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# Serum Sphingosine 1-Phosphate as a Biomarker for Post-Stroke Cognitive Impairment

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## ABSTRACT

**Background:** Stroke is a main cause of disability. Impaired cognition is an important aspect for stroke survivors. The discovery of laboratory biomarkers for post stroke cognitive impairment (PSCI) may help identification of those who are at risk of cognitive impairment and application of suitable therapeutic regimens.

**Objective:** This study aimed to measure S1P serum levels in a group of patients with severe ischemic stroke at admission and to determine if they are associated with post stroke cognitive state.

**Patients and Methods:** The study has been applied on sixty patients who had acute ischemic stroke in addition to 40 apparently healthy. The mean of age and gender in subjects and controls were matched. Serum sphingosine -1 phosphate (S1P) levels were analyzed by ELISA technique for all patients within 72 hours of admission and for healthy controls. The severity of the stroke has been evaluated based on the scale of the National Institute of Health Stroke (NIHSS). Patients also underwent cognitive assessment using Montreal Cognitive Assessment (MoCA) at admission and after 3 months.

**Results:** The level of serum S1P was apparently reduced in acute stroke patients by comparing with the healthy controls ($p< 0.001$). Furthermore, the decreased levels of the S1P serum were obviously with more disease severity as measured by high NIHSS score at admission and with more post stroke cognitive impairment as assessed by MoCA scale three months later after stroke onset.

**Conclusions:** The study came up with key findings that reported a clear step-down in the levels of serum S1P in the patients with acute stroke as compared to the healthy control and the same obvious reduction in S1P levels in cognitive impairment patients as compared to those with non-cognitive impairment.

**Keywords:** Post-stroke cognitive impairment, Sphingosine-1-phosphate, Montreal cognitive assessment scale.

## INTRODUCTION

PSCI is common among stroke survivors. Previous studies identified that prevalence of PSCI is 53.4% within 1.5 years of stroke [(1)]. Many cognitive domains are affected in PSCI; of these, impaired functions like attention appear to be the most prevalent. Cognitive deterioration is usually obvious immediately after stroke but most of these deficits resolve during the former recovery period. Nevertheless, longitudinal studies have demonstrated that long term-prevalence of impaired cognitive after stroke may be as high as 30-50% at 5 years [(2)]. Cognitive impairment results in poor long-term consequences on the daily activities and quality of life (QOL) and is associated with remote morbidity and disability. Therefore, finding a way to help in former detection and effective treatment for PSCI has become one of the priorities of modern neurological rehabilitation [(3)].

Detection of post stroke cognitive impairment depends basically on clinical manifestations, neuropsychological testing and neuroimaging. However, these methods depend on the cooperation of patients and are not accurate enough for former diagnosis of post stroke cognitive impairment. Therefore, the identification of objective former biomarkers for PSCI would be of significant clinical value in supporting the ability of physicians to tailor treatment regimens and improve outcome of PSCI, and give us better understanding of the pathophysiological mechanisms of the disease [(4)].

Sphingosine 1- phosphate (S1P) is a lipid metabolite that mediates several physiological processes, of which vascular endothelial cell function, coagulation thrombosis, angiogenesis and inflammation [(4)]. S1P is a pleiotropic lipid mediator. Several studies demonstrated that endothelial cells, thrombocytes, neutrophils, macrophages and erythrocytes are the main sources of S1P in blood [(5)].

About 50% of S1P in plasma is bound to high-density lipoprotein cholesterol (HDL-C), while about 40% is associated with albumin. S1P is a ligand of five G protein coupled cell surface receptors, S1PR1–S1PR5. These receptors are represented in different systems involving the immune, respiratory, cardiovascular, hepatic, and neurological systems [(6)]. By binding with different receptor subtypes, S1P regulates many physiological and pathological functions, such as blood flow, blood pressure, heart rate, vascular endothelial function, atherosclerosis, coagulation/thrombosis, and inflammatory responses, all of which play important roles in the pathogenesis and progression of stroke [(4)]. In addition, S1P is shown to execute various functions in the cells of the central nervous system, such as differentiation, survival and excitability of neurons, activation of astrocyte-mediated neuroinflammation, and processing of amyloid precursor protein [(7)]. Recently, serum S1P was found to



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be reduced in patients with an acute ischemic stroke [4] and its levels were obviously with a higher NIHSS score, increased infarction volume and poorer outcomes after 90 days.

