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Gene Polymorphism of Angiotensin II Type 1 Receptor (Agtr1) As A Risk Factor of Diabetic Nephropathy In Indonesian Patients With Type 2 Diabetes Mellitus

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Abstract: *There are many works reported that angiotensin II type 1 receptor (AGTR1) gene polymorphisms associated with diabetic nephropathy and has variable expression in different ethnic groups. However, the distribution data of AGTR1 gene polymorphism in patients with diabetic nephropathy in Indonesia is still lacking. This study aimed to determine the polymorphism of* **AGTR1 gene A1166C** *in patients with Type 2 diabetes mellitus in Yogyakarta, Indonesia. The subjects of the research were 60 patients with Type 2 diabetes, 30 diabetic nephropathy and 30 without nephropathy respectively. Gene polymorphisms were determined by PCR-RFLP method and the PCR products were digested with* ***BsuRI*** *restriction enzyme. The results showed the frequency distribution of genotypes of AGTR1 gene in patients with diabetic nephropathy were 86,7% for AA, 13,3% for AC, and 0% for CC respectively. It can be inferred that in Indonesian genotype AA is predominant compared to AC or CC.*

Keywords: *AGTR1gene, A1166C, diabetes mellitus,* Type 2 diabetes, diabetic nephropathy

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I. Introduction

Diabetes mellitus (DM) is a metabolic disorder that often leads to chronic complications such as atherosclerosis, diabetic nephropathy, blindness, end-stage renal failure or end-stage renal disease (ESRD). There are different types of DM including Type 1, Type 2 and gestational diabetes mellitus [1]. Nowadays, diabetes mellitus is one of the diseases with a high prevalence in the world, with a rate up to four percent. Among all types of diabetes, Type 2 diabetes mellitus reaches prevalence rate of 80-90%. Such prevalence will keep increasing and predicted to reach 5.4% in 2025. Mostly, diabetes patients are 40 years old and above and show symptom of obesity [2]. In United States, there are approximately 15 million people suffered from diabetes, 10% with Type 1 DM and 90% with Type 2 DM [3]. Though Type 1 and Type 2 DM can lead to ESRD, however Type 2 DM is known to be the major cause of ESRD and only Type 2 diabetes can develop to overt nephropathy [4]. Several researches on Type 1 and Type 2 diabetes mellitus suggested that genetic factor can increase the risk of diabetic nephropathy [5].

Renin Angiotensin System (RAS) plays important role in pathophysiology of diabetic nephrophaty (ND) [6]. One of the hormones involved in RAS is angiotensin II. Angiotensin II is a strong vasocontrictor and as mediator in cellular proliferation and extracelular protein synthesis [7]. Previous studies suggested that angiotensin II is an active metabolic hormone which directly induces insulin resistance, increases free radical formation, reduces leptin and adiponektin production and increases oxidative stress [8]. Angiotensin II plays a role as pro-mitotic, pro-proliferatif and possesses angiogenic effects in cancer etiology or progression [9].

Angiotensin II mostly work through receptor 1 angiotensin (AGTR1) located in smooth muscle of blood vessel, heart muscle and kidney. Therefore, AGTR1 coding gene is considered as ND10 candidate. The angiotensin II is functioning to increase glomerular permeability by different mechanism such as high intraglomeruler pressure [6]. In A1166c AGTR1 polymorphism, there is an increase in interchange between adenin (A) to cytocin (C) at nucleotide number 1166 at the 3' which is not translated.

II. Materials And Methods

Following informed consent the demographic and laboratory variables of the patients including age, gender, BMI, diagnostic period, systolic and diastolic (hypertension/ non hypertension), total colesterol, HDL and triglyceride were noted. Furthermore, peripheral blood lymphocytes were isolated by the standard phenol extraction method and used as DNA samples in genotype analysis in this study. The A1166C variant of the AGTR1 gene was detected using primers: 5'-GCACCATGTTTTGAGGTTG -3' as the forward and 5'-

CGACTACTGCTTAGCATA- 3' as the reverse primers. The PCR product was digested with the FastDigest BsuRI restriction enzyme (Thermo Fisher Scientific Inc.). The data were statistically analyzed using chi square, t-test, odds ratio test.

III. Results

There were 60 patients with Type 2 diabetes mellitus, 30 subjects with nephropathy and 30 subjects without diabetic nephropathy, were included in this research. The demographic and laboratory variables of patients participated in this are presented in Table 1. The results of RFLP analysis with *BsuR1*of PCR on AGTR1 A1166C gene are shown in Fig 1. Genotypes AA, AC and CC were found in case and control subjects.

