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Evaluation of salivary alpha-amylase activity after stroke

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Abstract
This study aimed to investigate the levels of sAA (Salivary Alpha-Amylase) in stroke patients. The H0 hypothesis of the study was established as the increase in sAA activity after stroke is not statistically significant when compared with the normal healthy population of the similar age group. Patients and Methods: This research is a case-control study. Forty-two patients who had a stroke during the period of February-November 2018 in the Department of Physical Medicine and Rehabilitation in Turkey and 40 healthy volunteers were included in the study. Saliva samples were collected within the first seven days after stroke. Saliva samples were taken by passive dilution (drool) technique with the standard method at certain hours. The concentration in U/mL of sAA in the samples is then calculated by comparing optical densities (OD) of the samples to the standard curve. Results: In the stroke group, a total of 42 patients and a total of 40 patients as volunteers were evaluated. The mean age was 69.4 ± 6.1 in the stroke group and 69.6 ± 6.4 in the healthy volunteers. The mean functional independence criterion in the stroke group was 64.7 ± 18.4. The sAA levels were found to be 142.18 ± 42.15 U / mL in the stroke group and 103.69 ± 36.52 U / mL in the healthy volunteers. There were statistically significant difference levels of the sAA between the two groups (p: 0.012). Conclusion: The levels of sAA in stroke patients were not clearly investigated in the literature, and the results indicate that patients with stroke have higher sAA activity compared to healthy subjects. The relationships between autonomic dysfunction and high sAA activity in stroke patients are new topics to be investigated.

Keywords: Salivary alpha-amylase, saliva, stroke, dysphagia

Introduction

Stroke is a disease caused by a disorder of cerebral functions, a sudden onset of death due to a decrease or stopping of blood flow to the brain. Death rates are increasing in patients with advanced intracranial pressure and stroke with an increase in intracranial pressure [1-4]. The two leading causes of non-neurogenic death after stroke are heart problems and infections. Autonomic nervous system dysfunction seen in patients with stroke appears to be the causative factors for cardiac problems and infections that cause post-stroke death, increased sympathetic nervous system activity and decreased parasympathetic nervous system activity [4]. Sympathetic nerves regulate brain artery structure against hypertension. When the blood pressure rises, the cerebral arteries are modified to have a lower lumen diameter and increased wall thickness than arteries from normotensive subjects [5]. Hypertrophy of the arterial wall serves to reduce high blood pressure and environmental stress applied to the wall components. In a study conducted in rats with essential hypertension, the development of vascular wall hypertrophy was prevented by sympathetic denervation of cerebral arteries after upper cervical ganglionectomy [6]. Denervation also impaired the ability of the cerebral arteries to regulate blood flow and increased the incidence of hemorrhagic stroke in rats [7,8]. Parasympathetic nerve fiber activation is thought to play a protective role in ischemic stroke, possibly related to its ability to induce arterial dilatation and increase blood flow to the ischemic region [9]. Thus, increased parasympathetic activity can potentially limit the damage caused by ischemia. The authors argue that there is a balance between sympathetic and parasympathetic activity and that this balance is essential for regulating brain blood flow. However, in stroke patients, this balance has been impaired, parasympathetic activity has been decreased, while sympathetic activity has been shown to increase. Plasma noradrenaline, adrenaline and dopamine levels as predictors of sympathetic activity (independent of age and blood pressure) were found to be higher in stroke patients compared to controls and transient ischemic attacks [10].

Therefore, objective evaluations should be preferred more frequently in stroke disease, as well as recognizing the

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symptoms of the disease. Salivary alpha-amylase (sAA) levels have recently been investigated as an indicator of sympathetic activity. Measurement of sAA levels may facilitate monitoring of sympathoadrenal medullary system activity. Changes in the autonomic nervous system are one of the major complications after stroke, but no markers have been identified to facilitate monitoring of sympatho-adrenomedullary system activation after stroke. In particular, the use of saliva samples has been shown in many studies as noninvasive and indirect determinants [11-14].

However, the assessment of SAA as a new and useful tool in the clinical evaluation of hypertension and the determination of sympathetic hyperactivity is thought to provide further information on the functional role of the hyperadrenergic condition in hypertension. It is also believed that non-pharmacological or pharmacological applications in the treatment of hypertension will offer new opportunities as a non-invasive determinant examining the effects on sympathetic dysfunction [15,16].

