Gender Differences of Gly972Arg Polymorphism of the IRS-1 Gene Related to Cardiovascular Disease Risk Factors Among Indonesians

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ABSTRACT

Background: *Cardiovascular disease is driven by traditional risk factors, sex, and genetic differences. The Asian population, specifically Indonesians, has been known at high risk of insulin resistance and endothelial dysfunction. A possible genetic risk factor related to cardiovascular diseases is Gly972Arg polymorphism of insulin receptor substrate 1 (IRS-1) gene, as this impairs endothelial function. To date, whether there is a gender difference in Gly972Arg polymorphism of the IRS-1 gene in Indonesians is unknown. This study aimed to to define whether there is a gender difference in Gly972Arg polymorphism of the IRS-1 gene in Indonesians.* ***Methods:*** *We studied adults living in two areas (rural and urban) in Indonesia. We collected demographic and clinical data from the study subjects. Gly972Arg polymorphism of the IRS-1 gene (rs1801278) was detected using TaqMan real-time polymerase chain reaction.* ***Results:*** *A total of 378 subjects were recruited. The wild-type allele (CC) was found in 86 (22.8%) subjects, heterozygous mutant allele (CT) in 245 (64.8%), and homozygous mutant allele in 47 (12.4%). The proportion of subjects with T alleles was significantly higher among women than men (54.6% vs. 45.4%, odds ratio: 1.89; p = 0.01). Subjects with T allele more often have hypertension (odds ratio: 1.69, p = 0.058).* ***Conclusion:*** *There were a higher proportion of women than men carrying the T allele of Gly972Arg polymorphism among Indonesians. Individuals with the T allele appeared to show a greater prevalence of hypertension. These results may explain a possible mechanism of the high prevalence of metabolic syndrome in Indonesia, especially in women.*

Keywords**:** *Gender, Gly972Arg polymorphism, IRS-1 gene, Indonesian.*

INTRODUCTION

The Indonesian population is facing an increasing challenge of growing rates of cardiovascular disease (CVD) and metabolic syndrome (MetS).[1] CVD has become an important health issue in Asia, particularly Southeast Asia and Indonesia, due to the increasing rates of dyslipidemia, diabetes, obesity, and hypertension.[2] In most Asia Pacific countries, nearly one-fifth of the adult population or more are affected by MetS, with a significant increase in prevalence.[1] The Asian population already faces a higher risk of diabetes mellitus than the Western population, and rapidly increasing rates of diabetes mellitus over short periods and with onset at a relatively

young age and low body mass index have been reported.[3] Meanwhile, findings of endothelial dysfunction, dyslipidemia, and hypertension are also increasing as a result of rapid urbanization, dietary changes, high smoking rates, and decreased physical activity.[2]

Insulin receptor substrate 1 (IRS-1) molecules are key mediators in insulin signaling. Polymorphisms of the IRS-1 gene are associated with endothelial dysfunction, insulin resistance, diabetes, dyslipidemia, and hypertension.[4–6] The most common polymorphism of the IRS-1 gene occurs at the Gly to Arg 972 substitution.[7] This polymorphism appears to play a pathogenic role in the development of CVD preceded by the aforementioned conditions.

Polymorphism of Gly972Arg of the IRS-1 gene is found in 5–6% of the normal population and 10% of the population with type 2 diabetes mellitus. A carrier of Gly972Arg polymorphism experiences a 25% increased risk of type 2 diabetes mellitus.[5] Wulandari et al. reported an increased risk of endothelial dysfunction in carriers of a homozygous mutant allele of this polymorphism with an odds ratio (OR) of 18.[8] Separately, a study of the Indonesian population by Syahrul et al. found Gly972Arg polymorphism is also a significant risk factor for ischemic stroke (OR= 2.6).[9]

CVD is reported to be driven not only by traditional risk factors but also by sex and genetic differences.[10] Recently, researchers have proven that sex greatly impacts the pathophysiology and clinical manifestations of cardiovascular events. However, the distribution of Gly972Arg polymorphism of the IRS-1 gene as a risk factor for CVD is not really quantified in the Indonesian population because there is limited research available regarding this gene. Therefore, a study to observe gender differences in Gly972Arg polymorphism of the IRS-1 gene as a CVD risk factor in the Indonesian population is needed.

