Clinical Research

Association between vaspin rs2236242 gene polymorphism and atherosclerosis in ischemic stroke patients with diabetes mellitus in the Indonesian population

Rivan Danuaji[1], Suroto[1], Bambang Purwanto[2], Dono Indarto[2], Faizal Muhammad[1], Diah Kurnia Mirawati[3], Vitri Widyaningsih[2], Soetrisno[2], Subandi[1], Pepi Budianto[3], Yetty Hambarsari[1], Baarid Luqman Hamidi[1], Hanindia Riani Prabaningtyas[3], Ervina Arta Jayanti Hutabarat[1], Ira Ristinawati[1], Teddy Tejomukti[1], Raden Andi Ario Tedjo[1]

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Authors' affiliations:
[1]Department of Neurology, Faculty of Medicine, Universitas Sebelas Maret, Dr. Moewardi Hospital, Surakarta, Indonesia, [2]Doctoral Program of Biomedical Medicine, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia, [3]Department of Neurology, Faculty of Medicine, Universitas Sebelas Maret, UNS Hospital, Surakarta, Indonesia

Corresponding author:
Rivan Danuaji
Department of Neurology, Faculty of Medicine, Universitas Sebelas Maret, Dr. Moewardi Hospital, Jalan Kolonel Sutarto No. 132, Jebres, Surakarta 57126, Central Java, Indonesia
Tel/Fax: +62-271-664178/ +62-271-637400
E-mail: rivan.danuaji@staff.uns.ac.id

ABSTRACT

BACKGROUND Stroke is the third leading cause of morbidity and second in mortality worldwide. Diabetes mellitus (DM) is a risk factor for stroke. Vaspin and single nucleotide polymorphism (SNP) rs2236242 involved DM pathogenesis. This study aimed to explore the correlation between SNP rs2236242, serum vaspin levels, and atherosclerosis in patients with ischemic stroke and DM.

METHODS This study was conducted in Dr. Moewardi Hospital from 2022 to 2023. The case group included patients with ischemic stroke and DM, while the controls were those with ischemic stroke only. Tetra-primer amplification refractory mutation system-polymerase chain reaction was used to determine the genotypes.

RESULTS There were 31 cases and 33 control. SNP rs2236242 showed that odds ratio (OR) (95% confidence interval [CI]) for AA-TT was 0.273 (0.241–0.305) and for TA-TT was 0.315 (0.298–0.341). The OR (95% CI) for allele A to T was 0.789 (0.373–1.669). The mean (standard deviation) serum vaspin level in the case group compared to the control was 1,570 (2,108) ng/ml *versus* 1,630 (1,428) ng/ml (p = 0.064). Higher vaspin levels were found in T allele of the TT (1,523 [2,269] ng/ml, p = 0.021) and TA (1,760 [1,349] ng/ml, p = 0.004) genotype groups than the A allele of the AA genotype group (0.914 [0.329] ng/ml).

CONCLUSIONS Vaspin gene polymorphism AA genotype or A allele significantly reduces vaspin levels in patients with ischemic stroke with DM.

KEYWORDS atherosclerosis, diabetes mellitus, Indonesian, single nucleotide polymorphism, stroke, vaspin

Individuals with diabetes mellitus (DM) are at a significantly higher risk of stroke, with studies such as INTERSTROKE indicating a 36% elevated risk. The prevalence of DM in Indonesia increased from 6.9% in 2013 to 8.5% in 2018, according to the 2011 Indonesian Society of Endocrinology consensus, and 10.9% in 2018, according to the 2015 consensus.[1] The discrepancy between the 8.5% prevalence reported in the 2011 Indonesian Society of Endocrinology consensus and the 10.9% prevalence in the 2015 consensus may reflect differences in diagnostic criteria or survey methodologies used during these periods. However, this increasing prevalence highlights the need to understand molecular factors contributing to DM and its complications. One such factor is vaspin, a serine protease inhibitor associated with insulin sensitivity and inflammation regulation.[2] Vaspin was first identified in the visceral adipose tissue of a mouse model of DM and has been shown to have anti-inflammatory and anti-apoptotic effects on vascular cells. Additionally, it

plays a role in improving insulin sensitivity, a key factor in managing DM.[3,4]