In this study we aimed to measure S1P serum levels in a group of patients with severe ischemic stroke at admission and to determine if they are associated with post stroke cognitive state.

## SUBJECTS AND METHODS

In a prospective study, 60 patients who had an acute ischemic stroke were recruited from Emergency Department and Neurology Outpatient Clinic, Al-Zahraa University Hospital. The assessment was done in the Neurology Department. Ischemic infarcts were verified in all patients by computed tomography (CT) performed at the time of admission within 48 h of symptom. The inclusion criteria also required age 40-65 years old.

**Exclusion criteria:** Diagnosis of dementia or significant impaired cognition before stroke, primary hemorrhagic stroke, decreased level of consciousness, severe aphasia or dysarthria, significant acute medical illness such as infection, autoimmune disease and cancer, significant acute neurological illness other than stroke, and the presence of a premorbid axis I psychiatric disorder.

The grade of neurological dysfunction was assessed at admission using NIH Stroke Scale (NIHSS). The cognitive status was measured using the Montreal cognitive assessment (MoCA), which has a cutoff score of 24 for cognitive impairment. The MoCA is a widely used, reliable, and validated instrument used in screening for cognitive impairment.

The patients were classified into two groups based on the MoCA scores. The first group consisted of post-stroke cognitively impaired patients (PSCI group: MoCA ≤ 24). The second group consisted of post-stroke non-cognitively impaired patients (PSNCI group: MoCA > 24). A forty healthy peoples were invited to participate as controls provided they had no history of stroke, transient ischemic attack (TIA), or other neurologic disorder. This ‘‘control’’ group were age and gender matched with the patient group. Blood samples were collected for serum level of S1P within 48 hours of admission. S1P levels were estimated by Sundered Biological Technology (China) by ELISA technique. This ELISA kit is based on the principle of double antibody sandwich technique to detect Human (S1P).

**Ethical approval:**

**All subjects or their care givers provided a written informed consents. The protocol of this study was reviewed and approved by the Research Ethics Committee, Faculty of Medicine for girls, Al-Azhar University (FMG-IRB), Cairo. The study was conducted according to the Declaration of Helsinki.**

***Statistical analysis***

It was performed using SPSS, the renowned statistics package software. The quantitative data were presented as mean ± standard deviation and ranges. Also qualitative variables were presented as number and percentages. Chi-square test was utilized to compare between two groups with qualitative data and Fisher exact test was utilized instead of the Chi-square test when the expected count in any call found less than 5. Independent t-test has been utilized to link between two clusters with quantitative data and parametric distribution. Mann-Whitney test was utilized in the judgment between two clusters with quantitative data and non-parametric distribution. Analysis of Variance (ANOVA) and Kruskall-Wallis tests were used to compare between other clusters with quantitative data and non-parametric distribution. We used Spearman correlation coefficient to assess the significant relation between two quantitative parameters in the same group. Probability (P-value) P-value ≤0.05 was considered significant, P-value < 0.001 was considered as highly significant and P-value > 0.05 was considered insignificant.

## RESULTS

Sixty patients diagnosed with acute ischemic stroke included in the study had mean age of 57.70 ± 6.22 years and 53.3% were females. In addition, 40 healthy people worked as control had mean age of 55.17 ± 10.01 years as shown in table (1)

**Table (1):** Demographic data of all groups including the patients and control groups

| Demographic data | Patients Group (n=60) | Control Group (n=30) | Test value | p-value |
|---|---|---|---|---|
| **Age (years)** | | | | |
| Mean ± SD | 57.70 ± 6.22 | 55.17 ± 10.01 | $t$=1.438 | 0.154 |
| Range | 42-65 | 40-65 | | |
| **Sex** | | | | |
| Male | 28 (46.7%) | 19 (63.3%) | $x^2$=2.227 | 0.136 |
| Female | 32 (53.3%) | 11 (36.7%) | | |

*Using: t-Independent Sample t-test, p-value >0.05 non-significant.*

The clinical data of the patient group were presented in table (2).