Elevation of amylase levels in saliva in stroke survivors might be an indicator for sympathetic dysfunction. This study aimed to investigate the levels of salivary alpha-amylase (sAA) as a marker for autonomic nervous system dysfunction causing death in stroke patients.

Material and Methods

Study population

This study was conducted in the Department of Physical Medicine and Rehabilitation in Turkey and between February-November 2018. Participants consisted of patients with stroke. In the stroke group, a total of 42 patients (16 female and 26 male) and a total of 40 patients (16 female and 24 male) as volunteers were evaluated. The study was conducted following the Declaration of Helsinki 2013 Brazil version and was approved by the Kutahya–Dumlupinar University Ethics Research Committee in Turkey [2018-KAEK-03/9). All of the participants are over the age of 18. The control group consisted of healthy volunteers, who have without the chronic disease (diabetes mellitus, liver disease, cardiovascular and cancer).

Selection and exclusion criteria

Participants were included from the study when routine clinical assessment identified the following reasons for stroke; (a) patients with ischemic cerebrovascular event (ICE) and related right or left hemiplegia, (b) Patients in the early stages of ICE (first 7 days), (c) Patients undergoing ICE for the first time. Healthy volunteers were included; (a) The control group was matched to the study group as age and gender, (b) No cancer or neurodegenerative disease, (c) Patients with chronic diseases such as irregular hypertension, diabetes mellitus, (d) Those who do not have an illness that may cause moderate-severe pain (those who do not have moderate / severe pain (VAS>60) VAS: Visual Analog Scale.

Participants were excluded from the study when routine clinical assessment identified the following reasons for stroke; (a) Transcranial ischemic attack cases recovering without damaging the first 24 hours, (b) Cases of hemorrhagic ICE or sinus vein thrombosis, (c) Tumor-induced ICE cases, (d) Patients with cancer or a neurodegenerative disease prior to ICE, (e) Patients with insufficient communication to give saliva, (f) Patients who voluntarily refuse to participate in the study. Healthy volunteers were excluded; (a) Persons who intentionally refuse to participate in the survey.

Sample Collection

Samples collected from the study group (n = 40) and a healthy control group (n = 40) were obtained from Salivette® (Sarstedt AG & Co, Germany). It is not recommended for use under three years. Samples (no particular case) were taken before brushing the teeth in the morning or 30 minutes after eating and/or drinking.

Detection Technique

Free flow & Intraoral collection

(Participants were asked to wake up at 08.00 in the morning and were sampled at 08.30).

Applications before sampling following steps; (a) Wake up at 08.00 am, (b) After waking up the mouth is shaken with plain water, (c) Sample collection time at 08.30, (d) Nutrition and high acidic content and sweetened fluid intake, (e) No Alcohol / caffeine / nicotine in the last 12 hours, (f) High-medium intensity physical activity within the previous 6 hours.

Applications after sampling following conditions; (a) Sample should be at least 20 µl, (b) Must not contain dense particles, (c) Store at -80 ºC in 10 min. An apparatus is used to absorb saliva samples from salivate tubes. This cylindrical apparatus is removed from the tube. Soak in the mouth until you are sure that it is wet enough (5 µL of sample is diluted with 1000 µL sample buffer. However, it is ideal to collect approximately 20 µL sample). After removal of the particles, the remainder was stored at -80 ° C until assay was carried out.

Dilution: 1000 µL sample buffer is put into a clean tube. Pipette 5 µl of this sample into the tube collected in Salivette® (salivate tubes). This final 1/201 dilution is used in the kit protocol.

The alpha-amylase activity in the saliva samples was determined using the BioVendor Alpha-Amylase Saliva Assay ELISA Kit (BioVendor, USA). The assay was performed according to the manufacturer's kit protocol. The color change was measured spectrophotometrically at a wavelength of 450 nm by using a microplate ELISA reader. The concentration of sAA in the samples was then determined in U/mL by comparing the OD of the samples to the standard curve.

Functional Independence Measurement (FIM)

FIM is a disability scale that has proven validity and reliability for use in neurological diseases, including stroke. The Turkish version was used to evaluate functional independence within the first seven days after stroke. FIM analyzes two different aspects of disability, motor, and cognitive functions. It consists of six functional sections including self-care, sphincter control, mobility, locomotion, communication, and social perception. A total of 18 activities in the FIM are evaluated for functional independence using a seven-point scale for each. The highest possible score is 126. [17].