METHODS

This was a cross-sectional observational study involving urban subjects seen at medical checkup clinics and the employee outpatient clinics of the National Cardiovascular Center Harapan Kita, Jakarta, Indonesia. Subjects from rural population were recruited as part of our community service program in Bogor, Indonesia. The inclusion criteria were an age of ≥18 years and the presence or absence of a history of cardiovascular events. The exclusion criteria includes those with any symptoms of cardiac disease, or unstable condition. We performed a convenient sampling so that the number of study subjects are almost equal between male dan female subjects.

Ethics Statement

This study was approved by the institutional review board and ethics committee for research of the National Cardiovascular Center Harapan Kita. All procedures were performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki, and written informed consent was obtained from all participants included in this study.

Blood and Baseline Data Collection

The polymorphism of Gly972Arg of the IRS-1 gene (rs1801278) was detected with TaqMan probe-based assay quantitative real-time polymerase chain reaction (qRT-PCR) (Applied Biosystems 7500 Fast, California, USA). Genomic DNA was taken from ethylenediaminetetraacetic acid–anticoagulated blood samples. PCR was performed on 4.5 μL of genomic DNA in the final volume of 10 μL containing 0.5 μL TaqMan Genotyping Assay (rs1801278), and 5 μL TaqMan genotyping mastermix. The thermal cycle protocol consisted of an initial cycle that lasted for 20 seconds at a temperature of 95 °C, followed by 40 cycles with each cycle lasting for 33 seconds, at a temperature of 95 °C for 3 seconds, and at 60 °C for 30 seconds.

Data Analysis

Statistical analysis was performed using the SPSS Statistics program (version 23.0; IBM). The Kolmogorov–Smirnov normality test was performed on the numerical data. Numerical data were deemed as exhibiting a normal distribution if the value of p from the Kolmogorov–Smirnov test was >0.05. Numerical data with normal distribution were presented as means, and numerical data with abnormal distribution were presented as medians. Gly972Arg polymorphism

of the IRS-1 gene as an independent variable was statistically analyzed together with CVD and sex difference as dependent variables during chi-squared analysis if the conditions for chi-squared analysis were fulfilled. If the chi-squared requirements were unmet, then Fisher's exact test was used. Spearman correlation analysis was adopted for analysis between two continuous parameters. A p-value of <0.05 was considered to be statistically significant.

Study Objective and Endpoint Definition

This study sought to observe gender differences in Gly972Arg polymorphism of the IRS-1 gene. The definitions for the various study endpoints are as follows: a wild-type allele is present when the RT-PCR code is CC, a heterozygous mutant allele is present when the RT-PCR code is CT, and a homozygous mutant allele is present when the RT-PCR code is TT. In this study, a mutant subject was defined as one having double T alleles. Meanwhile, hypertension was when the systolic brachial pressure is ≥140 mmHg or the diastolic brachial pressure is ≥90 mmHg, whereas dyslipidemia was when the fasting blood total cholesterol was ≥200 mg/dL or the low-density lipoprotein was ≥140 mg/dL. Diabetes mellitus was treated when the subject displayed a fasting blood glucose of ≥160 mg/dL or was already using antidiabetic drugs. A history of coronary artery disease included a history of hospitalization for acute coronary syndrome or an electrocardiogram showing pathologic Q-waves, poor R progression, depressed ST segment, or inverted T-waves. Obesity was defined as a body mass index of ≥25 kg/m^2. A heightened CVD risk was confirmed to exist in patients with at least one of these conditions (i.e., hypertension, dyslipidemia, diabetes mellitus, history of coronary artery disease, or obesity).

RESULTS

Patient Characteristics

The characteristics of patients participating in this research are presented in **Table 1**.

The mean age of the study subjects was 52 SD ±11.4 years, and the group included 187 (49.5%) men and 191 (50.5%) women. Hypertension and obesity were two of the most common risk factors for cardiovascular events found in this study and identified in 68.8% and 59.3% of the subjects, respectively. The wild-type allele (CC) was found in 86 (22.8%) subjects, the heterozygous mutant allele (CT) was found in 245 (64.8%) subjects, and the homozygous mutant allele was found in 47 (12.4%) subjects. Subjects with at least one T allele were associated with a greater prevalence of hypertension (OR: 1.69; p = 0.058) (**Table 2**).

