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GAS6/sAXL ratio correlates with National Institutes of Health Stroke Scale (NIHSS) and infarction size in patients with acute ischemic stroke

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Abstract

Background & Objective: Ischemic stroke is the leading cause of death and long-term disability worldwide. In patients with ischemic stroke, both cell loss and inflammation are observed. GAS6/sAXL signaling is effective in both inflammation and clearance of dead/dying cells. This study investigated the GAS6/sAXL pathway and its role in patients with acute stroke. Specifically, we evaluated whether GAS6/sAXL was associated with stroke severity and infarct volume. *Methods:* This study involved 53 patients with acute ischemic stroke (AIS) and 49 healthy controls. GAS6 and sAXL proteins were collected in the first 24 hours in the acute stroke. NIHSS scores, GCS, and demographic data of the patients at the time of admission to the hospital were recorded. The infarct area was calculated using cranial magnetic resonance imaging. *Results:* Mean age of the patients was 64±12 years, 60% were female. HDL was lower in AIS group (40.5±13.01 mg/dl) than in the control group (55.4±14.9 mg/dl) ($p<0.05$). The GAS6 levels of patients with ischemic stroke (30.58 [1.58-162.33] ng/dL) were significantly lower than the control group (83.33 [10.71-181.96] ng/dL) ($p < 0.001$). There was a significant difference in the GAS6/sAXL ratio between the AIS (8.60 [0.55-48] ng/mL) and control groups (14.78 [1.82-53.71] ng/mL) ($p < 0.001$). The serum GAS6 level and MR infarct area was positively correlated ($r = 0.381$ $p = 0.005$). The GAS6/sAXL ratio was positively correlated with the NIHSS and infarct area ($p = 0.004$). The GAS6/sAXL ratio and GCS showed a negative correlation ($p = 0.001$).
Conclusion: Plasma GAS6 levels were positively correlated with infarct size, and the GAS6/sAXL ratio was positively correlated with the NIHSS score and infarct area in patients with AIS. Plasma GAS levels and GAS6/sAXL ratio can be used as an indicator of severity of AIS.

Keywords: Acute ischemic stroke, GAS6/sAXL, infarct area

INTRODUCTION

An ischemic stroke occurs when a cerebral blood vessel is occluded, the tissue is deprived of oxygen and nutrients, and cell death occurs within minutes at the center of the infarction. After ischemic infarction, inflammation is an important secondary injury mediator.[1,2] Following brain ischemia, microglia become activated, producing a significant production of neurotoxic molecules and proinflammatory cytokines, resulting in additional tissue damage.[2] The inflammatory response helped to remove the dying/dead cells by phagocytosis. The efficient efferocytosis of the brain's microglia reduces neural inflammation, helps restore brain homeostasis, and prevents further cell death.[3,4] Growth arrest-specific protein 6 (GAS6) is a vitamin K-dependent plasma protein that binds to Tyro3, AXL, and Mer (TAM) receptors. It has the highest binding affinity to AXL, followed by Tyro 3 and Mer.[5] AXL is a transmembrane protein, but the extracellular part of the AXL receptor tyrosine kinase can be shedded from cells, resulting in a soluble receptor (sAXL). Ligands of TAM receptors can be inactivated by binding to soluble receptors, known as decoy receptors, which are formed by shedding the extracellular domains of these receptors.[6]

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GAS6 and AXL are expressed in endothelial cells, which are essential for endothelial activation.[7] GAS6 is also involved in the phagocytosis of apoptotic cells[8,9], and increased circulating GAS6 may be a sign of up-regulated expression of GAS6 because of increased apoptosis. sAXL has been shown to bind and inhibit GAS6 in various experimental situations[10], and increased GAS6/sAXL ratios observed in plasma could be associated with less inhibition by sAXL and stronger GAS6 mediated signaling.

The alterations in the levels of the GAS6/sAXL system components play important roles in the pathogenesis of many diseases such as; cancer[11], chronic renal failure[12], and cardiovascular diseases.[13] GAS6/sAXL signaling is effective in both inflammation and clearance of dead/dying cells. It has been shown that GAS6 reduces the release of proinflammatory cytokines.[14] TAM receptors have been reported to reduce inflammation. In the absence of TAM receptors, inflammation is uncontrolled, leading to decreased clearance of apoptotic cells, autoimmune disease[15], increased response to endotoxin[16], and increased inflammatory cytokines.[17]

This study investigated the GAS6/sAXL pathway and its role in patients with stroke. Our study's primary aim was to evaluate the relationship of serum GAS6/sAXL concentrations to NIHSS scores and infarct volume in patients with stroke.

