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Serum Clusterin Level Associated with Post-ischemic Stroke Cognitive Dysfunction

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Abstract

The exploration of potential blood-based biomarkers that could be useful in the early detection of cognitive impairment associated with ischemic stroke is still being studied. The objective of this study was to examine the correlation between serum clusterin levels and the prevalence of cognitive impairment in individuals with ischemic stroke. Methods: A total of 86 outpatients with mild ischemic stroke within the first three months of its onset were recruited from three primary hospitals in Mataram, Indonesia. ELISA was used to measure the serum clusterin level. Patients' sociodemographic and clinical data were also collected as covariates. The Montreal Cognitive Assessment-Indonesian version (MoCA-INA) instrument was used to evaluate cognitive status. The study used multivariate logistic regression analysis to investigate the effect of clusterin on the occurrence of cognitive impairment associated with ischemic stroke while controlling for other variables. Results: The multivariate logistic regression analysis revealed a significant correlation between elevated serum clusterin levels and a higher prevalence of cognitive impairment in ischemic stroke patients (odds ratio [OR] 3.56, 95% confidence interval [CI] 1.04-12.16, p = 0.043). Conclusion: Elevated serum clusterin levels have been associated with a higher occurrence of cognitive impairment in ischemic stroke patients.

Introduction

One of the most serious outcomes of ischemic stroke is cognitive impairment. Post-stroke dementia (PSD) is a significant contributor to dependence in those who have survived a stroke and is the second most prevalent type of dementia, following Alzheimer's disease (Fawal *et al*., 2021). It is estimated that between 25% and 44% of people who have had an ischemic stroke will have cognitive problems (Kalaria *et al*., 2016). In Indonesia, there is a three times more higher prevalence of cognitive dysfunction after a stroke (Pinzon *et al*., 2018). The optimal therapy to prevent or treat post-stroke dementia (PSD) is still not optimal due to the unclear mechanisms of PSD (Fawal *et al*., 2021). The currently accepted theory suggests that disruptions in oxygen and blood supply to the brain tissues and chronic inflammation lead to damage and changes in cortical connectivity, axonal tracts, and the number of neurons (Fawal *et al*., 2021). This highlights other comorbidities, such as hypertension and diabetes mellitus, which impact vascular dysfunction in the brain and are crucial factors in worsening cognitive dysfunction in post-stroke patients (Rasmussen & Langerman, 2019). Early detection of PSD is important to implement

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intervention strategies aimed at altering several risk factors, including hypertension, smoking, obesity, and diabetes. This approach facilitates avoiding and minimizing the disease's progression (Rasmussen & Langerman, 2019). As a result, not all post-stroke individuals in the Mild Cognitive Impairment (MCI) stage, who have not yet experienced a decline in the quality of life, will develop dementia. In the form of dementia, this cognitive dysfunction will decrease patients' quality of life, making them a burden to their families (Sharma *et al*., 2020).

Imaging examinations, for example, advanced magnetic resonance imaging (MRI), play a crucial role in diagnosing and detecting cognitive impairment associated with stroke by providing more precise information about the anatomical structures affected. (Mijajlovic *et al*., 2017). Nevertheless, the accessibility of this evaluation for stroke patients is hindered by its restricted availability and relatively expensive cost. Current studies are being conducted to identify biomarkers that can be used to diagnose stroke-related cognitive dysfunction at an early stage. However, the findings have not been sufficient. Optimal biomarkers for this objective should possess exceptional reliability and accuracy, cost-effectiveness, minimal invasiveness, and simple procedures (Giau *et al*., 2019). Blood-based biomarkers have various characteristics that meet those criteria, including cost-effectiveness, minimal invasiveness, and simple procedures. Alzheimer's disease (AD) possesses adequate reliability and accuracy to establish the diagnosis of the disease (Zetterberg *et al*., 2013). At the same time, in stroke-related cognitive dysfunction, there remains a lack of evidence about the reliability and accuracy of blood-based biomarkers.

