ORIGINAL ARTICLE

CONTENTS

Serotonin levels in children with cognitive impairment in delayed speech development in non-alcoholic fatty liver disease with obesity and Covid-19

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ABSTRACT

Aim: To identify features of changes in serum serotonin and tryptophan levels in children with cognitive impairment (CI) and delayed speech development (DSD) at non-alcoholic fatty liver disease (NAFLD) and COVID-19.

Materials and Methods: The study included 108 children with CI and DSD at NAFLD. The children were divided into 2 groups: group 1 included children with COVID-19 (n=58); group 2 consisted of 50 children without COVID-19.

Results: All the examined children were diagnosed the weight gain and NAFLD (non-alcoholic steatohepatitis), mainly of minimal to moderate activity. The results indicate a decrease of serotonin and tryptophan levels in the examined children with CI and DSD and NAFLD. However, in children of group 1 (after COVID-19), the level of ST was 2.4 times (p-value <0.01) higher than in children of group 2. Tryptophan levels were also 1.4 times higher in children from group 1 (p-value <0.05).

Conclusions: In children with CI and DSD at NAFLD, a decrease in serum levels of ST and Trp was diagnosed, which is more pronounced after COVID-19. A direct relationship between the decrease in serum Trp and serotonin levels has been established, which directly depends on the functional state of the liver and negatively correlates with the severity of insulin resistance.

KEY WORDS: children, cognitive impairment, delayed speech development, non-alcoholic fatty liver disease, obesity, COVID-19, insulin resistance, diagnostics, serotonin, tryptophan

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INTRODUCTION

Metabolic dysfunction-associated fatty liver disease (MAFLD) is a term proposed in 2020 to refer to fatty liver disease associated with systemic metabolic disorders instead of non-alcoholic fatty liver disease (NAFLD) [1].

The World Health Organization (WHO) estimates that non-alcoholic fatty liver disease (NAFLD) affects 25% of the world's population, and approximately 80 million people, both children and adults, in the United States have NAFLD. The prevalence of NAFLD in the paediatric population is estimated to be 13% (9.8% adjusted) with an age-related increase in prevalence from less than 1% in children aged 2 to 4 years to 17% in adolescents. The incidence of NAFLD in children has increased dramatically from 36/100,000 in 2009 to 58.2/100,000 in 2018, in parallel with the worsening childhood obesity epidemic. However, given the low level of adherence to NAFLD screening guidelines, the true prevalence and incidence of NAFLD in children is likely underestimated. In a study using histological assessment of children with chronic hepatitis, more than one-third of obese children had NAFLD. Male children are at higher risk of developing NAFLD than female children, with males predominating among obese adolescents [2].

The metabolic risk factors for MAFLD/NAFLD are type 2 diabetes mellitus and overweight/obesity according to ethnic body mass index (BMI) classifications. Risk factors include increased waist circumference, high blood pressure, plasma triglycerides, plasma high-density lipoprotein cholesterol, prediabetes, insulin resistance model score, and high-sensitivity C-reactive protein in the blood. The combination of liver steatosis with one of these three metabolic risk stratifications leads to the diagnosis of NAFLD [3, 4].

The COVID-19 pandemic has also had a negative impact on the lives of children and young people around the world. Public health measures aimed at reducing the transmission of SARS-CoV-2 in the community have included school closures and stay-at-home orders. The role of social inequality in exacerbating the negative impacts of quarantine on children's health and well-being was already evident after the first wave of

COVID-19. The new findings highlight the impact of the pandemic and socioeconomic impact on childhood obesity and, as a result, NAFLD [5].

COVID-19 has caused numerous stressful situations for many families due to job losses and has negatively impacted already vulnerable communities. The closure of schools has had a particularly negative impact on children living in poverty, for whom school provides access to healthy food, physical activity, medical and social care, and social networks. Stay-at-home orders and restrictions on outdoor recreation have led to an increase in sedentary lifestyles and screen time, particularly affecting children living in densely populated urban areas without access to green spaces. Maintaining healthy behaviours requires a high level of personal commitment, time, and cognitive, psychological and material resources, which vulnerable families struggled to manage before the COVID-19 pandemic. Stress and parental control, mental illness, and disrupted social environments in childhood are associated with weight gain and obesity in children, and the combination of COVID-19-related stressors has led to an increase in obesity prevalence among children as well [5-7].

