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Results Of The Study Of Brain Metabolism In Patients With Multiple Sclerosis According To ^{1}H-MRS Data

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Abstract:
^{1}H-MRS may be applied to measure the relative concentrations of many chemical compounds in the area of interest, such as N-acetylaspartate (NAA), choline (Cho), creatine (Cr), myoinositol (ml), glutamate (Glu) and glutamine (Gln), macromolecules and lipids. (lip), lactate (Lac).
The purpose of our study was to investigate regional features of biochemical processes occurring in the structurally unchanged substance of the brain in the realapsing-remitting and secondary progressive forms of multiple sclerosis, according to ^{1}H-MRS data.
The study looked at 58 MS patients aged 18 to 58 (of whom 29 were women), disease severity on the EDSS scale ranged from 1 to 6.5 points. The study also involved a group of 20 healthy volunteers of the same gender and age (14 of them were women).
All patients underwent multivoxel ^{1}H-MRS of the supraventricular white matter of the semioval centers and the medial cortex of the large hemispheres using the volume-selection PRESS (Point-RESolved Spectroscopy) method at TE=144ms and TE=53ms. The following ratios were evaluated: for TE=144ms IP - NAA/Cr, Cho/Cr, NAA / Cho, lac / Cr; for TE=56ms IP - NAA/Cr, Cho/Cr, NAA/Cho, mI/Cr, glx/Cr, lip/Cr.
According to the results of our study, we can conclude that metabolism changes from the norm within the brain of MS patients are of diffuse nature, according to the ^{1}H-MRS data, they are represented on the structural tomograms in the white matter ($p<0.05$) and in the gray matter of the medial cortex of the large brain hemispheres ($p<0, 05$), and are observed in the early stage of the disease in the group of patients with relapsing-remitting MS with a disease duration of up to four years ($p<0.05$). The nature of metabolism changes in different types of multiple sclerosis is not the same and has a specific pattern in the course of the secondary-progressive type, widespread, including in the form of a decrease in the NAA/Cr ratio in the gray matter of the medial cortex ($p<0.05$), which can become an additional criterion to confirm the progressive course of the disease.

Key words: *Multiple sclerosis, remitting form, secondary-progressive form, white and gray matters, atrophy, proton magnetic resonance spectroscopy, metabolite, axons and neurons, resonance peaks, McDonald's criteria, contrast agent, echo time TE, voxels, N-acetylaspartate, choline, creatine, myoinositol, glutamate and glutamine, macromolecules and lipids, lactate.*

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I. Introduction

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive method of magnetic resonance study that allows to define biochemical changes in the tissues *in vivo* in various diseases. In the diagnosis of MS, ^{1}H-MRS makes it possible to trace the course of the disease, predict the development of the disease and evaluate the effectiveness of therapy. In addition, metabolic disorders occur, usually, before structural changes, which allows to reveal pathological changes in the tissue before the formation of foci [19].

^{1}H-MRS may be applied to measure the relative concentrations of many chemical compounds in the area of interest, such as N-acetylaspartate (NAA), choline (Cho), creatine (Cr), myoinositol (ml), glutamate (Glu) and glutamine (Gln), macromolecules and lipids. (lip), lactate (Lac). The metabolite peak position, which is determined by the excitation frequency of protons in matter, depends on the strength of the magnetic field. With a view to standardizing, the pars per million (ppm) scale is used, which corresponds to the peak frequencies of matter at 1.5 Tesla [15].

According to accumulated data from existing studies, biochemical changes in a normal-appearing white matter of the brain are often observed in MS patients. Using the capabilities of large spatial coverage of multivoxel ^{1}H MRS, we managed to demonstrate the diffuse nature of metabolic changes in the brain tissues in MS patients [19, 12, 17].

Data from ^{1}H-MRS studies show a decrease in NAA levels in the apparently unchanged white matter, which indicates a dysfunction, loss of axons and/or neurons. The decrease in the concentration of NAA on ^{1}H MRS was confirmed by axonal loss in histopathological studies [6]. Increased levels of Cho and lipid resonance peaks were also found in those areas of the unchanged white matter where the visible structural foci were formed later on MRI [29].

A ^{1}H-MRS study in patients with PPMS compared with data from studies of patients with RRMS and control groups showed increased concentrations of Cr both in visualized foci and in the normal-appearing white matter [28]. The NAA values in the structurally unchanged white matter in the MS patient groups were lower than in the control group. Even compared to the results of the RMS group, the significant increase of Cr in the foci during PPMS is considered by the authors of the study to be a manifestation of marked gliosis changes in the course of the progressive type.

