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 REVIEW on PhD Thesis

METABOLIC CHANGES IN CHILDREN AND YOUNG PEOPLE WITH AUTISM SPECTRUM DISORDERS REVIEW on PhD Thesis

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Objectives and purpose of the work

Through documentary study and realized papers, the PhD thesis proposes to bring a contribution to the well-being of children with Autism Spectrum Disorder. 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 who, 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 and 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.

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 in research 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.

The objectives of these researches were established in accordance with the purpose of the work and will be the basis of further scientific researches regarding the influence of biochemical imbalances on the symptoms in autism.

The purpose of this research paper is to determine some biochemical markers that may lead to new links between neurochemical reactions and the onset and worsening of symptoms of autism spectrum disorders.

These links can be useful in achieving the goals of intervention in treatment and therapy, represented by: reducing symptoms, facilitating development and learning, promoting socialization and improving behavioral deviations.

Thus, our research focused on 10 main objectives:

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.

10. Knowing the opinions of parents of children with ASD regarding the influence of biochemical imbalances on autism.

This thesis has a multidisciplinary approach: psychology, special psychopedagogy, biology, biochemistry, medical sciences, which was accomplished through a complex collaboration in research activity. Therefore, the realization of the paper was possible through the contributions of Faculty of Medicine from University Ovidius from Constanta, Bioclinica laboratories of Constanta and Emergency Military Hospital *Dr. Alexandru Gafencu* of Constanta.

The PhD 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, the knowledge degree and the last two chapters have a practical - applicative character, personal contributions.

In the first part of the PhD thesis are described the main aspects related to autism spectrum disorders, essential aspects of this serious problem that unfortunately more and more children are facing. In this part of the paper 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 development 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 [1].

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. Analyzing the microbiome is extremely important for people with autism. Recently, the medical world has become increasingly aware of the importance of the populations of microorganisms that colonize the human gut, known collectively as the microbiota.

Recent studies have linked the imbalance of microbiota composition not only to inflammatory bowel diseases, but also to chronic diseases such as depression, autism, obesity, multiple sclerosis, cancer, rheumatoid arthritis or Parkinson's disease and many others.

The last part of the paper is continuing with the obtained results from the biochemical analysis and determination of the intestinal microbiome. The research results are presented in tables and figures. The conclusions argue the realization of the objectives and also open the new research in ASD domain.

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

INTRODUCTION

Children with autism do not look different from others, but they communicate, behave and learn differently than most other children. According to the report published in 2023 by the Centers for Disease Control and Prevention USA (CDC), 1 child in 36 is diagnosed with autism spectrum disorder. The data show an increase in prevalence compared to the previous report, published in 2021, where the prevalence was 1 child in 44. Autism is a common neurodevelopmental disorder characterized as a neuropsychiatric condition, diagnosed based on three behavioral criteria: abnormal social interactions, problems of communication associated with language deficits and restrictive and repetitive behaviors (stereotyped behaviors) [2].

The problem of autism is extremely serious. Although the number of children born with autism has increased, researchers have not yet precisely identified the causes of autism. In recent years, promising researches have been conducted to better understand autism, including its possible causes or the development of new treatments and interventions. Many people are interested in how the gut microbiome might be related to autism spectrum disorder (ASD). Children with autism spectrum disorders need attention, love and patience just like any other child, even if they are not able to communicate it. Researches show 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 to 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 the 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 understand the importance of social integration, surely this integration would be achieved in an appropriate way.

PART I - STAGE OF KNOWLEDGE

In the theoretical part, important information is presented about the disorders of the autistic spectrum, as well as the relevant biochemical changes that can influence the aggravation of the symptoms in autism according to recent research.

A new study has shown that environmental factors are more important in the cause of autism than previously thought and may play as important a role as

genes. A team of medical professionals concluded that environmental factors that play this harmful role include older parental age, low birth weight, twin pregnancy, drugs used during pregnancy, as well as pollution, pesticides and some conservants. The dramatic increase in the number of children with the disorder was too sudden to be explained by genes alone. Genetic defects do not occur spontaneously without outside intervention, so experts blame changes in the environment that adversely affect our DNA. In appropriate, repetitive and ritualistic behaviour, e.g. destroying objects, spinning objects, repeating movements, rocking the body, endless series of jumps on the mat or spinning movements around one's axis, are common characteristics of children with autism [4]. A new study has shown that environmental factors are more important in the cause of autism than previously thought and may play as important a role as genes. A team of medical professionals concluded that environmental factors that play this harmful role include older parental age, low birth weight, twin pregnancy, drugs used during pregnancy, as well as pollution, pesticides and some conservants.

1.GENERAL NOTIONS ABOUT AUTISM

In present, the causes of the autism remain unknown, but intensive research is constantly being conducted in this area. The primary abnormality of this disease appears to be cognitive, particularly affecting symbolic thinking and language, with behavioral disturbances secondary to cognitive deficits. "Organic brain disorders are indicated by increased complications of pregnancy and childbirth, as well as a link with epilepsy, and some people have non-localized neurological disorders" [3].

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According to DSM IV, "Autistic spectrum disorders are pervasive developmental disorders characterized by severe impairment of mutual social interaction and communication skills, narrowing of the area of interests, the presence of behavioral disorders and stereotyped activities." The qualitative impairment that defines these conditions is distinctly different from an individual's developmental level or mental state [6].

The autistic child refuses to be in contact with external people and objects and escapes inside them, where he fulfills his imaginative desires, emotionally compensating for fantasies that can sometimes lead to hallucinatory illusions [7].

Autism is not a disease but a pervasive developmental disorder, a concept that better expresses what happens to these people and what are the stages in the application of therapy. This pervasive developmental disorder requires lifelong care facilities specialized in autism [8]. While the etio-pathogenesis of ASD is unknown, the clinical manifestations are diverse and many possible genetic and environmental factors have been implicated. As such, it has been a great challenge to identify key neurobiological mechanisms and develop effective treatments [9]. As mentioned by Cucuruz in 2014, "autism has its onset after the age of 1 year when there is an absence of waiting mobility while being held, lack of response to the mother's smile, apathy and lack of interest in what is around him" [10]. Autism includes some disorders or deviations that affect at least 3 areas of development: the inability to relate to those around; to show his feelings; impossibility of using language and communication (verbal or non-verbal); stereotypes, including the presence of restrictive and repetitive behavior [11]. Despite these common features that characterize the autistic syndrome, the clinical features and severity of these core symptoms vary widely among affected individuals. Some children with autism can recover more easily than others. An essential condition for a good recovery is the start of psychobehavioral and communication therapy immediately after the diagnosis. Goldstein sees autism as a secondary defense against organic deficiency. The dysfunction, according to him, is the inability of an autistic child to develop abstract thinking. However, the results of psychoanalytic therapy appear slowly, under these conditions the behavioral current takes shape [12]. In 1963 Goshen conducted a research with autistic children and extended it to a family. He emphasizes the influence of the mother's behavior, especially in the first 6-18 months of life, who failed to communicate with him enough and encourage him. As a result, the child may not understand the meaning of the language and end up with mental retardation [13]. Spitz (1964), who originally emphasized the deleterious effects of lack of stimulation, later showed an identical concern for the opposite condition, suggesting that autistic infants may be so hypersensitive to their mother's emotions that they defensively try to reject what is too destructive for them [14]. M. Fordham mentions that autism is a dysfunction of the Ego. In autism, we have no recognition of a primary self or a non-self. Primary autism would be due to the absence of a breakdown of the primary mental component of the self [15]. Some psychoanalysts developed different theories, focused on self-division, objectivity and relationality, concepts that no longer exist today, but we have found the importance given to them at that time perhaps also because the published theories were based on unscientific assumptions [16]. Another representative of this

