Interhemispheric Asymmetry of the Brain in Patients with Type 1 Diabetes Mellitus and Cognitive Impairment

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Research Article

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Abstract

With an ageing of population and a splurging epidemic of diabetes mellitus (DM), the prevalence of complications associated with pathology of the central nervous system are expected to increase, which in the future may have serious consequences for public health. It is known that one of the main manifestations of brain damage in type 1 diabetes is cognitive impairment, which is possibly associated with the peculiarities of vascularization and interhemispheric asymmetry, which requires in-depth analysis using modern neuroimaging methods. The aim of the study is to assess the symmetry of structural, metabolic and neurovascularization changes in the brain in patients with type 1 diabetes and cognitive impairment. The study included 120 patients with type 1 diabetes aged 18 to 45 years suffering from cognitive impairment, and 30 people without cognitive decline which constituted the control group. Neuropsychological testing included the Montreal Cognitive Dysfunction Assessment Scale (MoCA test). For neuroimaging methods, standard magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), contrast and non-contrast-enhanced perfusion were used. Statistical processing was carried out using the SPSS Statistic 2020 software. In patients with type 1 diabetes with cognitive impairment, as manifested by impaired memory and/or attention, perfusion imaging revealed the presence of brain asymmetry zones. Standard MRI allowed to demonstrate changes in the white, gray matter and hippocampus in the right hemisphere. The results obtained were refined taking into account the topical localization, so during the perfusion study, regions with asymmetric blood flow were identified - namely, the white matter of the frontal lobe and the gray matter in the occipital lobe. Spectroscopy of the brain revealed that it was in these areas of the brain that the most significant metabolic disorders were noted – in the form of significantly altered ratio of N-acetylaspartate (NAA) / choline (Cho) on the left, along with the asymmetry in phosphocreatine level (Cr 2) on the right. In conclusion, early preclinical predictive diagnostics with the use of modern neuroimaging methods allows for timely detection of impaired vascularization and brain metabolism in this group of patients.

Introduction

Diabetes mellitus (DM) is a metabolic disease associated with the development of acute and chronic complications (Mc Crimmon, Ryan & Frier 2012; Sharafova et al. 2019). Among the complications of diabetes, relatively less attention is paid to cognitive impairments, which are verified in some patients with type 1 and 2 diabetes. (Kodl & Seaquist 2008). Interestingly, in the early 20th century, researchers and doctors recognized that diabetic patients often complain of poor memory and lack of attention. In 1922, Miles *et al.* have shown, examining memory and attention, that diabetic patients performed poorly on cognitive tasks (Miles & Root, 1922). The term diabetic encephalopathy was introduced in 1950 to describe complications of diabetes associated with the central nervous system (Dejong, 1950). Other terms, such as functional disorders of the brain and central neuropathy, have also been used in the literature to describe the cognitive dysfunctions associated with diabetes (Mijnhout et al., 2006). Modern methods make it possible to non-invasively study the morphology and functioning of the brain in diabetic patients, determining the possibilities of predictive diagnostics.

Currently, there is a sufficient number of descriptive studies of the brain in type 1 diabetes, which describe the phenomenon of focal atrophy of the white and / or gray matter, more often the frontal, temporal and occipital lobes (Biessels & Reijmer, 2014; Filip et al., 2020). Another neuroimaging method is Magnetic resonance spectroscopy (MRS) - an advanced biochemical analysis technique that detects changes in metabolic neurochemical levels of metabolites in various areas of the brain in vivo. In type 1 diabetes, changes in the levels of NAA, Cho and the NAA / Cr (creatine) ratio are most often recorded (Hansen et al., 2018; Samoilova et al., 2020; Zhao et al., 2018). Considering the peculiarity of the development of microangiopathies in patients with type 1 diabetes, the assessment of cerebral perfusion also plays an important diagnostic role, allowing for the demonstration of changes in cerebral blood flow and other characteristics of the cortical and subcortical formations of the brain (Emanuel et al., 2019). Therefore, the use of these methods in a complex way allows us to clarify the features of morphofunctional associations in patients with cognitive impairments. At the same time, there are data in the literature that indicate a possible connection between the asymmetry of the cerebral hemispheres and various pathological conditions, which are not caused by genetic prerequisites (Eyler et al., 2014; Samoylova *et al.*, 2021).