Many works have been written on these subjects. Studies of brain metabolism with MS using the ^{1}H-MRS method made a significant contribution to the study of the problem. Most of these studies include only local metabolic disorders, mostly in foci of demyelination [21, 24], do not take into account the ongoing changes in the structurally unchanged white matter and gray matter, or assess these changes in isolation from the types of course.

To assess diffuse changes in metabolism with MS, the method of assessing the averaged data of the entire brain is used [11, 9, 14], which does not allow taking into account local changes and regional characteristics of metabolism. This reinforces the need for developing a new methodology for further study of brain metabolism with MS and determining the metabolite profiles for different types of MS. Based on the above, the purpose of our study was to investigate regional features of biochemical processes occurring in the structurally unchanged brain substance with remitting and secondary progressive types of multiple sclerosis, according to ^{1}H-MRS data.

II. Materials and research methods.

The study looked at 58 MS patients aged 18 to 58 (of whom 29 were women), disease severity on the EDSS scale ranged from 1 to 6.5 points. The study also involved a group of 20 healthy volunteers of the same gender and age (14 of them were women).

The course of MS was defined as relapsing-remitting type in 28 patients (12 men and 16 women; aged 21 to 46, mean age – 32years; disease duration was 4 years ± 3 years 4 months; EDSS score – from 1 to 4 points, mean EDSS = 2.27) and relapsing-progressive in 30 patients (17 men and 13 women; aged 22 to 58, mean age - 44 years; disease duration was 13 years 6 months ± 8 years 6 months; EDSS score varied from 5 to 6.5 points, mean EDSS = 5.89).

Diagnosis was made according to Macdonald criteria [20] based on preliminary clinical examination and MRI data. During the collection of anamnesis and physical examination of the patients, no significant pathology of internal organs and other diseases, except for MS, were detected.

Multivoxel ^{1}H-MRS of supraventricular white matter and medial cortex of frontal and parietal lobes was performed before contrast injection during structural scanning using the PRESS volume-selection method (Point-RESolved Spectroscopy), echo time - TE = 57 ms as the minimum possible for PRESS multivoxel spectroscopy with a wide range of metabolites and 144 ms lactate visualization for ization. The remaining parameters of the spectroscopic sequences were identical: TR = 2000ms, voxel size 10*10*15mm, location in the supraventricular parts of the frontal and parietal lobes of the brain. Spectroscopic splitting was performed in parallel with transversal splitting, with the lower edge of splitting was touching the apical walls of the lateral ventricles of the brain. The spectroscopic study environment was 8x9 voxels, and it included the white and gray matter of the brain. 10 bands of saturation were used to suppress signal of the external environment, the study was conducted with second-order automatic Pencil Beam (PB-auto) and water suppression exitation (window=140Hz; second pulse angle=300). The technical characteristics of the MR spectroscopy sequences are given in Table 1 .

The software package SpectroView was used to view and evaluate the obtained spectroscopic data.

Table 1
Technical characteristics of the MR spectroscopy pulse sequences

Pulse sequence	TE, ms	TR, ms	FOV, mm		VOI, mm	
2D_PRESS_sh	53	2000	AP	190-220	AP	110
			RL	160-190	RL	100
			FH	15	FH	15

2D_PRESS_144	144	2000	AP	190-220	AP	110
			RL	160-190	RL	100
			FH	15	FH	15

In the data obtained at each TE, indicators of the main metabolites were important: N-acetylaspartate (NAA, the peak combines signals from N-acetylaspartate, and about 7% from N-acetylpartylglutamate), choline (Cho), creatine (Cr). Other metabolites were evaluated at a short (56 ms) TE time: myo-inositol (mI), total glutamine-glutamate complex (glx), lipids (lip). The position of the metabolite peak on the pars per million (ppm) scale is independent of the magnetic field induction.

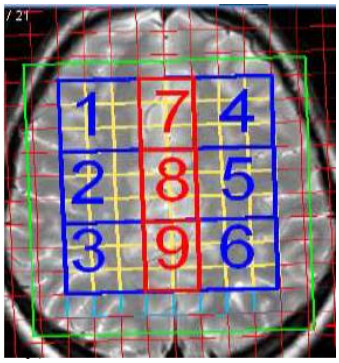
The following ratios were evaluated: for TE=144ms IP - NAA/Cr, Cho/Cr, NAA / Cho, lac / Cr; for TE=56ms IP - NAA/Cr, Cho/Cr, NAA/Cho, mI/Cr, glx/Cr, lip/Cr.