approach is F. Tustin, who essentially continued Winnicott's research. She argues, based on the autistic subject, that in order to combat the suffering caused by autism, the child organizes a relationship with the world, a true autistic "delusion"; it is about merging with a real world and feels the agony of the first rupture. For this purpose, the child would use "autistic objects" that he cannot part with. F. Tustin defines the two forms as: primary or "crustacean type", which corresponds to the global form of autism, which includes the entire personality of the child, and secondary or "segmental type", which corresponds to the partial form, which includes only his segments [17]. Typically, the diagnosis of autism is made by correlating informations obtained from a comprehensive clinical examination of the child with caregiver -reported data on the child's behavior and development, as well as with the results of specific autism psychometric scales. The disease begins before the age of 3 years, although it is quite difficult to make an early diagnosis [18]. About 33% of people with autism can manage partially on their own, but those with low intelligence and unable to speak need constant help. Adults with high-functioning autism succeed in their professions and are able to live independently, although they usually continue to have some difficulty interacting with other people. About 10% of these have some form of scientific ability, special but limited talents, such as memorizing lists, mental math, counting calendar dates, drawing, sculpting, dancing, or musical talent. Many young people with autism play the piano. It seems that this musical instrument helps them a lot [19]. The American psychiatrist L. Kanner (1943) in his original article "Disorders of Affective Contact in Autism" describes a clinical picture that differs from childhood schizophrenia. In his opinion, the inability of a small child to establish emotional connections with the environment is present from birth [20]. Kanner says that the main characteristics of autism, while similar to schizophrenia, are different in many ways. The children described by Kanner behaved as they had from birth and did not follow the gradual changes in behavior over time that children with schizophrenia do. As Kanner argued, the autistic child's behavior is driven by a strong desire to maintain uniformity and solitude [21]. In recent years, the number of children with autism spectrum disorders has increased. In the first year of life, there are usually no clear defining features, but it is necessary for parents to be vigilant. In the second and third year of life, a rapid examination by specialists is necessary for the following areas of interest: communication, especially understanding of language, its disorder, unusual use, inability to respond to names, non-verbal communication - insufficient (without hand pointing and difficulty in following a point), without social smiling to share good mood and respond to the smile of others [22].

Recent researches suggest that autism has a biological basis, but no pathogenic mechanisms have yet been identified. Autism is likely the end result of several causes. An important element is that approximately 10% of people with

autism also have a medical condition that appears to influence autistic symptoms. For the other 90% of individuals who do not have another disease, the genetic factor is very important. Evidence for this claim comes from a study of twin children. A study of families who have a child with autism showed that, although there is a high chance that the second child will also be born with autism, the rate is not much higher than the rate of autism in the rest of the population. A doctor in the United States of America researched twin children, one of whom had ASD, some were identical with the same genetic makeup, while fraternal twins are as different as siblings born several years apart. By comparing the prevalence of autism in two types of twins, researchers revealed the percentage by which environmental and genetic factors contribute to the development of autism. They showed that autism or brain dysfunction is inherited. "The presence of an autistic child in the family appears as a dramatic situation, his presence is also a warning. The risk of relapse, the problem of disorder between siblings, is imposed as a specific priority" [23]. Through socialization, individuals internalize the cultural norms and values of their community, which enables them to navigate the complexities of social interactions. This process contributes to the formation of social consciousness and the development of socially desirable behaviors that guide the way individuals behave and interact with others. Also, socialization facilitates the transmission of cultural knowledge, ensuring continuity and social cohesion. For the autistic child, socialization is very important because it is possible for him to "come out of his shell" and communicate better, thus improving the chances of a good school integration. The conclusion of these studies is that autism has a hereditary basis and it is the result of the interaction of several genes. Studies in the field of genetics have focused on trying to discover the gene or genes that would be responsible for autism in most cases, but no unanimous conclusion has yet been reached. Although it is believed that some areas of the human genome are breeding grounds for genetic defects, no genes have been identified that have an influence in autism, but there are situations where certain genetic defects are found. Surely future research will discover the causes of autism and new methods will be found to improve the lives of children diagnosed with this serious developmental disorder. Early therapy and psycho-educational interventions are vital and require an early diagnosis to be effective. Pharmacological and non-pharmacological interventions should complement each other. Their aim is to improve communication and social integration, to reduce behavioral problems and to determine the development of skills that allow the person in question to function independently. Specialists may prescribe medication to treat co-occurring symptoms of autism, such as anxiety, depression or obsessive-compulsive disorder. Some medications may be helpful in controlling symptoms such as self- and hetero-aggression, isolation, stereotypies, hyperactivity, reduced attention span, etc. In such situations, medication may

increase the effectiveness of educational-behavioral or other interventions. However, these do not cure autism, but help to improve the symptoms. Nutritional therapy regarding the exclusion of products rich in gluten, lactose and other alternative therapies do not have enough scientific evidence, but improvements in the condition of patients have been observed after eliminating certain products from the diet.

There is a clear link between nutrition and symptomatology, it is not for nothing that the intestine is called: the second brain. In order to follow and supervise the evolution of patients with ASD, it is necessary to: draw up the empowerment plan, monitor the empowerment measures, re-evaluate, adjust the empowerment plan [24]. Effective treatment methods for autism have been developed and used. In 2008, Dawson indicated that the intervention could improve social engagement and reciprocity in ASD [25]. At the same time, researchers have shown that music therapies are also an effective treatment for verbal communication. Because in the left side of ASD patients the inferior frontal gyrus is strongly activated when stimulated by songs, they have no response during speech stimulation [26]. There are several cases of ASD in which patients demonstrate extraordinary artistic or musical talents. Karimi et al. (2011) suggested Neurofeedback Training (NFT) and speech therapy in ASD patients, which are effective in improving patients' learning and speaking skills [27]. Cai et al. in 2013 reported the application of virtual reality technology as an effective intervention and treatment to promote learning and positive behavior in children with ASD. They invented Dolphin Therapy that teaches children to communicate through hand gestures with a virtual dolphin [28].

Table no. 1. Prevalence (rates per 100,000 inhabitants) of TSA, comparing the European Union and Romania, in the period 2007-2017

Year	European Union (UE)	Romania
2007	519,61	413,41
2008	519,65	412,85
2009	519,69	412,32
2010	519,69	411,80
2011	519,70	411,31
2012	519,81	410,71
2013	519,96	410,17
2014	520,18	409,63
2015	520,52	409,08

2016	521,18	408,56
2017	521,52	408,13

Source: [31]

These statistical data highlight the massive increase in the number of children diagnosed with ASD who have this developmental disorder. It is a worrying situation and researchers in the field all over the world should come together to find the causes and possible remedies to ameliorate this disability. Only through a joint effort can the desired goal be reached.