METHODS

This was a prospective single center study. This study was conducted at a University Medical Faculty, Department of Neurological Intensive Care Unit, and Neurology wards from January 2019 to January 2020 among 53 hospitalized adults with a diagnosis of AIS and 49 healthy volunteers. The University Ethics Committee approved the study, and all written informed consents were acquired from each participant or a health care proxy for those that were incompetent (No:2018/620). Forty-nine healthy people from the general population were recruited for the control group.

Participants

The inclusion criteria of the study subjects were hospitalized patients, who were: (1) aged 18 years or older and (2) hospitalized for AIS. Patients were excluded if they had any of the following: (1) Positive HIV serology; (2) hematological malignancy; or (3) received immunosuppressant therapy and agranulocytosis. The control consisted of healthy adults over the age of 18 without any medical illness.

The diagnosis of AIS was based on clinical assessment supported by magnetic resonance imaging (MRI) findings.

The disability and dependence of the patients were measured with the modified Rankin Scale (mRS) and National Institutes of Health Stroke Scale (NIHSS). The mRS score ranged from 0 to 6, with a score of 0 indicating no disability and higher scores indicating more severe disability. The NIHSS score ranged from 0 to 42, with higher scores indicating a more severe deficit. During the first 24 hours, the mRS and NIHSS scores of each patient were recorded.

The infarct size was determined based on diffusion-weighted imaging (DWI) of the MRI, as measured by one neurologist independently by using G3PACS software. According to a previous study, a small infarct volume was defined as less than 5 cm^3. The volume of small infarcts on DWI was calculated using the formula: $0.5 \times a \times b \times c$.[18] The largest lesion by eye was selected and marked. The longest lesion axis (an axis) on this slice was measured. A second line (b axis) was drawn perpendicular to the first line at the widest dimension. A third axis, the z (c) axis, was computed by multiplying the number of slices by slice thickness (7 mm). The categories of stroke etiologies were assessed using the TOAST criteria[19] and were classified as large vessel atherosclerosis, small vessel occlusion, cardioembolism, stroke with determined etiology, or stroke with undetermined etiology. After 24 hours of hospitalization, blood samples were collected and examined for GAS6 and sAXL.

Sample and measurement

Blood were collected from the study subjects in tubes with EDTA (3.2 mL, VACUETTE) and with gel separator (8 mL, VACUETTE). The tubes with gel separator were centrifuged at 2000xg for 10 minutes, and the serum was separated. After the autoanalyzer studies were completed, the remaining serum samples were aliquoted and frozen at -80°C for enzyme linked-immunosorbent assay (ELISA) studies. GAS6 and sAXL serum protein concentrations were analyzed by ELISA according to the manufacturer's protocols.

Statistical analysis

Statistical analysis was made by using the SPSS version 22.0 statistical package for MS Windows

(SPSS Inc., Chicago, IL, USA). Continuous variables were reported as the mean and standard deviation (SD) in the presence of a normal sample distribution, as confirmed by the Kolmogorov-Smirnov test. The median, minimum, and maximum were calculated in the presence of a non-normal distribution. The Mann-Whitney statistical test was used for the differences between the laboratory values of the AIS and control groups. Spearman's rank correlation test was used to evaluate the correlations between GAS6, GAS6/sAXL, NIHSS, infarct area, and GCS. Statistical significance was accepted as $p < 0.05$.

RESULTS

Mean age of AIS patients was 64±12 years and 60% of them were female. HDL was lower in patients (40.5±13.01 mg/dl) than in the control (55.4±14.9 mg/dl) ($p<0.05$). However, the white blood cell count was higher in AIS patients (8.997±3.404) than in the control group (7.855±2.612) ($p<0.05$). The demographic characteristics and laboratory values of the groups are shown in Table 1. The rate of smoking in patients with AIS was 40%, which was higher than in the control group ($p < 0.05$).

The mean NIHSS score of AIS patients was 7 ± 5, while the mean mRS score was 3.2 ± 1.4. Thrombectomy was performed in 12 (22.6%) patients with stroke, and thrombolytic therapy was given in 15 patients (28.3%). Forty patients (75%) received heparin treatment, and 12 patients (22.6%) received aspirin and clopidogrel.