Identifying the association between genotype and serum vaspin levels may help reduce the risk of atherosclerosis in patients with DM. Serum vaspin levels are influenced by various factors, including the single nucleotide polymorphism (SNP) rs2236242,[5,6] a variation on chromosome 14 of the human gene that affects serum vaspin levels. A study on the Iranian population revealed that SNP rs2236242 plays a significant role in serum vaspin levels.[6] SNP rs2236242 was selected for its known association with serum vaspin levels and its role in inflammatory and metabolic pathways linked to atherosclerosis. Studies, including those in the Iranian population, show its significant influence on vaspin levels, which help regulate vascular inflammation and insulin sensitivity. This study aimed to determine the relationship between SNP rs2236242, serum vaspin levels, and atherosclerosis in ischemic stroke patients with DM, specifically in the Indonesian population.

METHODS

Peripheral blood mononuclear cells (PBMCs) were isolated from 80 patients aged >18 years with ischemic stroke who were admitted to the Stroke Center of Dr. Moewardi Hospital between January 2022 and August 2023. Patients were diagnosed with acute infarction stroke within 6 days based on computed tomography or magnetic resonance imaging of the head and clinical examination. Vaspin levels (ng/ml) were measured using an enzyme-linked immunosorbent assay. We included 31 patients with both stroke and DM (fasting blood glucose [FBG] ≥126 mg/dl) and 33 patients with stroke only (also FBG ≥126 mg/dl) after excluding 16 patients with comorbidities, such as sepsis and tumors, that could affect vaspin levels.

Genomic DNA was extracted from PBMCs using the GF-1 Nucleic Acid Extraction Kit (Vivantis Technologies Sdn Bhd, Malaysia), following the manufacturer's instructions, with an estimated concentration of 50 ng/µl. Primers from https://primer1.soton.ac.uk were used to identify SNP rs2236242. The Basic Local Alignment Search Tool database and SnapGene program (Dotmatics, USA) were used to validate the gene sequence products (Table 1).[5] The size and characteristic for rs2236242 vaspin gene were shown in Table 2.

SNP rs2236242 was genotyped using the tetra-primer amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) method with an annealing temperature of 63°C. The results were analyzed by agarose gel electrophoresis and visualized using an ultraviolet transilluminator (300 nm wavelength). A 1 kb DNA ladder, which effectively separated fragments in the 200–1,000 base pair (bp) range, was used as a marker. Agarose gel electrophoresis was used to determine the product size (bp) and genotype.

In this study, body mass index (BMI) was categorized as underweight (<18.5 kg/m^2), normal weight (18.5–25.0 kg/m^2), and overweight or obese (>25.0 kg/m^2). This was used to explore the association between BMI and adipokine profiles, where both overweight and obese individuals are likely to exhibit significant adipokine dysregulation.

Table 1. T-ARMS-PCR primer design for rs2236242 vaspin gene

Primer	Sequences (5'3')
FI (allele T)	AAG ACG CCG CTT CTG TGC ACT
RI (allele A)	CAC AGG GAC CCA GGA TAA CTT GCT
FO	GGA GGC AGA CCA GGC ACT AGA AA
RO	ACC ATC TCT CTG GCT TCA GGC TTC

FI=forward inner; FO=forward outer; RI=reverse inner; RO=reverse outer; SNP=single nucleotide polymorphism; T-ARMS-PCR=tetra-primer amplification refractory mutation system-polymerase chain reaction

Statistical analysis

The case group consisted of patients with both ischemic stroke and DM, whereas the control group

Table 2. T-ARMS-PCR size and characteristic for rs2236242 vaspin gene

Genotype	Band-1	Band-2	Band-3	Phenotype
TT	174 bp	378 bp	-	Dominant homozygote
TA	248 bp	378 bp	-	Intermediate (heterozygote)
AA	174 bp	248 bp	378 bp	Recessive homozygote

bp=base pair; T-ARMS-PCR=tetra-primer amplification refractory mutation system-polymerase chain reaction

included patients with ischemic stroke without DM. The association between SNP rs2236242 and atherosclerosis was analyzed using the chi-square test. Independent *t*-tests and one-way analysis of variance with post-hoc tests were used to determine the significance of serum vaspin levels between the case and control groups and among the three genotypes. Statistical significance was set at $p<0.05$, and analyses were performed using SPSS software version 22.0 (IBM Corp., USA). Ethical approval for the study was obtained from the Health Research Ethics Committee, Dr. Moewardi Hospital (No: 1.319/X/HREC/2022).

