

## PLASMA LOW MOLECULAR WEIGHT AMINOTHIOLS IN ISCHEMIC STROKE PATIENTS WITH TYPE 2 DIABETES MELLITUS

Maksimova MYu<sup>1</sup>✉, Ivanov AV<sup>2</sup>, Nikiforova KA<sup>2</sup>, Virus ED<sup>2</sup>, Suanova ET<sup>3</sup>, Ochtova FR<sup>3</sup>, Piradov MA<sup>1</sup>, Kubatiev AA<sup>2</sup>

<sup>1</sup> Research Center of Neurology, Moscow, Russia

<sup>2</sup> Research Institute of General Pathology and Pathophysiology, Moscow, Russia

<sup>3</sup> Evdokimov Moscow State University of Medicine and Dentistry, Moscow, Russia

It was found that ischemic stroke (IS) results in decreased levels of a number of reduced forms of low molecular weight aminothiols (LMWTs). The study was aimed to assess the impact of type 2 diabetes mellitus (T2D) on the total content, reduced forms and redox status of LMWTs in patients with IS. A total of 175 patients with IS in the internal carotid artery basin (the average age was 62 (55–69) years) were assessed, who were admitted to the Center within the first 10–24 h since the onset of neurological disorder. The index group included 68 patients with IS and T2D (males made up 41.2%). The comparison group consisted of 107 patients with IS and stress hyperglycemia (males made up 57%), and the control group included 31 non-diabetic patients with chronic cerebrovascular disease (CCVD) (males made up 54.8%). The admission plasma levels of LMWTs were assessed by liquid chromatography in all patients. It was found, that IS in patients with T2D was associated with the rapid decrease in total cysteine (tCys), total glutathione (tGSH), total homocysteine (tHcy), reduced glutathione (rGSH), and glutathione redox status (GSH RS), along with the increase in cysteine redox status (Cys RS) and homocysteine redox status (Hcy RS). In contrast to patients with stress hyperglycemia developing during the acute period of IS, patients with T2D had lower tCys, tGSH, and tHcy levels. Thus, GSH RS of 4.06% or lower in the first 24 hours after the IS in patients with T2D was a predictor of poor functional outcome (mRS score was 3 or more 3 weeks after IS).

**Keywords:** ischemic stroke, type 2 diabetes mellitus, low molecular weight aminothiols

**Author contribution:** Maksimova MYu — concept formulation, data synthesis, manuscript writing; Ivanov AV — concept formulation, literature analysis, assessment of low molecular weight aminothiols; Nikiforova KA — primary laboratory data acquisition; Virus ED — laboratory data synthesis; Suanova ET — statistical data processing; Ochtova FR — clinical assessment of patients; Piradov MA — study management; Kubatiev AA — study management.

**Compliance with ethical standards:** the study was approved by the Ethics Committee of the Research Center of Neurology (protocol № 3-1/16 dated March 16, 2016), it was carried out in accordance with the basic principles outlined in the Declaration of Helsinki.

✉ **Correspondence should be addressed:** Marina Yu. Maksimova  
Volokolamskoe shosse, 80, Moscow, 125367, Russia; ncnmaksimova@mail.ru

**Received:** 25.10.2021 **Accepted:** 13.11.2021 **Published online:** 08.12.2021

**DOI:** 10.24075/brsmu.2021.057

## НИЗКОМОЛЕКУЛЯРНЫЕ АМИНОТИОЛЫ В ПЛАЗМЕ КРОВИ ПРИ ИШЕМИЧЕСКОМ ИНСУЛЬТЕ В СОЧЕТАНИИ С САХАРНЫМ ДИАБЕТОМ 2-ГО ТИПА

М. Ю. Максимова<sup>1</sup>✉, А. В. Иванов<sup>2</sup>, К. А. Никифорова<sup>2</sup>, Э. Д. Вирус<sup>2</sup>, Е. Т. Суанова<sup>3</sup>, Ф. Р. Охтова<sup>3</sup>, М. А. Пирадов<sup>1</sup>, А. А. Кубатиев<sup>2</sup>

<sup>1</sup> Научный центр неврологии, Москва, Россия

<sup>2</sup> Научно-исследовательский институт общей патологии и патофизиологии, Москва, Россия

<sup>3</sup> Московский государственный медико-стоматологический университет имени А. И. Евдокимова, Москва, Россия

Установлено, что ишемический инсульт (ИИ) приводит к снижению ряда восстановленных форм низкомолекулярных аминотиолов (НМАТ). Целью исследования было оценить влияние сахарного диабета 2-го типа (Т2СД) на общее содержание, восстановленные формы и редокс статус НМАТ у пациентов с ИИ. Обследованы 175 пациентов с ИИ в бассейне внутренних сонных артерий (средний возраст 62 (55–69) года, поступившие в центр в первые 10–24 ч с момента возникновения неврологических нарушений). В основную группу вошли 68 пациентов (41,2% мужчин) с ИИ и Т2СД. Группу сравнения составили 107 пациентов (57% мужчин) с ИИ и стрессовой гипергликемией, контрольную группу — 31 пациент (54,8% мужчин) с хронической цереброваскулярной патологией (ЦВП) без СД. Во всех случаях пациентам при поступлении проводили исследование НМАТ в плазме крови методом жидкостной хроматографии. Установлено, что ИИ у пациентов с Т2СД ассоциируется с резким снижением уровней общего цистеина (oЦис), общего глутатиона (oГлн), общего гомоцистеина (oГцис), восстановленного глутатиона (вГлн), редокс-статуса глутатиона (PC Глн), а также повышением редокс-статуса цистеина (PC Цис) и редокс-статуса гомоцистеина (PC Нсу Гцис). У пациентов с Т2СД, в отличие от пациентов с развитием стрессовой гипергликемии в остром периоде ИИ, отмечены более низкие показатели oЦис, oГлн и oГцис. Таким образом, уровень PC Глн, составляющий 4,06% и менее в первые сутки ИИ у пациентов с Т2СД, является предиктором неблагоприятного функционального прогноза (оценка по шкале mRS составляет 3 балла и более спустя 3 недели после ИИ).

**Ключевые слова:** ишемический инсульт, сахарный диабет 2-го типа, низкомолекулярные аминотиолы

**Вклад авторов:** М. Ю. Максимова — разработка концепции, обобщающий анализ полученных данных, написание статьи; А. В. Иванов — разработка концепции, анализ литературы, исследование низкомолекулярных аминотиолов; К. А. Никифорова — сбор и первичный анализ лабораторных показателей; Э. Д. Вирус — обобщающий анализ полученных лабораторных данных; Е. Т. Суанова — статистическая обработка полученных данных; Ф. Р. Охтова — проведение клинического обследования пациентов; М. А. Пирадов — организация исследования; А. А. Кубатиев — организация исследования

**Соблюдение этических стандартов:** исследование одобрено этическим комитетом ФГБНУ НЦН (протокол № 3-1/16 от 16 марта 2016 г.), проведено в соответствии с основными принципами Хельсинкской декларации.