2. BIOCHEMICAL CHANGES IN AUTISM

2.1. Autism and oxidative stress

The brain is particularly vulnerable to free radical attacks because of its high oxygen consumption and high levels of unsaturated fatty acids that make up neuron membrane lipids. It is highly oxygenated, making it susceptible to endogenous production of free radicals, and rich in unsaturated lipids, making it vulnerable to peroxidation. In addition, brain areas rich in catecholamines are particularly vulnerable to oxidative stress. However, results regarding the effects of oxidative stress in ASD are more difficult to interpret due to factors such as diet, medications, lifestyle, and even the subjects' ethnicity. The main markers of oxidative stress and the activity of the antioxidant defense system of the human body are a compound formed by the peroxidation of membrane lipids - malondialdehyde, glutathione and the enzymes glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase [32].

Neurobiological studies of ASD have traditionally focused on neurodevelopmental pathways and synaptic plasticity. However, some evidence suggests that immune system dysfunction may cause or at least contribute to ASD.

In addition, several studies have reported the role of oxidative stress in the pathogenesis of this group of diseases. Interestingly, there may be a link between the accumulation of oxygen radicals and immune dysfunction. Animal studies have advanced rapidly in recent years and numerous models have been proposed that show many of the hallmarks of autism. According to the AutDB database (http://autism.mindspec.org/autdb/Welcome.do, updated January 2020), there are currently 3145 animal models of ASD, including inbred, inducible, and genetic mouse models [33]. Genetic studies have shown that mutations in several genes encoding synaptic proteins, such as SHANK3 [34] are associated with ASD. In addition, ASD is syndromic with other neuropsychiatric diseases with single-gene

mutations, including fragile X syndrome (FMR1) [35], tuberous sclerosis (TSC1 or TSC2). Interestingly, several genes associated with TSA, such as PTEN, TSC1 and TSC2, all involved in the phosphoinositide-3-kinase (PI3K) pathway, show immunoregulatory functions. Genes involved in the ROS scavenging system are expressed in both ASD patients and ASD mouse models. In addition, oxidative stress may be associated with neuroinflammation, thus contributing to the ASD-like phenotype [36].

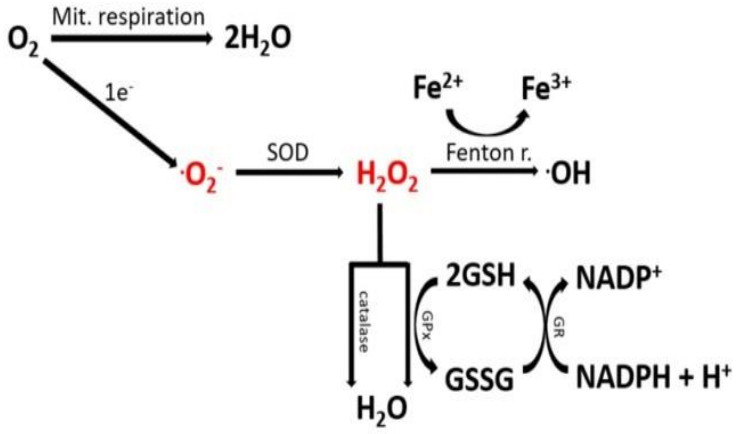

Figure no. II.2. Reactions required for detoxification from reactive oxygen species (ROS). Source: [37].

2.2. The influence of testosterone on the behavior of the autistic child

The implications of hormonal balance and imbalance have deeply permeated the language of human biology and physiology, its effects being the subject of much study not only among biochemists but also criminologists. One of the most famous positions was formulated by Konrad Lorentz, who mentioned the fact that aggression in humans has a spontaneous genetic basis. He says that a tension accumulates in the body which, if not removed, will amplify, and the aggressive behavior will intensify [38]. Although the etiology of ASD remains uncertain, studies suggest that hormonal imbalances such as testosterone may modulate the autism phenotype. There is limited literature on testosterone variations during adolescence in ASD [39].

Exploring the complex relationship between testosterone and autism reveals interesting insights into the characteristics and symptoms associated with autism spectrum disorders (ASD). Research shows that people with autism have higher testosterone levels than people without ASD. Testosterone, a hormone found

mainly in men but present in both sexes, plays a crucial role in various biological processes. Studies have shown that testosterone levels in people with autism are often higher during prenatal development. These high levels can affect the development of certain brain structures and contribute to the manifestation of autism spectrum traits. The effects of testosterone on autism symptoms are still under investigation, and the exact mechanisms are not yet fully understood. However, some researchers suggest that testosterone may influence certain behaviors and characteristics that are common in people with autism. One area of interest is the influence of testosterone on social communication skills, which may be impaired in individuals with ASD.

Some studies suggest that higher testosterone levels may be associated with difficulties in social interaction and communication. However, it is important to recognize that this is a complex relationship influenced by various factors and not all individuals with autism have the same patterns. 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 also important to recognize that not all individuals with autism identify as male or have typical male characteristics. Therefore, the assumption that all autistics have high testosterone levels or exhibit stereotypically "masculine" traits can be harmful and perpetuates harmful stereotypes. Finally, it is important to understand that treating high testosterone levels in children with autism can improve their symptoms [40].

A team led by Professor Simon Baron-Cohen and Dr Michael Lombardo, from the UK, and Bent Nørgaard-Pedersen, from Denmark, analyzed 19,500 amniotic fluid samples stored in a Danish biobank from babies born between 1993 and 1999. During pregnancy, amniotic fluid is found around the baby and the sample is taken when women decide to have an amniocentesis at 15 or 16 weeks of pregnancy. This coincides with a critical period of early brain development and sexual differentiation, allowing researchers to access this window of fetal development. The researchers identified amniotic fluid samples from 128 children who were later diagnosed with autism and corroborated the data with information from the Danish Central Psychiatric Diagnostic Register. They analyzed the 4 main male sex steroid hormones in amniotic fluid: progesterone, hydroxyprogesterone, androstenedione and testosterone. They also looked at the steroid cortisol and found that high levels of steroid hormones were closely associated with a group of children diagnosed with autism compared to a male group with normal hormone levels. According to Baron-Cohen, “it is one of the biological indicators-biomarkers that identifies children who will later develop autism. Although we know that high levels of prenatal testosterone are associated with slower social and language development, increased attention to detail and some autistic traits, we now show for the first time that these steroid hormones

can cause autism in male children and explains why it is more common in boys," he explained. In his opinion, these results are important given that they are found in all subgroups of the autism spectrum: Asperger's syndrome, classic autism and pervasive developmental disorder not specified. However, Baron-Cohen cautions that "these results should not be considered a reason to block steroid hormones as a possible treatment, as they would cause unwanted side effects and have little or no effect on changes in fetal steroid hormones", the first essential stages of brain development. He adds that the results should also not be taken as evidence of effective prenatal detection of this syndrome [41].