Thus, due to the lack of systematized data on the symmetry of brain structures, we formulated the aim of the study: to assess the symmetry of structural, metabolic and neurovascularization changes in the brain in patients with type 1 diabetes mellitus and cognitive impairments.

Methods

The study protocol was approved by the Ethics Committee of the Federal State Budgetary Educational Institution of Higher Education Siberian State Medical University of the Ministry of Health of Russia (conclusion No. 5265 of 05/02/2017). Written informed consent was signed by all participants prior to enrollment.

The study included 120 patients with type 1 diabetes with cognitive impairment. The control group (n = 30) was comparable in age (26 [23:39] years) and disease duration (13 [2:24] years). Screening for cognitive disorders was performed using the Montreal Cognitive Assessment Scale (MoCA test). The degree of cognitive impairment was established in strict accordance with generally accepted criteria, according to the classification of Academician of the Russian Academy of Medical Sciences N.N. Yakhno (2005), distinguishing between severe, moderate and mild cognitive impairments.

Standard MRI examination of the brain was performed in axial, sagittal and coronal projections using T2 (TR - time of repetition) 4932 ms, TE (Echotime) 90 ms, T1 (TR 280 MS, te 6.1 MS) modes, using the programs with free water signal suppression (Fluid Attenuated Inversion Recovery, FLAIR, TR 8000 ms, TE 105 ms, TI - time in version 2200 ms) on a Signa Creator "E" magnetic resonance scanner, GE Healthcare, 1.5 T, China.

Dynamic contrast MRI was performed, using Gadovist contrast agent administered as a 5 ml intravenous bolus with acquisition of images weighted by the inhomogeneity of the magnetic field (dynamic

susceptibility contrast MR), as well as the technique of arterial spin labeling (ASL), which does not require the administration of contrast agent and allows one to quantify cerebral blood flow.

To process the MRI results, the FreeSurfer program was used, which is designed to analyze and visualize the structural and functional parameters of neuroimaging from cross-sectional or longitudinal studies, which was developed by the Computational Neuroimaging Laboratory at the Center for Biomedical Imaging (USA, 2017). Proton spectroscopy of the brain was performed in a multivoxel mode; in the hippocampus region, the main spectra of NAA, Cho, Cr, and Cr2, as well as their ratios, were recorded.

For statistical analysis, the SPSS Statistic program version 25.0 and methods were used - analysis of genotype frequencies, Kendal's rank correlation coefficient for samples that do not obey the normal distribution law, and nonparametric Kruskal-Wallis analysis of variance to compare the medians of samples. P was considered significant at a <0,05 level.

Results

According to the study, in patients with type 1 diabetes, cognitive impairment was presented as mild in 36.9% (n = 24) of the patients, moderate in 30.76 (n = 20) and severe in 1.53% (n = 1).

Neuropsychological testing data showed a decrease in the overall score of the MoCA test and lower scores on the tasks for attention (serial subtraction) and memory (p <0.001).

The characteristics of the patients are presented in Table 1, the groups were comparable, with the exception of the level of fasting glycemia.

Table 1 Characteristics of patients with type 1 DM

Parameters	Patients with type 1 DM and cognitive impairment, n=120	Patients with type 1 DM without cognitive impairment, n=30	P
Age, years	27[18:45]	26 [23:39]	0,2
Disease duration, years	11[1:32]	13 [2:24]	0,2
Fasting plasma glucose, mmol/l	9.1 [6.4:16.4]	7,9 [5.5:18.3]	0,05
HbA1c,%	7.6[6:12.4]	6.9 [4.5:10.3]	0,2
Body weight index, kg/m ²	22.6[17.4:30.6]	21.8 [16:30.4]	0,2
Note: p≤0,05 – significant differences			

Interhemispheric asymmetry according to standard brain MRI

Initially, the method of segmentation was used to assess the volume of brain structures, as a result of which the total volumes of white, gray matter, including the hemisphere and hippocampus, were obtained (Table 2).