The selected regions of interest are shown in Figure 1. This principle of anatomical division has several advantages simultaneously:

1) makes it possible to compare the results of spectroscopic studies by linking them to the regions of interest, which, in turn, allows us to avoid the influence of the heterogeneity of the distribution of metabolites in the brain when assessing metabolic changes in MS;
2) allows to distinguish the gray matter of the medial cortex of the cerebral hemispheres and the supraventricular white matter;
3) allows to choose the same regions of interest regardless of the size of the study object;
4) retains the advantages of voxel-wise analysis, taking into account the lateralization of changes.

In order to achieve uniformity of the results in each region of interest, voxels falling on the tissue boundary (according to T2-WI) were not taken into consideration.

Fig. 1. Grouping of voxels in the supraventricular spaces of the brain according to 9 regions of interest: blue - white matter regions, red - voxels containing the medial cortex of the large hemispheres



It was decided to use a voxel of minimum size (10x10x15mm) with sufficient data quality (water peak width at half height after shimming <25Hz, SNR(NAA)>300 at more than 60% positions). The rather small voxel size made it possible to neglect data from the transitional anatomical zones (voxels containing less than 80% of the target substance) while retaining sufficient data from the target substance for statistical processing.

In addition, anatomical location of the voxel was taken into account: the study region was divided into 9 regions of interest, 6 of which included white matter, three regions in each hemisphere, and 3 regions included the medial cortex of the supraventricular space of the cerebral hemispheres (Fig. 1). Thus, a comparative assessment of changes in the metabolite ratios was performed separately for each region of interest.

III. Literature review.

When evaluating metabolic changes in pathological processes, it is important to take into account physiological processes occurring in a healthy brain, in particular, natural aging processes. For example, the early effects of normal aging have been shown by comparing data from a group of 16 volunteers aged 21 to 39 years with data from a group of volunteers aged 40 to 61 years: in the older age group, there were detected a significant decrease in the %NAA ratio, as well as a decrease in the NAA/Cho and NAA/Cr ratio, and a significant increase in the %Cho and %Cr ratio in the semioval and temporal regions compared to the younger age group [3]. In addition, significant reductions in %NAA and %Cho were observed in hippocampal and cortical areas. According to the authors, these data indicate that the natural aging of the brain is accompanied by a decline in neuronal function, membrane degradation, and/or an increase in the number of glial cells.

The absolute concentration of the main metabolites (NAA, Cho, Cr) in the frontal lobes of volunteers aged 20 to 70 years (10 people in each age decade) varied as follows: NAA decreased with age ($r = -0.42$, $P = 0.003$) by 12% between the 3rd and the 7th decade [7], while the concentrations of Cr and Cho did not change

significantly with age. The decrease in the absolute concentration of NAA with age likely reflects a decrease in neuronal volume.

An assessment of the metabolite concentrations in the supraventricular white matter spaces of the brain in 57 healthy volunteers aged 13 to 72 years (25 women, 32 men) made by Raininko and co-authors revealed a slight increase in mI and a slight decrease in NAA with increasing age. The change in glutamine/glutamate (Glx) group concentration had a U-age relationship, showing the highest concentrations in the youngest and the oldest volunteers. No significant dependence of Cho and Cr concentrations on age was detected, and gender differences were also not observed. The data show not only changes in the concentration of brain metabolites with age, but also confirm the non-linearity of changes for a group of neurotransmitters [22].

In chronic foci of MS, it is generally accepted that the decreased NAA levels indicate loss of neurons and/or axons, which is true for foci that have a hypointense signal on T1-weighted images (the so-called "black holes"). Decreased NAA concentrations in chronic foci in patients with relapsing-remitting MS (RRMS), secondary-progressive MS (SPMS), and primary-progressive MS (PPMS) compared to the control groups were accompanied by an inverse relationship between focal NAA levels and disease severity. An increase in Cr and Cho concentrations in foci indicates ongoing gliosis and, possibly, an active process of remyelination in isointense T1 foci [12, 21].

Active foci diagnosed with MR contrast enhancement have a decrease in NAA concentration, an increase in Cho concentration, and the presence of lipids in the spectrum is also possible [24]. Histopathological examination of biopsies of brain tissues confirms a decrease in NAA concentration in active foci during MS attacks, presumably as a result of axonal loss or possibly reversible disturbance of their function.

When performing MRI (with MR-contrast enhancement) and in MRS dynamics, temporal change in the NAA level in the acute focus was detected, which would be recovered after a while. In addition, it was noted that the minimum values of the NAA level were found at the moment of reaching the maximum volume of foci. It is also possible to increase the intensity of resonance peaks of lactate (Lac), myo-inositol (MI) and in some cases the presence of lipids [16, 26].