Table 1. Baseline Characteristics.

	Variables	Value (%)
Sex	Men	187 (49.5)
	Women	191 (50.5)
Age (years)		52 ± 11.4
Systolic BP (mmHg)		149.84 ± 33.165
Diastolic BP (mmHg)		88.98 ± 18.158
Hypertension		260 (68.8)
Diabetes mellitus		55 (14.6)
Dyslipidemia		91 (24.1)
History of coronary artery disease		104 (27.5)
Obesity		224 (59.3)
All-CVD risk (at least one CVD risk factor)		353 (93.4)
IRS-1 gene polymorphism (Gly972Arg)	CC (wild type)	86 (22.8)
	CT (heterozygous mutant)	245 (64.8)
	TT (homozygous mutant)	47 (12.4)

BP: blood pressure; CVD: cardiovascular disease

Table 2. Subjects with T allele (Mutant) vs. Wild Type and CVD Risk

Variables	With T allele (CT + TT) (%)	Wild type (CC) (%)	OR (95% CI)	p-value
Hypertension	208 (71.2)	52 (60.5)	1.69	0.058
Diabetes mellitus	37 (12.7)	18 (20.9)	0.5	0.056
Dyslipidemia	67 (23.8)	24 (28.6)	0.8	0.370
Obesity	173 (59.2)	51 (59.3)	1.0	0.993
All-CVD risk (at least one CVD risk factor)	271 (92.8)	82 (95.3)	0.6	0.405

CVD: cardiovascular disease; OR: odds ratio; CI: confidence interval

Gender differences in homozygous mutant (TT) subjects did not differ significantly between women and men with an OR of 1.25 (p = 0.483) (**Table 3**).

In this study, we found female subjects (54.6%) were more likely than male subjects (45.4%) to have at least one T allele (**Table 4**),

To provide illustration of the burden of CVD risk with the gender among study subjects **Table 5** was made. There were higher proportions of hypertension (OR: 1.88; p = 0.005) and also obesity (OR: 2.5; p < 0.001) among women subjects compared to the men.

DISCUSSION

To the best of our knowledge, there has been no similar studies considering the gender differences in Gly972Arg polymorphism of the *IRS-1* gene in the Southeast Asian population, particularly in Indonesia. Hence, this is the first study assessing sex differences in Gly972Arg polymorphism in Indonesia.

Our study revealed that subjects with at least one T allele are more often women, and a mutant (TT) allele profile can similarly occur in both sexes (**Table 2 and 3**). Meanwhile, men subjects with mutant allele profiles show an increased risk

Table 3. Homozygous Mutant vs Nonhomozygous Mutant

Variables	Women (N = 191) (%)	Men (N = 187) (%)	OR (95% CI)	p-value
Gly972Arg			1.25	0.483
Nonhomozygous mutant (CC + CT)	165 (86.4)	165 (88.2)		
Homozygous Mutant (TT)	26 (13.6)	21 (11.2)		

OR: odds ratio; CI: confidence interval

Table 4. Distribution of C and T Alleles

Allele	Women	Men	OR (95% CI)	p-value
Gly972Arg			1.89	0.01
C	199 (47.7%)	218 (52.3%)		
T	185 (54.6%)	154 (45.4%)		

OR: odds ratio; CI: confidence interval

Table 5. Sex and CVD Risk

Variables	Women (N = 191) (%)	Men (N = 187) (%)	OR (95% CI)	p-value
Hypertension	144 (55.4)	116 (44.6)	1.88	0.005
Diabetes mellitus	24 (43.6)	31 (56.4)	0.72	0.269
Dyslipidemia	40 (44.0)	51 (56.0)	0.67	0.103
History of coronary artery disease	9 (8.7)	95 (91.3)	0.05	0.000
Obesity	134 (59.8)	90 (40.2)	2.5	0.000
All-CVD risk (at least one CVD risk factor)	178 (50.4)	187 (49.6)	0.94	0.879

CVD: cardiovascular disease; OR: odds ratio; CI: confidence interval

of having dyslipidemia. This study also found that women subjects regardless of Gly972Arg polymorphism is related to the development of hypertension and obesity.

