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Association of *ELMO1* Genetic Polymorphism (rs741301) with the Progression of Diabetic Kidney Disease in Balinese Patients with Type 2 Diabetes Mellitus

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Background: Diabetic kidney disease (DKD) is one of diabetes mellitus microvascular complications. Engulfment and cell motility 1 (ELMO1) protein interacts with dedicator of cytokinesis 180 (DOCK180) and cyclooxygenase (COX)-2, which affects gene expression in extracellular matrix (ECM) and causes glomerular damage in several mechanisms, such as ECM accumulation and renal tubules thickening. Single nucleotide polymorphism (SNP) rs741301 is one of the *ELMO1* genetic polymorphisms involved in DKD. The aim of this study was to evaluate the association between *ELMO1* rs741301 polymorphism and DKD in type 2 diabetes mellitus (T2DM) among Balinese.

Materials and methods: This study was an observational analytical study with case-control method. Subjects were divided into control and case groups comprising 40 subjects each. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of DNA from T2DM patients were performed to detect the polymorphism in *ELMO1* rs741301. Genotype and allele distribution obtained from this study was analyzed by chi-square (χ^2) test and Hardy-Weinberg equilibrium law (p<0.05; CI: 95%).

Results: There was no significant difference between genotype or allele distribution of *ELMO1* rs741301 with DKD incidence. Genotype AA against GG had odds ratio (OR) of 0.793 (p=0.814), AG against GG had OR of 0.602 (p=0.674), and A allele against G allele had OR of 0.761 (p=0.509).

Conclusion: There is no significant relationship between *ELMO1* rs741301 polymorphism and DKD in T2DM patients among Balinese.

Keywords: *ELMO1 gene, diabetic kidney disease, polymorphism*

Introduction

Diabetes mellitus (DM) is a multifactorial and complex metabolic disorder identified by a decrease in body capability to maintain blood glucose level. This happens because of defects in insulin secretion and/or action.[1,2] According to International Diabetes Federation (IDF), Indonesia ranked

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fifth among the top 10 countries with the most DM cases in the 20-75 years group, with approximately 10.7 million cases. This number is predicted to increase by 3 million in 2030, resulting in 13.7 million cases.[3] Diabetes increases the risk of vascular complications, either microvascular or macrovascular. One of the microvascular complications that needs serious treatment is diabetic kidney disease (DKD).

According to several studies, the pathophysiology of DKD is correlated with genetics. Engulfment and cell motility 1 (*ELMO1*) is a gene candidate which plays an important role in the development and progression of DKD.[4] ELMO1 protein interacts with dedicator of cytokinesis 180 (DOCK180) and activates Rac1, an important element of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) holoenzyme, which plays a crucial role in stimulating reactive oxygen species (ROS) production.[5]

Overexpression of *ELMO1* increases gene expression that plays a role in accumulation of extracellular matrix (ECM), which leads to renal tubule and basal membrane thickening. *ELMO1* expression could also inhibit cell adhesion to ECM.[4] There is an increase of transforming growth factor-beta 1 (TGF-β1) expression when the gene expression of *ELMO1* is increased. The exact mechanism on how *ELMO1* influences TGF-β1 still remains unknown, since TGF-β1 has its own mechanism on ECM accumulation. On the other hand, there is also an increase in the expression of integrin-linked kinase (*ILK*).[6] Several studies also point out that ECM accumulation is influenced by ELMO1 and cyclooxygenase (COX-2), resulting in the increase in the expression level of fibronectin as one of the ECM main components.[7]

ELMO1 rs741301 polymorphism in Indonesia, especially in Bali has not been reported. Therefore, this paper will focus on the proposition of *ELMO1* rs741301 polymorphism and how this polymorphism would be linked to DKD. The aim of this study was to evaluate the association between *ELMO1* rs741301 polymorphism and DKD in T2DM among Balinese.

Materials and methods

Study Design

This study was an observational analytical study with case-control method. A total of 80 subjects which have been selected by simple random sampling methods were divided into control and case groups comprising 40 subjects each. Subjects in the case group were T2DM patients with urinary albumin-creatinine ratio (uACR) >30 mg/dL, while subjects in the control group were T2DM patients with uACR≤30 mg/dL. DNA samples used in this study were the DNA of T2DM patients extracted in the previous studies.[8–10] This study has been approved by The Research Ethic Committee, Faculty of Medicine, Universitas Udayana with letter of approval number: 2703/UN14.2.2.VII.14/LT/2021.