It is important to do hormonal analyzes in children because a hormonal dysfunction could be the cause of violent behavior. Boys tend to be more aggressive than girls. The male hormone testosterone is clearly linked to aggression in all primate species, including humans [42].

Identifying the level of testosterone in children with autism in order to try to reduce the severity of the symptoms and implicitly improve the state of health. The family must understand that autism is serious and many efforts must be made to improve it. Further research is needed to better understand the effects of testosterone on autism symptoms. Understanding the relationship between testosterone and autism spectrum traits is an ongoing but extremely important area of research. By unraveling the complex relationship between these factors, researchers hope to shed light on the causes of autism and possibly develop targeted interventions to improve the lives of people with autism and, why not, to reduce the number of children born with autism.

Research was carried out in 2018 on a group of aggressive but undiagnosed children with ASD. Scientists at the University of Iowa have identified a marker in the brain associated with aggression in children. The researchers studied children between two and a half and three and a half years old and measured the intensity of so-called "P3 brain waves" when they encountered a change in situation. The lower the intensity of these P3 brain waves, the more aggressive the child becomes, according to eurekalert.org. Children often tease each other, but while one child may see it as a game, another child sees it as a hostile gesture and reacts aggressively. Such things happen often among children, but it is not yet known why some do not respond to a challenge and others become aggressive. The results of the study could help identify tendencies toward aggressive behavior at an early age and help curb these impulses before adolescence, when aggression is much more difficult to treat. “There are all kinds of social cues, such as facial expression, tone of voice, body language, posture, gestures and proximity, and when children fail to notice and interpret them, they can become hostile. In addition, the little ones react differently, even if the factor is the same", explains the author of the study, professor of psychology Isaac Petersen. A question that arises is: what are P3 waves? The P3 wave is part of the waves that occur in the

brain when a person assesses and responds to changes in the environment, such as in social interactions. Previous studies, particularly those involving adults, have shown that people with low P3 waves become more aggressive when faced with change. As a result, experts say P3 is a key indicator of aggression, but it is also linked to depression, schizophrenia and autism. The researchers recruited 153 children who participated in individual sessions.

The children were fitted with a headset with sensors that measured brain activity and were forced to listen to a constant stream of sounds. As the children watched the cartoons without sound, the tones began to change in the background. The specialists measured the P3 waves, which changed their activity according to each change of sounds. These changes in tone are similar to changes in social interactions where the brain, both consciously and unconsciously, responds to the change. Children whose P3 did not reach a high level were rated as aggressive by their parents. The results applied equally to girls and boys. "Children who do not reach high levels of the P3 wave are not as able to detect changes in the environment, so they become confused and interpret the changes as hostile and react aggressively," says Petersen. The researchers followed the same participants at 30, 36 and 42 months to further test the hypothesis. "This marker has not been studied on a large scale, but it could be a tool we can use in the future to discover a tendency towards aggression. Early interventions are more important in the treatment of aggression. During adolescence, behavior is already much stronger, stable and harder to change," concludes Petersen. The study was published in the Journal of Child Psychology and Psychiatry, and the children were tested at Bloomington University in the state of Indiana – United States of America [43].

2.3. The importance of vitamin D for children with autism

Vitamin D is a fat-soluble vitamin essential for maintaining the health of the human body. It plays an important role in many physiological processes and has a crucial role in maintaining the health of the bone system, the immune system and muscle functions. Vitamin D is classified as a fat-soluble vitamin that is obtained from the diet or is produced by the skin from ultraviolet B (UVB, 290–320 nm) from sunlight [44]. Due to vitamin D deficiency and the risk of disease, in many countries there is a legal obligation to fortify certain products (dairy products, especially milk and other dairy drinks, margarine) [45]. The classic role of vitamin D is to regulate calcium and phosphate metabolism, which is 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 a potential increased risk for many other diseases, such as cancer [46].

Currently autism is a serious problem that unfortunately more and more people are facing and it is spreading throughout 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 began to increase at the same time as the number of children with vitamin D deficiency increased. This is concerning because there appears to be a malabsorption of this vitamin in the case of children with ASD. It was found that vitamin D deficiency is strongly associated with the severity of ASD and, in theory, affects the neurodevelopment 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 good development of the body.

Research has been conducted with increasing evidence highlighting the association between vitamin D and Autism Spectrum Disorders and the higher prevalence among children who lived in areas with low ultraviolet B rays compared to those living in sunny areas [47]. Vitamin D is physiologically transformed into its active form, 1,25(OH) D (Calcitriol), by two consecutive hydroxylation processes in the liver and kidney [48]. In addition, vitamin D plays an important role in modulating inflammation by regulating the production of inflammatory cytokines and immune cells, which are essential for the pathogenesis of many immune-related diseases [49]. Significantly higher concentrations of interleukin (IL)-1β, IL-6, IL-8, interferon-gamma (IFN-γ), eotaxin, and monocyte chemotactic protein-1 (MCP-1), along with significantly more transforming growth factors mici-β1 have been reported in individuals with ASD compared to their healthy controls [50]. Elevated IL-6 and TNF-α in children with ASD were positively correlated with ASD severity as measured by the Childhood Autism Rating Scale (CARS) ($R = 0.638$ and $R = 0.699$, respectively ($p < 0.0001$), and are also used as biomarkers of ASD diagnosis [51].

It is important to maintain an adequate level of vitamin D in the body through sun exposure, eating foods rich in vitamin D or supplementing with vitamin D, especially in cold seasons and for people at high risk of deficiency. It is also recommended to talk to your doctor before taking vitamin D supplements to determine the correct dosage and avoid possible drug interactions that could affect your health. Because autistic children do not spend much time outdoors becoming more agitated due to excessive heat, we find this high vitamin D deficiency in these children. They also have problems with food, sometimes they eat the same foods daily for a long time, which is a serious problem. A balanced food program could regulate the problems arising at the biochemical level and could lead to an improvement in the symptoms, a fact that would improve their health and increase the chances of a good social integration.

2.4. The role of vitamin B12 for the proper functioning of the nervous system

Vitamins of the B group include chemical substances of various nature, which participate in the activity of some enzymes, without which some syntheses and cellular activity could not take place. Some B vitamins play an important role in the formation of red blood cells, especially folic acid and vitamin B12. Low intake of vitamin B12 or insufficient absorption in the intestine becomes apparent after a longer time [52].

Vitamin B12 has a decisive role in lipid metabolism and in the regeneration and formation of myelin sheaths, which ensure the protection of nerves from the loss of electrical impulses, ensuring a correct and efficient nerve flow in the transmission of transmitted information. This "isolation" of the nerves responsible for tactile sensation and motor function is not only important, but simply necessary for the optimal functioning of the central nervous system, head and spinal cord. Vitamin B12 helps regulate the level of neurotransmitters that control brain activity and indirectly regulate perception and mood.