The levels of clusterin in the circulation, which have been observed to rise in individuals with Alzheimer's disease (AD) and are associated with the development of cognitive impairment (Jongbloed *et al*., 2015), have also been demonstrated to increase in patients with acute ischemic stroke (Romero *et al*., 2020; Song *et al*., 2019). Elevated clusterin levels in both brain tissue and blood circulation relate to the brain tissue's reaction to oxidative stress triggered by β-amyloid. (Woody & Zhao, 2016). The concentration of β-amyloid is elevated in the bloodstream of individuals experiencing acute ischemic stroke, hence playing a role in the development of cognitive impairment (Chi *et al*., 2019). Clusterin, functioning as a chaperone protein, is crucial in preventing brain β-amyloid accumulation and suppressing oxidative stress generated by β-amyloid. (Woody & Zhao, 2016). Nevertheless, the correlation between clusterin levels and the occurrence of cognitive impairment during the initial stage of ischemic stroke remains undetermined. The objective of this study was to examine the correlation between the occurrence of cognitive dysfunction-related ischemic stroke and serum clusterin levels in patients who have experienced ischemic stroke within three months. This study considered the clinical characteristics and socio-demographics of the participants.

Methods

This study used a cross-sectional design to investigate ischemic stroke patients who were consecutively recruited from a private hospital and two general hospitals in Mataram, West Nusa Tenggara Province, Indonesia, between January 2022 and December 2022. The subjects were included based on the following criteria: 1) had at least a 6-year education at the primary school level; 2) fully conscious; 3) between the ages of 40 and 70; 4) Individuals who have been diagnosed with a mild ischemic stroke within the initial three months following the onset of the stroke.; and 5) willingly participated. The exclusion criteria for participants were: 1) had aphasia; 2) had a hearing and visual impairment that could not be corrected; 3) had clinically significant depression; 4) a history of a diagnosis of psychiatric disorders and cognitive dysfunction before stroke; and 5) currently taking antipsychotic, antidepressant, and anti-anxiety medications before cognitive function test.

The diagnosis of ischemic stroke was established by analyzing the findings of a head CT scan, either during the initial three months following the stroke's onset or at the onset of the stroke. If an individual performed a second head CT scan that revealed a more obvious ischemic lesion, the second head CT

scan result was utilized. The minor ischemic stroke criteria for participants were based on the presence of a National Institutes of Health Stroke Scale (NIHSS) score of 8 or above during the cognitive function evaluation (Muchada *et al*., 2014). The participants' and their caregivers' information, as well as their medical records, were used to gather the history of a diagnosis of cognitive dysfunction or psychiatric illnesses before stroke. The medical records were utilized to gather data pertaining to the prior administration of antipsychotic, antidepressant, and anti-anxiety medications. Clinically severe depression was defined as the presence of a Beck Depression Inventory – II (BDI-II) score ranging from 14 to 63 among the participants (Wang & Gorenstein, 2013). The selection of participants based on the exclusion and inclusion criteria, as well as the evaluation of BDI-II and NIHSS scores, was conducted by a neurologist during the initial outpatient appointment. The study was carried out after acquiring written informed consent from the participants regarding their involvement. The Ethics Committee of Health Research at the University of Mataram approved this study (Register number: 387/UN18.F7/ETIK/2021).

The participants data were surveyed using a questionnaire to gather socio-demographic data, such as age, gender, education levels, and employment status. The variables used to measure the age of the subjects in this study were both continuous and categorical. As continuous variables, age was expressed in years, while as a categorical variable, it was classified into older (60-70 years) and young adult (40-59 years) groups. Gender was classified into female and male categories. The education level categorization included college graduates, high school, and elementary school. Employment status was categorized as employed and unemployed.

The clinical data, including the vascular risk factors (hypertension, diabetes mellitus, cigarette smoking, and body mass index (BMI) status) and stroke characteristics (infarct diameter and infarct side), were collected from the participants based on their medical records. Infarct diameter was classified into larger (≥15 mm in diameter) and small (<15 mm in diameter), while the infarct side was categorized into right, left, and bilateral hemispheres (Jaillard *et al*., 2009). Information regarding the infarct side and infarct size in the brain on a head CT scan of the participants was confirmed by a radiologist. Hypertension status was categorized as hypertensive and non-hypertensive. Diabetes mellitus status was categorized as diabetic and non-diabetic. The presence of hypertension and diabetes mellitus was confirmed based on information regarding the diagnosis documented in their medical records and/or information provided by the participants. The classification of cigarette smoking status was divided into two groups: smokers and non-smokers. To determine the BMI of the participants, the weight in kilograms (kg) was divided by the height in meters squared (m^2). The BMI status was classified as overweight/obesity (BMI ≥25 kg/m^2) and norm weight (BMI <25 kg/m^2)(Kauranen *et al*., 2014).

