.20, *p*=0.008). VEGF level with genotype of CG, CC and GG, not found any difference (*p*=0.245). Logistic regression shows that the most influential variable as the risk factor of DR is VEGF level (*p*= 0.007), polymorphism gen VEGF C(-634)G (*p*=0.022) and systolic blood pressure (*p*=0.023). **Conclusions:** Polymorphism of VEGF C(-634)G gene and high level of VEGF as the risk factor of DR, and can be used as a reference in handling early stage of DR patient to prevent blindness.

Keywords: diabetic mellitus; retinopathy; polymorphism; VEGF levels.

INTRODUCTION

Diabetic retinopathy (DR) is one of complications of diabetes mellitus (DM) patients. This was marked by retina macro vascular disorder caused by long term of hyperglycemic and as a cause of blindness worldwide.

DR risk factors are multi factors one of them is genetic factor. VEGF C(-634)G polymorphism gene was studied due to VEGF as an important factor cause of retina neo-vascularisation. In addition, research regardless of VEGF gene in some countries were varies and inconsistent. Researchs by Awata, *et al* (2002) in Japan, Suganthalakshmi, *et al* (2006) in India, and Yang, *et al* (2010) in China reported that VEGF C-634G gene polymorphism a risk factor of DR.[1-3]

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Research by Freathy, *et al* (2006) in UK, Nakamura, *et al* (2009) in Japan, and Chun, *et al* (2010) in Korea results in insignificant risk factor of DR.[4-6]

This study aims to support DR pathogenesis theory through the role of genetic factor of VEGF C(-634)G polymorphism gene on promoter region to induce DR. Specifically this study observed VEGF C(-634)G Balinese variances gene and VEGF levels for evaluating risk factor of DR.

MATERIALS AND METHODS

This study applied two designs, i.e. cross sectional and case control. Case and control were recruited from cross-sectional research. Case was DMT2 with DR complication patient and control was DMT2 without DR complication. This research was carried out at Internal Disease Division of Endocrine and Eyes Clinic Sanglah General Hospital, Bali-Indonesia. Sequencing technique was applied to evaluate polymorphism and ELISA for observing VEGF levels.

DMT2 of Balinese patients age of 40-60 years, 5-15 years suffering from the disease and willing to follow the research by signing informed consent were included in this study. Patient with glaucoma, eye pressure ≥ 21 mmHg, infection, intra ocular trauma, uveitis, vitreous blooding, intra ocular operation history, intravitreal injection of (anti VEGF, steroid), laser photocoagulation <6 month, stroke, and myocardial infarction were excluded. DR diagnosis was performed on the basis of slit-lamp bio-microscopic observation applying 78 or 90 dioptri condensing lens and retinal photo-fundus.

RESULTS

Hospital based cross sectional in this study reveals that 57 (58.7%) of 97 patients were DR. Average age of patients was 52.78±7.59 years, 59.8% of patients were male, DM duration was 6.62±2.33 years, 66% of family history with DM, systolic blood pressure of 131.55±14.09 mmHg, diastolic blood pressure of 89±8.10 mmHg, BMI 24.67±3.7 kg/m^2, and HbA1C of 8.5±2.55 %. This study obtained the present of genetic variants on VEGF C(-634)G gene with the most frequent genotype was CG (51.5%).

In the case control study, age, sexes, DM duration, BMI and HbA1C parameters were not different between case and control, except for blood pressure is different significantly (Table1).

Table1
Subject Characteristic between Case and Control Groups

Characteristic	Case (n=36)	Control (n-=36)	*p*
Age (years)	52.33±7.94	52.89±8.78	0.640
Sexes			
Male	22	23	0.500
Female	14	13	
DM (years)	6.32±3.86	5.71±5.11	0.580
BMI (kg/m^2)	24.46±3.54	24.52±4.03	0.640
Blood pressure (mmHg)			
Systolic	134.17±12.16	125.42±11.98	0.004
Diastolic	86.39±7.13	81.81±7.09	0.005
HbA1c (%)	8.61±2.51	8.13±2.52	0.970