The GAS6 levels of AIS patients (30.58 [1.58-162.33] ng/dL) were significantly lower than those of the control group (83.33 [10.71-181.96] ng/dL) ($p < 0.001$) (Figure 1). There was no difference in the sAXL levels between the stroke (3.7 [1.07-47.86] ng/dL) and control groups (10.63 [1.55-17.80] ng/dL) ($p = 0.51$). Table 2. GAS6, sAXL, GAS6/sAXL Values in AIS and Control Groups are shown. There was a statistically significant difference in the GAS6/sAXL ratio between the stroke (8.60 [0.55-48] ng/mL) and control groups (14.78 [1.82-53.71] ng/mL) ($p < 0.001$) (Figure 2).

Outcome

The median infarct volume in patients with ischemic stroke was 1950 (145-111,694) mm^3. A large infarct area was detected in 23 patients, and a small infarct area was detected in 30 patients. In patients with a large infarct area, the GAS6 value was higher ($p = 0.01$). The serum GAS6 level and MR infarct volume was positively correlated ($r = 0.381$, $p = 0.005$).

The GAS6/sAXL ratio was positively correlated with the NIHSS score and infarct area ($p = 0.004$). In addition, the GAS6/sAXL ratio and GCS showed a negative correlation ($p = 0.001$) (Figure 3).

DISCUSSION

In our study, we showed that the serum GAS6 and GAS6/sAXL ratio of patients with stroke is lower than that of healthy control. There was a positive correlation between the size of the infarct area and GAS6 levels in patients with AIS. The fact that the GAS6/sAXL ratio has a positive correlation with the NIHSS score and infarct area is an important result, since the NIHSS score and the infarct area are also prognostic factors in stroke.[20]

The protein GAS6 is found in many different cell lines and tissues, such as bone marrow stromal cells, endothelium, fibroblasts, monocytes, vascular smooth muscle cells, and the central nervous syste.[21] In early brain development, GAS6 is found in high concentrations and continues to be found in maturity.[22] TAM receptors are found in neuroglia, such as astrocytes, neurons, oligodendrocytes or myelinating glia, and microglia/macrophages.[21] The primary damage is caused by brain cell ischemia, while the secondary damage is due to inflammation in patients with AIS.[1,3,23] GAS6/sAXL signaling has many roles, including regulating inflammation[24], regulating ischemia[25], and maintaining homeostasis.[26]

GAS6 plasmatic concentrations in lupus[27], Behcet's disease[28], and inflammatory bowel diseases[29] were lower in comparison with healthy controls. The high susceptibility to thrombosis in Behçet's disease may be due to low GAS6 level. Rothlin *et al*.[30] TAM agonists reported that GAS6 contributes to the restoration of vascular integrity. In conclusion, the anti-atherosclerotic effect of GAS6 can be achieved by inhibiting the inflammatory response, anti-apoptosis, eliminating apoptotic cells, and promoting vascular repair. A clinical study by Holden *et al*.[31] found that GAS6 was inversely associated with maximum plaque height and total plaque area, and lower GAS6 may be related to higher increased atherosclerosis in men, particularly diabetics. Also, Fan *et al*. found that subjects with atherosclerosis in diabetes mellitus exhibited low levels of GAS6 and that reduced GAS6 is an independent risk factor for atherosclerosis in diabetes mellitus.[32]

When GAS6 binds to the soluble sAXL receptor, its activity is reduced, although it is expressed with elevated values in inflammatory

Table 1: Characteristics of acute ischemic stroke (AIS) patients

	AIS (n:53)	Control (n:49)	p
Demographic variables			
Age (years) mean±sd	64±12,81	51±8	0.055
Sex (M/F) n(%)	32/21 (60/40)	19/30 (39/61)	
Diabetes mellitus n(%)	19(36)	0(0)	
Hypertension n(%)	24(45)	0(0)	
Coronary artery disease n (%)	16 (30)	0(0)	
Smokers n(%)	21(40)	11(22)	**<0.05**
Alcohol habit n(%)	5(9)	0(0)	
Laboratory values			
Triglycerides mg/dl mean±sd	111,58±38,13	133±78,6	0.207
HDL mg/dl mean±sd	40,5±13,01	55,4±14,9	**<0.05**
LDL mg/dl mean±sd	111,58±38,13	118±49,1	0.439
Hemoglobin mean±sd	13,2±2,2	14,1±1	0.173
White cell Count per mm^3 mean±sd	8.997±3.404	7.855±2.612	**<0.05**
Thrombocyte count per mm^3 mean±sd	262.795±83.007	254.555±77.191	0.547
TSH mU/ml median (min-max)	1,25(0,001-12)	1,8(0,8-6,4)	0.242
B12 pg/ml median (min-max)	317(100-1191)	474(260-922)	0.912
Folic acid median(min-max)	9,3(2,2-15)	8,4(3,2-12)	0.793
Infarct volume mm3 median(min-max)	1950 (145 - 111.694)		
Stroke subtype OCSP n(%)			
TACI	4(7)		
PACI	38(72)		
POCI	1(2)		
LACI	10(19)		
Stroke subtype TOAST n(%)			
CS	8(15)		
LAA	9(17)		
SAA	9(17)		
SOE	3(7)		
SUE	24(44)		

