REPUBLIK INDONESIA
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Nomor dan tanggal permohonan	:	EC00201800679, 18 Januari 2018
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Nama	:	**Dr.Riani Erna, Sp.M**
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Kewarganegaraan	:	Indonesia
Jenis Ciptaan	:	**Laporan Penelitian**
Judul Ciptaan	:	**Pengaruh Polimorfisme Alel Gen RAGE-429T/C Dan Gly8Ser Pada Penderita Retinopati Diabetic Di RS.DR.Moh.Hoesin Palembang**
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Effect of RAGE -429T / C and Gly82Ser Gene Polymorphism in Diabetic Retinopathy in General Hospital Mohammad Hoesin Palembang

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ABSTRACT

The development of diabetic retinopathy varies in a number of populations, suggesting genetic factors contribute to its pathogenesis, compatible with the severity of hyperglycemia that occurs. One of the underlying theories is the increased glycosylation of enzymatic proteins that are irreversible to the formation of AGE. To know is there any effect of RAGE Genetic allele polymorphism in diabetics retinopathy in General Hospital Mohammad Hoesin Palembang. This study was an observational analytical study comparative study (case control study) on 90 subjects consisting of 30 people with type 2 diabetes with retinopathy, 30 people with DM without retinopathy and 30 normal control people who had a family relationship with case group. Frequency of RAGE -429T / C & Gly82Ser alelle gene is performed by PCR amplification and RFLP (Restriction Fragment Length Polymorphism) by using Alu1 enzyme. Frequency of C alleles is 20% from the DR group, 15% from the DNR, and 5% from control group. Odds Ratio was 7,85 with p = 0.028. Frequency of wild genotype alleles in the Gly82Ser RAGE gene was found 40% in DR, 53.33% in DNR, 70% in control group, whereas the mutant allele were 60% in DR, 46.67% in DNR, 30% in control group. Frequency of Gly allele is 70% in DR, 23% in DNR, 85% in control group and frequency of Ser DR allele (30%), RND (70%), 15% in control group, Chi Square test with p = 0.065, α=0.05, Odds Ratio 5,57, CI: 95%. There is the effect of variant RAGE -429T / C and Gly82Ser allele polymorphism in diabetic retinopathy patients.

Key words: Alu1, diabetic retinopathy, receptors for advanced glycation end product,.

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INTRODUCTION

Diabetic retinopathy is all abnormalities in the retina, caused by DM and its prevalence is closely related to chronic hyperglycemia[1, 2]. Diabetic retinopathy is a disease that causes blindness in people with diabetes mellitus. More than 135 million individuals suffer from diabetes or 2-4% of the population in the world [2]. WHO estimates that by 2025 there will be 300 million people worldwide suffering from Diabetes Mellitus both type 1 and type 2 [3].

Diabetic retinopathy is the most common type of retinopathy. The mechanisms underlying microvascular disorders are not known, but are thought to be related to duration of exposure to hyperglycemia as a major risk factor for the development of diabetic retinopathy[4, 5]. Exact data on the prevalence of diabetic retinopathy in Indonesia has not yet been established. But from several research professions mentioned between 10-32% [6]. In Policlinic General Hospital Mohammad Hoesin Palembang for 5 years obtained visits of DM patients to the Eye Polyclinic based on medical records are 2005: 98 cases, 2006: 186 cases, 2007 : 235 cases, 2008: 461 cases, from 2009 to July 2009 there were 275 new cases.

Kumaramanickavel *et al*, study on 2002 in the Indian population showed that the development of diabetic retinopathy varied in some individuals, and was significantly correlate with the Gly82Ser gene polymorphism of RAGE gene in Indians who suffered from diabetic retinopathy [6]. The presence of polymorphism correlation between the RAGE Gene (Gly82Ser, G11704T, A2184G, G2242A, -429T / C, -374T / A) and diabetic retinopathy was also found in both Chinese and

European populations.[7] Until now, there is no study have shown any correlation between RAGE gene polymorphism with the incidence of diabetic retinopathy in mutant or polymorphic malay populations, it is not yet known how RAGE gene contributes to the pathogenesis of Diabetic Retinopathy.