The serum clusterin levels were determined by analyzing serum samples from the subjects using the ELISA technique. Serum samples were collected from 5 ml blood samples and subjected to centrifugation at 3000 r/minute for 10 minutes. The tubes were then stored at -80°C in the Immunology Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram. The examination of serum clusterin levels using ELISA was carried out by BioTechnology Human CLU (Clusterin) ELISA Kit (Catalog No. E1189Hu). As a continuous variable, serum clusterin levels were expressed in mcg/ml, while as a categorical variable, the levels were categorized as higher and lower based on their cut-off point determined by a receiver operating characteristic (ROC) curve analysis.

The Montreal Cognitive Assessment-Indonesian version (MoCA-INA) test assesses the participants' cognitive function, which served as the outcome variable. The MoCA-INA is a validated instrument to assess global cognitive function among the Indonesian population. (Husein *et al*., 2010). This instrument examines several cognitive domains, including visuospatial/executive function, naming, attention, language, abstract thinking, delayed memory, and orientation. In this test, each participant was asked to do several structured tasks representing the cognitive domains

mentioned earlier on a printed instrument sheet guided by an examiner. During the acute phase of ischemic stroke, the presence of cerebral edema in patients leads to the occurrence of delirium, which subsequently influences the evaluation of cognitive function in these individuals (Balami *et al*., 2011; Dostovic *et al*., 2016). The edema process gradually subsides in the first few weeks, so to prevent bias due to post-ischemic stroke edema, optimal assessment of cognitive function is carried out during the sub-acute stage, which is three months after the onset of ischemic stroke (Lo Coco *et al*., 2016). Each task done correctly was given a score according to the scoring system provided in this instrument. This instrument has a total score of 30, and a score of 26 is used as a cutoff value to categorize participants as having normal cognitive status or cognitive dysfunction. Individuals with a MoCA-INA test score of 26 or higher were classified as having normal cognitive functioning, whereas those who obtained a MoCA-INA test score below 26 were classified as having cognitive impairment.

The neurologist evaluated the eligibility of patients who visited the neurology outpatient department of the hospital where the study took place. The assessment was based on predetermined inclusion and exclusion criteria. Eligible participants and their caregivers were then referred to a trained general practitioner to acquire details regarding the study protocol and get their consent to partake in the study. On a designated day, the participants engaged in an interview session with a trained general practitioner in order to gather data related to sociodemographic variables using structured questions. The medical records of the participants were also available for the collection of clinical characteristics of the participants, including hypertension, diabetes mellitus, cigarette smoking, and BMI status. Confirmation of the clinical data obtained from each participant was carried out through interviews and physical examination of the participant. Any data reported by the participant through interviews and obtained from the medical record is recorded carefully and in detail on the participant's case report form by the general practitioner who is responsible for the completeness of the participant's data. After the collection of sociodemographic and clinical characteristics was completed, the participants were directed by a general practitioner to a separate room for fasting venous blood sampling. Fasting venous blood sampling of the participants was carried out by well-trained laboratory staff using aseptic techniques. A total of 5 ml of fasting venous blood samples were drawn from each participant and then put into a serum tube by the laboratory staff.

After blood collection, the participants will undergo stroke characteristic data collection, including infarct diameter and infarct side in the brain and assessment of cognitive function. During the cognitive function assessment with the MoCA-INA instrument, each participant was given clear instructions to work on tasks representing various cognitive domains, including visuospatial/executive, naming, attention, abstract thinking, delayed memory, and orientation. Due to the complexity of the task, each participant was given the right to rest if necessary or even not to continue the cognitive function test if so desired for any reason. Sheets of cognitive function test results for each participant were given a complete identity and kept together with the patient's questionnaire sheet and case report form by the neurologist who collected the data. All blood samples taken on the examination day were brought by the laboratory staff to the Integrated Laboratory of the Faculty of Medicine, University of Mataram, for further processing to obtain a serum sample. The process of transporting blood samples from the research site to the Integrated Laboratory of the Faculty of Medicine, University of Mataram, uses bags containing ice packs to keep the sample temperature below 8°C before further processing. All serum samples obtained were put into Eppendorf tubes, each coded for each patient, and stored at -80°C. After all research data collection activities are completed in accordance with the previously prepared timeline, all serum samples stored at the Integrated Laboratory of the Faculty of Medicine, University of Mataram, West Nusa Tenggara Province, were then brought to the Immunology Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram, using dry ice transport media to maintain the sample temperature below -20°C.