Statistical Analysis

The data were evaluated by using SPSS 16.0 (SPSS Inc. Released

2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.) program. Continuous data are distributed normally (tested with the Kolmogorov-Smirnov Goodness of Fit Test (K-S test)). The values (mean and median) and spread (standard deviation and coefficient of variation) of continuous data were calculated. For the comparison of parametric data between groups, the Student-t-test was used. The relationship between the two variables was studied with "Pearson Correlation Test". p values <0.05 was considered to be significant.

Results

This study was conducted in the Department of Physical Medicine and Rehabilitation in Turkey. Participants consisted of patients with stroke. Sixteen of the stroke patients were female (38.1%) and 26 were male (61.9%). The control group consisted of 16 women (40%) and 24 men (60%). The mean age (p-value of K-S test is 0.187 for age as data) of the patients with stroke was 69.6 ± 6.4 years, while the mean age of the control group was 69.6 ± 6.9 years (p>0,05). sAA levels (p-value of K-S test is 0.207 for sAA levels as data) were 142.18 ± 42.15 U / ml in stroke group and 103.69 ± 36.52 U / ml in control group. The values of the patient group and the control group are given in Table 1. The increment in saliva amylase activity after stroke was a statistically significant increase when compared to the normal healthy population of the same age group (Table 1) (p= 0.012). sAA levels of patients with left hemisphere lesions were 150, 34 ± 42.75 U / mL, while salivary alpha-amylase levels of patients with right hemisphere lesions were 136.15 ± 56.19 U / mL. sAA levels were not statistically different in patients with right and left hemisphere lesions (p=0,067) (Table 2). There was a weak correlation between total functional independence scores and sAA scores (r=0.259) (Table3).

Table 1. Comparison of demographic variables and salivary alpha-amylase activity between the two study groups.

	Stroke (n:42)	Healthy control (40)	P value
Male	0.71± 0.14	0.45± 0.06	P< 0.001
Female	26 (% 61.9)	10.35± 1.74	P< 0.001
16 (% 38.1)	24 (%60)	8.94± 1.51	P< 0.001
16 (%40)	0.256	0.92± 0.25	P< 0.001
Age (year)	69.6±6.4	69.6±6.9	0.532
sAA (U/mL)	142.18±42.15	103.69±36.52	0.012

sAA: salivary alpha-amylase

Table 2. CsAA levels in patients with right and left hemisphere lesions

Affected part		sAA (U/mL)	P value
Right	26 (% 61.9)	136.15±56.19	0.067
Left	16 (% 38.1)	150. 34±42.75	

sAA: salivary alpha-amylase

Table 3. Comparison of demographic variables and salivary alpha-amylase activity between the two study groups.

FIS (mean±SD)	sAA (U/mL)	
Motor	42.3±14.1	r=0.284
Cognitive	22.4±8.4	r=0.211
Total	64.7±18.4	r=0.259

sAA: salivary alpha-amylase

Discussion

The World Health Organization defines stroke as sudden cerebral dysfunction lasting 24 hours or more without any apparent reason other than vascular causes [18]. In the world, stroke is ranked 1st in the disease ranking and the second most common cause of death is reported. It is stated that deaths from direct strokes occur mostly in the first week after brain damage [1,4,19]. Although most risk factors are known and not changed (age, gender, race, family history, heredity); new risk factors such as obesity, nutritional habits, hormone use, as well as definite irreversible risk factors such as hypertension, diabetes, heart diseases, gradually take place in the pathogenesis of stroke [19]. Increased sympathetic activity or decreased parasympathetic activity in patients with acute ischemic stroke have been associated with deteriorating outcomes [20-23].