The molecular biology approach allows to detect the presence of suspected polymorphisms and contribute to the onset of early diabetic retinopathy. So it can be used as an early detection marker and precise preventive action and more targeted therapy.

MATERIAL AND METHODS

Research Design

This study is an observational analytical type of comparative study (case control) with cross sectional approach. The study participants were diabetic retinopathy patients in South Sumatera, patients with type 2 diabetes without retinopathy and control populations without diabetes mellitus who met the acceptance and rejection criteria that came to General Hospital Mohammad Hoesin PalembangVitreo Retinal subdivision during the period November 2009-March 2010. After examination of BSN, BSPP, BMI (body mass index), age, sex, weight, height, blood pressure, duration of DM. (Table 1), an ophthalmological examination was performed with direct and indirect ophthalmoscopy. Sample criteria were diagnosed with DM type 2 > of 5 years. Retinopathy criteria was set based on ETDRS. 30 subjects DM with retinopathy, 30 subjects DM without retinopathy, 30 subjects control group expressed without diabetes or retinopathy. The independent variables are the RAGE -429T / C and Gly82Ser genetic alleles variants. All subjects were explained for the purpose of the study and signed the informed consent with the ethical feasibility test based on the Helsinki declaration.

Laboratory Techniques

Blood samples which taken through 2 ml antecubital venous puncture were inserted into tubes containing anti-coagulant ethylene diamine tetra acid (EDTA) for DNA and PCR extraction. DNA isolation by Chelex-100 method. Gen RAGE -429T / C on promoter amplified PCR RGR 1 Forward 5'-GGGGGCAGTTCTCTCCTC-3 'and RGR 1Reverse5'TCAGAGCCCCCGATCCTATTT-3' primer's (Eurogentec AIT biotech from Singapore) Gen Gly82Ser on exon 3 amplified PCR RGR 2 Forward 5'-CACTGTTTAGGCCCTGCTTC -3'and RGR2 reverse 5'GGAATTCTTACGGTAGACACGG-3'. Amplification consisted of 30 cycles, each cycle consisting of 95^{0}C for 1 minute, annealing reaction at 59,5^{0}C and 60^{0}C for 1 min, extension primer for 1 min at 72^{0}C, followed by final extension for 5 min at 72^{0}C.

Restriction Enzyme Technique Endonuclease (RFLP)

The polymorphism of Gen-429T / C is recognized as a change (AG CT) from Timin (T) to Cytosine (C). The site can be identified by the Alu1 restriction enzyme. Alu1 enzyme is an enzyme used to cut the primary PCR product that recognizes the AGTC site. While the Gly82Ser gene at codon number 82 (GGC / AGC), restriction enzyme was optimized at 10u / 5µl PCR product (20 µl) by Alu1 enzyme for 2 hours at 37^{0}C. The results of DNA amplification and PCR technique quality were seen using agarose electrophoresis technique (3% concentration) with electroporesis apparatus (Horizontal MiniSubDNA Biorad) containing TBE 1x (Tris-Boric acid-EDTA, 10.8 g / L Tris pH 8.0 containing 5.5 g / l of Boric Acid and 0.5 M EDTA pH 8.0) and 0.1% Ethidium Bromide intercalator added. At a voltage of 110 volts (figure 1, 2). The results of the restriction enzyme was analyzed and detected by using Gel Doc 1000 (Biorad, USA) to visualize with ultraviolet light at a wavelength of 300 nm and recorded.

Table 1: Characteristics of Study Subjects DR and DNR

Group	Sample Size	Mean Age	% Male	Mean BSN	Mean BSPP	Mean BMI	Mean systolic / dyastolic
Diabetic Retinopathy	30	60,60± 8,45	46,7	210,47± 111,46	243.57±84,82	21,79±2,79	80,67±11,43/131,00±20,23
DM + Non Retinopathy	30	55,37±7,01	43,3	234,03±111,69	255,83±79,74	23,11±4,27	81,00±8,449/131,00±18,45
Control	30	32,40±11,88	56,7	110.93±25.62	115,73±21,31	22,06±2,72	76,67±21,23/109,67±11,885

RESULTS

This study showed that the RAGE-429T / C gene polymorphism with genotype detection of RAGE -429T / C gene with the distribution of T type alloys in the retinopathy group was 80%, and in the non-retinopathy group there were 85% and 95% normal group of people , and a mutant allele or C is 20%, and mutants in the non-retinopathic DM group are 15%. The Odds ratio of 7.85 means

that the genotype of the wild type allele acts as a protector, it can be said that the risk of the patient for retinopathy is greater than 7.85 times in patients with a mutant allele (C).