After arriving at this laboratory, the serum samples were stored at -80°C until assay.

For normally distributed continuous variables, the data were provided as the mean value ± standard deviation (SD). For continuous variables with non-normal distribution, the median (minimum–maximum) was used. For categorical variables, the frequency (%) was provided. The analysis was conducted in two distinct stages. Initially, the study used independent t-test, Mann-Whitney test, or chi-square test to examine the notable disparities in mean, median, and/or frequency of sociodemographic, clinical, and serum clusterin levels between the groups with cognitive dysfunction and those with normal cognitive status. Additionally, the researchers performed multivariate logistic regression analysis to ascertain the relationship between serum clusterin levels and the occurrence of cognitive dysfunction while accounting for other confounders, such as sociodemographic characteristics (gender, age, education levels, and employment status) for Model 1; plus stroke characteristics (infarct side and infarct diameter) for Model 2; plus identified vascular risk factors (cigarette smoking, hypertension, diabetes, and overweight/obesity) for Model 3 and reported as adjusted odds ratio (OR) with 95% confidence interval (CI). The statistical significance was set at $p < 0.05$.

Results and Discussions

A sample of 86 individuals with ischemic stroke were enrolled. The results of a ROC analysis indicated that the cut-off point for serum clusterin level was determined to be 6.39 mcg/ml, with an AUC of 0.590 (FIGURE 1). Table 1 describes a summary of the clinical and socio-demographic characteristics of the participants. Sixty-one (70.9%) participants were assigned to the cognitive dysfunction group, and 25 (29.1%) participants were assigned to the normal cognitive group. The mean age of participants with and without postischemic stroke cognitive dysfunction was 54.6 ± 6.9 years and 52.5 ± 6.9, respectively ($p = 0.209$). However, the median of serum clusterin levels and the frequency of higher serum clusterin levels were not significantly different between cognitive dysfunction and normal cognitive groups. Between the cognitive dysfunction and normal cognitive groups, there were significant differences in the variables of employment status ($p = 0.049$), infarct diameter ($p = 0.044$), and MoCA-Ina scores ($p < 0.001$). There were no significant differences in age, gender, education level, infarct side, and vascular risk factors for

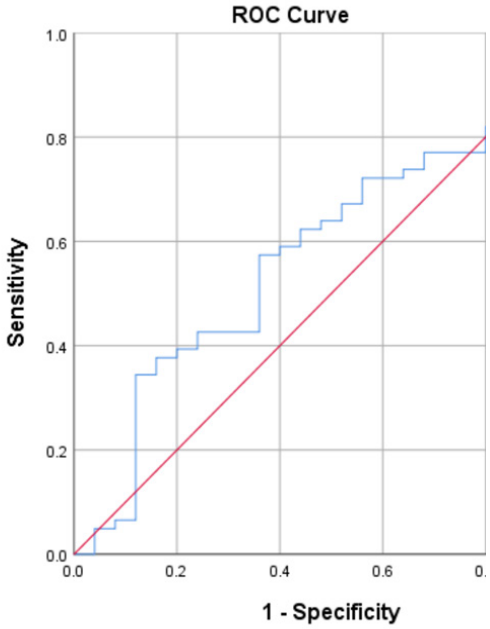

FIGURE 1. ROC Curve for the Discrimination Quality of Clusterin Level in Participants (Weak Categorization). Serum Levels of Clusterin at the Cut-Off Point of 6.39 mcg/ml with AUC 0.590 Were Used

TABLE 1. Socio-Demographic and Clinical Characteristics of the Participants (n = 86)

