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ABSTRACT OF THE DOCTORAL THESIS

METABOLIC CHANGES IN CHILDREN AND YOUNG PEOPLE WITH AUTISM SPECTRUM DISORDERS

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KEYWORDS

Autism spectrum disorders, oxidative stress, biochemistry, glutathione peroxidase, glucose, vitamin D, vitamin B12, total protein, testosterone, gut microbiome, control group

INTRODUCTION

At this moment we can say that medical science has evolved a lot, but in terms of autism, its causes and remedies, we do not have very good results. Autism unfortunately does not seem to be understood. In recent years, more and more investments have been made in identifying the causes and treatment of this disease, but the final results do not seem to be achieved. The sad fact is that statistics have shown that the incidence of autism has increased. About 200,000 children are born in Romania every year and about 2,000 new families are affected by this tragedy. Why has the number of children born with autism spectrum disorders increased? It is a question that we want to find out the answer in this research and we want to find new ways to improve the lives of these people.

Children with autism spectrum disorders need attention, love and patience just like any other child, even if they are not able to communicate it. Research shows that involving parents and other close people in the therapy of sick children produces very good results. Intensive therapy from the age of two, with the participation of parents, can significantly reduce the symptoms associated with autism, due to the plasticity of the brain, which is characteristic of the early period of development. Autism does not have an antidote, but early diagnosis of the disease and intensive intervention using behavioral and communication therapy help improve quality of life. Balancing the body from all points of view, especially biochemically, can lead to an increase in the chances of improving symptoms.

We believe that it would be important for members of society to understand what autism means and to help people with this developmental disorder to integrate more easily into society. If every citizen would understood the importance of social integration, surely this integration would be achieved in an appropriate way.

Motivation of the research

This doctoral thesis stems from the desire to shed some light on the complexity of autism problems, to help the families of children with autism spectrum disorders to overcome the situation in which they find themselves more easily, and children with autism to have an appropriate school and social integration.

The present research aims to present a scientific study on the metabolic changes and biochemical markers involved in ASD. We wanted to make the dosages of certain markers that appear, according to recent research, to be involved in autism. The paper identifies essential elements that can be useful in the evolution of children with autism, insisting on the achievement of a good collaboration between psychologist, psychopedagogue, psychiatrist, family and community. The approach of the work is innovative because it presents an overview of the autism phenomenon, capturing defining elements that influence the symptomatology, which can be the basis for specialists to develop an individualized therapeutic plan, which must have as its main objective the improvement of the health status of the child with autism. A novelty element of the thesis is the analysis of some biochemical markers that can be the basis for assessing the impact of autism on children's development.

The original contribution to the Romanian specialty literature is represented by the analysis of the intestinal microbiome in two groups of participants, one made up of children and young people with autism and another made up of neurotypical children and young people, clearly observing differences at this level. So far, at international level, the causes that lead to the appearance of autism spectrum disorders have not been precisely identified, and this research can be a small step in detecting the elements that influence autism.

All partners involved in the recovery of children with ASD must have the well-being of children as a common interest.

SUMMARY PRESENTATION OF THE CHAPTERS OF THE DOCTORAL THESIS

The doctoral thesis entitled *"Metabolic changes in children and young people with autism spectrum disorders"* is balanced structured in four chapters, of which the first two have a theoretical character, and the last two chapters have a practical - applicative character.

The main objective of the first chapter entitled: *"Conceptual delimitations in autism"* is to review the main aspects related to autism spectrum disorders, essential aspects of this serious problem that unfortunately more and more children are facing. It is made up of eight subchapters.

In the first subchapter which is called *"The definition of autism and its history"* are mentioned the main definitions given to this phenomenon, which seems to be growing and provide information on how children with autism can be helped to integrate at school and socially. Autism is an impairment of the developmental process and has an early onset. It affects language, cognition, social development and adaptation to the environment, which lead to large differences with other children (Leaf et al., 2010).

Second subchapter named: *"Etiology of autism spectrum disorders"* contains information about the main causes that are presumed to be the bases of the appearance of autism spectrum disorders. To this day, the causes of autism remain unknown, but intensive researches are constantly being carried out in this area. The primary abnormality of this disease seems to be cognitive, affecting in particular symbolic thinking and language, with behavioral disorders secondary to cognitive deficits. "Organic brain disorders are indicated by increased complications of pregnancy and childbirth as well as a link to epilepsy, and some people have non-localized neurological disorders" (Gelder, 1983).