Thus, the study of cognitive impairment (CI) and delayed speech development (DSD) in children with NAFLD during COVID-19 is still an urgent issue for the medical community.

AIM

The aim to identify features of changes in serum serotonin and tryptophan levels in children with CI and DSD in NAFLD and COVID-19.

MATERIALS AND METHODS

A comprehensive examination and treatment of children conducted on the clinical basis of the Department of Neurology, Neurosurgery and Psychiatry at the Medical Faculty of state higher educational establishment «Uzhhorod National University». The study included 108 childrens with NAFLD and cognitive dysfunction.

Among the examined children there were 60 boys (55.6 %) and 48 girls (44.4 %). The control group consisted of 20 children (12 boys (60.0%) and 8 girls (40.0%)). The average age at children of group 1 was 11.4±3.6 years, and 12.7±4.3 years – at children of group 2.

Children with CI and DSD at NAFLD were divided into two clinical groups: group 1 (n=58) included children with COVID-19, and group 2 included 50 children without COVID-19. All children's in group 1 had a previous confirmed diagnosis of COVID-19 (positive polymerase chain reaction (PCR) result for SARS-CoV-2 RNA (SARS-CoV-2 RdRP gene, SARS-CoV-2 gene E) in anamnesis.

Criteria for exclusion from the study were the presence of autoimmune, viral (hepatitis B, C, D viruses) liver damage, Wilson-Konovalov disease, haemochromatosis, acute traumatic brain injury, type 1 diabetes mellitus, type 2 diabetes mellitus (decompensation stage).

All studies were carried out with the consent of the parents of the children examined (they gave written consent to the relevant diagnostic and treatment measures), and the research methodology was in line with the Helsinki Declaration of 1975 and its revision of 1983, the Convention on Human Rights and Biomedicine developed by the Council of Europe, as well as Ukrainian legislation.

A general clinical and neurological examination was carried out, which included the collection of anamnesis, complaints, standard clinical, laboratory and instrumental examination, examination of the neurological status according to the generally accepted scheme, as well as neuropsychological testing of cognitive functions. The following methods were used for the latter: Montreal Cognitive Assessment (MCA); Mini-Mental State Examination (MMSE); 10-Word Memory Test by Luria A.R. (for the study of auditory-verbal memory). The Spielberger-Hanin method was used to determine the level of anxiety, and autonomic dysfunction was determined by the Wayne Questionnaire. Children underwent electroencephalography (EEG).

All examined children were subjects to research carried out in accordance with the general clinical, anthropometric, instrumental and laboratory methods. In order to verify the diagnosis attention was paid to the nature of complaints, medical history. In anthropometric research, height, weight, waist circumference were measured, and the body mass index (BMI) was calculated [8].

The ultrasound examination of all the children abdominal organs was carried out according to the generally accepted procedure. There were done standard general and biochemical tests based on the blood serum to determine the functional state of the liver (alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBL), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT)), indicators of lipid metabolism (total cholesterol (TT), triglycerides (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), very-low-density lipoproteins (VLDL)), carbohydrate metabolism (glucose, insulin, glycated hemoglobin (HbA1c,%)).

Table 1. Indicators of the liver functional state in the examined children

Indicator	Control group (n=20)	Examined children	
		group 1 (n=58)	group 2 (n=50)
ALT, U/L	22.1±1.4	97.4±3.9**,+	63.1±3.1**
AST, U/L	19.7±1.1	78.0±2.7**,+	53.4±3.4*
TBL, mmol/l	15.2±0.7	34.4±2.1**	30.6±0.9*
ALP, UI/L	163.9±5.4	339.6±6.3**,+	277.0±5.5**
GGT, U/L	17.1±2.6	56.3±2.5**	41.2±2.9*

Note: the difference is statistically reliable between the parameters of the control group and the examined children of groups 1 and 2:* – p<0.05; ** – p<0.01; the difference between the indicators in group 1 and 2 children is statistically reliable: + – p<0.05.