Characteristics	Cognitive Status		p-value
	Normal (n = 25)	Dysfunction (n = 61)	
Socio-Demographic Characteristics			
Age in years, mean±SD	52.5 ± 6.9	54.6 ± 6.9	0.209[a]
Age groups, n (%)			0.149[b]
Olders	3 (15.8)	16 (84.2)	
Young adults	22 (32.8)	45 (67.2)	
Gender, n (%)			0.366[b]
Female	5 (21.7)	18 (78.3)	
Male	20 (31.7)	43 (68.3	
Education levels, n (%)			0.217[b]
Elementary school	3 (15.8)	16 (84.2)	
High school	11 (28.2)	28 (71.8)	
College	11 (39.3)	17 (60.7)	
Employment status, n (%)			0.049[b]*
Unemployed	4 (14.8)	23 (85.2)	
Employed	21 (35.6)	38 (64.4)	
Clinical characteristics			
Infarct diameter, n (%)			0.044[b]*
Larger	2 (10.5)	17 (89.5)	
Small	23 (34.3)	44 (65.7)	
Infarct side, n (%)			0.996[b]
Bilateral hemisphere	5 (29.4)	12 (70.6)	
Left hemisphere	10 (28.6)	25 (71.4)	
Right hemisphere	10 (29.4)	24 (70.6)	
Hypertension, n (%)			0.581[b]
Yes	22 (28.2)	56 (71.8)	
No	3 (37.5)	5 (62.5)	
Diabetes mellitus, n %)			0.842[b]
Yes	10 (30.3)	23 (69.7)	
No	15 (28.3)	38 (71.7)	
Cigarette smoking, n (%)			0.955[b]
Yes	10 (29.4)	24 (70.6)	
No	15 (28.8)	37 (71.2)	
Overweight/obese, n (%)			0.240
Yes	12 (36.4)	21 (63.6)	
No	13 (24.5)	47 (75.5)	
Clusterin (mcg/ml), median (min-max)	6.1 (0.0 – 251.0)	7.1 (0.0 – 204.0)	0.334[c]
Clusterin level, n (%)			0.204[b]
Higher	11 (35.9)	36 (61.4)	

Low	14 (23.4)	25 (76.6)	
MoCA-Ina test score, median (min – max)	26.0 (25.0 – 29.0)	19.0 (10.0 – 24.0)	<0.001c*

MoCA-Ina = Indonesian version of Montreal Cognitive Assessment.

both groups.

The findings of a multivariate logistic regression study, specifically focusing on the relationship between clusterin and the frequency of cognitive dysfunction in participants with ischemic stroke, are presented in Table 2. This analysis was conducted after correcting for several factors. In Model 1 and Model 2, there was no significant association seen between elevated serum clusterin levels and cognitive deterioration in persons with ischemic stroke. Nevertheless, in Model 3, taking into account all independent variables such as stroke characteristics, vascular risk factors, and sociodemographic factors, it was observed that individuals with ischemic stroke who had elevated levels of serum clusterin were 3.56 times more likely to have cognitive dysfunction in comparison to those with lower levels of serum clusterin (OR 3.56, 95% CI 1.04 – 12.16, p = 0.043). In addition, larger infarct diameter was consistently associated with the frequency of cognitive dysfunction in ischemic stroke participants both in Model 2 (OR 7.39, 95% CI 1.36 – 40.14, p = 0.021) and Model 3 (OR 6.40, 95% CI 1.15 – 35.50, p = 0.034).

The objective of this study was to examine the correlation between serum clusterin levels and the occurrence of cognitive impairment in individuals with ischemic stroke within a three-month period following the onset of the stroke. This study revealed that ischemic stroke participants with higher serum clusterin levels were more likely to develop cognitive dysfunction. It was the first study demonstrating that higher serum clusterin levels contribute to the increased risk of cognitive dysfunction in ischemic stroke participants. In Alzheimer's disease, the relationship between the production of clusterin in the brain, cerebrospinal fluid, and circulating blood and the development of cognitive decline is relatively well understood. Increased levels of clusterin in the blood are significantly associated with an increased risk for the progression of Alzheimer's disease (Weinstein *et al.*, 2016), which is indicated by an increase in the rate of brain atrophy in patients with this disease (Thambisetty *et al.*, 2012). Clusterin is secreted in response to chronic oxidative stress-induced neuronal β-amyloid deposition, a pathological hallmark of AD (Woody & Zhao, 2016), and its peripheral concentrations appear to reflect those within brain regions susceptible to this pathology (Thambisetty *et al.*, 2012). Nevertheless, this protein is inherently neuroprotective since its secretion is intended to prevent further deposition of β-amyloid peptides in the brains of patients with AD. This fact indicates that the high production of clusterin actually represents the severity of the inflammatory response and oxidative stress triggered by beta-amyloid deposition in the brain, which is not successfully controlled by its neuroprotective effect (Woody & Zhao, 2016). Thus, increasing clusterin levels is more appropriate to be used as a predictor of worsening rather than improving the clinical outcome of Alzheimer's disease.

