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The effect of additional protein, phosphatidylcholine, phosphatidylserine, and inulin on S100β levels of acute ischemic stroke patients at Dr. Kariadi Central Hospital, Semarang

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ABSTRACT

Background: The brain releases biochemical substrates, such as S100β protein, into circulation in response to ischemic conditions as a sign of damage in nerve cells and disruption of the blood-brain barrier's integrity. Thrombolytic therapy has led to the development of many neuroprotective therapies such as protein, phosphatidylcholine, phosphatidylserine, and inulin, which can be added to food products. Protein, phospholipids, and inulin, have a neuroprotective impact on nerve cells in the brain and blood-brain barrier.
Objective: To prove the effect of protein, phosphatidylcholine, phosphatidylserine, and inulin on S100β levels and clinical outcomes in patients with acute ischemic stroke.
Materials and Methods: This study was done in a single-blind RCT. Eighteen ischemic stroke patients were randomly divided into nine subjects for the intervention group and nine subjects for the control group. The Control group received 250 ml conventional formula milk (11.8 g protein) 3 times/day. The intervention group received 250 mL commercial milk 3 rimes/day which contained 15 g protein with 128 mg phosphatidylcholine, 32 mg phosphatidylserine, and 3 g inulin. All of the groups were given hospital-standard therapy for ischemic stroke. S100β levels were measured at pre and post-intervention.
Results: Pre and post S100β levels in intervention and the control group did not show any statistically difference (p = 0.777 and p = 0.096), but there was a trend of decreasing levels of S100β in the intervention group (-24.6 ± 252.0 pg/mL) versus control group (135.8 ± 216.2 pg/mL).
Conclusions: The addition of protein, phosphatidylcholine, phosphatidylserine, and inulin did not have a significant effect on S100β levels.

Keyword: Protein; Phosphatidylcholine; Phosphatidylserine; Inulin; S100β; Stroke

BACKGROUND

Ischemic stroke is the most common stroke that occurs when blood vessels in the brain are clogged by plaque/embolism. Clogged blood vessels interfere with blood flow going to the brain, reducing the supply of oxygen and glucose to the brain. This condition could lead to the death of nerve cells in the brain (apoptosis).[1,2] Damaged and dead nerve cells will cause some biochemical substrates to leave the brain into the circulatory system in response to ischemic conditions. This clog could also result in reduced proteins that play a role in maintaining the integrity of the blood-brain barrier membrane, thus the membrane increases its permeability. This, in turn, causes several biochemical substrates that should stay in the components of the brain to move into the circulatory system.[1–6]

Various biochemical substrates released in response to ischemic conditions play important roles in triggering brain tissue damages[6], one of them is S100β protein. This protein helps to regulate intracellular calcium levels[3], where excessive intracellular Ca^{2+} levels will lead to apoptosis (death of nerve cells).[2,5] Excessive intracellular Ca^{2+} caused by the decrease in the supply of blood that carries oxygen and nutrients to the brain, so the brain will lack energy (ATP) to be function normally. The Ca^{2+} ATPase pump which normally picks up Ca^{2+} into the

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organelle becomes inactive due to lack of ATP in the brain, resulting in the accumulation of intracellular Ca^{2+}. Accumulation of intracellular Ca^{2+} will removes Mg^{2+} ions in charge to keep N-methyl-D-aspartate (NMDA) receptors from being active. Activation of the NMDA receptor will cause stimulation of the post-synaptic membrane, where this membrane is 5 times higher in permeability for Ca^{2+}, so the intracellular Ca2+ ion becomes excessive, which will lead to nerve cell apoptosis. Apoptosis will cause the release of S100β protein in brain components to move into the circulation. S100β protein secretion increases along with the response of glial cells due to metabolic disorders such as head injury, damaged blood-brain barrier, and ischemia.[6]

Damage to cells in the brain will also trigger the activation of catabolic hormones that the patient will experience hypercatabolism.[7] Hypercatabolism, if not treated promptly, will worsen the patient's nutritional status.[7] Also, stroke patients also experience intestinal dysbiosis through immunological pathways.[8–11] Dysbiosis is a condition in which qualitative and quantitative changes occur in the composition, distribution, and metabolic activity of microbes in the intestines, causing adverse effects and worsening the patient's clinical outcome.