**Table (2):** Clinical data of patients group

| **Clinical data** | |
|---|---|
| - Sex, n (%) | |
| -- Cognitive impairment among females, n (%) (n=32) | 29 (90.6%) |
| --Cognitive impairment among males, n (%) (n=28) | 22 (78.6%) |
| **Vascular risk factors:** | |
| -Diabetes mellitus, n (%) | 30 (50%) |
| -Hypertension, n (%) | 25 (41.7%) |
| -Hypercholesterolemia, n (%) | 40 (61.7%) |
| -Current cigarette smoking, n (%) | 20 (33.4%) |
| **Stroke character** | |
| -Previous stroke or TIA, n (%) | |
| -Symptom locations | 26 (43.4%) |
| Right, n (%) | 25 (41.7%) |
| Left, n (%) | 26 (43.4%) |
| Bilateral, n (%) | 9 (15%) |
| **Assessment** | |
| -NIHSS (0–42) at admission, Mean ±SD | 8.02 ± 1.92 |
| -MOCA at admission, Mean ±SD | 20.78 ± 2.61 |
| -MOCA after 3 months , Mean ±SD | 20.45± 2.73 |

Table (3) showed that serum levels of S1P (estimated within 48 hours of symptoms) were obviously lower in acute stroke patients in comparison with cool subjects.

**Table (3):** Comparison between patients and controls as regards serum S1P level

| **Sphingosine 1 phosphate (ng/ml)** | **Patients Group (n=60)** | **Control Group (n=30)** | **z-test value** | **p-value** |
|---|---|---|---|---|
| Mean ± SD | 52.76 ± 11.21 | 121.55 ± 18.43 | -7.703 | <0.001** |

*Using: z-Mann-Whitney test **p-value <0.001 highly significant*

Serum S1P levels were obviously lower in the subgroup of patients with post stroke cognitive impairment (PSCI) in comparison with those with post-stroke non-cognitively impaired (non-PSCI_ patients (Table 4).

**Table (4):** Comparison between post-stroke cognitively impaired and non-post-stroke cognitively impaired patients as regards serum S1P level

| **Sphingosine 1 phosphate (ng/ml)** | **Cognitively Impaired (n=51) 85%** | **Non Cognitively Impaired (n=9) 15%** | **Test value** | **p-value** |
|---|---|---|---|---|
| Mean ± SD | 50.42 ± 10.48 | 66.03 ± 2.70 | t=-4.441 | <0.001** |

*Using: z-Mann-Whitney test; t-Independent Sample t-test** p-value <0.001 highly significant.*

In this study, based on the ROC curve analysis, the optimum cut off value of the concentration of the S1P serum to be chosen at ≤ 62.2 ng/ml, with 84.3% Sensitivity, 88.9% Specificity and 86.6% Accuracy (Table 5, figure 1).

**Table (5):** Diagnostic performance of serum sphingosine 1 phosphate level (ng/ml) in discrimination of cognitively impaired group versus non-cognitively impaired group.

| **Cut-off** | **Sen.** | **Sep.** | **PPV** | **NPV** | **AUC [95% C.I.]** | **Accuracy** |
|---|---|---|---|---|---|---|
| **≤62.2** | 84.3% | 88.9% | 97.7% | 50% | 0.967 [0.886-0.996] | 86.6% |

***Cut-off,*** *cut off point of****; ROC,*** *receiver operating characteristic;* ***Sen,*** *Sensitivity;* ***Sep.,*** *Specificity;* ***PPV****, Positive predictive value;* ***NPV,*** *Negative predictive value;* ***AUC,*** *area under the curve*