Table 2. Indicators of carbohydrate ьetabolism in the examined children in blood serum

Indicator	Control group (n=20)	Examined children	
		group 1 (n=58)	group 2 (n=50)
Fasting blood glucose, mmol/l	4.5±0.3	5.2±0.2	4.9±0.5
HbA1c, %	4.2±0.4	5.0±0.3*	4.7±0.4
Insulin, U/L	8.8±0.7	33.2±1.7**,+	26.1 ±1.5**
C-peptide, ng/ml	4.0±0.5	17.8±0.8**	13.4±1.1**
HOMA-IR	1.5±0.4	8.6±0.6**,+	6.9±0.4**

Note: the difference is statistically reliable between the parameters of the control group and the examined children of groups 1 and 2:* – p<0.05; ** – p<0.01; the difference between the indicators in group 1 and 2 children is statistically reliable: + – p<0.05.

Table 3. Levels of Serotonin and Tryptophan in the in the examined children in blood serum

Indicator	Control group (n=20)	Examined patients	
		group 1 (n=58)	group 2 (n=50)
ST, mcg/L	344.7±15.6	98.1±5.4**,++	235.4±7.7*
Trp, nmol/ml	53.4±1.1	28.2±0.8**,+	40.7±1.0*

Note: the difference is statistically reliable between the parameters of the control group and the examined children of groups 1 and 2:* – p<0.05; ** – p<0.01; the difference between the indicators in group 1 and 2 children is statistically reliable: + – p<0.05.

Table 4. Correlation of ST and Trp indicators and functional state of the liver, carbohydrate metabolism in the examined children

Indicator	Indicator			
	ST, mcg/L		Trp, nmol/ml	
	group 1 (n=58)	group 2 (n=50)	group 1 (n=58)	group 2 (n=50)
ALT, U/L	r= 0.84; p<0.01	r= 0.72; p<0.05	r= 0.76; p<0.01	r= 0.66; p<0.05
AST, U/L	r= 0.68; p<0.05	_	_	_
Insulin, U/L	r= -0.80; p<0.01	r= -0.62; p<0.05	r= -0.72; p<0.05	r= -0.56; p<0.05
HOMA-IR	r= -0.86; p<0.01	_	r= -0.76; p<0.01	_

NAFLD was diagnosed in accordance with the criteria of the unified clinical protocol and the EASL-EASD-EASO clinical recommendations on the diagnosis and treatment of NAFLD [9]. The degree of liver damage was calculated using surrogate markers of fibrosis by means of online calculators NAFLD fibrosis score (NFS), the Fibrosis 4 calculator (FIB-4) as well as FibroTest and the liver elastograpthy results.

Quantitative determination of tryptophan (Try) levels in blood serum was performed by reversed-phase high-performance liquid chromatography in isocratic elution mode with electrochemical detection. Serum serotonin (ST) levels were determined by high-performance liquid chromatography on an Agoilent 1100 chromatograph using the Agilent Technologies (USA) test system.

The analysis and processing of the results of examining the patients was performed using the computer program STATISTICA 10.0 (StatSoft Inc, USA) using parametric and non-parametric methods of evaluating the received results.

RESULTS

All the examined children with CI and DSD at NAFLD were diagnosed the weight gain, namely overweight and obesity. All the children included in the study were, diagnosed the NAFLD, namely, non-alcoholic steatohepatitis, mainly of minimal to moderate activity. At the same time, more pronounced changes in transaminase levels were found in children of group 1. In children with CI and DSD at NAFLD during COVID-19 infection, a significant increase in the activity of transaminases, as well as TBL and GGT levels, was diagnosted (Table 1).

The determination of carbohydrate metabolism indicates insulin resistance in children with CI and DSD at NAFLD, which is more pronounced in group 1 at COVID-19 (Table 2).

Indicators of the neurohormone serotonin and the amino acid tryptophan in the blood serum of children included in the study were determined (Table 3).