Appropriate therapy is the key in ischemic stroke patients.[12,13] One of the pharmacological therapies given is thrombolytic therapy (rTPa).[14] This therapy cannot be given to all acute ischemic stroke patients due to very strict indication criteria, especially in terms of duration (time window). The best timeframe for the administration of this therapy, which can provide the benefit of brain functional improvement and can reduce mortality, is <3 hours and ranged between 3-4.5 hours after symptom onset.[15,16] If the therapy is given not according to the guidelines, it can cause side effects such as the risk of bleeding in the brain and gastrointestinal tract, and angioedema.[14]

This situation has led to the development of many neuroprotective therapies, namely the addition of phospholipids which can be given directly in the form of supplements (cytolin) or fortified in food products such as milk. Phospholipids are a type of fat found in many nerve cell membranes. There are several types of phospholipids, the common ones are phosphatidylcholine and phosphatidylserine. Phosphatidylcholine increases the biosynthesis of membrane phospholipids which is degraded by an increase in free radicals during brain ischemia (neuroprotection).[12,17] Phosphatidylcholine also inhibits the activation of enzymes that trigger apoptosis of nerve cells,[12,17] thus affecting S100β levels. Also, the protein contained in milk will increase protein intake, muscle mass, and possibly increase body mass index (BMI). Therefore, it can improve the patient's motoric function and prevent the patient from experiencing a decrease in nutritional status.[18–20]

One of the nutrients that need to be given to ischemic stroke patients is inulin. Studies regarding the effect of inulin in improving ischemic conditions are still very limited in ischemic stroke patients and there are no studies about the direct effect of inulin on blood biomarkers of brain damage such as S100β protein. So far research has proven that Short-Chain Fatty Acid (SCFAs) acts as neuroprotective agents in the nerve cells. Inulin will be quickly fermented by *Bifidobacteria* and *Lactobacilli* (probiotic bacteria) and will produce SCFA in the form of acetic acid, propionic, butyrate, L-lactate, CO2, and hydrogen as fermentation products.[21,22] SCFA can be used as the source of energy (ATP) by the central nervous system because long-chain fatty acids cannot cross the blood-brain barrier membrane. SCFA has been proved to play a neuroprotective role, synthesizing neurotransmitters, and modulating the immune system. Thus, SCFA can improve dysbiotic conditions due to ischemic stroke.[23–25]

Dr. Kariadi Central Hospital is an "A-Accredited" Hospital that has a Stroke Unit with an average of two new stroke patients every day. The number of stroke patients in this hospital continues to increase.[26] This research aimed to prove the effect of additional protein, phosphatidylcholine, phosphatidylserine, and inulin on S100β levels and clinical outcomes of acute ischemic stroke patients at Dr. Kariadi Central Hospital, Semarang.

MATERIALS AND METHODS

A randomized control trial with single-blind was used as the design of this research. The effect of additional protein, phosphatidylcholine, phosphatidylserine, and inulin was seen through S100β levels. The research subjects were acute ischemic stroke patients in the inpatient room of Rajawali 1A of Dr. Kariadi Central Hospital Semarang amounted to 18 people (control = 9, intervention = 9), 10 subjects were male and 8 subjects were female. The research lasted for 4 months (February-May 2020). Acute ischemic stroke patients were selected to participate if their attack onsets were <72 hours and aged > 18 years.

Also, the researchers had randomized the subjects through Consecutive Sampling and provided informed consent to the patient/patient's family. During the implementation of the research, 7 patients dropped out or lost to follow-up with the following details: 4 patients not domiciled in Semarang, 2 patients died during the intervention, and 1 patient was forced to move out from hospital because of cost limitation.

The physical and neurological examinations had been performed by the doctor in charge of treating those patients. Supporting data, such as weight, blood pressure, blood glucose, lipid profile, smoking history, rTPa therapy status, and history of recurrent stroke had been taken from the patients' medical records once when the patient entered the stroke ward only to determine the risk factor data such as obesity, hypertension, diabetes mellitus, dyslipidemia, and smoking history. The researchers assessed the subject's dietary intake of energy, carbohydrate, protein, fat, phosphatidylcholine, phosphatidylserine, and inulin every day using the 24 hours recall method. The intake data consumed by the subjects will be compared with the patient's requirement to obtain the adequacy of nutritional intake which is expressed in percentage (%) with the following formula:

$$\text{Adequacy intake:} \frac{nutrient\ intake}{nutrient\ need} \text{x } 100\%$$

