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(RESEARCH ARTICLE)

Glutamine in the cerebellum by magnetic resonance for the diagnosis of Autism

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Abstract

Background: Brain metabolites are involved in processes of excitation and cellular transmission and can provide the sites and indices of synaptic activity and plasticity in addition to supporting inferences about the ongoing pathophysiology. Among them, changes in glutamate levels are involved in the regulation of neurotransmitter activity and are responsible for several behavioral and electrophysiological phenotypes in autism including deficits in cognitive and motor behavior and social reward.

Objective: To study the changes in biochemical metabolites in the cerebellum by magnetic resonance spectroscopy (MRS) for diagnosis of autism.

Methods: One hundred patients with ASD, we randomly selected and submitted to imaging exams in their CNS by magnetic resonance with nuclear proton spectroscopy. All selected patients were examined by the same doctor and their imaging exams were performed by the same neuro-radiology professional.

Conclusions: Magnetic resonance imaging with cerebellar spectroscopy is a new tool for the diagnosis and management of autism and can serve all physicians, even if they are not specialists, as a guide for this difficult to manage condition. It can serve both as a means of diagnosis and as a clinical follow-up for the treatment of ASD.

Keywords: Autism spectrum disorder; Magnetic resonance spectroscopy; Cerebellum; Diagnosis; Glutamine

1. Introduction

Autism is a developmental disorder that is characterized by impaired social interaction, impaired verbal and non-verbal communication, limited activities and interests, and stereotyped behavior patterns. Manifestations vary in severity (mild to severe) and are called autism spectrum disorders [1-3].

Evidence for cortical dysfunction in autism has been shown by a proton magnetic resonance spectroscopic imaging study [4,5].

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Investigating etiologies for ASD, it was observed that abnormalities in neurochemistry alter brain function. These changes affect the function of specific areas, but also cause connectivity dysfunction with other areas, impacting social and cognitive functions [6,7].

Magnetic resonance imaging (MRI) is the best way to study brain anatomy and metabolism, in a non-invasive way with a low rate of complications. It can be used for brain morphology evaluation and for functional and metabolic evaluation when associated with proton spectroscopy [4,8].

As a result of the difficulties inherent in obtaining absolute confidence concentrations, most in vivo MRI studies with spectroscopy report relative measures in brain studies, assuming the calculated creatinine concentrations as a constant and having their signal as a reference [9,10].

Brain metabolites are involved in processes of excitation and cellular transmission and can provide the sites and indices of synaptic activity and plasticity in addition to supporting inferences about the ongoing pathophysiology. Commonly studied metabolites include:

1.1. N-acetylaspartate (NAA)

NAA, one of the prominent peaks consistently shown in the exam, is present almost exclusively in neurons and is remembered for being a marker of neuronal functional integrity and axonal mitochondrial metabolism. Its concentration decreases in proportion to the cellular damage. Its reduction is an indication of neuronal insult [11,12].

1.2. Creatine (Cr1)

The peak of creatine is composed of substances of the brain metabolism that remain stable in most situations. It is related to the cellular energy system. Its concentration is relatively constant in brain tissue. It serves as an internal reference for the other peaks of the spectral graph [13].

1.3. Choline (Cho)

Participates in the metabolism of cell membranes and reflects your turnover. Its increase reflects an increase in membrane synthesis or an increase in the cell population, as seen in some tumors [14].

1.4. Lactate (Lac)

Brain lactate levels, under normal conditions, are minimal or practically absent and therefore are not identified in the spectrum in normal situations (except in newborns). Its presence denotes that anaerobic mechanisms are present. It can be detected in situations of cerebral ischemia [15].

1.5. Glutamine / Glutamate (Glu/Glu)

Glutamate is an excitatory neurotransmitter involved in mitochondrial metabolism and glutamine participates in the regulation of neurotransmitter activity. The resonance frequencies of these metabolites are awfully close [16].

1.6. Myo-inositol (MI)

It is a product of myelin decomposition and a possible glial cell marker [17].

Magnetic resonance through spectroscopy (1H-MRS) allows non-invasive and simultaneous measurement of several neuro-metabolites and has been used to study the pathophysiology of ASD in a significant number of studies [9,10,17].