Because of both sympathetic and parasympathetic nervous system dysfunction after stroke, many organs and systems are inevitable after stroke. Complications due to direct autonomic nervous system dysfunction such as stroke-related depression, deep vein thrombosis, and pulmonary edema besides gastrointestinal bleeding may develop. Neurological complications are the most common causes of post-stroke deaths, especially in cardiac and immune systems [4,24]. There are several ways to detect impaired autonomic nervous system function after stroke [25]. Noradrenaline plasma levels are indicative of increased sympathetic activity. Noradrenaline levels in cardiac arrhythmia and heart rate changes have been shown to be a determinant for autonomic nervous system dysfunction [26]. In a similar study, Myers and colleagues conducted a study of the control of increased sympathetic activity after stroke. In this study, Norepinephrine for sympathetic activity, the activity of the adrenal epinephrine and dopamine levels were considered to be decisive. They found an increase in plasma NE levels, regardless of age and hypertension in stroke patients. They also suggested that the NE levels did not correlate with stress because they could not find a relationship between stress hormone cortisol and NE levels [27]. The identification and use of disease-specific biomarkers in recent years have become increasingly important, particularly in the early detection and prognosis of cancer and neurological diseases [28, 29]. Nowadays, saliva is widely used as a method of diagnosis for diseases as well as for treatment and use of malicious drugs [29]. Salmon glands in the amount of 0.75-1.5 L per day in response to neurotransmitter stimulation in the saliva release of the sAA level shows sympathetic activity, although individual differences and have a diurnal rhythm [6,7,29,30]. In the host defense of the sAA, some bacteria have the ability to prevent both growths and sticking to intact tissue [31]. In addition, TAA was considered to be a good biomarker for systemic diseases, especially oral diseases [29]. In a study on rats, it was shown that salivary amylase increased with sympathetic stimuli and parasympathetic stimulation and salivary amylase decreased [32]. Studies have shown that sAA is a marker for changes in adrenergic activity in response to psychological stress and may be an indicator of psychosocial stress [30,33,34]. Although there are some ways of detecting impaired autonomic nervous system function after stroke, there are very few studies that directly measure the activity of the autonomic nervous system [4]. However, there was no study evaluating the level of sAA after stroke. The level of sAA in our study was detected to be high (Table 1). An increase in sAA level is likely to be associated with increased sympathetic nervous system activation after stroke. In

our study, the elevated sAA level is likely to be associated with increased sympathetic nervous system activation after stroke. Also, our study is paralleled with studies showing increased sympathetic system activation of cerebral ischemia. On the other hand, the stroke of the right side of the brain has been shown to have greater effects on the autonomic nervous system than on the left side [35, 36]. In a study of 29 patients with stroke, plasma NE and E levels were higher in patients with infarction on the right side of the brain [37]. In our study show that there was no difference between the right and left hemisphere lesions in terms of sAA level (Table 2). However, according to the MR images, our study could not be classified according to the affected localization. In our study indicate that there was a weak correlation between functional independence scores and sAA scores (Table 3). In a study in which patients with stroke were classified according to urine epinephrine and norepinephrine levels, 50% of the patients who died were high catecholamines [38]. However, in our study, the correlation between the functional independence scale and the sAA levels in both the motor and cognitive scores was found to be weak in the early period of stroke patients. sAA activity shows antimicrobial properties [39, 40]. In our study show that sAA level was found to be increased in stroke patients, but this study should be carried out on antimicrobial properties in patients with stroke to prevent pneumonia risk. In the host defense of the sAA, some bacteria have the ability to prevent both growths and sticking to intact tissue [31]. As a result of dysphagia seen in patients with stroke [12.4%), aspiration pneumonia is a condition that can change the prognosis of patients [41,42]. Therefore, it is important to investigate dysphagia in patients with stroke and to start treatment rapidly. The high salivary amylase in stroke patients, in addition to being a determinant of autonomic dysfunction, is a protective factor in aspiration pneumonia in patients with dysphagia [41,43,44]. To facilitate early feeding in strokes, it is customary to use foods with increased consistency with starch. However, it should be shown by further studies that the increased sAA level may increase the risk of dysphagia by breaking down these foods in the mouth.

Conclusions

Our study was the first to evaluate sAA in stroke patients and was the first step to investigate whether a sAA level would be used as an indicator of the sympathoadrenal medullary system in stroke patients.

Conflict of interest
The authors declare that there are no conflicts of interest.

Financial Disclosure
All authors declare no financial support.

Ethical approval
The study was conducted following the Declaration of Helsinki 2013 Brazil version. Permission was approved by the Kutahya–Dumlupinar University Ethics Research Committee in Turkey (2018-KAEK-03/9) and written informed consent was collected from the patients.

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