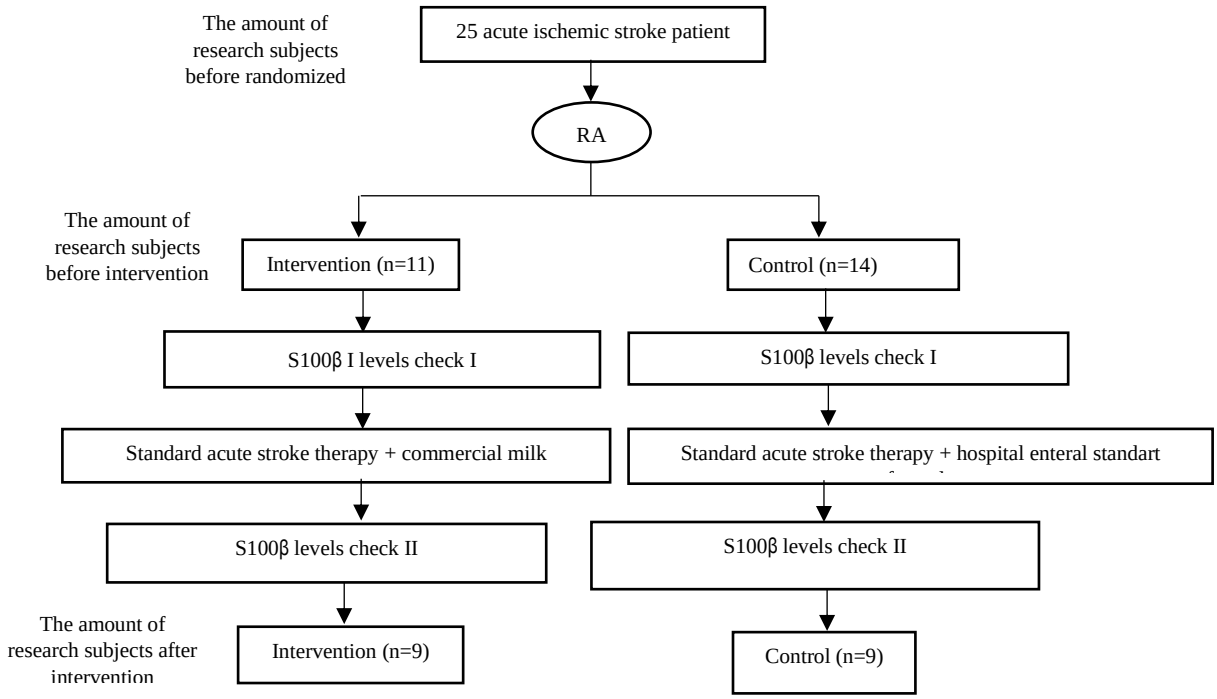

Figure 1. The Flow Chart of the Amount of Research Subjects (Start to Finish)

The nutritional requirement has been adjusted to the nutritional requirement for ischemic stroke patients: 30 kcal/kg BW for energy, 60% TEE for carbohydrate, 20% TEE for protein, 20% TEE for fat, 1000 mg/day for phosphatidylcholine, 100 mg/day for phosphatidylserine, and 12 mg/day for inulin. All data collection has been recorded on the research sheets that had been prepared by researcher.

The control group was given milk based on the enteral standard formula from the hospital that contains 11.8 g protein (from hospital database) without the addition of phosphatidylcholine, phosphatidylserine, and inulin as much as 250 ml.

The intervention group was given 69 g of commercial milk powder which contains higher protein than the control group (15 g) with the addition of 128 mg phosphatidylcholine, 32 mg phosphatidylcholine, and 3 g inulin. The commercial milk given to the intervention group contains a moderate glycemic index, therefore, it is necessary to adjust the dose and the duration of the intervention for subjects with diabetes. In the intervention group, subjects with diabetes mellitus received 34.5 g of commercial milk powder (half the original dose) so the duration of the treatment becomes longer (14 days). Researchers cannot exclude subjects with

diabetes because of the limited number of study subjects. All the treatment was given 6 times a day during the acute phase (1-3 days according to the patient's swallowing ability) by enteral tube and then given orally 3 times a day during the recovery phase until the end of treatment (day 7). During the administration of the intervention, the researchers observed functional and biochemical changes as well as possible side effects.

Whole blood vein samples were collected from the mediana cubiti vein. A sampling of 6 mL was carried out using a red *vacutainer* (blood clot without the addition of EDTA). The duration between blood sampling to storing should not exceed 8 hours. Blood samples were sent to the central laboratory of Dr. Kariadi Central Hospital Semarang for centrifugation and stored in a deep freezer (-80ºC) until the number of research, samples were met. After meeting the sample size, blood serums were sent to the GAKI Laboratory of the RSND-FK UNDIP. Serum S100β levels were checked using the Human S100β ELISA (Enzyme-Linked Immunosorbent Assay) kit Elabscience with catalog number E-EL-H1297. The inspection method was following the manual found in the kit. The analysis of serum S100β levels has been carried out twice; when the patients were admitted to Dr. Kariadi Hospital Semarang before given intervention (day 1) and after given intervention (day 7). This research has received Ethical Clearance approval by the Medical Research Ethics Commission of FK UNDIP / RSDK with registration No. 479 / EC / KEPK-RSDK / 2020.

Statistical analysis was performed using SPSS version 22.0 for windows. Data with categorical scales were expressed in the distribution of frequencies and proportions. The numerical scale data [levels of S100β and changes (Δ) of S100β] were tested for normality and then tested using Paired T-test and Independent T-Test. Also, different levels of S100β and changes (Δ) of S100β in the DM and Non-DM subgroups have been carried out using Paired T-Test/ Independent T-test for normally distributed data and Willcoxon/ Mann Whitney for data that were not normally distributed.

RESULTS

Research Subject Characteristics

There was no significant difference in the category of age (p = 0.347), gender (p = 0.637), mean BMI (p = 0.468), obesity status (p = 1.000), hypertension (p = 0.576), diabetes mellitus (p = 1.000), dyslipidemia (p = 1.000), smoking status (p = 1.000), rt-PA therapy (p = 1.000), inpatient duration (p = 0.637), intervention duration (p = 0.206), and history of recurrent stroke (p = 1.000).