Table 1. Demographic and laboratory characteristics of the studied patients

Variables	DN (n=30)	Non-DN (n=30)	p<0.05
Age (yr)	59.63 ± 8.15	58.17 ± 7.96	ns
Gender (F/M)	14/16	13/17	ns
Total Cholesterol (mg/dl)	199.41 ± 39.94	191.40 ± 53.49	ns
Triglyceride (mg/dl)	159.85 ± 69.45	145.21 ± 74.96	ns
HDL (mg/dl)	42.64 ± 14.37	45.48 ± 13.87	ns
BMI (kg/m²)	24.62 ± 4.46	23.79 ± 2.94	ns
Systolic (mmHg)	134.67 ± 22.70	122.83 ± 14.00	s
Diastolic (mmHg)	84.67 ± 13.06	79.83 ± 7.71	ns
DN: Type 2 diabetes patients with nephropathy; Non-ND: Type 2 diabetes patients without nephropathy			

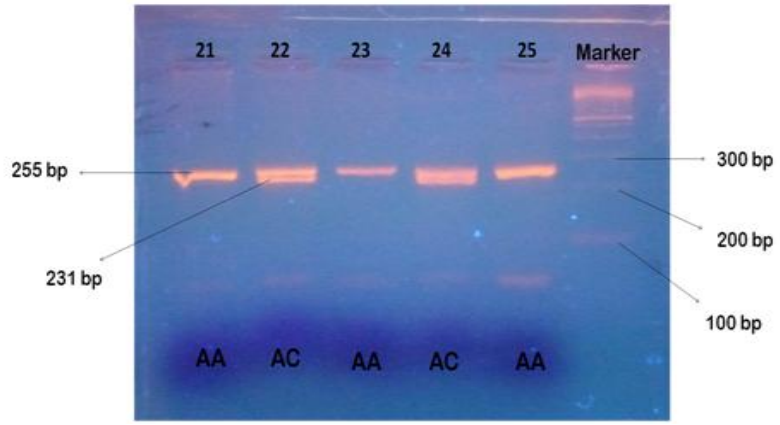

Figure 1. A1166C polymorphism of AGTR1 gene: BsuRI digestion of the PCR product. The wild type gene was 255 bp and the mutant was 231 bp.

The frequency distribution of AGTR1 A1166C genotypes in Type 2 diabetes mellitus patients with and without nephropathy are presented in Tabel 2.

Table 2 Distribution of AGTR1 A1166C genotypes in Type 2 diabetes mellitus patients with and without nephropathy

Genotype	DN patients (n=30)	Non-DN patients (n=30)	P<0.05
AA	26 (86.7%)	24 (80%)	ns
AC	4 (13.3%)	3 (10%)	
CC	0 (0)	3 (10%)	
A allele	56 (93.3%)	51 (85%)	ns
C allele	4 (6.6%)	9 (15%)	

DN: Type 2 diabetes patients with nephropathy; Non-ND: Type 2 diabetes patients without nephropathy

Frequency distribution of AGTR1 A1166C genotypes in the DN group are as follows, 86,7% (n=26) of the subjects with AA genotype, 13,3% (n=4) with AC genotype, and none of the patient with CC genotype. Whereas in Non-DN group there were 80% (n=24) subjects with AA genotype, 10% (n=3) with with AC genotype and 10% (n=3) with CC genotype. The frequency distribution of AGTR1 allele A and allele C in DN group are 93.3% (n=56) and 6.6% (n=4) respectively. Whereas in non-DN group the frequency of

allele A and allele C consecutively are 85% (n= 51) and 15% (n=9). However, between the two groups there is no statistical difference in the both gene and allele frequency distribution at *P*=0.05.

IV. Discussions

Current findings suggest that among Indonesian patients, AGTR1 gene A1166C genotype AA is predominant compared to AC or CC and such trend occured both in DN and non-DN groups. When these results are compared to the data from other countries, referred to Zhang et al. [10] as shown in **Table 3**, it seems that A1166C genotype polymorphism of AGTR1 gene in Indonesian patients are similar to that of Chinese. The similarity patterns are the same in both case group and control group.

Year	Country (Ethnicity)	Cases Group			Control Group			
		AA	AC	CC	AA	AC	CC	ρ
Current	Indonesian (Asian)	83,30%	11,70%	5%	80%	10%	10%	0,20
2006	Rep.Bashkortostan (Caucasian)	62,90%	33,87%	3,23%	71,79%	23,72%	4,49%	0,10
2008	Canada (Caucasian)	39,66%	56,90%	3,45%	43,64%	44,55%	11,82%	0,93
2008	China (Asian)	82,43%	14,86%	2,70%	94,41%	5,59%	0,00%	0,63

The data showed that in Asian ethnicity (Indonesia and China), the frequency of AA genotype tend to higher than that of Cacusian (Rep.Bashkortostan and Canada). However in both ethnic group (Asian and Caucasian) frequency of CC is the lowest. Patients with CC homozygote AGTR1 polymorphism seem to increase sensitivity of angiotensin II [11]. Angiotensin II is vasoconstrictor and mediator for cell proliferation and synthesis of protein extracellular matrix. The effect of intra renal RAS is kidney hemodynamic change such as the increase in intra glomeruler pressure, stimulation from mesangial cell proliferation and matrix production [12]. The function of angiotensin II is to increase efferren arteriol pressure and afferren or preglomeruler strand that causes autoregulation response in systemic blood pressure increase causing the increase in glomerulus capillar pressure. Beside, angiotensin II also plays a role in growth stimulation, fibrogenesis induction and regulates endotel function [13].

V. Conclusions

In comparison to the polymorphism data of AGTR1 gene in Chinese, Rep.Bashkortostan and Canadian patients, the frequency distribution of A1166C genotype in Indonesian patients with diabetic nephropathy is similar to that of Chinese. It suggests that AA genotype, in comparison to AC or CC, is predominantly expressed in Mongoloid patients than that of Caucasoid.

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