Significant at $p < 0.05$

C(-634)G polymorphism as a risk factor of DR was 1.815 times as can be seen in Table 2 within OR=1.815, CI 95% = 1.077-3.057, p = 0.025). In this study, it was obtained that there was no different between distribution of allele frequencies CC, CG, and GG for case and control groups (p=0.365). However, allele C was higher in DR(+) compare to DR(-), in contrast allele G was higher in DR(-) compare to DR(+) as can be seen from Table 3. High VEGF levels is 3.75 times as a risk factor of DR (OR=3.75, CI 95% = 1.34-10.2, p=0.008) (Table 3). In this study, it was also gained that average VEGF levels with CG, CC and GG were not different significantly (p = 0.245), however, VEGF level of CG genotype was higher compare to other genotypes.

Table 2
VEGF C-634G Polymorphism Gene for Case and Control Groups as Risk Factor of DR

VEGF Polymorphism	Case N=36	Control N=36	*p*
CG	26	15	0.023
CC	1	5	
GG	9	16	

Significance at $p < 0.05$

Table 3
Allele Distribution of VEGF C(-634)G Polymorphism Gene

Variable	Allele G	Allele C	*p*
DR(+)	44 (61.10%)	28 (38.90%)	0.365
DR(-)	47 (65.30%)	25 (34.70%)	

Significance at $p < 0.05$

Logistic regression analysis indicates that the most influential variables as risk factor of DR were VEGF levels (p = 0.008), followed by VEGF C(-634)G polymorphism gene (p = 0.036) and systolic blood pressure (p = 0.038) as indicates by Table 4 and Figure 1.

Table 4
High VEGF Levels in Case and Control Groups as Risk Factor of DR

VEGF levels	Case	Control	*p*
High	20	9	0.008
Low	16	27	

Significance at $p < 0.05$

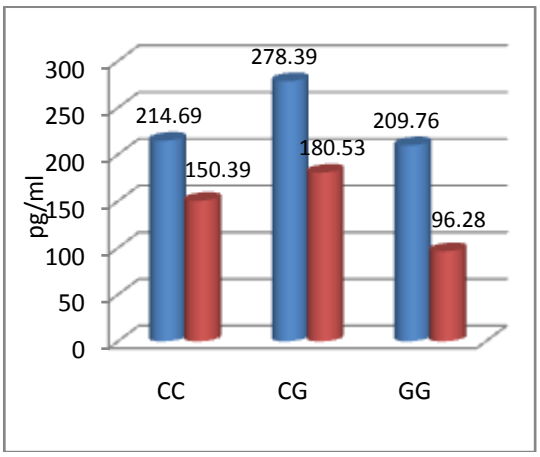

Figure 1
Comparison of VEGF Serum Levels to Polymorphism of VEGF C(-634)G gene, genotype of CC, CG and GG
case control

DISCUSSION

Results of VEGF C(-634)G polymorphism gene research worldwide were inconsistent. This is probably due to ethnic and environment difference.[1,2] DR hospital based research in others countries on the basis of DMT2 resulted in similar results to this research, i.e. > 50%. It has already known that 1/3 of DMT2 population will gradually suffer from DR. Therefore, number of people with DR will be increase in line with increasing of DMT2 patients.[7,8]

This study found that 66 % of the samples had history of DM. It has already known well that patients with DM family history will have a risk to gain DR. Duration of suffering to DM has also a risk to gain DR. It was reported that 2/3 of DM patients above 15 years will gain DR.[7,8]

Blood pressure was also affect the present of DR. DM patients frequently have high blood pressure (hypertenssion). WESDR reported that 17% of DMT2 patients will have hypertension and this will icrease to be 25% during 10 years. Undercontrol hypertension decreases retinopathy progresivity risk until 34%.[6]

Research by Chun, *et al* (2010) and Nakamura, *et al* (2008) obtained that insignificant results between the present of DR and m VEGF C-(634)G gene.[5,6] However, research by Awata, *et al* (2002), Suganthalaksmi, *et al* (2006) and Yang, *et al* (2010) gained a significant results between the present of DR and VEGF C(-634)G gene. They reporten that VEGF C(-634) gene is a risk factor of DR.[1-3]

This study resulted that VEGF C(-634)G gene CG genotype is a risk factor of DR (OR=1.815, CI 95% = 1.077-3.057, p = 0.023). Regardless of varies results, it was probably due to ethnic and environment different.