When there are imbalances of vitamin B12 in the body, some health problems can occur, such as megaloblastic anemia, which is characterized by anemia (decreased number of red blood cells) and extreme fatigue. Also, vitamin B12 deficiency can lead to neurological problems, such as peripheral nerve damage, memory impairment and difficulty concentrating. In addition, the lack of vitamin B12 can affect mood, causing depression and anxiety.

2.5. The importance of minerals for the body

From maintaining electrolyte balance to participating in vital enzyme reactions, minerals are involved in numerous physiological processes and are considered essential to our health.

The human body contains almost all 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 body, mineral substances are present in clearly defined proportions, in certain tissues and humors (intercellular fluid, lymph and blood). Maintaining these proportions is one of the most important for the proper functioning of the body. Some substances are involved in the structure of the body, so calcium and phosphorus are involved in the formation of bone tissue, iron enters the constitution of red blood cells. Serum iron is a form of iron found in the blood and used by the body to carry oxygen to the body's cells and tissues. When there is an imbalance in the level of serum iron in the body, various conditions such as anemia, chronic fatigue, difficulty concentrating and memory,

decreased immunity and digestive problems can occur. Therefore, it is important that serum iron levels are maintained within normal limits by including a balanced and iron-rich diet (meat, green leafy vegetables, legumes, nuts, seeds, etc.) as well as by iron supplementation under supervision a doctor in case of deficiencies or imbalances.

Although it is found in small quantities in the human body, magnesium is of particular importance at a structural and functional level. Determining and analyzing serum magnesium in people diagnosed with ASD is of major scientific and clinical importance. Magnesium is an essential mineral for the health of the human body, being involved in over 300 biochemical reactions. This mineral is necessary for the proper functioning of the muscles, heart, nerves and immune system. Magnesium also plays an important role in regulating blood pressure, maintaining bone health and energy metabolism [52].

Serum sodium is the main cation that is found outside the cellular space, influencing the absorption of water in the body. Serum sodium is a measure of the amount of sodium in the blood. Sodium is an important electrolyte in the body, responsible for maintaining water balance and osmotic pressure in cells. Sodium also plays a crucial role in the proper functioning of muscles and nerves. Potassium has the main role inside the cells, being frequently brought into the cell by Na/K - which acts as a transporter. It penetrates very slowly through the cell membrane and it is bound to the phosphate ion and the proteins in the cell. When imbalances occur in serum potassium levels, severe health problems can occur. Low potassium (hypokalemia) can lead to muscle cramps, fatigue, abnormal heart rhythms, or even arrhythmias, and high potassium (hyperkalemia) can cause muscle weakness, nausea, stomach pain, or even fatal arrhythmias.

Potassium imbalances can be caused by various factors, such as diet, use of certain medications, kidney or endocrine disease, vomiting or severe diarrhea. It is important to monitor the level of serum potassium and take measures to maintain its proper balance in the body. 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 [53].

Imbalances in the level of ionic calcium can lead to serious ailments. When the level of calcium in the blood is too low (hypocalcemia), symptoms such as muscle cramps, nausea, dizziness, muscle spasms or even convulsions may occur. On the other hand, too much calcium in the blood (hypercalcemia) can cause fatigue, confusion, muscle weakness or even kidney damage.

Mineral-rich foods are important for maintaining optimal health. There are many foods that are rich in these essential minerals, such as: spinach, black beans, pumpkin seeds and nuts, which provide plenty of iron. In addition, seafood such as tuna and salmon are excellent sources of selenium and zinc, which play an important role in the proper functioning of the immune system. Green leafy

vegetables such as lettuce and cabbage are rich in calcium, which is vital for bone health. Bananas and sweet potatoes provide plenty of potassium, which is essential for maintaining the body's electrolyte balance. We also have almonds, which are an excellent source of magnesium, which contributes to the proper functioning of the heart and nervous system. Sesame seeds are a rich source of calcium, which is essential for healthy bones and teeth. Tomatoes and carrots are rich in potassium and vitamin A, which are essential for maintaining water balance and healthy skin [52].

2.6. Nutritional needs of the child

Carbohydrates are an essential element of the diet and an important energy resource for the body, ensuring the energy requirement only for half a day and at rest [54].

Table no. 3 - The need for nutrients in children aged between 0-7 years

	PROTEINS	**GLUCIDES**	**LIPIDS**
0-1 year naturally fed	2-3 g/ kg body/ day	10-12 g/ kg body/ day	5-6 g/ kg body/ day
0-1 year artificially fed	3-4 g/ kg body/ day	12 g/ kg body/ day	6 g/ kg body/ day
1-3 years	3-4 g/ kg body/ day	10-12 g/ kg body/day	4-5 g/ kg body/ day
3-7 years	2-2,5 g/ kg body/ day	10 g/ kg body/ day	2-3 g/ kg body/ day

Source: [54].

Children with ASD have food intolerances, especially gluten. Ketogenic diet could improve symptoms in autism. This diet has very good results for epilepsy patients. In many cases of children with ASD, we also have epilepsy as an associated diagnosis, so this diet would be indicated.

The ketogenic, high-fat, adequate-protein, low-carbohydrate diet has had a resurgence of interest in the past decade for the treatment of difficult-to-control seizures in children. This review traces its history, looks at uses and side effects, and discusses possible alternatives and possible mechanisms of action of the diet. Finally, this review looks at possible future uses of the ketogenic diet for conditions other than epilepsy. In medicine, it is mainly used to relieve severe epileptic seizures. Carbohydrates in food are normally converted into glucose, which is then released into the blood throughout the body, which is especially important for use as fuel for the brain. However, if the diet is very low in carbohydrates, the liver will convert it to fat [55]. The results of a meta-analysis show that appropriate eating behavior is 4 times more difficult in children with autism. They are inattentive, anxious or agitated during meals and have extreme food selectivity (with preferences for certain colors, textures) and eating rituals.

The relative influence of each food can be revealed by assessing nutrient exposure. Reviews can be found in the literature describing the nutritional effects of individual foods, such as vegetables [56], nuts [57], and whole grains [58].

For example, the beneficial effects of nuts and seeds can be attributed to their high levels of unsaturated fat, soluble fiber, plant protein, vitamins, minerals, and phytochemicals, although disentangling the effects of individual components is extremely difficult due to synergistic effects from multiple components.

THE INTESTINAL MICROBIOME AND ITS IMPORTANCE

The pathogenesis of autism spectrum disorder is not fully understood, but 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 it is a two-way communication system. A growing body of evidence suggests that the gut-brain axis participates in the pathogenesis of autism spectrum disorder [59]. Despite its intrinsic complexity, the microbiome is partially inherited, in a process likely involving "small-world" power-law construction dynamics in neonates [60]. Research on the human microbiome has gone from a new field to a booming area of medical research, with more than $1.7 billion spent in the last decade alone [61]. According to the National Institutes of Health, the microbiome or balance of organisms in the digestive tract is responsible for postnatal development. Our children inherit two essential sets of genetic material from their parents. The first is the combined genetic information of the mother and the father and the second is the genetics of the thousands of microbial strains that make up the balance of the digestive tract - MICROBIOME.