✉ **Для корреспонденции:** Марина Юрьевна Максимова  
Волоколамское шоссе, д. 80, г. Москва, 125367, Россия; ncnmaksimova@mail.ru

**Статья получена:** 25.10.2021 **Статья принята к печати:** 13.11.2021 **Опубликована онлайн:** 08.12.2021

**DOI:** 10.24075/vrgmu.2021.057

Stroke is a major cause of morbidity, disability and mortality in the populations of many countries. Type 2 diabetes mellitus (T2D) increases the risk of ischemic stroke (IS) by 2–2.5 times, and the risk of death from stroke by 3 times [1, 2]. Hyperglycemia during the first hours of stroke could be the body's stress response to brain ischemia [3]. Severity of neurohormonal and metabolic disorder reflects the IS severity and contributes to the outcome of the disease [4].

High prevalence of the internal carotid artery (ICA) atherosclerosis in patients with T2D compared to non-diabetic patients has been reported. That is why atherothrombotic stroke is the most common IS pathogenetic subtype in individuals with T2D [5].

Oxidative stress is one of the key factors involved in the pathogenesis of IS. Accumulation of free radical oxidation products results in the enzyme SH group blockage and inactivation, DNA hydroxylation, DNA fragmentation, and the resulting destabilization of cell membranes [6, 7].

Low molecular weight aminothiols (LMWTs) are highly sensitive to oxidative stress. Oxidized forms of LMWTs predominate in blood plasma; redox status (RS) is the ratio between the reduced forms and the total content of each thiol. Experimental studies and clinical trials report the decrease in the levels of reduced forms during the acute period of IS, which could indicate activation of oxidative processes in case of insufficient antioxidant defence [8, 9].

Both the increase in total homocysteine content (tHcy) and the decrease in the total glutathione content (tGSH) in females were revealed in patients with T2D [10]. Other papers report the decreased glutathione redox status (GSH RS) in blood plasma [11] and whole blood [12] of patients with T2D. Negative correlation between hyperglycemia and plasma tGSH levels was observed in individuals with coronary artery disease (CAD) ( $r = -0.328$ ;  $p = 0.011$ ), furthermore, it was shown that T2D was a factor, having an additional negative effect on the tGSH levels [13]. Perhaps low plasma tGSH levels are associated with the increased activity of  $\gamma$ -glutamylcysteine transferase in hyperglycemia [10]. The decreased GSH synthesis along with the possible increase in GSH consumption in red blood cells of patients with T2D were also reported [14].

The study was aimed to assess the impact of T2D on the total content (t), reduced forms (r) and RS of plasma LMWTs in patients with IS.

## METHODS

The study included 175 patients with IS in the ICA basin (89 males and 86 females aged 46–84 (the average age was 62 (55–69) years)), admitted to the Research Center of Neurology (Moscow; Russia) within the first 10–24 h since the onset of the neurological disorder. The index group consisted of 68 patients with IS and T2D (males made up 41.2%). The comparison group included 107 patients with IS and stress hyperglycemia (males made up 57%), and the control group consisted of 31 non-diabetic patients with chronic cerebrovascular disease (CCVD) (males made up 54.8%, the average age was 69 (60–75) years).

Inclusion criteria: age 45–85 years; the first IS; admission within the first 6–24 h since the onset of neurological symptoms; cerebral infarction in the ICA basin confirmed by neuroimaging data; T2D or stress hyperglycemia on admission; submitted informed consent to participation in the research study.

Exclusion criteria: T1D; acute myocardial infarction; decompensated kidney disease, liver failure or respiratory failure; class III–IV heart failure.

General medical examination and neurological examination were performed in all patients.

The neurological impairment severity was assessed on admission and in the end of the IS acute period using the National Institutes of Health Stroke Scale (NIHSS) [15]. Neurological impairment was considered mild when NIHSS < 7, moderate when  $7 \leq$  NIHSS < 14, and severe stroke when  $14 \leq$  NIHSS. The activities in daily living and the ability to function independently were evaluated on admission and on day 21 of IS using the Bartel Index (BI) [16], and the functional status was assessed with the use of the modified Rankin scale (mRs). The ability to perform all the routine tasks and to be engaged in daily activities corresponded to the mRs score of 0–2 by day 21, and poor functional outcome corresponded to the score of  $\geq 3$  [17].

IS was diagnosed based on the clinical features and magnetic resonance imaging data (Magnetom Symphony and Magnetom Avanto, 1.5 T) acquired using standard sequences (T2, T1, T2-FLAIR, T2\*). Cerebral arteries were examined by 3D TOF MR angiography. Duplex ultrasonography of the cerebral arteries was performed using the Philips iU22 ultrasound system (Philips; Netherlands).

Pathogenetic subtype of IS was defined in accordance with the internationally accepted TOAST (Trial of ORG 10172 in Acute Stroke Treatment) criteria [18].

Fibrinogen assay was carried out with the ACL 9000 coagulation analyzer (Instrumentation Laboratory; USA).

Glucose (hexokinase method), glycated hemoglobin (HbA1c) (immunoturbidimetric assay), total cholesterol, low-density lipoprotein, urea, and creatinine levels were assessed with the Konelab 30i clinical chemistry analyzer (Thermo Fisher Scientific; Finland) using the reagent kits (Randox; UK).

All patients with IS underwent blood glucose monitoring on admission. The HbA1c test was performed in patients with admission blood glucose levels of 6.1 mmol/L or more. The HbA1c levels reflect the blood glucose levels over the last 3 months. The diagnosis of T2D was established based on the American Diabetes Association criteria [19].

All patients with IS received backbone therapy, which included hypotensive, antithrombotic and lipid-lowering agents. Glucose-lowering therapy was used along with the blood glucose monitoring.

The content of LMWTs was defined as described previously [8]. Venous blood was collected into tubes containing sodium citrate (0.38%) and centrifuged at 3,000 g for 3 min. Blood plasma for assessment of total LMWTs was collected and stored at  $-20$  °C. For derivatization, 100  $\mu$ L of blood plasma were added to 10  $\mu$ L of 50 mM dithioeritol and 10  $\mu$ L of the internal standard (0.45 mM penicillamine). Dithioeritol and penicillamine solutions contained 10 mM Na-EDTA. The mixture was incubated at 37 °C for 15 min. Subsequently, the 5,5'-dithiobisnitrobenzoic acid (600  $\mu$ L, 20 mM) dissolved in ethanol was added, and the mixture was incubated at 4 °C for 30 min. After centrifugation at 15 000 g for 5 min, supernatant was dried under a vacuum at 60 °C for 2 h. The pellet was resuspended in 30 mM NaOH prior to analysis.