RESULTS

A total of 64 patients with ischemic stroke were selected based on the inclusion and exclusion criteria (Table 3). Genotyping of SNP rs2236242 was performed for both patients with and without DM (Figure 1). Statistically significant differences were observed in the genotype and allele frequencies of rs2236242 between the case and control groups (Table 4). The genotypes of rs2236242 and vaspin levels in patients with ischemic stroke were associated (p = 0.045), and an association was observed between rs2236242 vaspin allele frequencies and ischemic stroke (p = 0.036).

The mean (standard deviation) of serum vaspin levels were compared between the case (1,570 [2,108] ng/ml) and control (1,630 [1,428] ng/ml) groups (p = 0.064). The A allele of the AA genotype group (0.914 [0.329] ng/ml, p = 0.076) was associated with significantly lower vaspin levels compared to the T allele, which was more dominant in the TT (1,523 [2,269] ng/ml, p = 0.021) and TA (1,760 [1,349] ng/ml, p = 0.004) genotypes. Furthermore, post-hoc analyses showed that only the AA (homozygous recessive) comparison did not yield significant differences, whereas the TT and TA genotypes were not statistically significant (p = 0.076).

DISCUSSION

As stroke ranks third in global morbidity and second in mortality, this study explores the correlation between SNP rs2236242, serum vaspin levels, and atherosclerosis in ischemic stroke patients with DM, a key risk factor. According to the traditional theory, adipose tissue is considered inert and primarily

Table 3. Sociodemographic and medical history profile of the subjects

Variables	Patients with both stroke and DM, n (%) (N = 31)	Patients with stroke only, n (%) (N = 33)
Sex		
Male	20 (65)	17 (52)
Female	11 (35)	16 (48)
Age (years)		
<40	3 (10)	7 (21)
40–59	15 (48)	11 (33)
60–79	12 (39)	10 (30)
≥80	1 (3)	5 (15)
Javanese race	31 (100)	33 (100)
Consciousness level		
Alert	31 (100)	33 (100)
Drowsy or worse	0 (0)	0 (0)
BMI (kg/m^2)		
<18.5	2 (6)	4 (12)
18.5–25.0	15 (48)	11 (33)
>25.0	14 (45)	18 (55)
History of hypertension		
Yes	25 (81)	20 (61)
No	6 (19)	13 (39)
History of stroke		
Yes	13 (42)	19 (58)
No	18 (58)	14 (42)
History of heart disease		
Yes	20 (65)	21 (64)
No	11 (35)	12 (36)
Extremities weakness		
Right hemiparesis	9 (29)	14 (42)
Left hemiparesis	10 (32)	15 (45)
Tetraparesis	12 (39)	4 (12)
Type of stroke		
Thrombotic infarct	7 (22)	15 (45)
Embolic infarct	10 (32)	10 (30)
Recurrent thrombotic infarct	13 (42)	5 (15)
Recurrent embolic infarct	1 (3)	3 (9)
Infarct volume (cc)		
<1.5	0 (0)	0 (0)
≥1.5	31 (100)	33 (100)
NIHSS score		
≤5	5 (16)	7 (21)
6–14	12 (39)	15 (45)
15–24	11 (35)	9 (27)
≥25	3 (10)	2 (6)