Our microbiome is made up of trillions of microbes. These microbes play a key role in promoting development. Analyzing the microbiome is extremely important for people with autism. Recently, the medical world has become increasingly aware of the importance of the populations of microorganisms that colonize the human gut, known collectively as the microbiota. Recently, the medical world has become increasingly aware of the importance of the populations of microorganisms that colonize the human gut, known collectively as the microbiota. Recent studies have linked the imbalance of microbiota composition not only to inflammatory bowel diseases, but also to chronic diseases such as depression, autism, obesity, multiple sclerosis, cancer, rheumatoid arthritis or Parkinson's disease and many others [62]. Recent studies have linked the imbalance of microbiota composition not only to inflammatory bowel diseases, but also to surprising chronic diseases such as depression, autism, obesity, multiple sclerosis, cancer, rheumatoid arthritis or Parkinson's disease and many others. Because the microbiome can influence human homeostasis, it can be used as a target for various nutritional interventions to support and promote health (eg,

optimizing fiber intake by administering prebiotics and probiotics) [63]. On the other hand, substances that disrupt the microbiome (eg, unbalanced diet) also attract attention because they can cause dysbiosis, which can have negative effects on human health in the long term [64]. Much of the current interest stems from the ability of food additives, chemical residues (pesticides and veterinary drugs), antibiotics or other environmental contaminants to cause biologically relevant microbiome disruptions [65]. There is now clinical and biological evidence that stress can influence gut microbiota and the development of neurological diseases [66]. In particular, the changes at the neurological level that occur during stress are reversed by probiotics [67]. An imbalance of the maternal flora during pregnancy with insufficient colonization of the fetal flora can lead to the development of neurological diseases such as autism or schizophrenia [68].

The strong correlation of gastrointestinal symptoms with autism severity indicates that those children with more severe autism are likely to have more severe gastrointestinal symptoms and vice versa. It is possible that the symptoms of autism are accentuated because of the gastrointestinal problems they have [69]. Microbiomes are complex and dynamic networks of microorganisms (bacteria, viruses, fungi, archaea) that adapt to their specific habitats (e.g. humans, soil, plants, water, animals, growth sites along the food chain) and live functional [70]. Microbiome ecosystems exert mutual influence, even when they are physically separated (eg, animals and soil) [71]. In addition, microbiomes are very sensitive to environmental conditions and exposure to different types of substances. In humans, various factors (eg, genetics, diet, drugs, lifestyle, oxygen, pH) contribute to the formation of microbiome subpopulations in different parts of the gastrointestinal tract [72].

In recent years, a very large increase of the children with autism spectrum disorders has been observed, and these persist throughout life, so early diagnosis and therapeutic intervention immediately after diagnosis are recommended to increase the chances of improving the associated symptoms and to avoid the onset problems with school integration, problems that can lead to school dropout. Thus, to confirm the diagnosis and the severity of the form, a multidisciplinary team consisting of pediatricians, psychiatrists and psychologists (specializing in autism) is required. Parents must be aware of the seriousness of the diagnosis and understand that autism therapies, although very expensive, help the child a lot. Biochemical balance and an intestinal microbiome with a composition within normal limits are extremely important for proper development.

PART II - PERSONAL CONTRIBUTIONS EXPERIMENTS

DESCRIPTION OF THE PROCEDURE FOR FORMING GROUPS OF SUBJECTS

In these research 81 children and young people participated, some of whom were diagnosed with disorders of the autistic spectrum who are included in the 1st research group and the control group consisting of neurotypical children and young people who are included in the 2nd group of research.

The participants in batch 1 are between 3 and 23 years old. After we identified the children and their parents agreed to be part of the scientific study we wanted to carry out, we handed them a consent agreement that they signed. The next step in the elaboration of the work consisted in carrying out some biochemical analyzes and the analysis of the microbiome. Due to the fact that some of the children with autism cannot have blood tests taken due to the severity of the symptoms, they only performed certain tests or only microbiome analysis. There are also subjects who did not done all the investigations for other reasons. Thus, we divided the participants into groups according to the analyzes investigated, being separate experiments but all incorporated in the thesis.

The control group consists of neurotypical children and young people aged between 3 and 23 years and their parents signed the consent agreement before the research began. The children and young people in the control group did not perform all the analyzes for various reasons. All the data obtained during the medical analyzes were statistically analyzed with the SPSS 20 program. I also used in the research a questionnaire with 48 questions that I created in Google Forms and posted it to be completed on WhatsApp groups for children with ASD. By completing it by the parents, we wanted to see what are the frequent symptoms of these children, what investigations and recuperative plans they have carried out. A total of 45 parents completed the questionnaire. All the data obtained from the google forms questionnaire were analyzed statistically with the SPSS 20 program.

Description of subject groups

Group 1 of subjects is represented by children and young people diagnosed with autism spectrum disorders and group 2, the control group, is made up of neurotypical children. The group of subjects with ASD was divided in certain studies into two groups, group 1 who were following the treatment given by the psychiatrist and group 2 who did not receive treatment. The age of the participants in the subject groups is between 3 years and 23 years. More male than female children participated in the research.

A number of 8 experiments were carried out regarding blood tests and intestinal microbiome, correlations between the markers detected in autism, as well as the presentation of parent's answers to a questionnaire consisting of a number of 48 questions. After analyzing the results obtained, in accordance with the 10 general objectives of the research paper, I will present the general conclusions. In the first practical part of our research, we considered it important to carry out the statistical interpretation of the results of the biochemical analyzes of some markers which, according to the specialized literature, if they present values outside the normal limits, these could be the basis of the aggravation of the symptoms in autism. We obtained relevant results in the analysis of glutathione peroxidase data, glucose, total proteins, vitamin D and testosterone analysis. In the first research, regarding oxidative stress and combating it, we observe that in the group of children and young people with ASD who take medication, we have values outside the normal limits in a percentage of 27.78%, i.e. 5 participants. In the case of children and young people with ASD who do not take medication, only one participant has low glutathione peroxidase values. We also observe that in the group formed by neurotypical children and young people, no participant has glutathione peroxidase values outside the normal limits. The occurrence 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 received from the specialist doctor, due to its chemical composition, can influence the biochemical balance and worsen the symptoms. The low level of glutathione shows us the existence of imbalances in the mechanisms that the human body uses to fight against the negative effects of oxidative stress. In the experiment regarding the dosing of biochemical values of glucose, following the statistical interpretation of the data and according to the result of the Mann-WhitneyU test where $p = 0.02 < 0.05$, it is observed that we have statistically significant differences regarding the distribution of glucose values in children and young people diagnosed with ASD compared to neurotypicals.The group formed by children and young people with ASD has a percentage of 23.33%, i.e. 7 participants with values outside the normal range and in the group formed by neurotypical children and young people, no participant has values outside the normal range. A cause of increased glucose values in the group of children and young people with ASD could be inadequate nutrition.