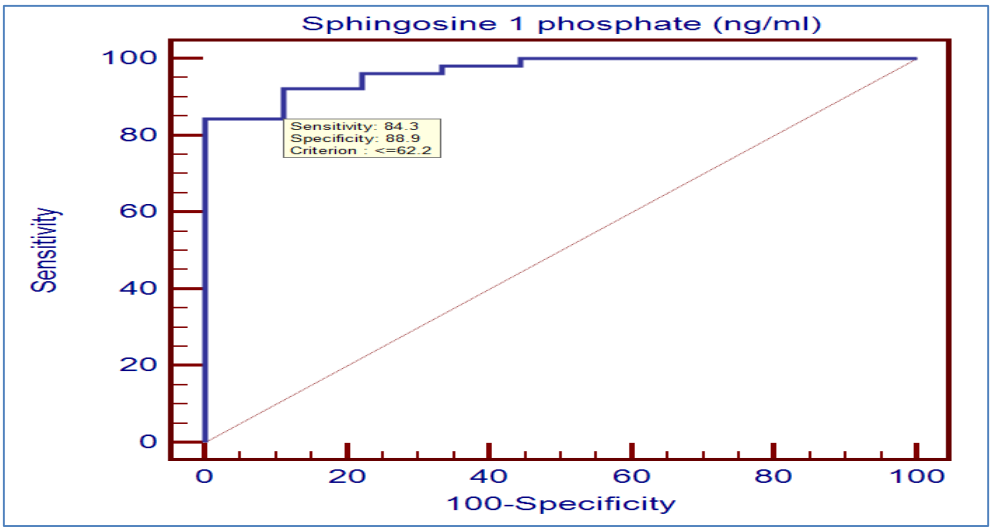


**Figure (1):** Receiver-operating characteristic (ROC) curve for cognitively impaired of stroke patients using the sphingosine 1 phosphate (ng/ml).

As regards, functional disability assessed by NIHSS score, patients with PSCI were more functionally impaired than non-PSCI patients (Table 6).

**Table (6):** Comparison between cognitively impaired and non-cognitively impaired stroke patients according to NIHSS.

| | Cognitively Impaired (n=51) 85% | Non-Cognitively Impaired (n=9) 15% | Test value | p-value |
|---|---|---|---|---|
| **National Institutes of Health Stroke Scale (NIHSS)** | | | | |
| Mean ± SD | 8.98 ± 1.83 | 2.56 ± 0.53 | U=-4.618 | <0.001** |

*Using: z-Mann-Whitney test; t-Independent Sample t-test**p-value <0.001 highly significant*

Regarding, correlation between serum S1P levels and MoCA score (after 3 months) among stroke patients, there was positive significant correlation (Figure 2)

There was negative significant correlation between SIP serum levels and NIHSS score in the patients group (Figure 3)

Serum S1P level does not differ significantly among different sites of lesions (Figure 4).