BMI=body mass index; NIHSS=National Institutes of Health Stroke Scale

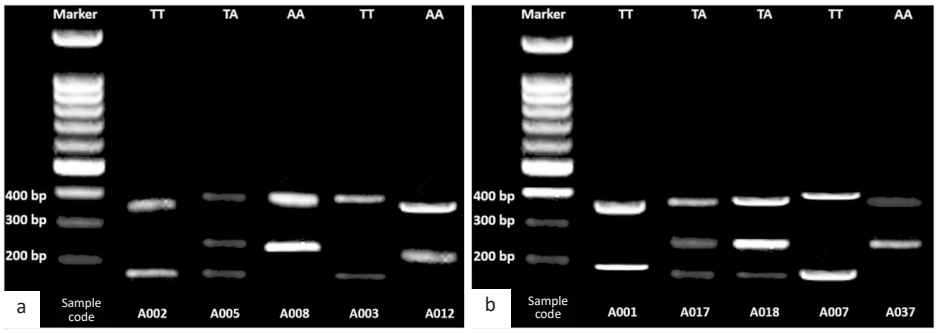

Figure 1. T-ARMS-PCR product identification of the vaspin gene. It showed SNP rs2236242 comparison between the case (a) and control (b) groups which give each representative three polymorphism genotypes: TA, TT, and AA. bp=base pair; SNP=single nucleotide polymorphism; T-ARMS-PCR=tetra-primer amplification refractory mutation system-polymerase chain reaction

Table 4. The association of SNP rs2236242 vaspin gene in ischemic stroke patients with DM (case) and without DM (control)

SNP	Case (N = 31)	Control (N = 33)	OR (95% CI)	*p*
Genotypes				**0.045**
AA	3 (10)	1 (3)	0.273 (0.241–0.305)	
TA	15 (48)	17 (52)	0.315 (0.298–0.341)	
TT	13 (42)	15 (45)	1.00	
Alleles			0.789 (0.373–1.669)	**0.036**
A	21 (34)	19 (29)		
T	41 (66)	47 (71)		

CI=confidence interval; DM=diabetes mellitus; OR=odds ratio; SNP=single nucleotide polymorphism

serving functions such as temperature regulation, fat storage, energy supply, shock absorption, and insulation. However, recent research has shown that adipose tissue is an endocrine organ with multiple functions and active metabolism.[7,8] Vaspin, also known as SERPINA12, is an insulin-sensitive adipocytokine discovered in 2005. It is associated with inflammation and metabolic syndrome.[9] It was first discovered in visceral adipose tissue from Otsuka Long-Evans Tokushima fatty mice, an animal model of type 2 DM and central obesity. The vaspin gene is located on chromosome 14q32.13 and consists of six exons and five introns.[10] The A allele of the SNP rs2236242 in the vaspin gene has been identified as a risk factor for atherosclerosis in obesity and DM, as it is associated with lower vaspin levels, while the T allele is linked to higher serum vaspin levels.[11]

A gene consists of coding (exons) and non-coding (introns) regions.[12] Introns, while non-coding, play important roles in regulating gene expression, alternative splicing, and maintaining genomic stability, among other functions.[12] Exons carry the information required for protein synthesis. The role of the T allele in vaspin rs2236242 varies depending on the biological pathway involved. If the T allele is associated with inflammation and stroke, this could explain its use as a reference. Studies often explore how different alleles (T *versus* A) influence gene function, particularly in terms of insulin sensitivity, inflammation, and other metabolic processes.[13]

The TT genotype was the most prevalent in the study population, making it a suitable reference for comparison with other genotypes. The group with the T allele of the TT and TA genotypes exhibited significantly higher vaspin levels than the group with the A allele of the AA genotype. However, the AA group had fewer participants, which made it less balanced than the TT and TA groups. This finding in the Javanese population reflects the dominant nature of the T allele over the A allele.[14]

These results can be partly explained by Mendel's law of dominance and uniformity. According to these laws, when multiple alleles are present, such as the vaspin gene *rs2236242* variants at the same locus on chromosome 14q32.13, the dominant allele (e.g., the T allele) typically overrides the effect of the recessive allele (e.g., the A allele).[15] However, human genetics often involves more complex inheritance patterns that deviate from simple Mendelian principles. Factors such as incomplete dominance, codominance, polygenic inheritance, and gene-environment interactions can