In this study found the highest frequency of allele G is 85% in control group while allele S gene is 9 peoples or 30% highest in retinopathy group. This shows mutant allele polymorphism is more than 10% in retinopathy group.

From Odds ratio = 5,57 (5,38-5,47) it means that genotype of Gly82ser allele (wild type) function as protector, it can be said that patient risk for retinopathy is bigger 5,57 times in patient with allel GS / SS (mutant).

Table 2: Frequency Genotypes of RAGE -429T / C and Gly82Ser Gene Polymorphisms

		Group		
	Genotype	**DM + Retiopathy**	**DM + Non Retinopathy**	**Control Goup**
	Wild type	18(60%) /12 (40%)	21(70%) /16 (53,33%)	27(90%)/21 (70%)
	Mutan	12(40%) /18 (60%)	9(30%) /14 (46,67%)	3(10%) 9 (30%)
Total	90	30/30	30/30	30/30

Table 3: Frequency Allele Polymorphism of RAGE -429T / C and Gly 82Ser gene alleles

		Group		
	Allele	**DM + Retinopathy**	**DM + Non Retinopathy**	**Non Retinopathy**
Allele Frequency	T/G	0.8/0.70	0,85/0.23	0,95/0.85
Allele frequency	C/S	0.20./30	0,15/0.7	0,05/0,15
Total		1,0	1,0	1,0
RAGE allele gene -429T/C		*Odds ratio=7,85 (7,16-6,83) CI;95% p=0,028*		
Gly82 Ser allele gene		*Odds ratio=5,57 (5,38-5,47) CI 95%, p=0.065*		

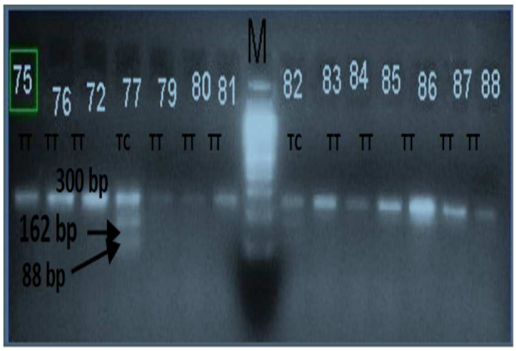

Figure 1: Gene polymorphism RAGE -429T / C with electrophoresis (Gel Agarose 3%)

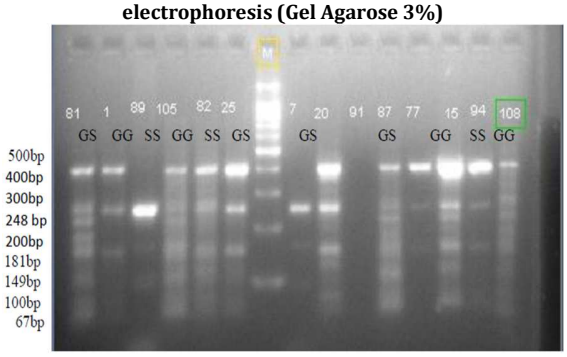

Figure 2: RAGE Gly82Ser gene polymorphism with electrophoresis (3% Agarose Gel)

DISCUSSION

The RAGE-429T / C and Gly82Ser gene polymorphisms in the retinopathy patients obtained the genotypic frequency of RAGE-429 T/C is TT (wild-type alleles) 18 people (60%) DR, 21 non-retinopathic DM patients, and control group 27 people.