In the third subchapter entitled: *"The biological basis of autism"*, recent researches are presented suggesting that autism has a biological basis, but no pathogenic mechanisms have yet been identified, autism likely being the result of several biological causes and pathways, with the genetic factor being very important.

Fourth sub-chapter, called: *"The main theoretical directions in autism"*, addresses the issue of autism from the perspective of "theory of mind" which includes two components, namely: social-perceptual – in which mental states are evaluated based on available information and socio-cognitive – based on the level of perception, having a close relationship with it. The first component appears at the beginning of development and depends on the subcortical and cortical domains. The second component of the theory of mind corresponds to

a higher level according to the other cognitive systems such as memory and language in order to "measure" social judgment in the areas of the prefrontal cortex (Secară, 2007; Sparrevohn and Howie, 1995). In this subchapter is also presented *Central Coherence Theory* which was developed in 1989 by Uta Frith. According to this theory, children with autism have a special way of perceiving and thinking, which is characterized by the detachment of information processing systems, the absence of context, the fragmentation of the gestalt. In 2003 Uta Frith considered that this theory explains the existence of isolated special abilities, the reduced influence of the social context and the obsessive interest in details. Uta Frith believes that people with autism "make a plan and carry out fragmentary actions" (Frith, 2003). It is also presented here *Executive Dysfunction Theory* covering a wide range of higher cognitive processes such as: working memory, anticipation, planning, impulse control, inhibition, cognitive flexibility or the ability to adapt to change, initiation and monitoring of action (Secară, 2007).

In the fifth subchapter entitled: „*Clinical picture of autism and diagnostic criteria*", the defining elements present in children with autism spectrum disorders as well as the DSM-IV-TR diagnostic criteria are shown.

Sixth subchapter which is called: "*Treatment, evolution and prognosis of autism*" presents the role of early therapy and psycho - educational interventions that are vital and require an early diagnosis to be efficient. Despite the different treatment methods for autism spectrum disorders, none of them has a specific and long-lasting effect. Pharmacological and non-pharmacological interventions must complement each other. The aim of treatment is to facilitate social and language development, to reduce the amplitude of behavioural problems (rituals, aggression, hyperactivity) and to determine the development of skills that allow the person concerned to function independently (Hadjiu, Heel 2020,p. 20). Nutritional therapy to exclude products rich in gluten, lactose and other alternative therapies does not have enough scientific evidence, but improvements have been observed in the condition of patients after certain products have been eliminated from the diet.

In the seventh chapter, named: „*Research in the field*", are presented recent studies on autism and its social implications. The mechanisms of autism have been extensively investigated using different methods. Neuroanatomical studies have shown macrocephaly and abnormal neuronal connectivity in people with autism, and genetic studies in mice implicate different neuronal proteins in the development of ASD (Stoner et. colab., 2014).

Eighth subchapter entitled: "*Autism Statistics*" presents recent statistical data on autism. According to WHO data from April 2017, 1 in 160 children worldwide suffers from

one of the autism spectrum disorders (ASD). However, some well-controlled studies have shown that the numbers are much higher. The prevalence of ASD in many low- and middle - income countries is still unknown. According to epidemiological studies conducted in the last 50 years, the prevalence of ASD is increasing worldwide. There are many possible explanations for this apparent increase, including increased awareness, expanded diagnostic criteria, better diagnostic tools, and improved reporting.

The main objective of chapter II entitled: *"Biochemical changes in autism"* is to present the main biochemical markers whose imbalances can influence the symptomatology in autism, leading to the worsening of the health condition and implicitly to a deficient school and social integration. This chapter is structured into nine subchapters.

In the first subchapter, called: *"Oxidative stress – general considerations"*, the defining elements of oxidative stress are presented. Oxidative stress can be defined as an imbalance that occurs in the body between the action of reactive oxygen species and its defense mechanisms that play an antioxidant role. This imbalance can result from the formation of some excess reactive oxygen species or from a decrease in the efficiency of antioxidant defense mechanisms. The reasons for such a decrease in the efficiency of specific defence mechanisms can be diverse, including, for example, genetic reasons in the case of enzyme diseases. It has been frequently shown to be an essential element in the onset of certain diseases (Şerban and Roşoiu, 2003).