Table 2 Brain segmentation in patients with type 1 DM

Anatomic region	Patients with type 1 DM, n=150		<i>P</i>
	With cognitive impairment, n=120	Without cognitive impairment, n=30	
Grey matter, mm ³	478009 [457669,1-511273,8]	497704 [442993,1-586559,6]	0,106
Grey matter, left hemisphere, mm ³	225046 [212392,9-232197,2]	252441 [224292,9-271860,4]	0,0004
Gray matter, right hemisphere, mm ³	235085 [219200,2-254713,2]	253587 [223200,3-270387,4]	0,015
White matter, mm ³	455968 [421138,4- 473940,5]	503517 [440036,6-509720,7]	0,005
White matter, left hemisphere, mm ³	232213 [217884,9-239910,2]	241831 [217516,8-264238,8]	0,639
White matter, right hemisphere, mm ³	235509 [211609,8-237965,5]	270207 [228287,6-310513,8]	0,046
Left hippocampus, mm ³	73 [72,1-73,4]	73 [72,9-74,8]	0,141
Right hippocampus, mm ³	72 [71,1-73,2]	73 [72,4-75,0]	0,005
Note: $p \leq 0,05$ – significant differences			

In patients with type 1 diabetes and cognitive impairments, changes were noted in the volumes of white matter of both hemispheres, gray matter and the hippocampus on the right, compared to the patients from the control group, which indicate signs of atrophy in aforementioned areas.

Interhemispheric asymmetry according to contrast and non-contrast cerebral perfusion

We defined the null hypothesis as the absence of significant differences between the perfusion indices of the right and left cerebral hemispheres, while the alternative hypothesis is the presence of significant differences between the samples. To accept or reject this paradigm, the Mann-Whitney test was applied. Table 3 shows the values of the Mann-Whitney U-statistics for which the alternative hypothesis is accepted, that is, statistically significant differences between the samples are proved.

Table 3 Asymmetry of neurovascularization according to contrast and non-contrast perfusion in patients with type 1 diabetes mellitus and cognitive impairment

Localization and indices of perfusion	U-statistics	P value
Frontal lobe, white matter, MTT/sec	201,5000	0,032032
Temporal lobe, grey matter, ASL/CBF	201,5000	0,032032
Note: U-Mann-Whitney test, p-value ≤ 0.05 ; MTT - mean transit time, AS, arterial spin labeling - method of spin labeling of arterial blood, CBF - cerebral blood flow.		

Thus, during contrast perfusion in the area of the white matter of the frontal lobe, asymmetry was observed in the parameter of the average time of blood passage. When assessing non-contrast perfusion, differences were shown in the main parameter of cerebral blood flow in the occipital lobe of the gray matter in patients with type 1 diabetes and cognitive impairment. Apparently, this is an example of how the processes of neuroplasticity are implemented, aimed at compensating for cognitive impairments.

Interhemispheric asymmetry as assessed using proton spectroscopy of the brain

Based on the initial data of proton spectroscopy, a correlation matrix was built to check the presence of variables that have a high degree of connection with each other during proton spectroscopy of the brain (Fig. 1).

The figure shows that the relationship between the variables exists, but it is not pronounced enough to exclude any variable, since the maximum coefficient does not exceed 0.8. Therefore, based on the correlation matrix, no feature can be rejected as uninformative. The Kruskal-Wallis test was used to check the informativeness of the features (Table 4).