Foci of spinal cord demyelination are characterized by a decrease in NAA concentration, an increase in Cr concentration [5] and a decrease in Cho level [10].

Also, in a 3D multivoxel ^{1}H-MRS study of the normal-appearing white matter of the brain of MS patients, an increase in the concentration of Cho by 32% and Cr by 22% was noted [13]. At the same time, the concentration of NAA decreased by 9% compared to the control. It is important to note that elevated Cho levels in MS patients compared to the control groups had a specificity of 100% and a sensitivity of over 90%.

During the observation of 24 patients with PPMS, it was found that without structural changes in the white matter on traditional MRI, the NAA/Cr ratio in the brain of MS patients is significantly lower than in the control group, which may indicate axonal loss, even in the absence of focal changes, and be a factor associated with the rapid progression of this type of course.

In a multicenter study [19], 40 patients with PPMS were examined using the ^{1}H-MRS method, and a significant decrease in the NAA/Cr ratio was detected in the structurally unchanged white matter of the brain of MS patients compared to healthy volunteers.

In addition, changes in the concentration of other metabolites were also observed. For example, a ^{1}H-MRS study of normal-appearing white matter in the brains of patients with clinically proven multiple sclerosis revealed an increase in glx (peak sum of glutamine and glutamate) compared to the group of healthy volunteers [31]. At the same time, a decrease in glx of apparently unchanged white matter was observed during a two-year observation of patients with SPMS [18].

There are data on ^{1}H-MRS changes in white matter metabolism in patients with clinically isolated syndrome (CIS) who do not have structural MRI changes. For example, 14 of 42 patients with a single episode of optic neuritis had the decreased NAA levels, increased Cho levels, and a lipid peak.

IV. Study results.

All patients underwent multivoxel ^{1}H-MRS of the supraventricular white matter of the semioval centers and the medial cortex of the large hemispheres using the volume-selection PRESS (Point-RESolved Spectroscopy) method at TE=144ms. The following ratios were evaluated in the selected regions: NAA/Cr, Cho/Cr, Lac/Cr. These indicators were compared separately for the apparently unchanged brain matter, focal changes, and the perifocal regions.

The concentration of N-acetylaspartate in the apparently unchanged brain matter, as the overall mean value of the NAA/Cr ratio (TE = 144 ms), in the group of patients with relapsing-remitting MS type (RRMS) is 9% lower (Mann-Whitney test, $p<0.05$) than in the group of healthy volunteers. In the secondary progressive type of MS (SPMS) group, compared to the control group, this indicator is 17% lower (Mann-Whitney test, $p<0.05$).

The mean values of choline concentration in the apparently unchanged brain matter of MS patients in the form of Cho/Cr (TE=144ms) are insignificantly changed when comparing the values of patient groups (RRMS and SPMS) with the healthy control groups.

The concentration of lactate in the apparently unchanged matter of the brain in MS patients in the form of average values of the lac/Cr ratio (TE=144 ms) varies slightly in all studied regions of the MS patient groups. The average value of the lac/Cr ratio in the patient groups does not exceed the value of the healthy control group. This is indirect evidence of the absence of active foci of demyelination in the voxels selected to evaluate the apparently unchanged brain matter.

21 patients from the multiple sclerosis group underwent multivoxel ^{1}H-MRS of the supraventricular white matter of the semioval centers and the medial cortex of the large hemispheres using the volume-selection PRESS (Point-RESolved Spectroscopy) method at TE=53ms. These indicators were compared separately for the apparently unchanged brain matter, focal changes, and perifocal regions.

The concentration of N-acetylaspartate in the apparently unchanged brain matter, in the form of the overall mean value of the NAA/Cr ratio (TE = 53 ms) in all studied regions, in the group of patients with relapsing type of MS (RRMS) is 6% lower than in the group of healthy volunteers. In the secondary progressive type MS (SPMS) group, this indicator is 4% lower compared to the control group.

The concentration of choline in the apparently unchanged brain matter in RMS patients in the form of mean values of the Cho/Cr ratio (TE=53ms) is slightly changed compared to the mean values of healthy control group. At the same time, the difference in overall mean values between SPMS groups and healthy volunteers group was 9%.

The mI/Cr ratio (TE=53ms) in the apparently unchanged brain matter in the MS patient groups was 50% lower in the RMS group and 53% lower in the SPMS group compared to the healthy control group.

The concentration of glutamine-glutamate groups in the apparently unchanged brain matter of the MS patient groups, in the form of the mean value of the glx/Cr ratio (TE = 53ms), was 15% lower in the RMS patient group and 23% lower in the SPMS group compared to the healthy control group.