After analyzing the results, we notice that there are more participants who have glucose values outside the normal limits in the group formed by children and young people with ASD who take medication, i.e. a percentage of 26.67%, i.e. 4 participants, compared to the group formed by children and young people with ASD who do not take medication, in which we have a percentage of 20.00%, i.e. 3

participants. Since the difference is small, we can say that the medication taken by people with ASD does not seem to influence the glucose values in our case.

In the case of determining the blood parameter - total proteins, following the statistical interpretation of the data, according to the result of the Mann-WhitneyU test where $p = 0.023 < 0.05$, it follows that there are statistically significant differences between the results of the biochemical analysis of total proteins obtained by children and young people with ASD and between the results obtained by neurotypical children and youth from the control group, so we have statistical significance. Determining the level of total proteins is important to make an assessment of the nutritional status.

In the following experiment, we considered it important to carry out the determination of biochemical analyzes for vitamin D because this vitamin has an essential role in the good development of children. Following the statistical interpretation of the data, according to the result of the Mann-WhitneyU test where $p = 0.000 < 0.05$, we observe that we have statistically significant differences between the results of the vitamin D biochemical analyzes obtained by the children and young people with ASD and the neurotypical ones from the control group. After comparing the vitamin D values, it can be observed that in the group formed by children and young people with ASD who take medication, we have a percentage of 73.33%, i.e. 11 participants who have values outside the normal limits, more than in the group formed by children and young people with ASD who do not take medication, where we have a percentage of 40% i.e. 9 participants who have values outside the normal values.

Medicines are prescribed by the psychiatrist to improve the symptoms in autism, but they can influence the values of vitamin D, which has an essential role in the good physical and especially mental development of children.

In the group formed by children and young people diagnosed with ASD, we have a percentage of 66.67%, i.e. 20 participants who have values outside the normal values, and in the control group we have a percentage of 20.00%, i.e. 6 participants who have values outside the normal values, a fact that worries 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 values of vitamin D are found in large numbers in all groups of participants, which is not good.

In the following research experiment, we determined the biochemical values of the electrolytes. Following the analysis and statistical interpretation of the data, we obtained relevant results for total calcium and serum sodium.

Following the statistical interpretation of the data, for total calcium, by using the non-parametric Mann-WhitneyU test where $p = 0.049 < 0.05$, we observe that there are statistically significant differences between the total calcium values for

participants with ASD who take medication and those who do not take medication, so we have statistical significance.

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 skeletal system, the cardiovascular system, the muscular system, the central nervous system and other vital organs, and it is important to correct them in time, because they can have serious health consequences.

After comparing the serum sodium values, we notice that in the group formed by children and young people with ASD who take medication we have a percentage of 33.33%, i.e. 5 participants who have values outside the normal limits and in the group formed by children and young people with ASD who do not take medication, we have only one participant who has values outside the normal range. We can say that the medication administered to children with autism can influence serum sodium values. When there are imbalances in the serum sodium level, various health problems can occur and in the case of children with autism, the symptoms are accentuated. These imbalances can be treated with intravenous fluids or by adjusting dietary sodium intake. It is important to regularly monitor serum sodium levels to prevent more serious health problems.

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

In the following experiment, we performed the determination of testosterone values, because we considered that there is a strong connection between the level of testosterone and the aggravated symptomatology of the participants with ASD. In the case of children with autism, a high level of this biochemical marker leads to the appearance of aggression, a fact that hinders the chances of a good school and social integration. Following the statistical interpretation of the data, according to the result of the T test, we observe that the value of $p = 0.003 < 0.05$, from which we can conclude that we have statistically significant differences regarding the distribution of testosterone values in children and young people diagnosed with ASD compared to those neurotypical.

After analyzing the data, we can say that high testosterone values influence the behavioral manifestations of children and young people with ASD, because a fairly large percentage, 38.89%, i.e. 7 participants from the group of children and young people with ASD have high testosterone values and, in the group, formed by neurotypical children and young people only one participant has a high value for this blood parameter.

The specialized literature mentions the fact that a high level of testosterone leads to the appearance of aggression in boys, a fact confirmed by our research.

Analyzing the intestinal microbiome

In order to better understand the structure of the intestinal microbiota in children with autism spectrum disorders of different ages, we considered that it is important to evaluate the intestinal microbial population. Through this experiment we wanted to identify if, in the case of children and young people with ASD, there is a causal link between intestinal transit disorders such as chronic constipation, the 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 the bioindicators, the LPS-positive bacteria and the fibers that degrade the microbiota found in the intestinal microbiome.

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.

After comparing the values of the faecal pH bioindicator, it is observed that in the group formed by children and young people with ASD who take the medication prescribed by the psychiatrist, we have values outside the normal limits in a percentage of 40.00%, i.e. 6 participants, and in the case children and young people with ASD who do not take medication, a percentage of 20%, i.e. 3 participants have values outside the normal limits.

After analyzing the fecal pH results, we notice that in the group of children diagnosed with ASD, we have values outside the normal limits in a percentage of 30.00%, i.e. 9 participants, and in the control, group consisting of neurotypical children and young people, a percentage of 16.67%, i.e. 5 participants have values outside the normal limits.

We observe the fact that in the group of 30 nerurotypical children and young people we have 5 high values for faecal pH, which is quite worrying. Children should have a balanced diet in order to have the best possible physical and cognitive development.

Following the statistical interpretation of the results obtained with the biodiversity bioindicator, because with the Mann-WhitneyU test we have $p = 0.014 < 0.05$, it follows that we have significant differences regarding the distribution of Biodiversity values in children and young people diagnosed with ASD and in neurotypical subjects from the control group. After comparing the biodiversity values, we notice that in the group of children and young people with ASD we have values outside the normal limits in a percentage of 40.00%, i.e. 12 participants, and in the group of neurotypical children and young people we have a percentage of 13, 33%, i.e. 4 participants who had values outside the normal

limits. The large number of children and young people with ASD who have values outside the normal limits should make us sound the alarm.

Also, in the group formed by children and young people with ASD who take the medication prescribed by the psychiatrist, we have values outside the normal limits in a percentage of 66.67%, i.e. 10 participants and in the group of children and young people with ASD who do not take medication, we have values outside the normal limits in a percentage of 13.33%, i.e. 2 participants. We can thus observe that the medication influences the biodiversity bioindicator values.

Following the statistical interpretation of the results obtained in Lactate Production by subjects with ASD and by neurotypical subjects, in the Mann-WhitneyU test we have $p = 0.003 < 0.05$, so it follows that we have significant differences regarding the distribution of values in children and young people diagnosed with ASD and in subjects neurotypicals from the control group. The median test indicates the same thing, where $p = 0.004 < 0.05$, so we have statistically significant differences between the median values of the two groups.

After analyzing the results, we notice that we have a large imbalance at the level of the Lactate Production bioindicator in the group of children and young people diagnosed with ASD, because a percentage of 66.67%, i.e. 20 participants have values outside the normal limits. In the group formed by neurotypical children and young people we have a percentage of 20%, i.e. 6 participants who have values outside the normal limits.