To define the levels of the LMWTs reduced forms, plasma (100  $\mu$ L) was added to 25  $\mu$ L of the 5-sulfosalicylic acid solution (230 g/L) immediately after plasma collection, frozen and stored at  $-80$  °C. The samples were centrifuged at 15,000 g for 5 min prior to derivatization. Subsequently, 40  $\mu$ L of supernatant were mixed with 40  $\mu$ L of 20 mM 5,5'-dithiobisnitrobenzoic acid and 2.5  $\mu$ M penicillamine in 0.4 M sodium phosphate buffer (pH 8.0). Then 10  $\mu$ L of 1 M NaOH were added, the solution was mixed for 5 s, and subsequently 12.5  $\mu$ L of 1 M HCl with 20 mM N-ethylmaleimide were added to quench the reaction.

Analysis was performed using the Waters ACQUITY UPLC system (Waters, Milford; USA), equipped with the photodiode array ( $\lambda$ PDA) UV detector (absorbance 330 nm; resolution 10.8 nm; frequency 5 s<sup>-1</sup>) and the Poroshell 120 SBC18 column (2.8  $\mu$ m, 150  $\mu$ m  $\times$  2 mm) (Agilent; USA). The temperature of the column and samples of 50 and 10 °C, respectively, was maintained. The volume of the sample injection was 10  $\mu$ L, and the flow rate was 0.2 mL/min. Eluent A: 0.1 M ammonium acetate with 0.12% (v/v) HCOOH; eluent B: acetonitrile. Chromatography involved gradient elution using a linear gradient of solvent B of 2.5–10% for 5 min. Regeneration was performed with the use of 70% B for 1.5 min, and equilibration was carried out using 2.5% B for 4 min.

Statistical data processing was performed with the IBM SPSS Statistics Version 20.0 software package (IBM Corp.; USA). The Descriptive Statistics module was used to obtain the discriminative model. The quantitative characteristics were presented as median, 25th and 75th percentiles (Me (Q<sub>1</sub>–Q<sub>3</sub>)), qualitative data were presented as absolute frequencies and percentage. The Kruskal–Wallis test and Mann–Whitney U test were used to compare the groups based on their quantitative characteristics. The logistic regression procedures were used to identify the prognostic factors. The variables were selected by conditional inclusion. The quality of the logistic regression model was assessed by ROC analysis and calculation of statistical characteristics of the tests (sensitivity, specificity). To evaluate the predictive power of the model, the Area Under Curve (AUC) was assessed. To define the optimal cutoff value, the importance of maintaining the sensitivity-specificity balance was taken into account. The two-tailed critical p-value, used in all comparisons and tests, was set at 0.05.

## RESULTS

The main characteristics of patients with IS and CCVD are presented in Table 1. The groups of patients showed no differences in age, gender, and indicators of lipid and protein metabolism. The patients with IS had been constantly taking antihypertensive drugs in 46 cases (26.3%), antiplatelet drugs in 22 cases (12.6%), anticoagulants in 11 cases (6.3%), and statins in 8 cases (4.6%) before being included in the study. The group with CCVD received no preventive therapy before inclusion in the study.

Stroke, caused by the ICA atherothrombosis, was diagnosed in 35 cases (20%), cardioembolic stroke was diagnosed in 50 cases (28.6%), and lacunar stroke due to small-artery disease was diagnosed in 90 cases (51.4%).

In patients with atherothrombotic stroke, the developing step-by-step neurological deficits and the occurrence of single large infarctions affecting the cortical and subcortical regions outside the neighboring vascular territories were observed.

The heart-related causes of the cerebral artery thrombosis were as follows: embologenic forms of CAD (paroxysmal atrial fibrillation in 29 cases (58%), permanent atrial fibrillation in 17 cases (34%), postinfarction cardiosclerosis in 4 cases (8%). In 34 patients, atrial fibrillation was diagnosed for the first time during the acute period of IS. Sudden emergence of persistent neurological symptoms was a clinical feature typical for cardioembolic stroke. Based on the MRI data, the cortical and subcortical infarctions were localized mostly in the middle cerebral artery basin.

IS, resulting from the hypertensive, small, deep cerebral infarctions, was characterised by the gradually increasing

**Table 1.** Characteristics of patients with IS and chronic cerebrovascular disease

Parameter	Patients with IS (n = 175)	Non-diabetic patients with CCVD (n = 31)	p
Age, years; Me (Q <sub>1</sub> –Q <sub>3</sub> )	62 (55–69)	63 (58–69)	0.583
Gender (males/females) (%)	89/86 (50.9/49.1)	17/14 (54.8/45.2)	0.702
IS subtype:			
Atherothrombotic stroke (ATS), n (%)	35 (20.0%)		
Cardioembolic stroke (CES), n (%)	50 (28.6%)		
Lacunar stroke (LS), n (%)	90 (51.4%)		
Stroke severity on admission:			
NIHSS < 7 (mild)/	92 (52.6%)		
7 ≤ NIHSS < 14 (moderate)/	67 (38.3%)		
14 ≤ NIHSS (severe)	16 (9.1%)		
NIHSS on admission; Me (Q <sub>1</sub> –Q <sub>3</sub> )	6 (3–11)		
NIHSS on day 21; Me (Q <sub>1</sub> –Q <sub>3</sub> )	3 (2–7)		
mRs on admission; Me (Q <sub>1</sub> –Q <sub>3</sub> )	3 (2–4)		
mRs on day 21; Me (Q <sub>1</sub> –Q <sub>3</sub> )	2 (1–3)		
Bartel Index on admission; Me (Q <sub>1</sub> –Q <sub>3</sub> )	70 (20–90)		
Bartel Index on day 21; Me (Q <sub>1</sub> –Q <sub>3</sub> )	85 (60–98)		
Good recovery (mRs 0–1), n (%)	79 (45.1%)		
Glucose, mmol/L (on admission); Me (Q <sub>1</sub> –Q <sub>3</sub> )	6.50 (6.22–7.72)	5.6 (5.3–6.0)	< 0.0001
Total cholesterol, mmol/L (on admission); Me (Q <sub>1</sub> –Q <sub>3</sub> )	5.9 (5.0–6.9)	5.9 (4.9–6.6)	0.277
LDL cholesterol, mmol/L (on admission); Me (Q <sub>1</sub> –Q <sub>3</sub> )	2.33 (1.80–2.97)	2.2 (1.4–3.0)	0.433
Creatinine, $\mu$ mol/L	91.0 (79.0–103.0)	86.0 (75.0–97.0)	0.059
Urea, mmol/L (on admission); Me (Q <sub>1</sub> –Q <sub>3</sub> )	6.5 (5.9–6.9)	6.2 (5.6–6.8)	0.841
Fibrinogen, g/L (on admission); Me (Q <sub>1</sub> –Q <sub>3</sub> )	3,40 (2,90–4,13)	3,58 (2,97–4,06)	0,119