The results indicate a decrease of serotonin and tryptophan levels in the examined children with CI and DSD and NAFLD. However, in children of group 1 (after COVID-19), the level of ST was 2.4 times (p-value <0.01) higher than in children of group 2. Tryptophan levels were also 1.4 times higher in children from group 1 (p-value <0.05).

We analysed the relationship between the level of neurohormone ST and tryptophan in the blood serum and indicators of the functional state of the liver and carbohydrate metabolism in the examined children. It should be noted that there was a strong correlation between ST and tryptophan in both groups, namely, r= 0.88; p<0.01 in children of group 1 and r= 0.76; p<0.01 in children of group 2 with CI and DSD at NAFLD.

Statistical analysis revealed a correlation between the progression of liver damage and a decrease in the levels of ST and Trp. The decrease in serum levels of ST and Trp correlated significantly with insulin and HOMA-IR levels at children with Ci and DSD at NAFLD (table 4).

Thus, the studies indicate changes in neurohormone ST and amino acid Trp levels in the blood of children with CI and DSD at NAFLD, which are more pronounced after COVID-19.

DISCUSSION

Clinical studies have defined COVID-19 as a multisystemic infection that can affect various systems, such as cardiovascular, hematologic, renal, gastrointestinal, neurologic and hepatobiliary, and endocrinologic [10]. Children and/or adolescents tend to generally have a symptomatic but mild COVID-19 course with few requiring intensive care treatment and a very low rate of mortality [11-13]. However, cognitive impairment is often reported after SARS-CoV-2 infection [14].

New evidence suggests that SARS-CoV-2 can infect the brain, leading to CI. It can spread to other parts of the brain via transsynaptic neurons, including the olfactory, optic and vagus nerves. It can also enter the central nervous system via the bloodstream or lymphatic system. However, the mechanisms underlying COVID-19-associated CI are currently under active investigation. They include non-immune effects, such as viral proteins, tissue hypoxia, hypercoagulation and pathological changes in neuronal cells, and immune effects, such as microglial and astrocyte activation, peripheral infiltration by immune cells, blood-brain barrier disruption, dysregulation of the cytokine network and gut microbiota. Inflammation is a central feature, and both central and systemic inflammation can cause acute and persistent neurological changes, including CI [15].

Thus, the study of mechanisms that influence the formation and progression of existing cognitive impairment in children, especially in metabolic diseases such as NAFLD, obesity, type 2 diabetes, is still an important and controversial issue in medicine.

The relationship between NAFLD and mood disorders may be bi-directional in nature as metabolic liver disease has been reported to be an independent risk factor for emerging anxiety and depression [16, 17].

The relationship between mental illness in children and metabolic liver disease is an under-researched issue. NAFLD in children and adolescents is the most common cause of liver disease and has a high comorbidity rate with childhood obesity. NAFLD in children depends on many factors, including genetics, low birth weight, and male gender [18-20].

Serotonin is a multifunctional bioamine that plays an important role in the regulation of numerous physiological and pathological pathways. Approximately 90-95% of the total 5-HT content in the body is synthesised and secreted from enterochromaffin cells found in the epithelium of the gastrointestinal tract (GIT). 5-HT exerts its biological effects primarily by binding to 5-HT receptors (HTRs), from where it is then taken up by 5-HT transporters (SERT, slc6a4) and metabolised by monoamine oxidase. As a gastrointestinal hormone, 5-HT is able to directly regulate liver function, thus mediating liver regeneration [21].

The results of our examinations indicate a decrease of serum serotonin levels at children with CI and DSD at NAFLD, which is more pronounced in group 1 (with COVID-19) and directly depends on the level of tryptophan in the blood.

The mechanism of interaction between the GIT and NAFLD remains poorly understood. A deeper understanding of the role of 5-HT in the pathogenesis of NAFLD could lead to significant progress in pharmaceutical development, especially in children with cognitive impairment, which is an extremely relevant issue during the COVID-19 pandemic.

CONCLUSIONS

In children with CI and DSD at NAFLD, a decrease in serum levels of ST and Trp was diagnosed, which is more pronounced after COVID-19. A direct relationship between the decrease in serum Trp and serotonin levels has been established, which directly depends on the functional state of the liver and negatively correlates with the severity of insulin resistance.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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