The lip/Cr ratio (TE=53ms) in the apparently unchanged brain matter of the groups of MS patients in none of the studied groups exceeded the mean values of the healthy control group by more than two times, indicating the absence of a significant change in lipid metabolism.

The mean value of NAA/Cr brain correlates negatively with disease duration (Spearman's correlation coefficient=-0.63, $p<0.05$) when comparing TE=144ms sequence data. A significant negative dependence on TE=53ms (Spearman's correlation coefficient=-0.44, $p<0.05$) was detected only in the case of patients with disease duration up to 20 years. In addition, no changes in other metabolic ratios ($p>0.05$) were detected.

The severity of the patients was assessed by the scores of neurological disorders according to the Expanded Disability Status Scale (EDSS). When assessing the two groups of patients together, the mean value of cerebral NAA/Cr correlates negatively with EDSS scores (Spearman's correlation coefficient = -0.69 (TE = 144) and = -0.49 (TE = 53ms), $p<0.05$).

Focal changes were assessed by viewing T2, FLAIR, and T1 images. By comparing the geometry of the slices, the voxels of the spectroscopic sequences, with the regions of changed signal corresponding to foci of demyelination filled up by more than 20%, were marked as “focal” ones. The voxels containing demyelinating foci or less than 20% of the surrounding volume were marked as “perifocal”. Comparison of metabolic changes in the structurally changed regions was made according to the location of 9 selected regions.

In the perifocal regions, the metabolic parameters of NAA/Cr are significantly lower (Wilcoxon test, $p<0.05$), and Cho/Cr are significantly higher (Wilcoxon test, $p<0.05$) according to the MRI data of the corresponding parameters in the structurally unchanged regions of the same patients, however, the differences in all regions are not significant, which can be attributed to the heterogeneity of metabolic changes. It is important to note that the differences when comparing the mean values of metabolites are not significant (Wilcoxon test, $p>0.05$), which shows the advantages of the regional approach. An example of metabolic changes in the perifocal region is shown in Figure 2.

Other metabolic parameters (Lac/Cr, mI/Cr, glx/Cr, Lip/Cr) in the perifocal and normal-appearing regions do not differ significantly.

The NAA/Cr characteristic does not show significant differences between the metabolism of foci and normal-appearing substances of the same patients (Wilcoxon test, $p>0.05$). However, we have a significant increase in the Cho/Cr ratio (Wilcoxon test $p<0.05$) both at TE=144ms and TE=53ms. In addition, a significant increase in the Lip/Cr ratio was detected (Wilcoxon test, $p<0.05$).

No significant changes in the values of other metabolites (Lac/Cr, mI/Cr, glx/Cr) were found in the voxels containing foci of demyelination, compared to those with normal appearance.

The multivoxel ^{1}H-MRS of supraventricular white matter and gray matter of the medial cortex of the large hemispheres was performed at TE=144ms and TE=53ms, with identical other values. The voxels corresponding to structural images of the apparently unchanged brain matter were taken into account. The TE

144 ms sequences in the selected regions were evaluated for the following indicators: NAA/Cr, Cho/Cr, Lac/Cr. With the sequences at TE=53 ms, the following indicators were evaluated: NAA/Cr, Cho/Cr, mI/Cr, glx/Cr, Lip/Cr. An example of obtained metabolic maps is shown in Figure 3.

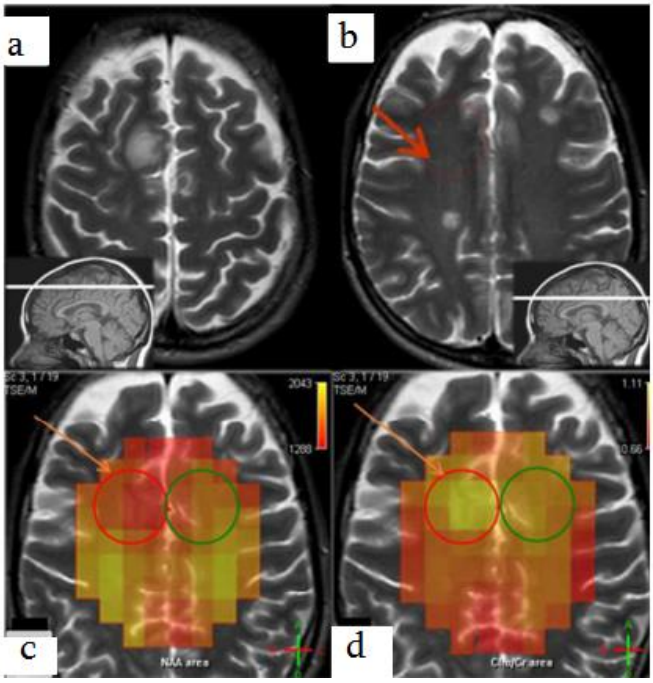


Fig. 2. Metabolism changes in the perifocal region: a) large juxtacortical focus of demyelination is visualized on T2 WI; b) there are no noticeable changes in the white matter below it; c, d) metabolic maps clearly show a decrease in NAA (c) and an increase in Cho/Cr (d)