**Table 2.** Comparative analysis of the low molecular weight amino thiols plasma levels ( $\mu\text{M}$ ) and redox status (%) in patients with IS and controls (Mann–Whitney U test)

Indicator	Patients with IS ( $n = 175$ ); Me ( $Q_1$ – $Q_3$ )	Non-diabetic patients with CCVD ( $n = 31$ ); Me ( $Q_1$ – $Q_3$ )	$p$
tCys ( $\mu\text{M}$ )	332.3 (226.8–375.9)	267.1 (230.4–321.5)	0.72
tGSH ( $\mu\text{M}$ )	1.64 (1.02–2.52)	3.09 (2.10–3.66)	< 0.0001
tHcy ( $\mu\text{M}$ )	13.54 (8.64–18.93)	13.01 (11.45–15.93)	0.932
rCys ( $\mu\text{M}$ )	6.28 (4.98–7.19)	6.32 (5.57–8.36)	0.33
rGSH ( $\mu\text{M}$ )	0.038 (0.024–0.058)	0.242 (0.177–0.374)	< 0.0001
rHcy ( $\mu\text{M}$ )	0.16 (0.13–0.23)	0.14 (0.10–0.17)	0.018
Cys RS, %	2.03 (1.64–2.67)	2.49 (1.98–2.97)	0.066
GSH RS, %	2.04 (1.51–3.47)	8.36 (6.68–15.96)	< 0.0001
Hcy RS, %	1.18 (0.90–1.91)	1.09 (0.83–1.33)	0.041

intensity of neurological deficits in the form of lacunar syndromes. Small, deep cerebral infarctions were localized in the basal ganglia, white matter of cerebral hemispheres, and internal capsule. Stage III hypertension was found in 71 patients (78.9%) with lacunar stroke, and stage II hypertension was observed in 19 patients (21.1%).

The baseline IS severity assessment made it possible to reveal mild neurological impairment (NIHSS < 7) in 92 cases (52.6%), moderate neurological impairment ( $7 \leq \text{NIHSS} < 14$ ) in 67 cases (38.3%), and severe neurological impairment ( $14 \leq \text{NIHSS}$ ) in 16 cases (9.1%) (see Table 1).

The admission blood glucose level in patients with IS was 6.50 (6.22–7.72) mmol/L.

Analysis of LMWTs showed that the tGSH, rGSH, and GSH RS levels in the group with IS were significantly decreased

( $p < 0.0001$ ), and the rHcy and Hcy RS values were significantly increased ( $p = 0.018$  and  $p = 0.041$ ) compared to the group with CCVD (Table 2).

Analysis of the total sample revealed higher rHcy values in males with RS compared to females (0.17 (0.14–0.24)  $\mu\text{M}$  vs. 0.15 (0.12–0.21)  $\mu\text{M}$ ;  $p = 0.026$ ).

In the group of IS patients enrolled, T2D was diagnosed in 68 cases, and stress hyperglycemia was diagnosed in 107 cases (Table 3). The duration of T2D of less than 5 years was observed in 22 patients (32.4%), and the disease duration exceeding 5 years was observed in 46 patients (67.6%). In the group of patients with a combination of IS and T2D, the HbA1c level was 7.8% (6.8–9.6). The levels of less than 6.5% were defined in 107 patients (61.1%), the levels of 6.5–6.9% were found in 32 patients (18.3%), 20 patients (11.4%) had the

**Table 3.** Characteristics of patients with a combination of IS and T2D, and patients with a combination of IS and stress hyperglycemia (Mann–Whitney U test)

Parameter	Patients with a combination of IS and T2D ( $n = 68$ )	Patients with IS and stress hyperglycemia ( $n = 107$ )	$p$
Age, years; Me ( $Q_1$ – $Q_3$ )	68 (55–75)	62 (54–67)	0.046
Gender (males/females) (%)	28/40 (41.2/58.8)	61/46 (57.0/43.0)	0.045
IS subtype:			
Atherothrombotic stroke (ATS), n (%)	4 (5.9%)	31 (29.0%)	< 0.0001
Cardioembolic stroke (CES), n (%)	34 (50.0%)	16 (15.0%)	< 0.0001
Lacunar stroke (LS), n (%)	30 (44.1%)	60 (56.1%)	0.162
Stroke severity on admission:			
NIHSS < 7 (mild)/	32 (47.1%)	60 (56.1%)	0.278
$7 \leq \text{NIHSS} < 14$ (moderate)/	30 (44.1%)	37 (34.6%)	0.264
$14 \leq \text{NIHSS}$ (severe)	6 (8.8%)	10 (9.3%)	1.000
NIHSS on admission; Me ( $Q_1$ – $Q_3$ )	7 (4–11)	6 (3–10)	0.238
NIHSS on day 21; Me ( $Q_1$ – $Q_3$ )	3 (2–8)	3 (2–7)	0.390
mRs on admission; Me ( $Q_1$ – $Q_3$ )	70 (28–81)	70 (20–90)	0.790
mRs on day 21; Me ( $Q_1$ – $Q_3$ )	85 (60–100)	85 (60–95)	0.957
Bartel Index on admission; Me ( $Q_1$ – $Q_3$ )	3 (2–4)	3 (2–4)	0.922
Bartel Index on day 21; Me ( $Q_1$ – $Q_3$ )	2 (1–3)	2 (1–3)	0.762
Good recovery (mRs 0–1), n (%); Me ( $Q_1$ – $Q_3$ )	30 (44.1%)	49 (45.8%)	0.877
Duration of T2D, years; Me ( $Q_1$ – $Q_3$ )	7 (5–10)	–	
Glucose, mmol/L (on admission); Me ( $Q_1$ – $Q_3$ )	6.50 (6.18–8.11)	6.40 (6.16–7.08)	0.083
HbA1c, %; Me ( $Q_1$ – $Q_3$ )	7.8 (6.8–9.6)	5.5 (5.4–5.8)	< 0.0001
Total cholesterol, mmol/L (on admission); Me ( $Q_1$ – $Q_3$ )	5.50 (5.00–6.63)	6.00 (5.00–7.00)	0.353
LDL cholesterol, mmol/L (on admission); Me ( $Q_1$ – $Q_3$ )	2.33 (2.16–2.94)	2.27 (1.72–2.98)	0.432
Creatinine, $\mu\text{mol/L}$ (on admission); Me ( $Q_1$ – $Q_3$ )	94.0 (80.0–107.0)	88.0 (78.0–100.0)	0.075
Urea, mmol/L (on admission); Me ( $Q_1$ – $Q_3$ )	6.40 (5.25–6.93)	6.60 (6.10–6.90)	0.233
Fibrinogen, g/L (on admission); Me ( $Q_1$ – $Q_3$ )	3.61 (3.05–4.11)	3.39 (2.83–4.13)	0.267