Adequacy Level of Energy, Protein, Fat, and Carbohydrate Intake

The adequacy level was obtained from the total intake per day compared to the actual needs and was presented in percentage. Table 2 shows that there were significant differences in the mean level of adequacy of energy intake (p = 0.008), protein (p = 0.002), carbohydrate (p = 0.002), phosphatidylcholine (p=0.000), phosphatidylserine (p=0.000), and inulin (p=0.000) however there was no significant difference in the mean level of adequacy of fat intake between groups (p = 0.912). For the control group, all adequate levels of nutrient intake were deficits, whereas in the intervention group only adequate levels of intake of fat and phosphatidylcholine were deficits.

Serum S100β Levels Distribution Before and After Interventions

The results of statistical tests showed that there was no significant difference in the distribution of serum S100β levels both before (p = 1.000) and after the intervention (p = 0.576) between the intervention group and the control group. The serum S100β level category <236.7 pg/mL indicates that the patients tended to have a good improvement in clinical outcome. Serum S100β levels> 236.7 pg/mL are associated with poor clinical outcomes and even death. Based on the aforementioned results, one subject in the intervention group before treatment had a high-risk S100β level category, but it went down to the low-risk category after the treatment compared to one subject in the control group who had a low-risk S100β level category before treatment and went up to being categorized as high risk after the treatment. This shows that there was a trend of improvement in S100β levels in the intervention group compared to the control group.

Differences in S100β levels and changes (Δ) of S100β levels

The mean serum S100β levels in baseline of the control and intervention groups (358.8 $\pm$ 215.3 and 512.3 $\pm$ 343.9 pg / mL, respectively) were not statistically significant (p = 0.273). This illustrates that the condition of the two groups before the intervention was homogeneous. In table 4, it is shown that there was a trend of decreasing mean serum S100β levels in the intervention group from 512.3 $\pm$ 343.9 pg / mL to 487.7 $\pm$ 366.8 pg / mL, while in the

control group there was an increasing trend in the mean serum S100β level from 358.8 ± 215.3 pg / mL to 494.6 ± 296.4 pg / mL. Also, the pre and post S100β levels in both the intervention and control groups were not statistically significant (p = 0.777 and p = 0.096, respectively). There was no significant difference in the mean change (Δ) of serum S100β levels in the intervention group (-24.6 ± 252.0 pg / mL) and control group (135.8 ± 216.2 pg / mL), but in the intervention group, the tendency of decline was better than the control group although it was not statistically significant (p = 0.166).

Table 1. The Characteristics of Research Subjects in Research Groups

Characteristic	Group		*p*
	Intervention (n=9)	Control (n=9)	
Gender			
- Male	4 (44.4%)	6 (66.7%)	0.637[b]
- Female	5 (55.6%)	3 (33.3%)	
Mean age (year)	50.6 ± 14.8	62.6 ± 8.8	0.053[a]
Age category			
- >60	3(33.3%)	6(66.7%)	0.347[b]
- <60	6(66.7%)	3(33.3%)	
Mean BMI (kg/m^2)	25.3 ± 4.1	24.1 ± 2.9	0.468[a]
Obesity Status			
- Yes (BMI ≥ 25 kg/m^2)	4 (44.4%)	3 (33.3%)	1.000[b]
- No (BMI < 25 kg/m^2)	5 (55.6%)	6 (66.7%)	
Hipertension			
- Yes (BP ≥ 130/80 mmHg)	8 (88.9%)	6 (66.7%)	0.576[b]
- No (BP < 130/80 mmHg)	1 (11.1%)	3 (33.3%)	
Diabetes Mellitus			
- Yes	3 (33.3%)	4 (44.4%)	1.000[b]
- No	6 (66.7%)	5 (55.6%)	
Dislipidemia			
- Yes	7 (77.8%)	8 (88.9%)	1.000[b]
- No	2 (22.2%)	1 (11.1%)	
Smoking Status			
- Yes	5 (55.6%)	6 (66.7%)	1.000[b]
- No	4 (44.4%)	3 (55.6%)	
rt-PA Therapy			
- Yes	2(22.2%)	1(11.1%)	1.000[b]
- No	7(77.8%)	8(88.9%)	
Inpatient Duration			
- <7 days	6(66.7%)	4(44.4%)	0.637[b]
- ≥7 days	3(33.3%)	5(55.6%)	
Intervention Duration			
- 7 days	6(66.7%)	9(100%)	0.206[b]
- 14 days	3(33.3%)	0(0%)	
History of Recurrent Stroke			
- Yes	3(33.3%)	3(33.3%)	1.000[b]
- No	6(66.7%)	6(66.7%)	

[a] Independent T-Test; [b] Fisher-Exact Test
The values in table are: mean ± SD; n (%) Percentage shown in columns