In contrast to Alzheimer's disease, the association between higher serum clusterin levels and an increased risk of cognitive dysfunction in the early phase of ischemic stroke, as found in this study, is elusive. Previous studies have shown that serum clusterin levels are also found to increase in acute ischemic stroke. But unfortunately, none of these studies have analyzed the relationship between increased clusterin levels and the frequency of post-acute ischemic stroke cognitive dysfunction. Some studies were aimed at investigating the role of clusterin levels in the blood as a predictor of the severity of acute ischemic stroke (Iłżecka *et al.*, 2019; Nguyen *et al.*, 2020; Song *et al.*, 2019). Since the pathology of Alzheimer's disease (AD) is also found in 15–30% of stroke patients, leading to overlapping diagnoses of poststroke cognitive impairment and Alzheimer's disease in clinical practice (Yang *et al.*, 2018), our findings suggest that elevated clusterin levels in the early stages of acute ischemic stroke may also be considered as a predictor of poststroke cognitive impairment. However, since the

TABLE 2. Multivariate Logistic Regression Analysis Examining the Association Between and Frequency of Post-Ischemic Stroke Cognitive Dysfunction After Adjustment for Covariates (n = 86)

Independent Variables	OR	95% CI	p-value
Model 1			
Higher serum clusterin level	2.06	0.71 – 6.01	0.185
Older age	2.62	0.54 – 12.65	0.230
Male gender	1.17	0.30 – 4.65	0.823
Unemployed	2.03	0.48 – 8.62	0.337
Elementary school vs. college (education level)	4.08	0.83 – 20.12	0.084
High school vs. college (education level)	2.74	0.57 – 13.10	0.207
Model 2			
Higher serum clusterin level	2.94	0.93 – 9.31	0.067
Older age	2.91	0.57 – 14.74	0.394
Male gender	0.90	0.19 – 4.34	0.894
Unemployed	2.39	0.51 – 11.26	0.269
College vs. elementary school (education level)	5.06	0.94 – 27.31	0.059
High school vs. elementary school (education level)	3.66	0.70 – 19.12	0.125
Larger infarct size	7.39	1.36 – 40.14	0.021*
Bilateral vs. right hemisphere (side of lesion)	1.38	0.29 – 6.51	0.683
Left vs. right hemisphere (side of lesion)	0.90	0.27 – 3.01	0.866
Model 3			
Higher serum clusterin level	3.56	1.04 – 12.16	0.043*
Older age	3.10	0.58 – 16.67	0.187
Female gender	1.47	0.25 – 8.75	0.670
Unemployed	2.53	0.47 – 13.75	0.281
Elementary school vs. college (education level)	5.33	0.93 – 30.98	0.062
High school vs. college (education level)	4.64	0.79 – 27.40	0.090
Larger infarct size	6.40	1.15 – 35.50	0.034*
Bilateral vs. right hemisphere (side of lesion)	1.63	0.33 – 8.18	0.551
Left vs. right hemisphere (side of lesion)	0.88	0.25 – 3.07	0.835
Hypertension	1.70	0.25 – 11.60	0.586
Diabetes	1.85	0.52 – 6.55	0.339
Cigarette smoking	1.87	0.45 – 7.75	0.389
Overweight/obesity	1.68	0.56 – 5.11	0.358

Model 1: adjusted for age, gender, employment, and education levels; Model 2: Model 1 plus stroke characteristics (infarct size and infarct side); Model 3: Model 2 plus identified vascular risk factors (hypertension, diabetes, cigarette smoking, and overweight/obesity).

presence of vascular risk factors, such as diabetes mellitus and obesity, is independently associated with increased clusterin production and the development of Alzheimer's disease (Bradley, 2020; Cai *et al*., 2016; Ha *et al*., 2020), the presence of these vascular risk factors before stroke may also have contributed to the significant findings in this study. However, this possibility still requires confirmation through further investigation.