Abbreviations; CS; cardioembolism LAA; large vessel atherosclerosis, LACS; lacunar syndrome, PACS; partial anterior circulation syndrome, POCS; posterior circulation syndrome SAA; small vessel occlusion, SOE; stroke with determined etiology SUE; stroke with undetermined etiology, TACS; total anterior circulation syndrome

Table 2: GAS6, sAXL, GAS6/sAXL values in AIS and control group

Values	AIS	Control	p
GAS6 ng/dL median(min-max)	30.58 (1.58 - 162.33)	83.33 (10.71-181.96)	p< 0.001
sAXL ng/dL median(min-max)	3.7 (1.07 - 47.86)	10.63 (1.55 - 17.80	0.51
GAS6/sAXL	8.60 (0.55 - 48.40)	14.78 (1.82 - 53.71)	< 0.001

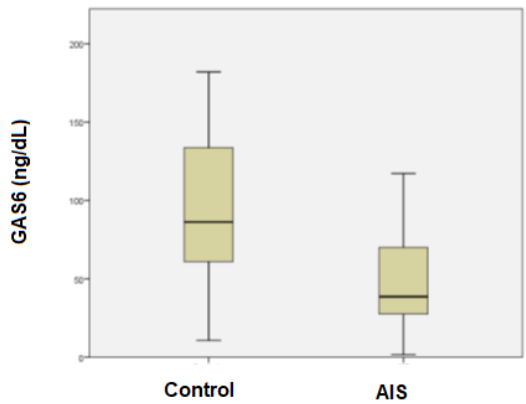

Figure 1. Serum GAS6 levels in AIS and control patients. GAS6 levels were significantly decreased in patients with AIS compared to controls (p<0,001).

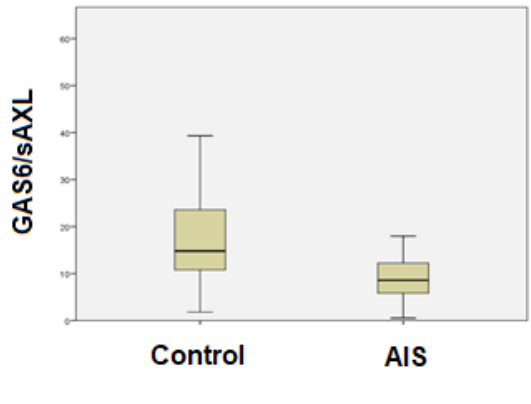

Figure 2. Serum GAS6/sAXL ratio in AIS and control patients. GAS6/sAXL ratio were significantly decreased in patients with AIS compared to controls (*p*<0,001).