Correlation of allele frequencies as a risk factor of DR was also obtained varies between some researches. Allele frequencies correlated to risk marker for development of DR and increase activity on promoter region. Bleda, *et al* (2011) reported that G allele was significantly higher in DR(+) group (p=0.004).[9] On the other hand, Nakamura, *et al* (2008) found insignificant risk factor in either C and G allele to develope DR (p=0.244).[5] Research by Awata, *et al* (2002) found that C allele is higher in case group (0.48) compare to C allele in control group (0.35).[1]

This study obtained that allele frequencies is insignificant different between two groups. However, we gained that C allele is higher in DR(+) group compare to DR(-) group. On the other hand, G allele is higher in DR(-) group compare to DR(+) group.

Resaerch by Awata, *et al* (2002) and Yang, *et al* (2010) reported that VEGF level is higher in DR(+) group compare to DR(-) group.[1,2] This study was in line with study by Awata and Yang *et al*. This study obtained that high VEGF levels were observed higher in case compare to control group (OR = 3.75, CI 95% = 1.34-10.2, p=0.008). DM patients with DR complication have an increased of VEGF levels, therefore, it can be used as a predictive factor and guidance to manage DM patients with DR complication.

VEGF levels, generally can be affected by many factors, such as tissue hypoxia, hyper-glycemic, stress oxidative, environment, and other gene polymorphism besides VEGF C-(634)G gene polymorphism. Plasma VEGF levels are not similar to VEGF levels in vitreous and aqueous tissues. VEGF expression in endothelial vascular leads to increase of permeability of blood vein that induce increased of angiogenesis activity. Awata, *et al* (2002) found that VEGF level increase on VEGF C(-634)G gene CC genotype. This finding makes the research consistent with strong genetic correlation on C allele. VEGF C(-634)G gene correlated to lipo-polysaccharide (LPS) that induce VEGF production from peripheral blood mononuclear cells (PBMCs) in which in gene level that can increase transcription factor.[1]

Research by Yang, *et al* (2010) found that VEGF C(-634)G gene CC genotype has VEGF levels significantly higher compare to other genotypes. Therefore, they concluded that for people of Han China, CC genotype as genetic risk factor and main factor to induce high VEGF levels.[2]

Research by Bleda, *et al* (2011) found that serum VEGF levels with CC, CG and GG genotypes were not insignificantly different as risk factors of DR. Research by Nakamura, *et al* ((2008) also found that there was insignificant different between VEGF with VEGF C(-634)G gene polymorphism in either on vitreous and plasma.[9]

In this study, it was found that serum VEGF levels were insignificant different between CG, CC, and GG genotypes. However, average VEGF levels on DR group with CG genotype has a higher trend compare to CC and GG genotypes.

Polymorphism of C(-634)G gene was a binding site of myeloid zinc finger protein, MZF1 a transcription factor. MZF1 has a function of to bind DNA promoter target and regulate transcription. Their mechanisms have not been understood properly. It could probably due to lucipherase reporter system.[1]

Further developed research is evaluating mRNA levels on VEGF gene. This was probably strongly correlated to DR development and progressivity. Finding of genetic predisposition on DR can be applied to manage therapy based on individual genetic history and preferably carried out at initial stage DM diagnoses. Therefore, it will be more effective to inhibit DR progressivity.[5]

Logistic regression analysis resulted in that for all predicted independent variables correlated to DR, such as blood pressure (systolic and diastolic),

VEGF levels, and VEGF C(-634)G polymorphism gene, in which the most significant was VEGF levels (p= 0.007), followed by VEGF C(-634)G polymorphism gene (p=0.022) and finally systolic blood pressure (p=0.023).

CONCLUSION

Polymorphism of VEGF C(-634)G gene and high VEGF levels are risk factors of DR on Balinese DMT2 patients.

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