This study demonstrated that infarct size was the stroke characteristic associated with the high frequency of cognitive dysfunction among participants in this study, whereas the lesion side in the brain was not. This finding was in line with the results of previous studies conducted

by Prodjohardjono *et al.* (Prodjohardjono *et al.*, 2020) and Sachdev *et al.* (Sachdev *et al.*, 2006). Since several consequences of stroke occur as a result of dysfunction of the anatomic structures with their complex functional networks for specific tasks, the larger size of the infarction contributes to the greater degree of damage to the anatomical structure and its consequences (Laredo *et al.*, 2018). Thus, considering that cognitive dysfunction is a consequence of ischemic stroke (El Husseini *et al.*, 2023), the extent of the degree of damage to the anatomical structures due to stroke will also increase the vulnerability of stroke patients to have this consequence. However, a previous study conducted by Nys *et al.* (Nys *et al.*, 2007) showed that stroke patients with a lesion side in the left hemisphere are more likely to have cognitive dysfunction than those with a lesion side in the right hemisphere. The finding of this previous study is supported by the results of other studies, which showed that stroke lesions involving structures in the left hemisphere, including the left frontotemporal region, left thalamus, left angular gyrus, and left basal ganglia, tend to increase the risk for cognitive dysfunction (Weaver *et al.*, 2021; Zhao *et al.*, 2018). The difference in results regarding the relationship between the site of the lesion and the incidence of ischemic stroke-associated cognitive dysfunction between this study and the previous studies is more likely due to differences in research methods. Since stroke patients with aphasia were excluded from this study, this may contribute to bias in the results of this study, particularly with regard to the relationship between the site of the lesion and the frequency of ischemic stroke-associated cognitive dysfunction.

This study also indicated that older age, male gender, low education level, unemployment, hypertension, diabetes mellitus, overweight/obesity, and smoking were not associated with the higher frequency of cognitive dysfunction in ischemic stroke patients. Prior studies have shown conflicting results regarding these associations. Prior studies conducted by Utomo and Pinzon (Utomo & Pinzon, 2023) and Kaddumukasa *et al.* (Kaddumukasa *et al.*, 2023) showed that older age and low educational level increased the risk of cognitive dysfunction among ischemic stroke patients. A multicenter study conducted by He *et al.* (He *et al.*, 2023) showed that, apart from older age and low education level, being unemployed and diabetes mellitus were risk factors for post-stroke cognitive dysfunction, whereas hypertension was not. A study conducted by Levine *et al.*(Levine *et al.*, 2018) showed that older age was a predictor of post-stroke cognitive dysfunction, while education level, gender, hypertension, diabetes mellitus, and smoking status were not. Similar to what has been stated in the discussion regarding stroke characteristics above, the differences in the results of this study and previous studies regarding the role of sociodemographic and vascular risk factors are more likely due to differences in research methods.

This research provides additional evidence that serum clusterin levels have a diagnostic value for detecting cognitive dysfunction associated with ischemic stroke in the early phases of the disease, specifically within the initial three months after the onset of the stroke. However, there are several limitations to this study. First, it is important to note that this study was conducted exclusively in a single location, representing a singular population, and utilized a relatively small sample size. Furthermore, the participants in our research were limited to patients who had suffered from minor strokes; our findings must be interpreted carefully upon generalization. Second, since biomarkers for Alzheimer's disease were not assessed in this study, patients with pathology for Alzheimer's disease may be found in this study. Third, the accuracy of determining the infarct diameter may be compromised by significant variability in the time of a head CT scan conducted for diagnostic purposes.

Conclusions

This study revealed a significant prevalence of cognitive dysfunction among individuals diagnosed with ischemic stroke. The findings indicated a positive correlation between elevated serum clusterin levels and the prevalence of cognitive impairment in these individuals. Furthermore, it was observed that a greater infarct diameter exhibited a positive correlation with the increased occurrence of

cognitive impairment in individuals diagnosed with ischemic stroke. These findings offer supplementary data for the development of early identification and intervention approaches for cognitive dysfunction associated with ischemic stroke.

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