Table 4 Values of the Kruskal-Wallis rank test for parameters of proton spectroscopy of the brain of patients with type 1 diabetes mellitus

Parameter	Statistics	p-value
NAA left	22,62228	0,0001
NAA right	26,53589	0,0003
Cho left	28,52084	0,0000
Cho right	14,18308	0,0027
Cr left	5,721290	0,1260
Cr right	33,81858	0,0000
Cr2 left	29,96852	0,0002
Cr2 right	34,03594	0,0001
NAA/Cr left	33,19992	0,0000
Naa/Cr right	31,45471	0,0001
NAA/Cho left	34,10368	0,0004
Naa/Cho right	33,72973	0,0001
Cho/Cr left	23,58656	0,0000
Cho/Cr right	18,70439	0,0003

Based on the data in the table, we can conclude that for each variable the hypothesis of significance does not change at the significance level of 0.5, because the p-value does not exceed this figure. Figure 2 shows a diagram of the distribution of the importance of features

Figure 3 demonstrates that the least informative trait is Cr on the left, with the most informative being NAA / Cho on the left and Cr2 on the right. Thus, the asymmetry of the hippocampal region on the right is associated with a change in metabolism depicted as the change in the NAA / Cho ratio.

Discussion

As a result of the study, it was revealed that type 1 diabetes is characterized by mild to moderate cognitive impairment with a predominance of impaired attention and memory. It should be noted that memory impairment in this type of diabetes has not been previously recorded, which may require the use of specific tests to understand the phenomenology of this result. These data contradict the results of many authors, who believe that with type 1 diabetes, the neurodynamic component of cognitive functions is mainly affected, namely the pace and the ability to concentrate (Sosina et al., 2017), although there are studies that focus on memory impairment primarily associated with diabetes (Arvanitakis et al., 2004; Okereke et al., 2008).

For example, a study conducted by Weigner *et al.* (2008) using magnetic resonance imaging as the diagnostic tool and with a focus on the cerebral white matter revealed that the participants who had type 1 diabetes had comparatively lower scores to the controls on one measure of executive function (Sorting Test), short-term memory, delayed recall, vocabulary, and psychomotor efficiency.

With regard to the issues of asymmetry of the cerebral hemispheres, many studies are devoted to the issues of sensorimotor integration (Biduła & Króliczak, 2015; Keller, Roberts & Hopkins, 2009). On the other hand, the different functioning of the cerebral hemispheres is an important phenomenon in injury, employing the rehabilitation potential and neuroplasticity of the central nervous system (Karolis *et al.*, 2019). The only large meta-analysis in neurology on hemispheric asymmetry was presented in 2019, consisting of 159 publications on voxel-based morphometry (registration of 4469 patients and 4307 controls), showing that asymmetry does exist in neurodegenerative diseases. Regions with asymmetric brain decline were located in areas primarily affected by neurodegeneration. Thus, with moderate cognitive impairment, the region of the right hippocampus is most vulnerable (Minkova *et al.*, 2017).

The data obtained in this study deserve attention from the perspective of preventive medicine, since early preclinical diagnosis of cognitive impairment and related dysfunctions, including microangiopathies, can reduce health care costs and improve the quality of life of patients with type 1 diabetes. Information on non-invasive techniques seems promising: non-contrast perfusion, in which the area of interest is the gray matter of the occipital lobes; and proton spectroscopy of the hippocampus, the informative signs for which are the NAA / Cho ratio on the left and the Cr2 content on the right.

Conclusion

In patients with type 1 diabetes with a disease duration of more than 10 years, the neurodynamic type of cognitive impairment can turn into cortical-subcortical one, taking into account the topical localization of the revealed changes. Asymmetry of the hemispheres is characteristic of patients with type 1 diabetes and cognitive impairment. Early preclinical predictive diagnostics with the use of modern neuroimaging methods allows for timely detection of impaired vascularization and brain metabolism in this group of patients.

However, further work is needed to validate the findings and provide a better understanding of the functional role of interhemispheric asymmetry, for example, in the context of cognitive reserve and compensation.

Declarations

o Funding (information that explains whether and by whom the research was supported)

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“The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.”

o Conflicts of interest/Competing interests (include appropriate disclosures)

The authors hereby declare that there are no conflicting interests.

o Availability of data and material (data transparency)

The data that support the findings of this study are available on request from the corresponding author, [ASO]. The data are not publicly available due to [restrictions e.g. their containing information that could compromise the privacy of research participants].