The second subchapter entitled: "*Autism and Oxidative Stress*" shows information about the link between autism and oxidative stress. The researchers have found that ASD may be caused by oxidative stress, a build-up of free radicals that can damage the cells that develop in children with ASD. Evidence has identified specific genetic variations in genes related to glutathione metabolism and genes related to oxidative stress and detoxification. Environmental factors such as heavy metals, infections, certain medications, and toxins such as cigarette smoke and pesticides may also play a role. Preventing further harm and improving outcomes for people with ASD may depend on identifying and correcting these discrepancies early in diagnosis. As Jenner noted in 1994, if there is an influence of oxidative stress on the onset of nervous system degeneration and if it turns out to have a primary or secondary role in etiological progression, the potential therapeutic benefits that will accompany the realization of this fact may be great. Among the biochemical compounds studied as markers of oxidative stress and the activity of the antioxidant defense system of the human body, we mention a compound formed by the peroxidation of membrane lipids - malondialdehyde, glutathione

and the enzymes glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase (Jenner, 1994).

In the third subchapter entitled: *"Glutathione and its importance"*, the important role of glutathione is highlighted in the defense of the human body against oxidative stress. Glutathione is the most popular antioxidant, it is a powerful antioxidant found in every cell in the human body and plays an important role in protecting cells from oxidative damage and supporting a healthy immune system. Glutathione is the main topic of many scientists (Kidd, 1997).

The fourth subchapter, which is called: *"The influence of testosterone on the behavior of autistic child"*, addresses the relationship between testosterone and behavioral changes in autistic children. While studies have shown a correlation between higher levels of prenatal testosterone exposure and an increased risk of developing autism, it is important to note that a correlation does not equal causation. It is important to understand that treating high testosterone levels in children with autism can improve their symptoms (Gialloreti, 2019). Their high level of aggression can be caused by several factors, but the biochemical imbalance of testosterone is definitely one of the causes. The implications of hormonal balance and imbalance have deeply penetrated the language of human biology and physiology, its effects being the subject of many studies not only among biochemists, but also among criminologists. Biological theories consider aggression as an innate tendency to act, an instinct, a predetermined pattern of responses that are genetically controlled (Dafinoiu, 2000).

In the fifth subchapter, named: *"The importance of vitamin D for children with autism"*, the major role of vitamin D for the proper functioning of autism is highlighted. Currently, autism is a serious problem that more and more people are unfortunately facing and is spreading around the world like an epidemic. Although the disease cannot be attributed to a single factor, it is important to note that the prevalence of autism has begun to increase at the same time as the number of children with vitamin D deficiency has increased. This is concerning because it seems to be malabsortion of this vitamin in children with A.S.D. It is found that vitamin D deficiency is strongly associated with ASD severity and theoretically affects the neurological development of children with ASD. Due to its anti-inflammatory properties, it stimulates the production of neurotrophins, reduces the risk of seizures and regulates glutathione and serotonin levels, being very important for the proper development of the body. The classic role of vitamin D is to regulate the metabolism of calcium and phosphate, which are essential for bone remodeling. Based on research in recent decades, it has been shown that low sun exposure, vitamin D limitation and deficiency are associated

with an increased risk potential for many other diseases, such as cancer (Bouillon et al., 2020).

Sixth subchapter entitled: *"The role of vitamin B12 for the proper functioning of the nervous system"* explains the importance of vitamin B12 in the cognitive development process of children with autism. B vitamins comprise chemicals of various nature, which participate in the activity of enzymes, without which some synthesis and cellular activity could not take place. The human body contains almost all the chemical elements found in nature. They are broken down by chemical processes and then eliminated as metabolic waste through urine, digestion or skin. To replace them, they must be introduced through food.

In the seventh subchapter named: *"The importance of minerals for the body"* We present theoretical notions about the role of minerals in maintaining health. From maintaining hydroelectrolyte balance to participating in vital enzymatic reactions, minerals are involved in numerous physiological processes and are considered essential for our health. The human body contains almost all the chemical elements found in nature. Ionic calcium has an important role in the prevention of diseases and in the processes of coagulation and regulation of muscle contractility and neuromuscular sensitivity (Roşoiu, 2010).

In the eighth subchapter entitled: *"Nutritional needs of the child"* it is shown the importance of a healthy and balanced diet that helps the body to be stronger, with high immunity and greater resistance against diseases. In the case of children with autism spectrum disorders the situation is more complicated due to the refusal of children to eat certain foods, they consume the same foods for a long time. It is quite difficult to convince an autistic child to eat certain foods if he does not want to. Parents are making great efforts to solve this rather complicated situation. They are aware that a healthy diet is the key to good health.