Nowadays, gender differences in the development of CVD risk factors have been documented. Age, hypertension, total cholesterol, and low-density lipoprotein cholesterol exhibit a great influence in men, whereas menopause, systolic arterial hypertension, smoking, diabetes, triglycerides, and high-density lipoprotein cholesterol mainly act in women.[10]

Women are protected from atherosclerosis during the fertile age range given that estrogen exerts beneficial effects on the cardiovascular system by acting through both genomic and nongenomic mechanisms.[11] However, the risk of CVD will rise exponentially after menopause due to a lack of estrogen. This demonstrates the importance of the role of the endothelium in cardiovascular events, in that women are protected from such by estrogen during the premenopausal period.

Endothelial dysfunction, one of the main risk factors of CVD, occurs by way of several mechanisms such as reduced endothelial nitric oxide synthase (eNOS) or lipotoxicity due to dyslipidemia. eNOS is mainly regulated by insulin. If there is impairment in insulin metabolism as seen in an insulin-resistance condition, the production of eNOS will be depleted and will lead to endothelial dysfunction.

Perticone et al. reported that carriers of Gly972Arg polymorphism exhibit reduced expression of eNOS due to chronic exposure to insulin. The reduced expression of eNOS results in endothelium-dependent vasodilation disorders, thus making the patient more prone to hypertension.[12] In our study, subjects with at least one T allele displayed an increased risk of developing hypertension. Therefore, this finding is in line with that from the study by Perticone et al.

Research also suggests that Gly972Arg polymorphism can also influence glucose homeostasis in premenopausal women.[13] In our study, there was no significant association between sex and diabetes mellitus, although we found that women subjects are at higher risk for obesity, which may be attributed to a certain degree to some imbalances in glucose homeostasis. Whether this glucose homeostasis imbalance is due to Gly972Arg polymorphism or not requires further study, but from our data, women with at least one T allele of Gly972Arg polymorphism and women with the mutant genotype were not significantly correlated with the presence of diabetes mellitus or obesity.

Lipotoxicity can cause endothelial dysfunction by way of oxidative stress, inflammation, mitochondrial dysfunction, and endoplasmic reticulum stress as well as cell death. Lipotoxicity is caused by dyslipidemia (i.e., abnormally high levels of triacylglycerol, nonesterified fatty acid, and cholesterol).[14] A genome-wide association study revealed that polymorphism in *IRS-1* is associated with an impaired metabolic profile, including decreased subcutaneous-to-visceral fat ratio, increased insulin resistance, dyslipidemia, heightened risk of diabetes and coronary artery disease, and decreased adiponectin levels.[15] According to the World Health Organization statistics, the prevalence of dyslipidemia (defined as TC ≥160 mg/dL) in adults aged ≥25 years in Indonesia was approximately 36% (33.1% for men and 38.2% for women).[2] Dyslipidemia in the Asian population is known to be ethnic specific,[16] and by 2011, the prevalence of dyslipidemia in all ethnic groups in Indonesia (defined as TC >240 mg/dL) was between 9.0% and 25%.[17]

Most studies from the Asian region have reported a higher prevalence of MetS in women, except in those conducted in rural Australia, rural India, urban Japan, and urban Pakistan and according to one national survey conducted in China and Macau.[1] The results of our study that show the T allele is more likely to occur in women support the revelation of a possible mechanism for the high rate of MetS in the Asian and Indonesian populations, especially among women.

The present study is limited by study subjects recruited only from those coming to Harapan Kita NCVC clinics and subjects from our community service program in Bogor, Indonesia. So the data of present study represented Indonesians recruited for this study

only. However, the findings of this study may provide basis for specifically designed study with the subjects recruited from various region an ethnics in Indonesia. Any data of genomic CV risk for Indonesia is important in this upcoming era of precision medicine.

CONCLUSION

There are a higher proportion of women than men carrying the T allele of Gly972Arg polymorphism among Indonesians. Those with the T allele appear to show a greater prevalence of hypertension. These results may suggest a possible mechanism for the high prevalance of metabolic syndrome in the Indonesian population, especially among women.

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