Code availability (software application or custom code)

Not Applicable.

o Authors' contributions

We hereby declare that the authors contributed equally to the following tasks:

☒ Conception or design of the work

☒ Data collection

☒ Data analysis and interpretation

☒ Drafting the article

☒ Critical revision of the article

☒ Final approval of the version to be published

**** Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals**

o Ethics approval (include appropriate approvals or waivers) and Consent to participate (include appropriate statements)

The study protocol was approved by the Ethics Committee of the Federal State Budgetary Educational Institution of Higher Education Siberian State Medical University of the Ministry of Health of Russia (conclusion No. 5265 of 05/02/2017). Written informed consent which include consent to participate and consent for publication was signed by all participants prior to enrollment.

o Consent for publication (include appropriate statements)

Not Applicable.

References

1. Arvanitakis Z, Wilson RS, Bienias JL, et al. (2004). Diabetes mellitus and risk of Alzheimer disease and decline in cognitive function. *Arch Neurol*. 2004 May;61(5):661-6. DOI:10.1001/archneur.61.5.661
2. Biduła SP, Króliczak G. Structural asymmetry of the insula is linked to the lateralization of gesture and language. *Eur J Neurosci*. 2015 May;41(11):1438-47. doi: 10.1111/ejn.12888.
3. Biessels GJ, Reijmer YD. (2014). Brain MRI in children with type 1 diabetes: snapshot or road map of developmental changes? *Diabetes*. 2014 Jan;63(1):62-4. doi: 10.2337/db13-1466.
4. Dejong, R. (1950). The nervous system complications of diabetes mellitus, with special reference to cerebrovascular changes. *J. Nerv. Ment. Dis*. 1950;111: 181–206.
5. Emanuel AL, van Duinkerken E, Wattjes MP, Klein M, Barkhof F, Snoek FJ, Diamant M, Eringa EC, IJzerman RG, Serné EH. (2019). The presence of cerebral white matter lesions and lower skin microvascular perfusion predicts lower cognitive performance in type 1 diabetes patients with retinopathy but not in healthy controls-A longitudinal study. *Microcirculation*. 26(3):e12530. doi: 10.1111/micc.12530.
6. Eyler LT, Vuoksima E, Panizzon MS, Fennema-Notestine C, Neale MC, Chen CH, Jak A, Franz CE, Lyons MJ, Thompson WK, Spoon KM, Fischl B, Dale AM, Kremen WS (2014). Conceptual and data-based investigation of genetic influences and brain asymmetry: a twin study of multiple structural phenotypes. *J Cogn Neurosci*. 2014 May;26(5):1100-17. doi: 10.1162/jocn_a_00531.
7. Filip P, Canna A, Moheet A, Bednarik P, Grohn H, Li X, Kumar AF, Olawsky E, Eberly LE, Seaquist ER, Mangia S. (2020). Structural Alterations in Deep Brain Structures in Type 1 Diabetes. *Diabetes*. 2020 Nov;69(11):2458-2466. doi: 10.2337/db19-1100.
8. Hansen TM, Brock B, Juhl A, Drewes AM, Vorum H, Andersen CU, Jakobsen PE, Karmisholt J, Frøkjær JB, Brock C. (2018). Brain spectroscopy reveals that N-acetylaspartate is associated to peripheral sensorimotor neuropathy in type 1 diabetes. *J Diabetes Complications*. 33(4):323-328. doi: 10.1016/j.jdiacomp.2018.12.016.
9. Karolis VR, Corbetta M, Thiebaut de Schotten M. (2019). The architecture of functional lateralisation and its relationship to callosal connectivity in the human brain. *Nat Commun*. Mar 29;10(1):1417. doi: 10.1038/s41467-019-09344-1.
10. Keller SS, Roberts N, Hopkins W. (2009). A comparative magnetic resonance imaging study of the anatomy, variability, and asymmetry of Broca's area in the human and chimpanzee brain. *J Neurosci*. 29(46):14607-16. doi: 10.1523/JNEUROSCI.2892-09.2009.
11. Kodl CT, Seaquist ER. (2008). Cognitive dysfunction and diabetes mellitus. *Endocr Rev* 2008;29:494–511. DOI:10.1210/er.2007-0034
12. McCrimmon RJ, Ryan CM, Frier BM. (2012). Diabetes and cognitive dysfunction. *Lancet* 2012;379:2291–9. DOI:[https://doi.org/10.1016/S0140-6736\(12\)60360-2](https://doi.org/10.1016/S0140-6736(12)60360-2)