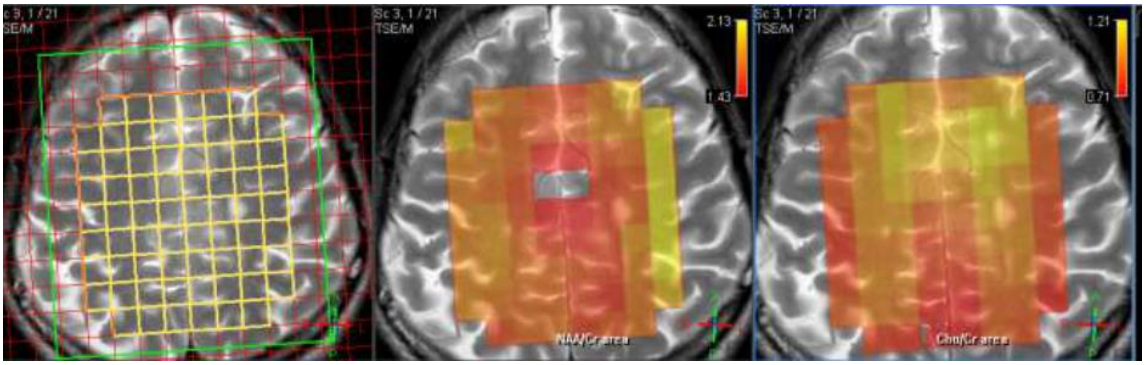


Fig. 3. RMS patient. An example of NAA/Cr and Cho/Cr metabolic maps of brain supraventricular spaces in RMS. There is an uneven decrease in NAA/Cr and an increase in Cho/Cr in the white matter of the frontal lobes.

In patients with RRMS or TE=144ms in MRS, a significant decrease in the NAA/Cr ratio was detected in comparison with the healthy control group in the regions 2-7 (according to the Mann-Whitney criterion, $p<0.05$). This suggests that NAA levels are reduced not only in the apparently unchanged white matter of the brain of RMS patients, but also in the gray matter of the medial cortex of the middle third of the frontal lobes. No statistically significant differences were detected in the Cho/Cr and lac/Cr ratios in the RRMS group compared to the healthy volunteers group. The absence of any significant changes in these indicators was expected for the apparently unchanged brain matter.

In patients with RRMS at TE=53ms with MRS, a significant decrease in the NAA/Cr ratio was detected in the regions 2, 5, 7, 9 (according to the Mann-Whitney criterion, $p<0.05$). There was no statistically significant difference in the Cho/Cr ratio in the RRMS group compared to the healthy volunteers group. The absence of any significant changes in the lip/Cr indicator was expected for the apparently unchanged brain matter. There were also no significant changes in the mI/Cr ratio.

A significant change in the glx/Cr ratio in the white matter of the left parietal lobe was detected, which may be indicative of the initial changes in neurotransmitter balance in the apparently unchanged white matter of the brain in patients with RRMS.

Consider the results of metabolic studies of patients with established diagnosis of multiple sclerosis, whose disease course was defined as the SPMS type.

The multivoxel ^{1}H-MRS of the supraventricular white matter and the medial cortex of the large hemispheres was performed at TE=144ms and TE=53ms, with is identical at other indicators.

In SPMS patients at TE=144ms in MRS, a significant decrease in the NAA/Cr ratio was detected compared to the healthy control groups in all regions (according to the Mann-Whitney criterion, $p<0.05$). This allows us to confirm that the reduction of NAA levels occurs not only in the apparently unchanged white matter of the brain of SPMS patients, but also in the gray matter of the medial cortex. An example of these changes is shown in Figure 4. No statistically significant differences were detected in the Cho/Cr and lac/Cr ratios in the SPMS group compared to the healthy volunteers group. The absence of any significant changes in the lac/Cr ratios was expected for the apparently unchanged brain matter.