Table 2. The Mean Adequacy Intake of Energy, Protein, Fat, and Carbohydrate between Groups

Adequacy Levels	Intervention Mean±SD	Control Mean±SD	*p*
Energy (%)	95.1±18.4	73.8±10.1	0.008[a]
Carbohydrate (%)	107.0±20.6	76.6±12.2	0.002[a]
Protein (%)	94.2±17.6	68.5±10.9	0.002[a]
Fat (%)	74.3±21.2	73.4±11.2	0.912[a]
Fosfatidilkolin (%)	72.7±8.1	25.3±3.4	0.000[a]
Fosfatidilserin (%)	112.4±12.3	48.7±10.3	0.000[a]
Inulin (%)	92.4±8.0	24.4±7.3	0.000[a]

[a] Independent T-Test

Table 3. The Distribution of S100β Levels before Intervention in Research Groups (n=18)

	Category of Serum S100β Levels (pg/mL)	Group		*p*
		Intervention (n=9)	Control (n=9)	
Before Intervention	Low risk (<236.7)	2(22.2%)	2(22.2%)	1.000[b]
	High risk (≥236.7)	7(77.8%)	7(77.8%)	
After Intervention	Low risk (<236.7)	3(33.3%)	1(11.1%)	0.576[b]
	High risk (≥236.7)	6(66.7%)	8(88.9%)	

[b]Fisher Exact Test

Table 4. The Difference in Serum S100β Levels (pg/mL)

Group	n	Before Mean±SD	After Mean±SD	*P*	Δ Mean±SD
Intervention	9	512.3±343.9	487.7±366.8	0.777[c]	-24.6±252.0
Control	9	358.8±215.3	494.6±296.4	0.096[c]	135.8±216.2
p		0.273[a]	0.965[a]		0.166[a]

[a]Independent T-Test; [c] *Paired T-Test*

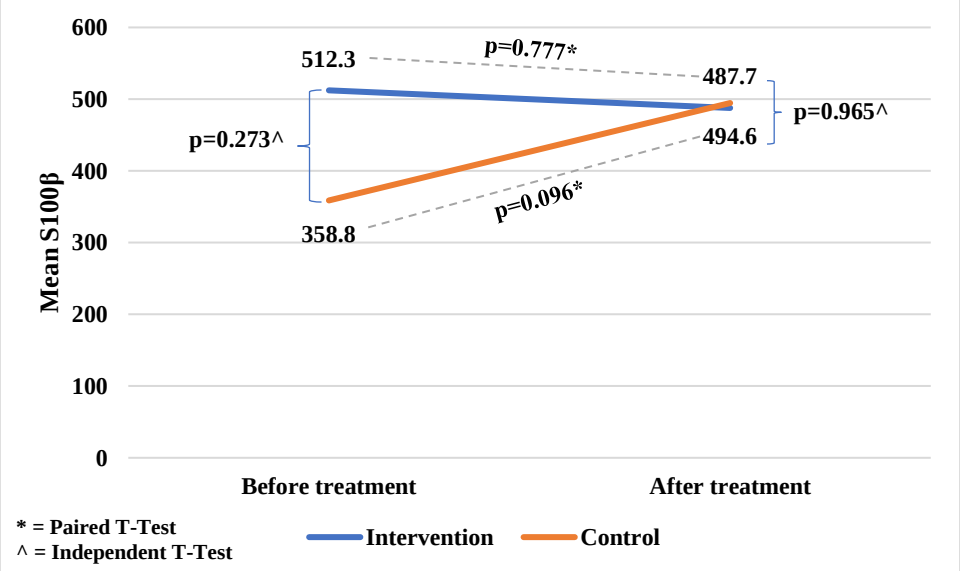

Figure 2. The Serum S100β Levels before and after Intervention

Sub-group analysis of S100β levels

In DM and non-DM sub-groups, the median serum S100β levels before treatment between the intervention and control groups were not statistically significant (p = 0.480 and p = 0.251, respectively). This illustrates that the condition of the two sub-groups before the intervention was homogeneous. There was no significant difference in the median

levels of S100β before and after the intervention in both DM (p intervention = 0.285; p control = 0.272) and non-DM sub-group (p intervention = 0.484; p control = 0.308).

In DM and Non-DM sub-groups, there was a trend of decreasing S100β levels in a better direction for the intervention group and the decrease (Δ) in the DM sub-group was bigger than Non-DM sub-group compared to the control group where there was an increasing trend (Δ) in S100β levels in DM and Non-DM sub-group and the increase (Δ) in DM sub-group was bigger than Non-DM sub-group.

DISCUSSION

The data of research subjects showed no significant difference in mean BMI, mean age, age category, gender, obesity, hypertension, diabetes mellitus, dyslipidemia, smoking status, rt-PA therapy, inpatient duration, intervention duration, and history of recurrent stroke (p>0.05).