13. Mijnhout, G.S., P. Scheltens, M. Diamant, et al (2006). Diabetic encephalopathy: a concept in need of a definition. *Diabetologia*. 49: 1447–8. doi.org/10.1007/s00125-006-0221-8
14. Miles, W.R. & Root. HF. (1922). Psychologic tests applied to diabetic patients. *Arch. Intern. Med.* 1922; 30: 767–777. doi:10.1001/archinte.1922.00110120086003
15. Minkova L, Habich A, Peter J, Kaller CP, Eickhoff SB, Klöppel S. (2017). Gray matter asymmetries in aging and neurodegeneration: A review and meta-analysis. *Hum Brain Mapp.* 2017 Dec;38(12):5890-5904. doi: 10.1002/hbm.23772.
16. Okereke O, Kang JH, Cook NR, et al. (2008). Cognitive decline in two large cohorts of community dwelling older adults. *J Am Geriatr Soc.* 2008 Jun;56(6):1028-36. DOI:10.1111/j.1532-5415.2008.01686.x. Epub 2008 Apr 1.
17. Samoilova J, Matveeva M, Tonkih O, Kudlau D, Oleynik O, Kanev A (2021). A Prospective Study: Highlights of Hippocampal Spectroscopy in Cognitive Impairment in Patients with Type 1 and Type 2 Diabetes. *J Pers Med.* 2021 Feb 19;11(2):148. doi: 10.3390/jpm11020148.
18. Samoilova, Y.G., Rotkank, M.A., Kudlay, D.A. *et al.* (2020). A Prognostic Model of the Development of Cognitive Impairments in Patients with Type 1 Diabetes Mellitus. *Neurosci Behav Physi* **50**, 1136–1139 (2020). <https://doi.org/10.1007/s11055-020-01015-9>
19. Sharafova MU, Sagdova S.C, Yusuf S.D. (2019). Сахарный диабет: современное состояние вопроса (часть 1). *Вестник Авиценны*. 2019; 21(3):502-512.2020).
20. Sosina VB, Zakharov VV, Stokhov IA, Vakhnina NV. (2017). Когнитивные нарушения при сахарном диабете. *Неврология, нейропсихиатрия, психосоматика*. 2017;9(1):90–95. DOI: 10.1017/S1355617713001483
21. Weinger K, Jacobson AM, Musen G, Lyoo IK, Ryan CM, Jimerson DM, Renshaw PF. (2008). *The effects of type 1 diabetes on cerebral white matter.* , 51(3), 417–425. doi:10.1007/s00125-007-0904-9
22. Zhao X, Han Q, Gang X, Wang G. (2018). Altered brain metabolites in patients with diabetes mellitus and related complications - evidence from ¹H MRS study. *Biosci Rep.* 2018 Sep 7;38(5):BSR20180660. doi: 10.1042/BSR20180660.

Figures

Figure 1

Correlation matrix for checking the normality of parameters of proton spectroscopy of the brain

Figure 2

Significance of MRS parameters Note: * - statistical significance ($p \leq 0,05$)

Image not available with this version

Figure 3

demonstrates that the least informative trait is Cr on the left, with the most informative being NAA / Cho on the left and Cr2 on the right. Thus, the asymmetry of the hippocampal region on the right is associated with a change in metabolism depicted as the change in the NAA / Cho ratio.