**Table 4.** Comparison of the low molecular weight aminothiols plasma levels ( $\mu\text{M}$ ) and redox status (%) in patients with a combination of IS and T2D, and in patients with a combination of IS and stress hyperglycemia (Mann–Whitney U test)

Indicator	Patients with IS and T2D ( $n = 68$ ; Me ( $Q_1$ – $Q_3$ ))	Patients with IS and stress hyperglycemia ( $n = 107$ ; Me ( $Q_1$ – $Q_3$ ))	$p$
tCys ( $\mu\text{M}$ )	135.6 (126.7–150.5)	342.6 (307.0–390.0)	< 0.0001
tGSH ( $\mu\text{M}$ )	0.84 (0.55–1.1)	2.13 (1.32–2.71)	< 0.0001
tHcy ( $\mu\text{M}$ )	5.48 (4.75–6.5)	15.24 (12.97–20.61)	< 0.0001
rCys ( $\mu\text{M}$ )	6.57 (4.44–7.72)	6.24 (5.03–7.14)	0.904
rGSH ( $\mu\text{M}$ )	0.038 (0.017–0.051)	0.036 (0.025–0.06)	0.223
rHcy ( $\mu\text{M}$ )	0.15 (0.12–0.19)	0.17 (0.13–0.24)	0.099
Cys RS, %	4.53 (2.85–5.69)	1.87 (1.46–2.27)	< 0.0001
GSH RS, %	4.47 (2.09–6.02)	1.96 (1.45–2.74)	< 0.0001
Hcy RS, %	2.61 (1.47–3.35)	1.08 (0.84–1.42)	<0.0001

HbA1c levels of 7–7.9%, and the levels exceeding 8% were found in 16 patients (9.2%).

Stroke, resulting from cerebral artery thrombosis with a cardiac source of embolism, was more common in patients with T2D ( $p < 0.0001$ ), and atherothrombotic stroke was more common in patients with stress hyperglycemia ( $p < 0.0001$ ). No intergroup differences in the prevalence of lacunar stroke were found ( $p = 0.162$ ). The examined groups of patients were comparable in the neurological impairment severity and their functional status during the acute period of stroke (see Table 3).

In the group of patients with a combination of IS and T2D, the significantly decreased tCys, tGSH, and tHcy levels were observed compared to the group of patients with IS and stress hyperglycemia ( $p < 0.0001$ ). However, there were no significant differences in the rCys, rGSH, and rHcy levels between the groups of patients with T2D and stress hyperglycemia. Probably, this entails higher Cys RS, GSH RS, and Hcy RS values in the group of patients with IS and T2D ( $p < 0.0001$ ) (Table 4).

No significant correlations between the blood glucose levels and the levels of LMWTs were revealed both in the total group of patients with IS and in the distinct groups of patients (T2D and stress hyperglycemia).

When the patients with a combination of IS and T2D were divided based on the degree of functional recovery by day 21, analysis of LMWTs showed that in the group of patients with slight limitations in performing the activities of daily living (mRS score 0–2), the Cys RS and GSH RS were significantly higher compared to patients with severe functional limitations (Cys RS 5.26 vs. 3.51  $\mu\text{M}$ ,  $p = 0.043$ ; GSH RS 4.92 vs. 2.79  $\mu\text{M}$ ,  $p = 0.018$ ) (Table 5).

The logistic regression procedures were used to identify the markers of poor outcome (mRS  $\geq 3$ ) in patients with a combination of IS and T2D. The predictive value of GSH RS

is presented in Fig. 1. ROC analysis of 68 patients with IS in the ICA basin and T2D showed that the threshold level GSH RS  $\leq 4.06\%$  during the first 24 h of IS is a predictor of poor IS outcome (mRS  $\geq 3$ , 21 days after IS). Sensitivity of the model was 63.6%, specificity was 69.6%, and AUC was  $0.74 \pm 0.09$ , which corresponds to the good quality in predicting the functional recovery in patients with IS.

## DISCUSSION

Chronic hyperglycemia, associated with T2D, is considered one of the risk factors for IS. Glucose is a chemical compound, which actively interacts with proteins and lipids, forming the advanced glycation end products [20, 21]. Hyperglycemia, chronic oxidative stress, and mitochondrial dysfunction, associated with T2D, result in endothelial dysfunction, impaired angiogenesis, activation of hemostasis, and the increased blood-brain barrier permeability [22]. Hyperglycemia enhances oxidative processes by lowering the levels of vitamins E, C, and other antioxidants (uric acid) [11], it is also an additional factor that stimulates and facilitates the reactive oxygen species formation [23].

Chronic hyperglycemia is defined based on the degree of glucose binding to hemoglobin, and the percentage of HbA1c. The higher the level of HbA1, the higher blood glucose levels were observed in the patient during the last 3 months. The normal levels do not exceed 6.5%. The increase in HbA1c by 1% increases the risk of stroke by 17% [24]. In our study, the duration of T2D was 7 years, and the level of HbA1c was 7.8 %.

In individuals with IS, the effects of hyperglycemia on the brain are mediated by both impaired cerebral microcirculation, and the toxic effects on brain tissue. Lactate accumulation, free radical formation, development of cytotoxic cerebral edema, and abnormal blood-brain barrier permeability are attributed to hyperglycemia [25, 26].

**Table 5.** Low molecular weight aminothiols plasma levels ( $\mu\text{M}$ ) and redox status (%) in patients with a combination of IS and T2D taking into account the functional recovery (Mann–Whitney U test)

Indicator	Patients with IS and T2D (mRS 0–2 on day 21). $n = 28$ ; Me ( $Q_1$ – $Q_3$ )	Patients with IS and T2D (mRS 3 or more on day 21); $n = 40$ ; Me ( $Q_1$ – $Q_3$ )	$p$
tCys ( $\mu\text{M}$ )	132.8 (125.7–140.0)	139.2 (132.2–57)	0.09
tGSH ( $\mu\text{M}$ )	0.85 (0.74–1.02)	0.61 (0.35–1.16)	0.416
tHcy ( $\mu\text{M}$ )	5.87 (4.78–6.38)	5.33 (4.53–7.01)	0.823
rCys ( $\mu\text{M}$ )	6.57 (4.18–8.53)	6.55 (4.72–7.72)	0.959
rGSH ( $\mu\text{M}$ )	0.042 (0.02–0.053)	0.026 (0.014–0.042)	0.129
rHcy ( $\mu\text{M}$ )	0.15 (0.12–0.17)	0.15 (0.12–0.23)	1,000
Cys RS, %	5.26 (3.20–6.10)	3.51 (2.06–5.04)	0.043
GSH RS, %	4.92 (3.40–6.43)	2.79 (1.97–4.63)	0.018
Hcy RS, %	2.7 (1.97–3.57)	2.17 (1.13–3.18)	0.158