ELMO1 rs741301 Genotyping

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the polymorphism in *ELMO1* rs741301. Genotyping was conducted by using forward primer 5'– CAC AAC TGT CTC AAC AGT CTG – 3' and reverse primer 5' – GCA ATA GAT TTT ATG AGG TGG TAG – 3'. Each PCR reaction contained 12.5 µL master mix, 2 µg forward primer, 2 µg reverse primer, 9.5 µL ddH_2O, and 2 µg DNA. DNA were amplified with steps as follows: Denaturation at 94°C for 5 minutes, followed by denaturation, annealing, and extension consecutively at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1.5 minutes repeatedly in 35 cycles, and final extension at 72°C for 10 minutes. Each 10 µL PCR result was then digested by 0.5 µL *Alu* I (Thermo Scientific™ West Bay Shore, NY, USA) in 30.5 µL reaction volume at 37°C for 16 hours. Two bands (164 bp and 24 bp fragments) were yielded from allele G, while 1 band (188 bp) was yielded from allele A. The amplification product was separated by electrophoresis in 3% agarose gel, stained by SYBR™ Safe DNA Gel Stain (Thermo Scientific™) and visualized on UV-transilluminator (UVITEC Ltd., Cambridge, UK).[11]

Data Analysis

Differences in characteristics of the subjects were analyzed using univariate analysis, while for the association between DKD risk and *ELMO1* rs7413011 polymorphism, the data were analyzed using chi-square (χ^2) test and odds ratio (OR) with confidence interval (CI) value of 95%. Data were also analyzed by Hardy-Weinberg equilibrium (HWE) law. Analysis is conducted by using SPSS 26 for Windows 64-bit software (IBM, Armonk, NY, USA) with p-value of <0.05 was considered as significant.

Results

Table 1 showed that there was no significant difference in the characteristics of the subjects between control and case group (p>0.05). Characteristics of the subjects included in

Table 1. Characteristics of subjects.

Variable	Group		*p* -value
	Case (n=40)	Control (n=40)	
Gender (male/female)	25/15	23/17	0.819
Age (years)	58.58±6.89	58.73±7.70	0.855
DM history (years)	11.10±4.99	10.88±6.14	0.437
Body height (cm)	163.05±7.76	163.41±8.24	0.699
Body weight (kg)	63.22±12.30	63.67±10.93	0.758
BMI (kg/m^2)	23.71±3.88	23.86±3.95	0.923
Systolic BP (mm/Hg)	139.55±28.11	127.95±17.13	0.052
Diastolic BP (mm/Hg)	77.30±10.63	76.05±8.42	0.566
Fasting blood glucose level (mg/dL)	148.15±43.15	168.25±52.33	0.065

this study were age, DM history, body mass index (BMI), fasting blood glucose, systolic and diastolic blood pressure (BP).

Table 2 and Table 3 showed that there were three genotypes (AA, AG, and GG) and two alleles (A and G) in T2DM patients in Balinese samples. G allele was found in 31 (54.4%) subjects with DKD, while A allele was found in 54 (52.4%) non-DKD subjects. There was no significant difference between genotype and allele with the occurrence of DKD (p>0.05) in T2DM patients.

Furthermore, this study also conducted an assessment of the suitability of the HWE law. In the total population, the frequency distribution of *ELMO1* gene genotype showed compliance with the HWE law (p>0.05) (Table 4).

Discussion

The results of this study shown in Table 2 and Table 3 suggested that there was no significant relationship between *ELMO1* rs741301 polymorphism and DKD in T2DM patients in Bali. Insignificant results were found in AA, AG, GG genotypes as well as A and G allele. These findings are in line with a study in Mexican-American ethnicity. The study uses 892 subjects, which is divided into 455 and 437 subjects in case and control group, respectively.[12] Another study also demonstrates that there is no significant relationship between single nucleotide polymorphism (SNP) rs741301 polymorphism and DKD in 141 and 416 subjects with American-Indian ethnicity in case and control group, respectively.[13]

A study demonstrates a relationship between *ELMO1* rs741301 polymorphism and DKD in control and case groups comprising 94 subjects each. It is found that GG and G as risk genotype and allele of DKD, respectively (p<0.05; OR=3.33).[4] Similar result is also found in a population in Iran (control and case groups comprising 100 subjects each), with the GG genotype having 2.5 times higher risk of DKD (OR=2.5) and the G alleles with OR=1.7.[14] In addition, G allele significantly affects the incidence of DKD in the north Indian population, consisting of 202 and 215 subjects in case and control group, respectively.[15] A study in Egyptian patients also found a significant relationship between *ELMO1* polymorphism and DKD with an OR=2.7 in GG genotypes.[16] Through a meta-analysis research, a significant relationship is also found between SNP and DKD.[17]

Differences between the results of this study and previous studies could be affected by several factors, including complexity of *ELMO1* gene pathway in affecting the incidence of DKD. *ELMO1* gene is not only interacts with a single pathway that causes the development of DKD,

Table 2. Genotype distribution of *ELMO1* rs741301.