In patients with SPMS at TE=53ms with MRS, a decrease in the NAA/Cr ratio was detected in the regions 1, 2 (according to the Mann-Whitney criterion, $p<0.05$). There was no statistically significant difference in the Cho/Cr and lip/Cr ratios in the SPMS group compared to the healthy volunteers group. The absence of any significant changes was expected for the apparently unchanged brain matter. There were also no significant changes in the mI/Cr and glx/Cr ratios.

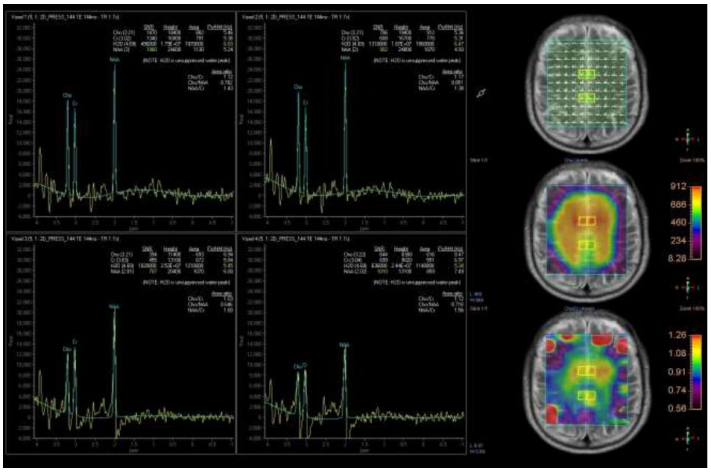


Fig. 4. SPMS patient. In the gray matter of the medial cortex, there is a decrease in the intensity of the NAA peak with the elevation of the Cho peak, which exceeds the Cr peak in the gray matter of the frontal lobes (diagrams above), while in the gray matter of the parietal lobes, the intensity of the peaks is Cho < Cr

Thus, the use of the ^{1}H-MRS method with this methodology of data collection and analysis made it possible to obtain indicators of brain metabolism in demyelination foci, perifocal matter, and white matter, which have no structural changes on MRI, and showed a decrease in NAA/Cr and an increase in Cho/Cr in the demyelination and perifocal regions in patients with multiple sclerosis. Moreover, metabolic changes were detected in the gray matter of the medial cortex and the apparently unchanged white matter of the cerebral hemispheres in the form of decreased NAA/Cr in the RRMS and SPMS groups. In addition, the correlation between the severity and duration of the disease was confirmed in the form of a decrease in the NAA/Cr ratio with an increase in the duration of the disease and an increase in neurological deficit.

V. Discussion of results.

The study presents metabolic characteristics of the brain of healthy volunteers, as well as a spectrum of changes in brain metabolic parameters in different types of MS.

A study of brain tissue metabolism with proton MRS in healthy volunteers revealed a significant heterogeneity of the metabolic index of neuronal function (NAA/Cr ratio) between the gray matter and the white matter of the medial cortex of the frontal and parietal lobes, which is consistent with the literature data [4]. In addition, significant regional heterogeneity of the NAA/Cr and Cho/Cr ratios of the white matter in the frontal and parietal lobes was revealed, indicating the need to compare these data in the anatomically similar regions of the brain matter.

The inclusion of regional heterogeneity of this indicator allows to increase the accuracy of the assessment of minimal changes in those regions of the brain tissues that do not have changes on the structural MRI image. This observation may explain the absence of changes in metabolic parameters in the normal-appearing brain matter of MS patients in a number of studies [19, 30, 23], as well as the absence of dependence between metabolic and clinical parameters [8, 2]. In addition, this may explain the lack of metabolic differences when comparing relapsing-remitting and progressive MS [1].

When studying metabolism of brain tissues in patients with MS using the proton MRS method, we divided the obtained data into two categories as follows: 1) the results of metabolic measurements during the structural scan in the regions with changes and in the regions adjacent to the places with structural changes; 2) the results of metabolic measurements in tissues without structural changes.

In foci with structural changes and surrounding regions, a significant increase in choline concentration was detected, indicating the presence of damaged cell membranes, as well as an increase in lactate, which has pointed to the accumulation of anaerobic glycolysis products. The obtained data do not contradict the results of studies of metabolism of inactive foci in multiple sclerosis [12, 25, 21]. These changes indirectly indicate a slow ongoing inflammation in demyelinating foci and perifocal regions.

In addition, a significant inverse correlation was obtained between the MS disease duration and the indicator of neuronal functional integrity in the form of the NAA/Cr ratio for brains without changes in structural MRI. These results indicate a continuous loss of nerve tissue during the course of the disease.