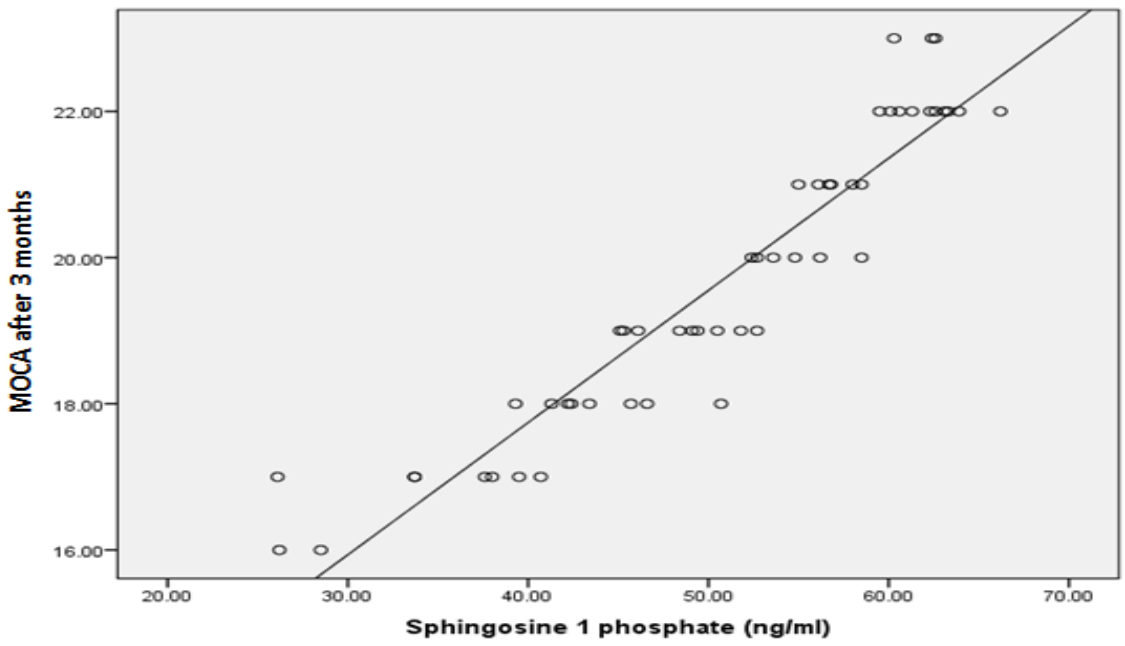


**Figure (2):** Correlation between S1P serum level and MOCA after 3 months.

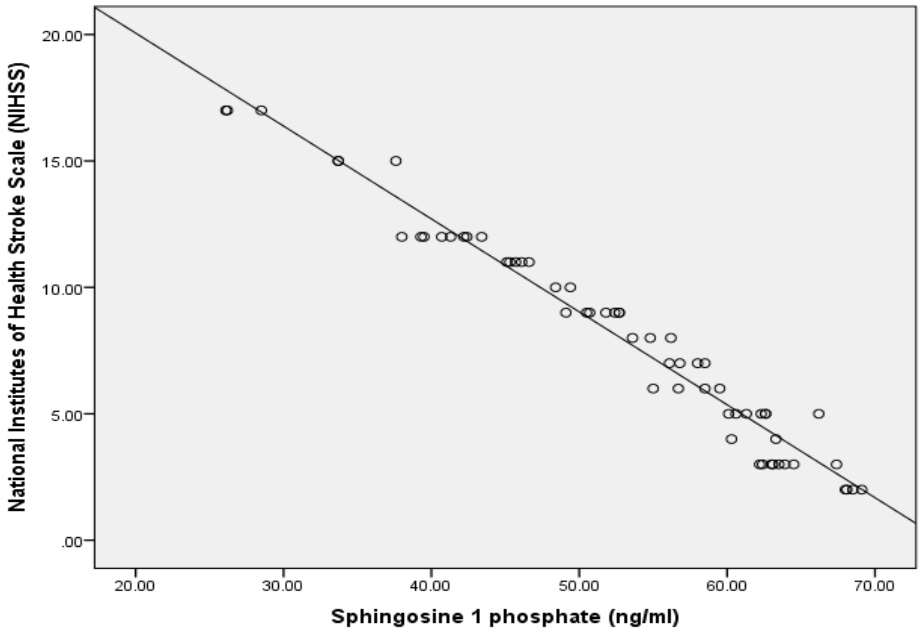


**Figure (3):** Correlation between S1P serum level and NIHSS.

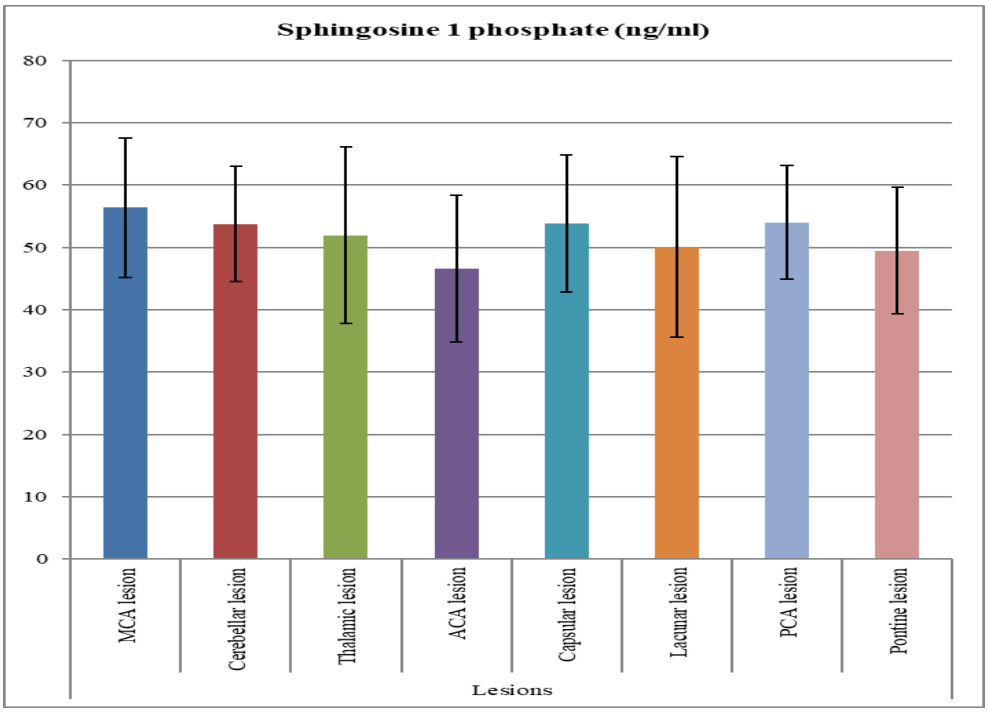


**Figure (4):** Serum level of S1P and different sites of lesions.

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## DISCUSSION

In this study we tried to explore the relation between S1P serum levels and post-stroke cognitive impairment, one of the most devastating complications of cerebrovascular stroke. As was reported in previous studies [4, 8], serum S1P level was apparently lower in patients with acute ischemic stroke than healthy controls. Furthermore, low serum S1P level was associated with more severe neurological disability and impaired cognitive at 3 months follow-up after ischemic stroke. Sphingosine-1-phosphate (S1P) regulates cell survival, lymphocyte migration, and endothelial barrier function all processes affected in acute ischemic stroke. Cerebral atherosclerosis is the main cause of stroke [9]. The main feature of that is a disturbance of vascular endothelial barrier, which leads to increased vascular permeability, which results in reduced S1P serum levels, as the endothelium is an important serum S1P source [10-11].

The reduction in S1P serum levels will stimulate endothelial inflammation by promoting pro-inflammatory endothelial markers such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1), which result in redistribution of the actin cytoskeleton and regulate the adherent junctions of the endothelial cells, ultimately