The frequency of the T allele in the RAGE -429T/C gene is 80% in DM + retinopathy, 85% in DM without retinopathy, 95% in control patients. C alleles in retinopathy is 20%, 15% in non-retinopathic DM group, and in the normal control group, there is 5% with odds ratio = 7.85 CI; 95% p = 0.028. Similarly, Hudson B et al. (2000) in the United Kingdom, T allele frequency in retinopathy group in white race is 56.6% in T allele and 43.4% in C allele in retinopathy group and allele non retinopathy T = 73.3% and allele C = 24.7% with p = 0.012.10. It is different from the research of JiXiong X et al (2003) that there is no significant relationship between -429T/C and -374T/A RAGE gene with diabetic retinopathy in Chinese ethnic with type 2 DM where T allele frequency is 90.1% in DR and NDR, 87.8% C allele 9.9% in DR, and 12.2% in DNR.[15] Similarly, a study by Goulart et al (2008) which found gene-429T/C allele polymorphism was not significant in white and black race in the USA in 2008 in type 2 DM

patients with diabetic retinopathy, the allele frequency of RAGE -429T/C gene was T 0.837 in the DR group, and 0.849 in NDR group. C allele in retinopathy group is 0.163 and 0,151 in non retinopathy group with p => 0.30. Compared to black race, allele T 0.878 in group retinopathy group and 0.888 in NDR. While the C allele on DR is 0.122 and 0.112 in NDR with p = 0.65.57.

In the study we found a significant relationship that the RAGE -429T / C gene polymorphisms in diabetic retinopathy in patients with type 2 diabetes in Dr. Moh Hoesin Palembang, with Odds ratio = 7.85. To see more complete point mutations in the DNA chain should be done DNA sequencing.

Frequency of Gly allele on Rage Gly82Ser Gene is 70% in patients with retinopathy and DM without retinopathy is 23%, and normal control is 85%. Ser allele on retinopathy group was 30% and 70% in DM without retinopathy and 15% in control group. From the statistical test obtained the value of Odds ratio 5,57 (5,383-5,465). Study by Kumaramanickavel et al in India (2005) found GlyGly 80% allele, GlySer 18%, and SerSer 2% (Gly 89%, S 11%) for DNR (diabetic non retinopathy), and GlyGly 98%, GlySer 7 %, SerSer 2% (Gly 94.5%, S 5.5%) in DR group, last from control group (GlyGly 98%, GlySer 2%, SerSer 0%) Gly 98% allele, S 2%) compared between group with p = 0.03.

If it is odds ratio = 5,57 (5,383-5,465), means that the frequency of Gly82ser (wild type) allele acts as protector, it can be said that patient risk for retinopathy is greater 5.57 times in patients with GlySer / SerSer allele mutants).

Unlike Yoshioka et al in Japan in 2005 that found no correlation between Rage Gly82Ser gene in diabetic retinopathy population in type 2 diabetes mellitus. The same was reported in several European studies by Kankova *et al*, and Liu in China [15,17,18] Likewise with Hudson BI et al (1998) reported no significant correlation between Gly82Ser in ethnic Caucasian populations and Asia [58]. The characteristic similarity with the research done by Kumaramanickavel is that of Asian ethnicity.

Diabetic Retinopathy has many genetic variants (polygenic). In a meta-analysis performed by Abhary *et al.,* (2009) in Australia who attempted to investigate the relationship of genetic variants to the development of RD, of 30 genes and 34 different variants in a study cohort study of most polymorphisms occurring in type 2 diabetes mellitus, that the significant polymorphisms are NOS3, VEGF, ITGA2, and ICAM1[57] Gene polymorphisms of RAGE Gly82Ser and -429T/C gene are expected for the therapeutic approach, is inhibitors of RAGE, currently still invitro in Phase 2, of the RAGE itself will be suppressed with Aminoguinidine which binds AGE binding to the arteriole capillaries and prevents the formation of abnormal blood vessels, resulting in the lost perissue being reduced. Similarly, cerivastatin and olmesartan preparat are still in the invitro stage reported by Yamagishi et al (2007) to suppress angiogenesis, and block AGE-RAGE receptor signaling. In the study there was no examination of AGE levels, which by measuring AGE levels (advanced glycation end product) in the blood can sort the sample with abnormal AGE levels and can be associated with the degree of retinopathy.

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