Table 5. The *Sub Group* Analysis of S100β Levels between Groups

Sub Grup	Kelompok	n	Sebelum Median(Min-Max)	Sesudah Median(Min-Max)	*p*	Δ Median(Min-Max)
DM	Intervention	3	971.9(115.5-977.9)	521.8(146.6-771.8)	0.285[c]	-200.1(-456.1 – 31.1)
	Control	4	428.8(252.1-794.2)	641.0(253.4-1015.9)	0.272[d]	117.8(-83.1 – 494.9)
	P		0.480[e]	0.589[a]		0.102[a]
Non-DM	Intervention	6	475.1(28.5-660.7)	392.7(25.6-1004.8)	0.484[c]	-39.9(-117.5 – 344.5)
	Control	5	255.9(107.1-426.5)	417.8(187.7-610.9)	0.308[c]	75.0(-178.8 – 355.0)
	P		0.251[a]	0.593[a]		0.584[e]

[a] Independent T-Test; [c] Paired T-Test; [d] Willcoxon Test; [e] Mann Whitney Test

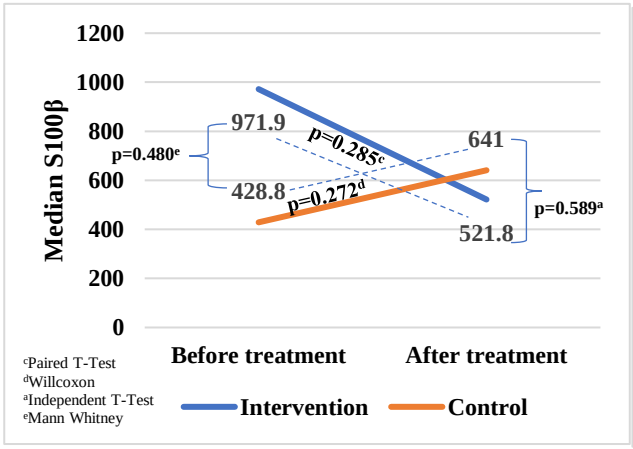

Figure 3. The S100β Levels before and after intervention in DM Sub Group

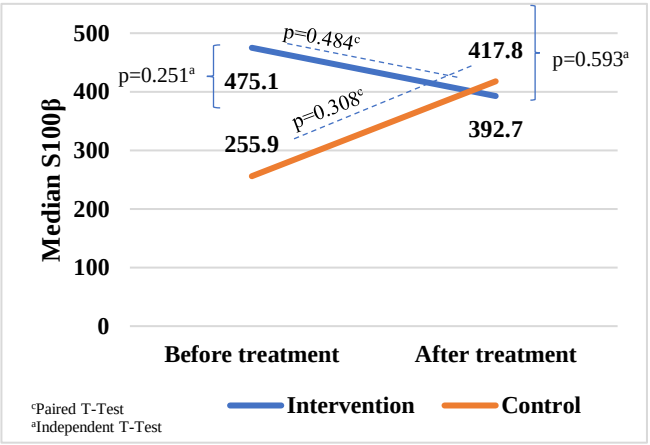

Figure 4. The S100β Levels before and after intervention in Non-DM Sub Group

Stroke does not only occur in the elderly, but also occurs in those at productive age under 45 years, and there are some cases where the sufferers are under 30 years old.[27,28] In this study, 2 subjects were under 30 years old. The habit of consuming food containing high fat and smoking has been shown to influence the incidence of ischemic stroke at an age less than 45 years.[29] People who consume high-fat foods ≥3 times/week have a 3.744 times more risk of suffering from ischemic stroke compared to people who consume high-fat foods <3 times/week.[29] High-fat food sources when consumed excessively can increase cholesterol levels in the blood. If the cholesterol level in the blood is abnormal (≥ 200 mg / dL) it will increase the accumulation of fat on the walls of blood vessels, causing narrowing of the blood vessels and disrupting the blood supply to the brain.[30] Cholesterol can form blood clots and plaques that clog the arteries and eventually cut off the blood flow to the heart and brain. This can lead to heart attacks and strokes.[31] Smoking is associated with a hypercoagulable state. It is characterized by an increase in thromboxane release which causes increased platelet activation and degeneration of the vascular endothelium, which promotes the formation of thrombus-producing plaque. Nicotine has no direct

effect on this mechanism, but it is possible that the burning of cigarette smoke causes thromboembolic formation, leading to ischemic stroke.[32]

The data on the adequacy of nutritional intake showed that there were significant differences in levels of the adequacy of energy, carbohydrate, and protein between groups ($p < 0.05$). There was no difference in the level of adequacy of fat between groups ($p > 0.05$). The nutritional content of commercial milk given to the intervention group had higher energy, carbohydrate, and protein content (Energy = 289.8 kcal, Protein = 15.18 grams, KH = 44.16 grams per 250 mL) compared to milk given to the control group (Energy = 222.2 - 246.4 kcal, Protein = 11 - 11.8 grams, CH = 28 - 29.2 grams per 250 mL), while milk given to the control group had higher fat content (9 gram / 250 mL) compared to the milk given to the intervention group (5.52 g / 250 mL). This was also influenced by nutritional requirement acceptance of each respondent, method of administration (enteral tube for acute phase and then orally for recovery phase), the role of medical team, especially nurses, and family members in motivating patients to eat, as well as the length of hospitalization of patients.