Genotype	Group		OR	*p* -value
	Case, n (%)	Control, n (%)		
AA	16 (45.7)	19 (54.3)	0.793	0.814
AG	17 (51.5)	16 (48.5)		
GG	7 (58.3)	5 (41.7)	0.602	0.674

Table 3. Allele distribution of *ELMO1* rs741301.

Allele	Group		OR	*p* -value
	Case, n (%)	Control, n (%)		
A	49 (47.6)	54 (52.4)	0.761	0.509
G	31 (54.4)	26 (45.6)		

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Table 4. Genotype and allele distribution of *ELMO1* rs741301 with HWE.

Group		Case (n)	Control (n)	HWE
Genotype	AA	16	19	0.329
	AG	17	16	
	GG	7	5	
Allele	A	49	54	
	G	31	26	

but also inhibits the metalloproteinase genes that function as an antifibrotic gene and stimulates TGF-β1 which causing the thickening of the glomerular membrane and producing renal tissue fibrosis.[6,18,19] Phosphorylation of COX-2 is mediated by FYN, which is a member of the Src family from protein tyrosine kinase, resulting in cytoplasmic localization of COX-2. Since ELMO1 is also localized in the cytoplasm, an interaction between ELMO1 and COX-2 may occur, which leads to an increase in fibronectin promoter activity, resulting in the accumulation of fibronectin.[7,20] DOCK180-ELMO complex causes an activation of Rac1, which in turn creates a binding between GTPase-Rac1, increases NOX activity and affects ROS production, hence a disturbance in the glucose-stimulated insulin secretion (GSIS) occurs.[5,21] Due to the complexity of the *ELMO1* gene pathway to cause a DKD, examination of ELMO1, COX-2, DOCK180, and TGF-β1 serum levels are necessary for the subjects to control the confounding variables that may contribute to the incidence of DKD. It is also necessary to monitor the interaction between SNPs in *ELMO1* gene, especially rs1345365 and rs10951509, whether it is affecting rs741301 in increasing the incidence of DKD.

DKD is characterized by a progressive increase in albuminuria and is the cause of end stage renal disease (ESRD).[22] Inflammation in DKD involves the increase of oxidative stress in the kidney.[6,23,24] Several studies have found that ELMO1 cytokine plays an important role in the increase of oxidative stress[6], although the specific mechanism that explains the role of SNP rs741301 polymorphism in *ELMO1* gene in DKD is still unclear. In a series of studies, it is found that an increase in ELMO1 serum level causes Rac1 activation through the RhoG-ELMO-DOCK180 pathway complex, which increases the NOX and ROS[5,21] as well as fibronectin gene expression through COX-2 activity.[7]

This study also assessed the consistency of the genetic proportion of *ELMO1* rs741301 polymorphism in Balinese by the HWE equation. This equation states that "genotype frequency in a population will remain constant from one generation to the next in the absence of disturbing factors". The basic principle of HWE is the absence of mutations, natural selection, non-random mating, genetic drift, and gene flow.[25,26] Results showed that the frequency distribution of *ELMO1* genotype showed conformity with the HWE law (p>0.05). However, the HWE value in this study may not be representative to the entire population of Bali due to the selection of subjects and the number of subjects in this study were relatively small compared to studies in other populations. The proportion of genetic polymorphism that depends on the population observed both racially and ethnically may also affect the HWE value obtained in this study.[27]

Conclusion

It can be concluded that there is no significant relationship between *ELMO1* genetic polymorphism (rs741301) and DKD in T2DM patients among Balinese. Additional number of subjects is required in further studies to increase the power of the study. It is also necessary to conduct another research that assesses several other genetic polymorphisms and other SNPs that are associated with *ELMO1* to investigate the interaction between genes.

Authors Contribution

AL, PANKP and AASAA were involved in concepting and planning the research. AAIKK, AL and AASAA performed the data acquisition. AAIKK calculated the experimental data, performed the analysis, and designed the tables. AAIKK and AL drafted the manuscript. AAIKK, AL and PANKP interpreting the results. All authors took parts in giving critical revision of the manuscript and have read and approved the final manuscript.

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