2. Material and methods

Between March 2016 and January 2019, we examined 300 patients with ASD, in our Unit of Gastroenterology, Food Allergy and Autism. All selected patients were diagnosed with ASD, based by the CARS score [18]. This scale was designed to diagnose ASD and differentiate it from other pathologies that lead to delayed development. Although there is no “gold standard” among the scales known for the detection of ASD, CARS is often used as part of the diagnostic process. The score on the scale varies up to 60 points and we consider patients with scores above 30 points as the cutoff point for the diagnosis of ASD. In addition to the CARS score above 30, we completed the diagnosis with two writing advices from a neurologist and a psychiatrist, using the DSM V criteria [19].

Careful assessment of the clinic of patients with ASD, when they came to our service, showed that they had, before the development of ASD, symptoms in other systems, involving the digestive, respiratory and skin. Among these patients with ASD, we randomly selected 100, in which we performed imaging exams, where we studied their CNS by magnetic resonance with nuclear proton spectroscopy. All selected patients were examined by the same doctor and their imaging exams were performed by the same neuroradiology professional.

The 1H-MRS studies were performed on a GE 3T (GE Healthcare, Milwaukee,WI) full body scanner with a high resolution 8-channel coil head (Invivo, Orlando, FL). The participant's head was positioned along the cantomeatal line and immobilized by means of a forehead brace. The spectra in 1H-MRS were obtained using the PRESS technique (PRESS; TE135 ms, TR12000 ms, spectral width15000 Hz, 4,096 data points used, voxels of 8**mL** (2x2x2 cm) centered to the left of the cerebellar hemisphere and the frontal lobe in all individualsstudied. This acquisition allowed the quantification of GG, N-acetyl aspartate (NAA), creatine (Cr), phosphocreatine (PCr), glycerophosphate line (GPC), phosphocholine (PCH), and myo-inositol (MI).

3. Results

Evaluation using magnetic resonance imaging (MRI) did not reveal any patient with brain morphological changes. Diffusion MRI assessment did not show acute ischemia, nor did it reveal neoplastic or inflammatory brain lesions in any of the patients. The evaluation of MRI with proton spectroscopy showed that allpatients have high levels of Glu/Glu in the cerebellar hemisphere (100%). Only one patient (1%) showed an increase in Glu/Glu in the frontal hemisphere. It is important to highlight that in all patients with ASD, an increase in CNS Glu/Glulevels was found. The direct measurement of the cerebral metabolite Glu/Glu is a potential strategy to identify individuals with ASD.

4. Discussion

The cerebellum previously considered an associative complex as a motor coordination and execution center, with current knowledge, has also come to be a center involved in the highest cognitive and emotional functions, even in the developing brain. Recent theories of cerebellar function advance with the idea that the cerebellum can act not only in the modeling of motor functions, but also in cognitive behavior [3, 20-23]

Histological and anatomical abnormalities of the cerebellum are the most consistent findings in the brain of patients with ASD. These findings reinforce the concept of prenatal onset with interaction with postnatal neurobiological processes. Cerebellar abnormalities are likely to contribute significantly to several of the clinical features of the autistic spectrum [24, 25].

The discovery of the lymphatic system present in the central nervous system led to a reassessment of the basic assumptions of neuroimmunology and shed new light on the etiology of neuroinflammatory and neurodegenerative diseases, associated with the dysfunction of the immune system, highlighting in this respect the immunological changes caused by food allergy [26 – 28].

Several recent immunological studies have suggested the involvement **of** cerebellum in the pathophysiology of ASD. Self-generated or maternal autoantibodies against neural cells, including cerebellar Purkinje cells, have been detected in the serum of children with ASD. A recent study showed that children with these autoantibodies had a higher degree of aberrant behavior and cognitive changes and their adaptive function decreased compared to children without autoantibodies [29-32].