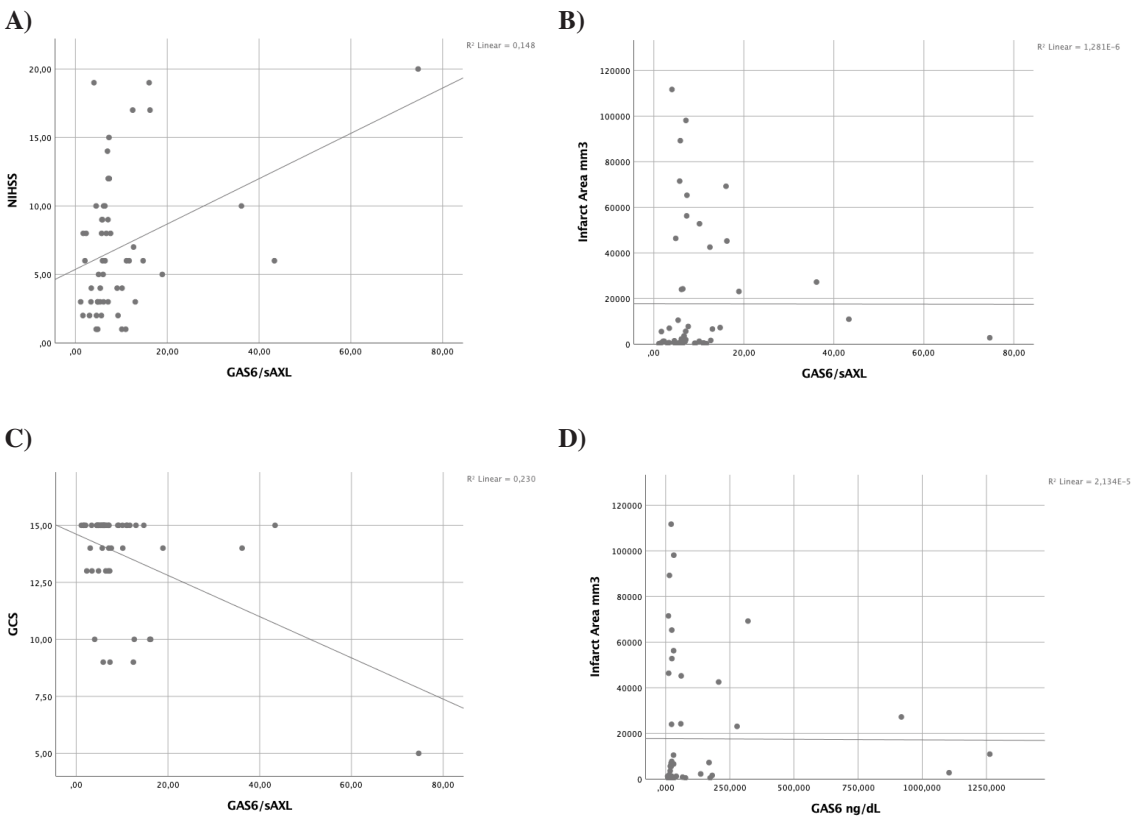

Figure 3. Correlation of GAS6 level and GAS6/sAXL ratio with disease severity (A; Correlation of GAS6/sAXL with NIHSS [r: 0.384, p:0.004], B; Correlation of GAS6/sAXL with Infarct Area [r: 0.391, p:0.004], C; Correlation of GAS6/sAXL with GCS [r: -0.480, p:0.001], D; Correlation of GAS6 with Infarct Area [r: 0.381, p:0.004]

situations. The binding of GAS6 by sAXL formed by the shedding of the cellular AXL receptor may hinder the physiological advantage of GAS6 in the inflammatory process.[24] In the study of Ekman et al., plasma concentrations of GAS6 and sAXL proteins were included in patients with abdominal aortic aneurysm (AAA). A significant correlation was found between GAS6/sAXL ratio and AAA size, similar to our study.[33] Fewer data in humans are available, and they have been collected mostly in *in vitro* models. Gruber *et al*.[34] reported that the inflammatory cytokines IL-6 and TNF-α were significantly increased and CNS injury and clinical scores were higher in mice with GAS6 deficits. The specific role of the GAS6/AXL pathway and the relationship between serum GAS6/sAXL levels and infarct area together with NIHSS scores in patients with stroke has not been previously addressed. Wu *et al*.[35] in their study in the rat model showed that endogenous GAS6 and AXL decreased significantly 24 hours after MCAO; however, the proinflammatory cytokines IL-1β, IL-6, and TNF-α were significantly increased. When these mice were treated with rGAS6, the infarct volume significantly decreased (indicating that rGAS6 can not only reduce neuroinflammation but also decrease infarct volume) and improved the function scores of rats 24 hours after. In our study, we found similarly low GAS6 values and low GAS6/sAXL ratio after 24 hours. In our study, since we found low GAS6 levels in patients with stroke, the GAS6/sAXL ratio was positively correlated with the NIHSS score. Our study is important because we demonstrated this finding in animal studies in humans and it is important in terms of allowing for the treatment of patients with AIS.

There were limitations in our study. First, the study population was not large. Second, GAS6, AXL, and sAXL levels and other inflammatory markers were not examined to indicate their association with inflammation in patients with AIS.

In conclusion, we showed that the serum GAS6 level and GAS6/sAXL ratio of patients with stroke are lower than those of healthy individuals. We showed that plasma GAS6 levels were positively correlated with infarct size, and the GAS6/sAXL ratio was positively correlated with the NIHSS score and infarct area in patients with AIS. GAS6, which represents a novel marker in patients with AIS, has a critical role in vascular biology. Thus, plasma GAS6 levels and GAS6/sAXL ratio can be used as an indicator of the severity of AIS. More clinical studies are needed to clarify the mechanisms involved.

DISCLOSURE

Ethics approval: Institutional ethics approval was obtained from Erciyes University ethics committee (2018/620). Consent from the patient/guardian has been taken.

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Conflict of interest: None

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