enhancing the injury of the endothelial barrier [12-13]. Moreover, after brain infarction, hypoxic ischemic mitochondrial dysfunction, excitotoxicity, and oxidative stress damage promoting the breakdown of vascular endothelial cells, resulting in disturbance of both function and integrity of the endothelial barrier [9, 14]. Hence, S1P receptor agonists have been studied in various experimental stroke models and also in acute ischemic stroke patients and was found to be useful by regulating endothelial adherent junctions, cerebrovascular responses, and blood–brain barrier functions by activation of S1P signaling [8, 15, 16]. In line with our results, decreased serum S1P levels were associated with adverse events after acute ischemic stroke. In the MARK-STROKE cohort, the deleterious effects included death, stroke, myocardial infarction, and re-hospitalization [8]. Moreover, In the CIRCULAS cohort, an obvious correlation of lower serum S1P levels with higher NIHSS scores, mortality and modified Rankin Scale was observed [8].

According to ROC curve analysis, the cut off value of serum S1P level in our study to discriminate PSCI patients and non-PSCI patients was ≤ 62.2 ng/ml, with 84.3% sensitivity, 88.9% specificity and 86.6% accuracy.

These results support the use of S1P as a probable diagnostic biomarker for PSCI. However, additional research is necessary to confirm the clinical utility of S1P as a PSCI biomarker and to clarify the complex pathobiology of S1P in PSCI.