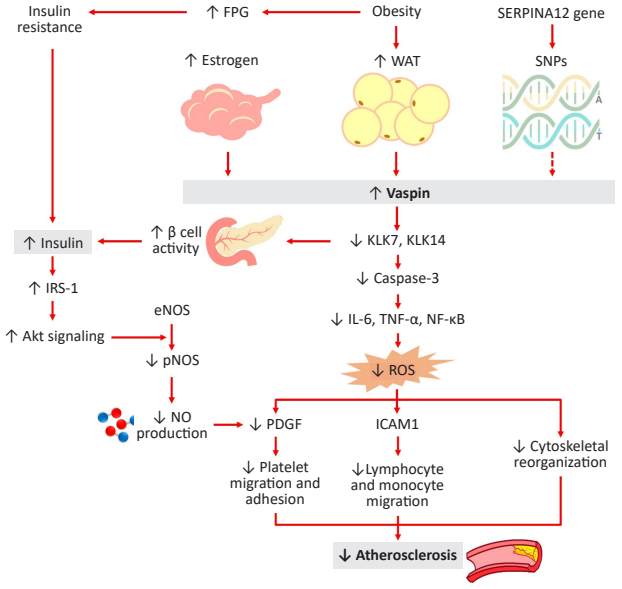

Figure 2. Serum vaspin affects the molecular pathways involved in DM compensating anti-inflammatory and anti-atherosclerotic processes. Vaspin has been shown to have a protective effect on endothelial cells from atherosclerosis. However, vaspin raises insulin levels as a consequence of DM. eNOS=endothelial nitric oxide synthase; FPG=fasting plasma glucose; ICAM1=intercellular adhesion molecule 1; IL-6=interleukin 6; IRS-1=insulin receptor substrate 1; KLK=kallikrein; NF-κB=nuclear factor-kappa B; NO=nitric oxide; PDGF=platelet-derived growth factor; pNOS=phosphorylated endothelial nitric oxide synthase; ROS=reactive oxygen species; SNPs=single nucleotide polymorphisms, which is refers to rs2236242; TNF-α=tumor necrosis factor-alpha; WAT=white adipose tissue. Reproduced from: Danuaji R, Suroto S, Purwanto B, Indarto D, Muhammad F, Mirawati DK, et al. Serum vaspin role in atherosclerosis and glucose tolerance disorders: a systematic review and meta-analysis. J Appl Pharm Sci. 2023;13(01):024–41. Under the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/)

significantly influence phenotypic expression. For instance, traits associated with serum vaspin levels may not solely depend on Mendelian dominance but also on interactions between multiple genetic and environmental factors.[16] Therefore, while the TA genotype may reflect increased vaspin protein activity associated with the T allele, the observed phenotype likely results from a combination of these complex influences.

Studies have shown that vaspin plays antiatherogenic and anti-inflammatory roles in smooth muscle and vascular endothelial cells, particularly in obesity-related inflammation and cardiovascular diseases. It helps protect these cells from apoptosis and inflammation (Figure 2).[10] Vaspin reduces the expression of adhesion molecules induced by tumor necrosis factor-alpha in vascular smooth muscle cells, thereby decreasing lymphocyte adhesion. This effect is achieved by reducing the activation of reactive oxygen species (ROS), protein kinase C, and nuclear factor-kappa B. Vaspin also inhibits the production of ROS-malondialdehyde and advanced glycation end-products, which in turn reduces cytoskeletal changes and the expression of factors such as platelet-derived growth factor, intercellular adhesion molecule 1, and E-selectin.[7]

A limitation of this study is that it cannot establish a causative relationship between the variables owing to its analytical observational design. Additional limitations need to be acknowledged including the relatively small sample size and the exclusion of patients with comorbidities such as sepsis and tumors, which may impact the generalizability of the results. Furthermore, potential confounders, such as differences in medication use, lifestyle habits, and genetic variability within the Indonesian population, could affect serum vaspin levels and should be considered.

In conclusion, SNP rs2236242 was significantly associated with atherosclerosis in ischemic stroke patients with DM in the Indonesian population. The AA genotype of SNP rs2236242 is linked to a significant reduction in serum vaspin levels in patients with ischemic stroke and DM compared to the TT and TA genotypes. However, no significant difference was found in vaspin levels between the case and control groups, and the sample size was small. Further research are recommended using a cohort study design with a larger number of participants, focusing on other adipokine SNPs, such as chemerin rs17173608 and omentin rs2274907, to better understand the potential pathomechanisms of atherosclerosis in patients with DM and ischemic stroke.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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