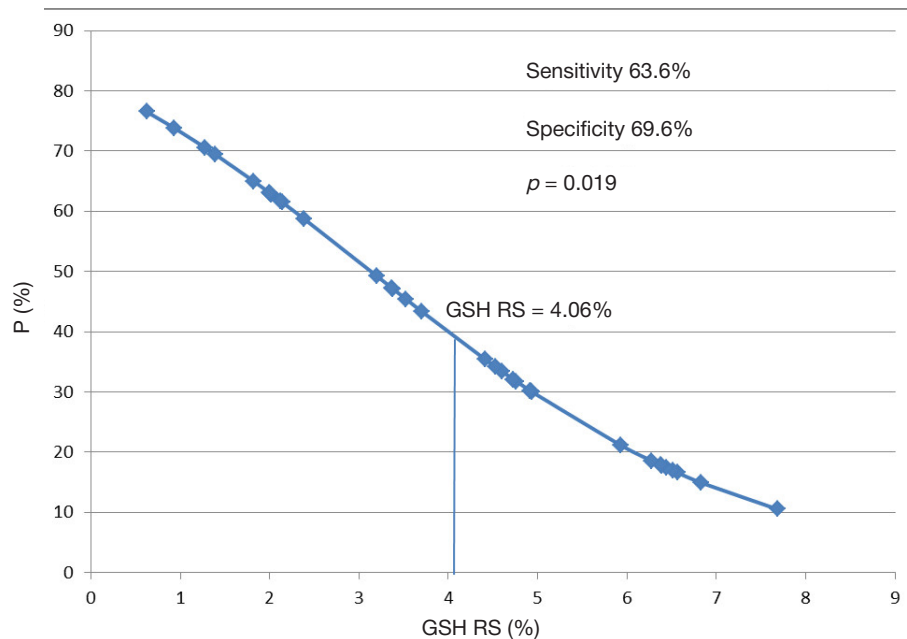


Fig. ROC curve for poor functional outcome ( $mRS \geq 3$ ) in patients with a combination of IS and T2D when assessing the glutathione redox status

Hyperglycemia in individuals with T2D and IS results in depletion of the antioxidant system and disorders of all types of tissue metabolism. The thiol/disulfide ratio (SH/SS) is the indicator of cellular and tissue redox homeostasis, as well as of the blood plasma antioxidant capacity [11, 27].

Glutathione is an endogenous thiol tripeptide, composed of cysteine, glutamic acid, and glycine. It is being synthesized continuously, but at a relatively low rate [28]. Glutathione is also a factor involved in regulation of glucose metabolism in patients with cardiovascular disorders [13]. Polymorphism of glutathione S-transferase, the enzyme, involved in the glutathione-mediated detoxification of xenobiotics, contributes to the early cardiovascular complications of diabetes mellitus [29].

In individuals with acute oxidative stress, the total content of glutathione (GSH) is decreased, and the levels of oxidized glutathione (GSSG) are increased, which results in the accelerated GSH/GSSG cycle [27].

Hyperglycemia during the acute period of IS is associated with poor functional outcome [4], however, the efficacy of insulin-based glucose lowering therapy and its effects on the severity and outcome of stroke have not been proven [1].

The findings show that T2D in patients with IS is associated with the rapid decrease in plasma tCys, tGSH, tHcy, rGSH, and GSH RS, along with the increase in Cys RS and Hcy RS.

Perhaps the chronic oxidative stress results in depletion of LMWTs in blood plasma. Irreversible disposal of GSH may

be associated with intense oxidative stress, when GSH is exported from cells in order to prevent the significant shift in the redox equilibrium [28]. Presumably, it is the main mechanism explaining the lack of correlations between tGSH, tCys and tHcy, as well as between tGSH and rGSH. No significant correlations between plasma glucose levels and LMWTs have been revealed. This indicates that alterations in the metabolism of LMWTs associated with T2D are mediated by non-glycemic mechanisms.

The GSH RS value of 4.06% or lower during the first 24 h of IS is a predictor of poor IS outcome ( $mRS \geq 3$ , three weeks after IS). Therefore, the search for approaches to glutathione metabolism correction in patients with T2D may be regarded as the potential therapeutic objective during the acute period of IS.

## CONCLUSIONS

T2D is a factor having a major impact on the metabolism of LMWTs in patients with IS. Regardless of the lack of correlations between the glucose levels and LMWTs, T2D was associated with reduced total content of homocysteine, cysteine and glutathione, and the glutathione redox status of 4.06% or lower during the first 24 h of IS was associated with poor functional outcome. Glutathione metabolism correction in individuals with a combination of IS and T2D may have a positive impact on the course of IS.

## References

- Hill MD Stroke and diabetes mellitus. *Handb Clin Neurol*. 2014;126:167-74. DOI: 10.1016/B978-0-444-53480-4.00012-6.
- Sarwar N, Aspelund T, Eiriksdottir G, Gobin R, Seshasai SR, Forouhi NG, et al. Markers of dysglycaemia and risk of coronary heart disease in people without diabetes: Reykjavik prospective study and systematic review. *PLoS Med*. 2010; 7 (5): e1000278. DOI: 10.1371/journal.pmed.1000278.
- Barth E, Albuszies G, Baumgart K, Matejovic M, Wachter U, Vogt J, et al. Glucose metabolism and catecholamines. *Crit Care Med*. 2007; 35 (9 Suppl): S508-18. DOI: 10.1097/01.CCM.0000278047.06965.20.
- Zhu B, Pan Y, Jing J, Meng X, Zhao X, Liu L, et al. Stress Hyperglycemia and Outcome of Non-diabetic Patients After Acute Ischemic Stroke. *Front Neurol*. 2019; 10: 1003. DOI: 10.3389/fneur.2019.01003.
- Tuttolomondo A, Pinto A, Salemi G, Di Raimondo D, Di Sciacca R, Fernandez P, et al. Diabetic and non-diabetic subjects with ischemic stroke: differences, subtype distribution and outcome. *Nutr Metab Cardiovasc Dis*. 2008; 18 (2): 152-7. DOI: 10.1016/j.numecd.2007.02.003.
- Zhao H, Han Z, Ji X, Luo Y. Epigenetic regulation of oxidative stress in ischemic stroke. *Aging Dis*. 2016; 2016; 7 (3): 295-306. DOI: 10.14336/AD.2015.1009.
- Khoshnam SE, Winlow W, Farzaneh M, Farbood Y, Moghaddam