The discovery of cerebellar atrophy in a group of celiac patients agrees with previous autopsy reports, which showed selective loss of Purkinje cells from the cerebellar cortex of patients with celiac disease and neurological disorders and also shows a functional and clinical correlation in which the vast majority of patients complains of balance disorder. The mechanisms underlying Purkinje cell loss in relation to gluten sensitivity are yet to be fully elucidated [33, 34].

However, current knowledge suggests cellular destruction mediated by an immune response, which may involve transglutaminase autoantibodies. Thus, it is reasonable to assume that the proliferation of glial cells and the decrease in the density of neurons is an important factor in the abnormal growth of the brain and decreased levels of NAA, observed in children with ASD [12, 35].

Elevated prostaglandins may be related to the recent record that immune dysregulation / inflammation is the first etiological factor in autism [36, 37].

The significant increase in PGE2 in autistic patients may be related to glutamate toxicity. PGE2 changes intracellular calcium (Ca2 +) homeostasis, glutamate release and activation of transcription factors. PGE2 by these actions increase the release of Ca2 +, with some extracellular calcium intake. This, in turn, induces the release of glutamate in astrocytes, causing an abnormal neuron-astrocyte interaction. This elevated glutamate is coupled with a decrease in GABA, due to a decrease in glutamic acid decarboxylase. The expression of this protein in the cerebellar cortex is partial in the brain of autistic individuals. Elevated PGE2 would thus be related to elevated glutamate and reduced GABA levels in autistic patients[38].

This may explain the possible correlation of food allergy with ASD, since food allergy is a continuous inflammatory process in the body, which places in the lymphatic circulation, appreciable amounts of CD4 and CD8 T lymphocytes and antibodies of the type IgE, IgA, IgM and IgG, all immuno-active, looking for a "homing" for their immune-allergic response. With the discovery of lymphatics in all systems, including the brain, it can become the centralizing organ of the immune-allergic response, if neurons suffer any type of inflammation, attracting the immune response to them [39, 40].

However, it is important to note that direct measurement of Glu/Glu neurotransmission is possible with high-field 1H - MRS, but this technique measures both vesicular and metabolic glutamate[9, 10]

Excessive glutamatergic activity is also associated with neuronal degeneration indicating that Glu/Glu levels are significantly correlated with NAA in the caudate nucleus and cerebellum in all groups with ASD [41,42].

Higher levels of NAA can be triggered by increased axonal mitochondrial metabolism to maintain axonal conduction. It is not surprising that an increase in Glu/Glu, an excitatory neurotransmitter, is associated with an increase in local neuronal metabolism [35].

Glutamate antagonists are well known to induce positive and negative psychotic symptoms more like schizophrenia than positive symptoms induced by dopamine agonists. It can be assumed that this course of deterioration can be partly explained by the cortical neuronal toxicity secondary to increased glutamate exposure, which in turn can induce a compensatory increase in cortical glutamatergic activity. Excessive glutamatergic activity is also associated with neuronal degeneration. [43, 44]

A significant increase in relation to Glu x creatine in the left cerebellar hemisphere is reported in patients with ADHD compared to the corresponding region in controls and there are differences in other metabolites measured in the left cerebellar hemisphere and there are no differences in proportions of any metabolites in the vermis and hemisphere right, these data are compatible with our findings of spectroscopic changes.

5. Conclusion

The findings of elevation of cerebellar glutamate in autistic patients with food allergy could be related factors, if the cerebellar region create an inflammatory reaction, becoming the "homing" of the lymphocytes from the food allergy process, leading to cell destruction with reduction of Purkinje cells. This factor becomes long-lasting, due to the feedback from the immune system through food allergy.

Magnetic resonance imaging with cerebellar spectroscopy is a new tool for the diagnosis and management of autism and can serve all physicians, even if they are not specialists, as a guide for this difficult to manage condition. It can serve both as a means of diagnosis and as a clinical follow-up for the treatment of ASD.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

All procedures for enrollment and conduction of this research project was reviewed and approved by the Institutional ReviewBoard of the UNIGRANRIO University under number CAAE66813917.0.0000.5283.

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Authors' contributions

All authors contributed equally in preparing all parts of the work and approved the version submitted for revision.

Statement of informed consent

Written informed consent was obtained from each subject.

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