An indicator of the functional integrity of neurons and their processes, in the form of the NAA/Cr ratio, negatively interacts with the severity of the disease. In this case, there is no doubt in this relationship, because the increase in neurological deficit is directly related to the decrease in the functional parameters of the nervous tissue. However, the interaction of these types of factors is characteristic only for the group with relapsing type of MS, and in the SPMS group, there was no connection between these indicators, which is also consistent with the results of studies of groups with progressive types of MS.

By linking axon metabolism to oligodendrocyte metabolism, NAA promotes myelination. In addition, changes in NAA concentration lead to changes in the histone H3 methylation levels, which regulate cellular energy, metabolism, and growth [27]. It is currently unclear whether the mitochondrial abnormalities reported in MS are primary or secondary to the inflammatory disease process.

In addition to the features of perifocal changes in metabolism, using the regional approach to data evaluation, the features of local changes were also noted. In RRMS patients, compared to the control group, a significant decrease was detected in the NAA/Cr ratio levels (according to the Mann-Whitney test, $p<0.05$) was detected in areas 2-7 (TE=144ms) and 2, 5, 7, 9 (TE=53ms). This allows us to prove that NAA levels decrease not only in the apparently unchanged white matter of the brain of patients with RRMS, but also in the gray matter of the medial cortex of the middle third of the frontal lobes (area 7) - in the anterior halves of the cingulate gyrus.

In addition, using a regional approach, a total nature of changes in SPMS patients at ^{1}H-MPC TE=144ms was confirmed. A significant decrease in NAA/Cr level (according to the Mann-Whitney test, $p<0.05$) was detected in all studied regions compared to this indicator of the control group. This allows us to confirm that NAA levels are reduced not only in the apparently unchanged white matter of the brain of SPMS patients, but also in the gray matter of the medial cortex of the middle third of the frontal lobes.

When comparing the results of the two studied groups of MS using the regional approach of ^{1}H-MRS data evaluation, significant differences were detected. For example, in the progressive course type group, signs of neuronal dysfunction were detected in all studied parts of the medial cortex of the cerebral hemispheres, which can be a helpful differential-diagnostic criterion in determining the type of MS course.

In addition, the NAA/Cr ratio was significantly reduced in absolutely all regions of interest and in the apparently unchanged white matter in SPMS patients, which was not typical of the RRMS group.

In addition, in the medial cortex of the frontal lobes, in the region of the anterior half of the cingulate gyrus, and in the surrounding regions of the white matter, which do not have structural changes, a local increase in choline concentration was detected in some cases of SPMS. However, no statistical deviation from the norm was found in the study group, and there was no any clinical sign to identify such patients.

No difference was found in the relative concentrations of mI/Cr and glx/Cr, which is consistent with the results of studies in the remission period.

The obtained results show that in the study of patients with MS in the absence of clinical exacerbations, the sequence of pulses using the regional method of evaluation of the results of “long” TE = 144 ms made it possible to detect a greater number of cases of deviations from the norm of metabolism. This allows us to recommend the TE=144 sequence for longer follow-up of patients. However, it should be noted that when exacerbations are observed, an additional PI should be performed at TE = 53ms or less to obtain mI and glx levels in addition to NAA, Cr, and Cho levels.

Diffuse reduction in functional capacity of neurons and their processes on structural MR tomograms in the unchanged white matter in MS patients leads to a delay in the normal transmission of the pulse, therefore, a violation of information transmission, which is the main task of nerve cells. In addition to obvious neurological deficits, mostly due to focal lesions, an increasing overall cognitive deficit is inevitable, which inevitably affects the quality of life of the patients. Correct early diagnosis, as well as regular monitoring of the development of the disease in patients, will allow us to slow down the development of the symptoms of the disease. In this sense, the development and implementation of new diagnostic methods is a priority for multiple sclerosis clinical researchers. The sophisticated methods for detecting, monitoring and treating demyelinating diseases will have a positive effect on the lives of MS patients, preserving their quality of life and prolonging their ability to work.

Thus, we can conclude that metabolism changes from the norm within the brain of MS patients are of diffuse nature, according to the ^{1}H-MRS data, are represented on the structural tomograms in the apparently unchanged white matter ($p<0.05$) and in the gray matter of the medial cortex of the large cerebral hemispheres ($p<0.05$), are observed already in the early stages of the disease, and the duration of the disease is up to four years ($p<0.05$) in the group of patients with relapsing-remitting MS with a disease duration of up to four years. The nature of metabolism changes in different types of multiple sclerosis is not the same and has a specific pattern in the course of the secondary-progressive type, widespread, including in the form of a decrease in the NAA/Cr ratio in the gray matter of the medial cortex ($p<0.05$), which can become an additional criterion to confirm the progressive course of the disease.

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