- HF Pathogenic mechanisms following ischemic stroke. *Neurol Sci.* 2017; 38 (7): 1167–86. DOI: 10.1007/s10072-017-2938-1.
8. Ivanov AV, Alexandrin VV, Paltsyn AA, Nikiforova KA, Virus ED, Luzyanin BP, et al. Plasma low-molecular-weight thiol/disulphide homeostasis as an early indicator of global and focal cerebral ischaemia. *Redox Rep.* 2017; 22 (6): 460–6. DOI: 10.1080/13510002.2017.1311464.
  9. Maksimova MY, Ivanov AV, Virus ED, Alexandrin VV, Nikiforova KA, Bulgakova PO, et al. Disturbance of thiol/disulfide amino thiols homeostasis in patients with acute ischemic stroke stroke: Preliminary findings. *Clin Neurol Neurosurg.* 2019; 183: 105393. DOI: 10.1016/j.clineuro.2019.105393.
  10. Duman BS, Oztürk M, Yilmazeri S, Hatemi H. Thiols, malonaldehyde and total antioxidant status in the Turkish patients with type 2 diabetes mellitus. *Tohoku J Exp Med.* 2003; 201 (3): 147–55. DOI: 10.1620/tjem.201.147.
  11. Tessier D, Khalil A, Fülöp T. Effects of an oral glucose challenge on free radicals/antioxidants balance in an older population with type II diabetes. *J Gerontol A Biol Sci Med Sci.* 1999; 54 (11): M541–5. DOI: 10.1093/gerona/54.11.m541.
  12. Forrester TE, Badaloo V, Bennett FI, Jackson AA. Excessive excretion of 5-oxoproline and decreased levels of blood glutathione in type II diabetes mellitus. *Eur J Clin Nutr.* 1990; 44 (11): 847–50. PMID: 2086214.
  13. Karolczak K, Kubalczyk P, Głowacki R, Pietruszyński R, Watała C. An inverse relationship between plasma glutathione concentration and fasting glycemia in patients with coronary artery disease and concomitant type 2 diabetes: A pilot study. *Adv Clin Exp Med.* 2017; 26 (9): 1359–66. DOI: 10.17219/acem/65441.
  14. Lutchmansingh FK, Hsu JW, Bennett FI, Badaloo AV, McFarlane-Anderson N, Gordon-Strachan GM, et al. Glutathione metabolism in type 2 diabetes and its relationship with microvascular complications and glycemia. *PLoS One.* 2018; 13 (6): e0198626. DOI: 10.1371/journal.pone.0198626.
  15. Fussner J, Velasco C. Stroke Coordinator Boot Camp. Assessing Stroke — Scores and Scales — American Heart Association. Available from: <https://www.heart.org>.
  16. Quinn TJ, Langhorne P, Stott DJ. Barthel index for stroke trials: development, properties, and application. *Stroke.* 2011; 42 (4): 1146–51. DOI: 10.1161/STROKEAHA.110.598540.
  17. Harrison JK, McArthur KS, Quinn TJ. Assessment scales in stroke: clinimetric and clinical considerations. *Clin Interv Aging.* 2013; 8: 201–11. DOI: 10.2147/CIA.S32405.
  18. Adams HP, Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993; 24: 35–41. DOI: 10.1161/01.STR.24.1.35.
  19. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* 2013; 36: S67–S74. PMID:15618111.
  20. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature.* 2001; 414 (6865): 813–20. DOI: 10.1038/414813a.
  21. Nishikawa T, Kukidome D, Sonoda K, Fujisawa K, Matsuhisa T, Motoshima H, et al. Impact of mitochondrial ROS production on diabetic vascular complications. *Diabetes Res Clin Pract.* 2007; 77 Suppl 1: S41–5. DOI: 10.1016/j.diabres.2007.01.031.
  22. Smith CJ, Lawrence CB, Rodriguez-Grande B, Kovacs KJ, Pradillo JM, Denes A. The immune system in stroke: clinical challenges and their translation to experimental research. *J Neuroimmune Pharmacol.* 2013; 8 (4): 867–87. DOI: 10.1007/s11481-013-9469-1.
  23. Poulsen RC, Knowles HJ, Carr AJ, Hulley PA. Cell differentiation versus cell death: extracellular glucose is a key determinant of cell fate following oxidative stress exposure. *Cell Death Dis.* 2014; 5 (2): e1074. DOI: 10.1038/cddis.2014.52.
  24. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ.* 2000; 321 (7258): 405–12. DOI: 10.1136/bmj.321.7258.405.
  25. Bruno A, Liebeskind D, Hao Q, Raychev R; UCLA Stroke Investigators. Diabetes mellitus, acute hyperglycemia, and ischemic stroke. *Curr Treat Options Neurol.* 2010; 12 (6): 492–503. DOI: 10.1007/s11940-010-0093-6.
  26. Venkat P, Chopp M, Chen J. Blood-Brain Barrier Disruption, Vascular Impairment, and Ischemia/Reperfusion Damage in Diabetic Stroke. *J Am Heart Assoc.* 2017; 6 (6): e005819. DOI: 10.1161/JAHA.117.005819.
  27. Jones DP. Redox potential of GSH/GSSG couple: assay and biological significance. *Methods Enzymol.* 2002; 348: 93–112. DOI: 10.1016/s0076-6879(02)48630-2.
  28. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. *J Nutr.* 2004; 134 (3): 489–92. DOI: 10.1093/jn/134.3.489.
  29. Etemad A, Vasudevan R, Aziz AF, Yusof AK, Khazaei S, Fawzi N, et al. Analysis of selected glutathione S-transferase gene polymorphisms in Malaysian type 2 diabetes mellitus patients with and without cardiovascular disease. *Genet Mol Res.* 2016; 15 (2). DOI: 10.4238/gmr.15025845.