Also, there were six subjects (66.7%) in the intervention group and four subjects (44.4%) in the control group who returned home before the 7th day. Patients in both control and intervention groups who returned home before the 7th day still had poor appetite, but patients in the intervention group have received the commercial milk to be consumed at home while the control group did not. This led to a higher level of energy, protein, and carbohydrate intake in the intervention group even though the patient did not have a good appetite. Also, patients who returned home before the 7^{th} day tended to have an increase in fat intake based on the results of the recall of intake by telephone. They tended to eat food that contains high fat rather than at the hospital, where the menu has been adjusted with the requirements and conditions of each respondent.

The results of statistical tests showed that there was no significant difference in the mean serum S100β levels between before and after the intervention or between groups. The causes of this insignificant difference were multifactorial, one of which was that the subjects in this research have different comorbidities such as hypertension, diabetes mellitus, obesity, and dyslipidemia. According to research, S100β levels in subjects who have comorbid like diabetes mellitus, hypertension, central obesity, and dyslipidemia will tend to be increased.[33,34] Two subjects in the intervention group and one subject in the control group in this research received rt-PA therapy. Some research proved the effectiveness of citicoline as a neuroprotective therapy, showing that rt-PA could improve blood flow in the penumbra area. This leads to becoming soluble and having difficulty improving its performance due to the effects of thrombolysis. Also, the higher the severity of the stroke will affect the performance of citicoline because the penumbra areas that are damaged are not fully reached by citicoline.[12,35] The results of the difference test (Δ) S100β levels in this research were not statistically significant ($p = 0.166$) between groups. This shows that rt-PA therapy does not affect the intervention given. Another research also stated that rt-PA did not significantly affect S100β levels.[36] The reason was that the response of each individual to rt-PA therapy was different. rt-PA therapy can exert a significant neuroprotective effect only when thrombolysis and reperfusion are achieved earlier, whereas in cases where reperfusion is not immediately achieved there may be side effects such as blood-brain barrier (BBB) disruption and hemorrhagic transformation.[36]

A study shows that there is a positive and significant correlation between age and S100β levels in study subjects with mild TBI (Traumatic Brain Injury). The study reported that the subjects aged >65 years had higher levels of S100β (> 0.10 μg / L) compared to subjects aged <65 years. This is because older subjects usually have chronic diseases or neurological diseases such as Alzheimer's and Parkinson's, but further research is needed to explain this association.[37] In this study, the mean age of subjects in the control group was older (62.6 ± 8.8) than in the intervention group (50.6 ± 14.8). This is one of the possible causes of S100β levels in the control group which tended to increase compared to the intervention group.

Research on the effect of giving protein, neuroprotective substances (phosphatidylcholine and phosphatidylserine), and inulin on brain damage markers, especially S100β, is still very limited. In this research, the interventions were administered orally in the form of powdered milk containing neuroprotectants (phosphatidylcholine and phosphatidylserine) and prebiotic inulin. In oral administration, phosphatidylcholine and phosphatidylserine are almost completely absorbed efficiently. Once absorbed, phosphatidylcholine and phosphatidylserine spread widely throughout the body through the blood-brain barrier and reach the central nervous system (CNS).[38,39] Inulin is soluble in

water and difficult to be hydrolyzed by enzymes in the digestive tract, thus it can reach the large intestine mostly intact. In the large intestine, inulin will be fermented by probiotic bacteria into short-chain fatty acids (SCFA) and some specific microflora that produces lactic acid.[40]

The results of different tests for S100β levels were not statistically significant, but in the intervention group, there was a trend of decreasing S100β levels compared to the control group. According to research, a high-protein diet given from the beginning provides regulation at the molecular level, which helps to increase SOD expression, suppresses MDA expression, and restores iNOS expression.[41] This suggests that a high-protein diet given from the beginning can inhibit oxidative stress, thereby helping the body to eliminate free radicals that play a role in the pathophysiological process of stroke.[41]

Vitamin B supplementation (folic acid, riboflavin, vitamin B12) and choline are effective in improving stroke recovery by suppressing the transcription factor p53 mechanism which causes apoptosis (death of nerve cells) where p53 activation is triggered by an increase in homocysteine. Also, there is an increase in neuroplasticity through increased BDNF (Brain-derived neurotrophic factor) expression and activation of anti-oxidative mechanisms characterized by increased levels of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) and SOD2 (Superoxide dismutase 2) enzyme. This means an increase in the flexibility of cells to manage oxidative damage.[42]