Several studies have pronounced the modulatory effects of S1P signaling on the central nervous system (CNS) inflammation. However, other studies advocate the neuroprotective effect of S1P signaling [17, 18]. The inflammatory part is crucial in vascular cognitive impairment (VCI) as immune related ischemia-reperfusion injury plays a critical role in the morbidity of cerebrovascular disease. Most of the knowledge about the role of S1P in CNS diseases comes from the studies of the roles of S1PRs in multiple sclerosis (MS). There is evidence that S1PRs regulation affects the glial cell function. For example, SPHK is enhanced by lipopolysaccharide in microglial cells, resulting in increased expression of inflammatory mediators [19]. Moreover, S1P signaling may also act directly on neurons besides the modulation of neuroinflammation through the regulation of astrocytes and microglia. This was revealed in the transient middle cerebral artery occlusion (tMCAO) model where administration of S1P receptor modulation by fingolimod reduced neuronal injury [20]. This may be arbitrated by two neuroprotective mechanisms, the S1P-phosphatidylinositol-3-kinase (PI3K)-Akt-FOXO3a axis [21] and the extracellular signal-regulated kinase (ERK)/Bcl-2 signaling pathway [22]. Also, Fingolimod may stimulate ERK signaling, resulting in anti-apoptotic protein Bcl-2 expression enhancement, which may result in limitation of destruction of neurons after cerebral ischemia [22].

The main pathological feature of VCI is loss of neurons [23], also this is the case for many types of dementia, including vascular dementia (VaD), post stroke dementia and AD [24, 25]. A clear correlation has been found between SPHK expression and neuronal density in the frontal as well as entorhinal cortices, signifying that the increased SPHK stimulated S1P signaling and enhanced neuronal survival as a response to disease processes [26]. Moreover, these effects were amplified in the presence of amyloid plaques, a neuropathological hallmark of AD. In addition, an important finding in neuroimaging of cerebrovascular disease is the presence of white matter lesions (WMLs), which is assumed to be due to axonal loss and demyelination [27]. Furthermore, in a study on aging quantified plasma S1P there was a negative although non-significant correlation between S1P and WML volume [28]. Moreover, a recent study demonstrated a direct correlation between S1P and VCI [29]. They showed that there is an apparent decrease in the 16-carbon, d16:1form of S1P. This decrease is specific for VCI and not found in AD. Excitingly, the ratio of d16:1 S1P to the more abundant d18:1 form verified a clear negative association with inflammatory cytokines, interleukin-6 (IL-6), IL-8, and tumor necrosis factor-alpha (TNF). Moreover, it shows that d18:1S1P has a proinflammatory effect on astrocytes in vitro, and that this influence can be diminished by d16:1 S1P. Meanwhile, as d16:1 S1P content is genetically regulated [30], it is likely that its dysregulation may result in worsening of VCI as a cause, rather than occurring as a result of cerebrovascular disease.

In this study we did not find an association between serum S1P and site of lesion and this could be due to small sample size. However, previous studies demonstrated that low serum S1P was associated with anterior circulation non-lacunar infarctions [8].

Our study has limitations, first the small sample size. Second, the short period of follow-up hindered us from detecting the fluctuations of serum S1P levels as the disease progressed. Third, we used ELIZA to detect serum S1P instead of the more sensitive mass spectrometry or LC–MS/MS technology. Lastly, the role of imaging (computed tomography (CT) and magnetic resonance imaging (MRI)) in acute stroke and its association with serum levels of S1P is needed to be analyzed.

## CONCLUSIONS

The study highlighted the apparent difference in the serum level of S1P measurements between the healthy and patient groups and found the same remarkable difference in NIHSS score and serum level of S1P between cognitive and non-cognitive impairment subgroups of the patient group. Thereby, the study reveals a harmful role for low serum levels of S1P in acute ischemic stroke patients as regards physical disability and cognitive disability. Furthermore, by the obtained results, it is concluded that

S1P could be utilized as a potential biomarker for PSCI, which will have implications for former detection and management.

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