In the ninth subchapter, which is called: *"The intestinal microbiome in people with autism spectrum disorders",* informations are presented about the specifics of the intestinal microbiome in children with autism and how its imbalance influences the severity of autism symptoms. The pathogenesis of autism spectrum disorder is not fully understood, but it involves a combination of genetic, environmental and immune dysfunction factors. The gut-brain axis is viewed as a communication pathway between the gut and the brain and is a two-way communication system. A growing body of evidences suggest that the gut-brain axis participates in the pathogenesis of autism spectrum disorder.

Part II of this paper contains Chapter III entitled: *"Material and methods",* which presents the way in which the biochemical analyzes have been performed, the place where the samples have been made and the materials used, as well as the way in which the questionnaire

addressed to parents with autism have been applied. It also includes Chapter IV called *"Results and researches"*, in which a number of nine experiments are presented, correlations between certain biochemical markers that influence the symptomatology in autism, as well as the interpretation of the answers to the questionnaire addressed to parents with autism. Through the experiments presented in the research we want to identify metabolic changes and biochemical markers involved in ASD.

Thus, we carried out the following experiments:

1. Determination of the value of glutathione peroxidase, which has an important role in the metabolism of neurotransmitters and oxidative stress.
2. Identification of biochemical values of glucose that can be altered in children with autism spectrum disorders.
3. Establishing the blood parameters of total proteins, as their role is very important in ensuring health.
4. Identify vitamin D values, as there are specialized studies that mention the link between vitamin D deficiency and autism.
5. Analyze the biochemical marker vitamin B12, because the appearance of an imbalance in it can influence the symptoms in autism.
6. Establishing the values of electrolytes: serum calcium, serum magnesium, serum iron, serum sodium, serum phosphorus, potassium and ionic calcium.
7. Determination of the biochemical concentrations of testosterone, since its increased values can cause an increase in agitation or aggressiveness.
8. Analysis of the intestinal microbiome, which is very important to be carried out in children with ASD, since an imbalance in it seriously affects health.
9. Correlations of biochemical markers in patients diagnosed with autism spectrum disorder, to help us better understand which are the biochemical markers in the blood that show changes in children with ASD.

GENERAL CONCLUSIONS

Following the analysis of the obtained results, in accordance with the 10 general objectives of the research paper, we will present the general conclusions.

In the first practical part of our research, we considered important to perform the statistical interpretation of the results of the biochemical analyses of some markers that, according to the specialty literature, if they present values outside the normal limits, could be the basis for the worsening of symptoms in autism. We obtained relevant results in the analysis of data on glutathione peroxidase, glucose, total protein, vitamin D and testosterone analysis.

The appearance of an imbalance in glutathione peroxidase can seriously affect the health of children, leading to poor cognitive development and could influence the symptoms of autism. The medication that children with autism receive from the specialist, due to the chemical composition, can influence the biochemical balance. The low level of glutathione shows us the existence of imbalances at the level of the mechanisms that the human body uses to fight against the negative effects of oxidative stress.

Determination of the level of total protein is important to make an assessment of nutritional status. A possible cause of increased biochemical imbalances in the group of children and young people with ASD could be inadequate nutrition, because children with ASD are inattentive, anxious or agitated during meals and have extreme food selectivity (with preferences for certain colors, textures) and eating rituals.

We also considered important to determine the biochemical analyses for vitamin D because this vitamin has an essential role in the proper development of children. In the group of children and young people diagnosed with ASD we have a large imbalance in vitamin D, that is we have a percentage of 66.67%, so 20 participants who have values outside the normal limits, and in the control group we have a percentage of 20.00%, that is 6 participants who have values outside the normal values. This situation concerns us and we believe that measures should be taken to improve this situation. Vitamin D is extremely important for the proper development of the body. We can conclude that low vitamin D values are found in large numbers in all groups of participants, which is not good. Medications are prescribed by the psychiatrist to relieve the symptoms of autism, but they can influence the values of vitamin D which has an essential role in good physical and especially mental development.

In the next research experiment we determined the biochemical values of electrolytes. Following statistical analysis and interpretation of the data, we obtained relevant results for total calcium and serum sodium. An adequate level of total calcium in the body is crucial for

the proper functioning of all systems and organs. Imbalances in total calcium can affect the bone system, cardiovascular system, muscular system, central nervous system and other vital organs and it is important to correct them in time, as they can have serious consequences on health.

The study done shows that the medication prescribed to children and young people with ASD can sometimes be associated with the appearance of a biochemical imbalance, with a worsening of the symptoms and not with a reduction of it as it should normally have been. These drugs also have side effects such as weight gain, which worsens the health of the child with autism.