Several kinds of research on the effects of phosphatidylserine supplementation in experimental animals and humans have been carried out on the improvement of cognitive and memory functions by largely unexplainable mechanisms. In essence, phosphatidylserine plays a role in the activation of signaling proteins and receptors that are important for neuron survival, differentiation, and regulation of neurotransmitters in the signaling process.[39]

Inulin is an example of a prebiotic. Prebiotics are components of food ingredients that the digestive tract cannot enzymatically digest. Thus, they will be fermented by microbiota in the large intestine. Inulin acts as a substrate to increase the diversity of beneficial microbiota in the intestine, increasing the production of SCFA (acetate, propionate, and butyrate) as a result of anaerobic fermentation.[43] SCFA can affect the gut-brain axis and brain function directly or indirectly. SCFAs are absorbed by colonic cells via H^+-dependent monocarboxylate transporter (MCT) or Na^+-dependent monocarboxylate transporter (SMCTs). SCFA then binds to G protein-coupled receptors (GPCRs) such as FFAR2, FFAR3, GPR109a / HCAR2 (hydrocarboxylic acid receptors), and GPR164 by inhibiting histone deacetylation that will affect intestinal mucosal immunity and intestinal barrier integrity. SCFA interaction with some of these receptors on enteroendocrine cells will cause indirect signaling to the brain through the systemic circulation by inducing the secretion of intestinal hormones such as glucagon-like peptide 1 (GLP1), peptide YY (PYY), γ-aminobutyric acid (GABA), and serotonin (5-HT). Colon-generated SCFAs reaches the systemic circulation and other tissues leading to activation of brown adipose tissue, regulation of mitochondrial function in the liver, increased insulin secretion by β-pancreatic cells, and energy homeostasis throughout the body. Systemically, SCFAs influence systemic inflammation by inducing differentiation of T regulatory (Treg) cells and regulating interleukin secretion. SCFA can cross the blood-brain barrier via monocarboxylate transporter (MCT) located on endothelial cells and improve the integrity of the blood-brain barrier by increasing the expression of tight junction proteins. Furthermore, in the central nervous system (CNS), SCFA fixes inflammation in the nerves by improving glial cell function and morphology, modulating levels of neurotrophic factors, increasing neurogenesis, contributing to serotonin biosynthesis, and increasing the function and homeostasis of nerve cells. SCFA interactions with the gut-brain axis can directly or indirectly affect the emotion, cognition, and pathophysiology of brain disorders.[44]

Since researchers can not exclude subjects with diabetes mellitus, then subgroup analysis was done. The results of DM and Non-DM sub-groups analysis showed that in DM and Non-DM sub-groups there was a trend of decreasing S100β levels in a better direction for the intervention group and a decrease (Δ) in the DM subgroup was bigger than Non-DM subgroup compared to a control group where there were a trend of increasing (Δ) levels of S100β in the DM and Non-DM subgroups and an increase (Δ) in DM subgroup was bigger than Non-DM subgroup. This reason was that in the DM subgroup the intervention duration was different from the Non-DM subgroup, especially in the intervention group. Milk given to the intervention group had a rather high glycemic index, so it was necessary to adjust the daily dose in patients with comorbid DM. This resulted in the intervention duration that was originally given for 7 days stretched to 14 days.

According to research, the pattern of S100β levels will increase from 0-2 hours of onset to 24-48 hours of onset (peak level) then S100β levels will decrease up to 2-3 weeks after onset.[45] This causes a decrease in (Δ) levels of S100β bigger in the DM subgroup compared to the Non-DM sub-group.

The limitation of this research was the number of samples which was 18 subjects (intervention = 9; control = 9). Dr. Kariadi Central Hospital is a referral hospital for COVID-19 patients, so the hospital limits the number of patients at the polyclinic every day. Also, many people are afraid of going to the hospital. Another reason might be the dose and duration of intervention. In some research, the minimum duration of 14 days[38,41,43,46,47] and a minimum dose that can provide a better clinical outcome is 1000 mg/day for phosphatidylcholine.[48,49] In this study, the mean phosphatidylcholine intake in the intervention group is still a deficit. The subjects receive the treatment by enteral tube in the acute phase (1-3 days after stroke onset) followed by oral administration. For administration through the enteral tube, it will be easier to ensure that the treatment is completed, whereas when given orally, some patients do not cooperate due to several factors such as the imperfect swallowing ability and the level of consciousness is still weak. Also, there were subjects in both the control and intervention group who returned home before the 7th day of treatment because some patients ask to continue the recovery phase at home after they have passed the acute period. This has to lead the researchers to recall those subjects regarding their intake by telephone. As a result, patients tend to forget about what they have consumed, also the (household size used by those subjects was different so that the recall results could be underestimating/overestimate. In the hospital, portion standards are already specified for each type of diet.

CONCLUSIONS

The addition of 15 g protein, 128 mg phosphatidylcholine, 32 mg phosphatidylserine, and 3 g inulin, three times a day, for seven days, in patients with acute ischemic stroke does not have a significant effect on S100β levels statistically, however, there is a decreasing trend of S100β levels for the intervention group.

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