## Литература

1. Hill MD Stroke and diabetes mellitus. *Handb Clin Neurol.* 2014;126:167-74. DOI: 10.1016/B978-0-444-53480-4.00012-6.
2. Sarwar N, Aspelund T, Eiriksdottir G, Gobin R, Seshasai SR, Forouhi NG, et al. Markers of dysglycaemia and risk of coronary heart disease in people without diabetes: Reykjavik prospective study and systematic review. *PLoS Med.* 2010; 7 (5): e1000278. DOI: 10.1371/journal.pmed.1000278.
3. Barth E, Albuszies G, Baumgart K, Matejovic M, Wachter U, Vogt J, et al. Glucose metabolism and catecholamines. *Crit Care Med.* 2007; 35 (9 Suppl): S508–18. DOI: 10.1097/01.CCM.0000278047.06965.20.
4. Zhu B, Pan Y, Jing J, Meng X, Zhao X, Liu L, et al. Stress Hyperglycemia and Outcome of Non-diabetic Patients After Acute Ischemic Stroke. *Front Neurol.* 2019; 10: 1003. DOI: 10.3389/fneur.2019.01003.
5. Tuttolomondo A, Pinto A, Salemi G, Di Raimondo D, Di Sciacca R, Fernandez P, et al. Diabetic and non-diabetic subjects with ischemic stroke: differences, subtype distribution and outcome. *Nutr Metab Cardiovasc Dis.* 2008; 18 (2): 152–7. DOI: 10.1016/j.numecd.2007.02.003.
6. Zhao H, Han Z, Ji X, Luo Y. Epigenetic regulation of oxidative stress in ischemic stroke. *Aging Dis.* 2016; 2016; 7 (3): 295–306. DOI: 10.14336/AD.2015.1009.
7. Khoshnam SE, Winlow W, Farzaneh M, Farbood Y, Moghaddam HF Pathogenic mechanisms following ischemic stroke. *Neurol Sci.* 2017; 38 (7): 1167–86. DOI: 10.1007/s10072-017-2938-1.
8. Ivanov AV, Alexandrin VV, Paltsyn AA, Nikiforova KA, Virus ED, Luzyanin BP, et al. Plasma low-molecular-weight thiol/disulphide homeostasis as an early indicator of global and focal cerebral ischaemia. *Redox Rep.* 2017; 22 (6): 460–6. DOI: 10.1080/13510002.2017.1311464.
9. Maksimova MY, Ivanov AV, Virus ED, Alexandrin VV, Nikiforova KA, Bulgakova PO, et al. Disturbance of thiol/disulfide amino thiols homeostasis in patients with acute ischemic stroke stroke: Preliminary findings. *Clin Neurol Neurosurg.* 2019; 183: 105393. DOI: 10.1016/j.clineuro.2019.105393.
10. Duman BS, Oztürk M, Yilmazeri S, Hatemi H. Thiols, malonaldehyde and total antioxidant status in the Turkish patients with type 2 diabetes mellitus. *Tohoku J Exp Med.* 2003; 201 (3): 147–55. DOI: 10.1620/tjem.201.147.
11. Tessier D, Khalil A, Fülöp T. Effects of an oral glucose challenge on free radicals/antioxidants balance in an older population with type II diabetes. *J Gerontol A Biol Sci Med Sci.* 1999; 54 (11): M541–5. DOI: 10.1093/gerona/54.11.m541.
12. Forrester TE, Badaloo V, Bennett FI, Jackson AA. Excessive excretion of 5-oxoproline and decreased levels of blood

- glutathione in type II diabetes mellitus. *Eur J Clin Nutr.* 1990; 44 (11): 847–50. PMID: 2086214.
13. Karolczak K, Kubalczyk P, Głowacki R, Pietruszyński R, Watała C. An inverse relationship between plasma glutathione concentration and fasting glycemia in patients with coronary artery disease and concomitant type 2 diabetes: A pilot study. *Adv Clin Exp Med.* 2017; 26 (9): 1359–66. DOI: 10.17219/acem/65441.
  14. Lutchmansingh FK, Hsu JW, Bennett FI, Badaloo AV, McFarlane-Anderson N, Gordon-Strachan GM, et al. Glutathione metabolism in type 2 diabetes and its relationship with microvascular complications and glycemia. *PLoS One.* 2018; 13 (6): e0198626. DOI: 10.1371/journal.pone.0198626.
  15. Fussner J, Velasco C. Stroke Coordinator Boot Camp. Assessing Stroke — Scores and Scales — American Heart Association. Available from: <https://www.heart.org>.
  16. Quinn TJ, Langhorne P, Stott DJ. Barthel index for stroke trials: development, properties, and application. *Stroke.* 2011; 42 (4): 1146–51. DOI: 10.1161/STROKEAHA.110.598540.
  17. Harrison JK, McArthur KS, Quinn TJ. Assessment scales in stroke: clinimetric and clinical considerations. *Clin Interv Aging.* 2013; 8: 201–11. DOI: 10.2147/CIA.S32405.
  18. Adams HP, Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993; 24: 35–41. DOI: 10.1161/01.STR.24.1.35.
  19. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* 2013; 36: S67–S74. PMID:15618111.
  20. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature.* 2001; 414 (6865): 813–20. DOI: 10.1038/414813a.
  21. Nishikawa T, Kukidome D, Sonoda K, Fujisawa K, Matsuhisa T, Motoshima H, et al. Impact of mitochondrial ROS production on diabetic vascular complications. *Diabetes Res Clin Pract.* 2007; 77 Suppl 1: S41–5. DOI: 10.1016/j.diabres.2007.01.031.
  22. Smith CJ, Lawrence CB, Rodriguez-Grande B, Kovacs KJ, Pradillo JM, Denes A. The immune system in stroke: clinical challenges and their translation to experimental research. *J Neuroimmune Pharmacol.* 2013; 8 (4): 867–87. DOI: 10.1007/s11481-013-9469-1.
  23. Poulsen RC, Knowles HJ, Carr AJ, Hulley PA. Cell differentiation versus cell death: extracellular glucose is a key determinant of cell fate following oxidative stress exposure. *Cell Death Dis.* 2014; 5 (2): e1074. DOI: 10.1038/cddis.2014.52.
  24. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ.* 2000; 321 (7258): 405–12. DOI: 10.1136/bmj.321.7258.405.
  25. Bruno A, Liebeskind D, Hao Q, Raychev R; UCLA Stroke Investigators. Diabetes mellitus, acute hyperglycemia, and ischemic stroke. *Curr Treat Options Neurol.* 2010; 12 (6): 492–503. DOI: 10.1007/s11940-010-0093-6.
  26. Venkat P, Chopp M, Chen J. Blood-Brain Barrier Disruption, Vascular Impairment, and Ischemia/Reperfusion Damage in Diabetic Stroke. *J Am Heart Assoc.* 2017; 6 (6): e005819. DOI: 10.1161/JAHA.117.005819.
  27. Jones DP. Redox potential of GSH/GSSG couple: assay and biological significance. *Methods Enzymol.* 2002; 348: 93–112. DOI: 10.1016/s0076-6879(02)48630-2.
  28. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. *J Nutr.* 2004; 134 (3): 489–92. DOI: 10.1093/jn/134.3.489.
  29. Etemad A, Vasudevan R, Aziz AF, Yusof AK, Khazaei S, Fawzi N, et al. Analysis of selected glutathione S-transferase gene polymorphisms in Malaysian type 2 diabetes mellitus patients with and without cardiovascular disease. *Genet Mol Res.* 2016; 15 (2). DOI: 10.4238/gmr.15025845.