After analyzing the data, we can say that high testosterone levels influence the behavioral manifestations of children and young people with ASD. It is important to do hormone tests in children because a hormonal dysfunction could be the cause of violent behavior.

Identification of testosterone levels in children with autism in order to try to reduce the severity of symptoms and implicitly improve health. The family must understand that autism is serious and a lot of effort must be made to improve it. The specialty literature mentions that a high level of testosterone leads to the appearance of aggression in boys, a fact also confirmed by our research.

Analyzing the gut microbiome

In order to better understand the structure of the intestinal microbiota in children with autism spectrum disorders of different ages, we considered important to evaluate the intestinal microbial population. Through this experiment we wanted to identify whether, in the case of children and young people with ASD, there is a causal link between intestinal transit disorders such as chronic constipation, increased level of psychomotor agitation and the presence of an imbalance in the intestinal microbiome. Recent studies have shown that gut microbes can be linked not only to gastrointestinal problems, but also to behavioral symptoms of autism spectrum disorder.

In this experiment we analyzed bioindicators, LPS-positive bacteria and microbiota-degrading fibers found in the gut microbiome. We noticed that there are imbalances in the following bacteria: Citrobacter spp., Enterobacter spp., Escherichia spp., Pseudomonas spp. and Serratia spp..

Regarding the analysis of bioindicators, we can mention that we obtained conclusive results for our research only for: fecal pH, Biodiversity, Lactate Production, Acetate Production, Mucin Degradation and LPS Bacteria – positive. The large number of children and young people with ASD who have values outside the normal limits makes us think that

the imbalance at the level of bioindicators in the intestinal microbiome may be the basis for the worsening of autism symptoms. The intestinal microbiome has a very important role in maintaining the health of the body and that is why it is important to perform the analysis of the intestinal microbiome if gastrointestinal problems or other diseases occur.

We can conclude that there is a fairly large imbalance in certain bacteria present in the intestinal microbiome, which can influence the state of health. Participants in the group of children and young people with ASD have greater imbalances in the microbiome than participants in the control group. This can be influenced by the diagnosis of autism but also by improper nutrition and further research should be carried out with an emphasis on the link between diet and the microbiome. Due to an inadequate diet, specific nutrients important for learning efficiency may be missing. Children with autism spectrum disorders tend to have a limited food repertoire and a greater reluctance to eat certain foods compared to neurotypical people. Diet plays an important role in determining gut microbial composition and function, therefore, a selective diet can influence the gut microbial community.

Children with autism spectrum disorder and gastrointestinal symptoms showed many imbalances in the gut microbiome. A probiotic approach should act as a means of restoring a healthy microbiota. To confirm the efficacy of probiotic therapy in children with autism spectrum disorders, larger studies with laboratory analyses of the microbiota are needed for better direction. Further research on the brain-gut-microbiome axis may lead to new methods for identifying gastrointestinal disorders in children with autism spectrum disorders and new treatments for reducing symptoms in autism.

Correlations between biochemical markers detected in children and young people diagnosed with ASD

Regarding the correlations between the analyzed biochemical parameters, we obtained in two cases a correlation between the variables, because the value of the probability coefficient p was smaller than 0.05. Between serum sodium and serum iron we have the Spearman's rho correlation coefficient of 0.578, a value between 0.5 and 0.7, which shows us that we have a moderate positive correlation, so it indicates that an eventual increase in sodium is associated with an increase in iron. We also have the value of the coefficient of determination R^2Linear = 0.209.

Between serum magnesium and serum phosphorus we have the Spearman's rho correlation coefficient of -0.402*, a value between -0.3 and -0.5, which shows that we have a weak to moderate negative correlation, indicating that a possible decrease in phosphorus is

associated with an increase in magnesium. We also have the value of the coefficient of determination R2 Linear = 0.111.

Questionnaire for parents

What we wanted to highlight after the development and application of this questionnaire was the fact that parents must be aware of how important it is for the body of children with autism to be in a biochemical balance and to understand that their health can be improved through teamwork.

In conclusion, it can be said that autism spectrum disorders appear more and more frequently in recent years. They occur in children and persist throughout life, so early diagnosis is recommended. Thus, a multidisciplinary team of pediatricians, psychiatrists and psychologists (specialized in autism) is needed to confirm the diagnosis and its severity. Potential treatments for manifestations of autism spectrum disorders include drugs that have been developed to treat other conditions that secondarily interfere with some of the symptoms of autism. Early and intensive intervention can significantly help the recovery of children with autism. Our findings contribute to the expansion of the current knowledge base on biochemical imbalances in autism and provide new motivation for further research.

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