After comparing the milk production values obtained by the group of children and young people with ASD who take the medication prescribed by the psychiatrist and the group of children and young people with ASD who do not take medication, we notice that in both groups we have the same percentage of 66, 67%, i.e. 10 participants who have values outside the normal limits. These results show us that the medication does not influence the level of bacteria in the intestinal microbiome.

We notice that in the control group we also have a percentage of 20%, i.e. 6 participants who have values outside the normal limits, which is a worrying fact. The percentages in both groups are quite high and because of this, measures should be taken to improve this imbalance. Parents should be more attentive to children's nutrition because imbalances in the level of bioindicators in the intestinal microbiome can affect their proper development.

After comparing the Acetate Production values obtained by children and young people with ASD and by neurotypical children and young people, we notice that in the group formed by children and young people with ASD we have a percentage of 36.67%, i.e. 11 participants who have values outside normal limits and in the group of neurotypical children and young people we have a percentage of 6.67%, i.e. 2 participants who have values outside the normal limits.

After analyzing the data, we notice that in the group formed by children and young people with ASD who take the medication prescribed by the psychiatrist, we have a percentage of 46.67%, i.e. 7 participants who have values outside the normal limits, compared to the group of children and young people with TSA who do not take medication where we have a percentage of 26.67%, so only 4 participants have values outside the normal limits for Acetate Production. In this case we can say that the medication can influence the values of the bioindicator Acetate production.

After the statistical interpretation of the results, at the Mann-WhitneyU test we have $p = 0.047 < 0.05$, so it follows that there are statistically significant differences between the values obtained after analyzing the Mucin Degradation bioindicator of subjects with ASD and neurotypical ones. The median test indicates the same thing because $p = 0.045 < 0.05$, so we have statistically significant differences between the median values of the two groups.

After carrying out the statistical interpretation of the data for LPS-positive bacteria, we notice that in the group formed by children and young people with ASD we have a percentage of 30.00%, that is, 9 participants have values outside the normal limits and in the group of neurotypical children and young people we have a participant who has values outside normal limits.

After comparing the values for LPS-positive bacteria, we notice that in the group formed by children and young people with ASD who take medication we have a percentage of 46.66%, i.e. 7 participants who have values outside the normal limits and in the group of children and young people with TSA who do not take medication, we have a percentage of 13.33%, that is, 2 participants who have values outside the normal limits, which shows us that the medication can influence the level of the LPS Bacteria bioindicator - positive. The rather large number of children and young people with ASD who have values outside the normal limits makes us think that the imbalance in the level of bioindicators in the intestinal microbiome can be the basis of the aggravation of symptoms in autism.

The intestinal microbiome has a very important role in maintaining the health of the body and that is why it is important to analyze the intestinal microbiome if problems occur at the gastrointestinal level or other diseases.

In this experiment, we analyzed and interpreted from a statistical point of view the results of the analysis of the bacteria in the intestinal microbiome. We noticed that there are imbalances at the level of the following bacteria: *Citrobacter* spp., *Enterobacter* spp., *Escherichia* spp., *Pseudomonas* spp. and *Serratia* spp. After comparing the values for the bacterium *Citrobacter* spp., it is observed that in the group consisting of children and young people with in ASD, we have a percentage of 53.33%, i.e. 16 participants who have values outside the normal limits and in the group of neurotypical children and young people we have a percentage of 40%, i.e. 12 participants who have values outside the normal

limits. We notice, however, that in both groups we have a large number of participants who have values outside the normal limits, which shows us that there are people in the two groups who present an imbalance at the level of the *Citrobacter* spp bacteria and this is worrying. A proper and balanced diet could help alleviate this problem. Probiotics can also restore the intestinal microbiome, leading in the case of children and young people with autism to an improvement in symptoms and to achieving a better intestinal transit.

After analyzing the *Citrobacter* spp. values, we notice that in the group of children and young people with ASD who take medication we have a percentage of 73.33%, i.e. 11 participants with values outside the normal limits and in the group of children and young people with ASD who do not take medication, a percentage of 33.33%, i.e. 5 participants have values outside the normal limits.

After comparing the values of *Enterobacter* spp, we notice that in the group formed by children and young people with ASD we have a percentage of 26.67%, i.e. 8 participants who have values outside the normal limits and in the group of neurotypical children and young people we have a percentage of 10.00%, i.e. 3 participants who have values outside the normal limits.

After comparing the values of Enterobacter spp., we notice that in the group formed by children and young people with ASD who take the medication prescribed by the psychiatrist, we have several values outside the normal limits, we have a percentage of 33.33%, i.e. 5 participants who have found values outside the normal limits compared to the group of children and young people with ASD who do not take medication, where we have a percentage of 20.00%, that is, 3 participants have values outside the normal limits. Regarding the results obtained with Escherichia spp bacteria, after they were interpreted statistically using the Mann-WhitneyU test where we have $p = 0.017 < 0.05$, it follows that there are statistically significant differences regarding the distribution of values for *Escherichia* spp. for the group of children and young people diagnosed with ASD and for neurotypical subjects in the control group. The median test indicates the same thing because $p = 0.020 < 0.05$, so we have statistically significant differences between the median values of the two groups.

Regarding the analysis of the values for the bacteria *Escherichia* spp., we notice that the group formed by children and young people with ASD presents a greater imbalance at the level of the bacteria *Escherichia* spp. than the control group formed by neurotypical children and young people. In the group formed by children and young people with ASD, we have a percentage of 23.33%, i.e. 7 participants who have values outside the normal limits and in the group of neurotypical children and young people we have a percentage of 10.00%, i.e. 3 participants who have values outside normal limits.

We also note that in the group of children and young people with ASD who take medication we have a percentage of 33.33%, i.e. 5 participants who have

values outside the normal limits and in the group of children and young people with ASD who do not take medication, we have a percentage of 13.33%, i.e. 2 participants who have values outside the normal limits.

After comparing the values of *Pseudomonas* spp., it is observed that in the group formed by children and young people with ASD we have a percentage of 23.33%, i.e. 7 participants who have values outside the normal limits and in the group of neurotypical children and young people we have a percentage of 13.33%, i.e. 4 participants who have values outside the normal limits.

Regarding the analysis of *Serratia* spp. values, we note that in the group of children and young people with ASD we have a percentage of 33.33%, i.e. 10 participants who have values outside the normal limits and in the group of neurotypical children and young people we have a percentage of 13.33%, i.e. 4 participants who have values outside the normal limits.

We can conclude that there is a rather large imbalance in the level of certain bacteria present in the intestinal microbiome, a fact that may underline the aggravation of symptoms in autism.

In this experiment we also wanted to identify if there is an imbalance in the fiber level that degrades the microbiota and if this imbalance could influence the state of health.

After comparing the values at the *Bifidobacterium* fiber level, we notice that in the group of children and young people with ASD there is an imbalance because we have a percentage of 33.33%, i.e. 10 participants who have values outside the normal limits compared to the control group of children and neurotypical young people where we have a percentage of 20%, i.e. 6 participants who have values outside the normal limits.

After analyzing the values obtained for *Bifidobacterium*, we notice that in the group of children and young people with ASD who take medication we have a percentage of 53.33%, i.e. 8 participants who have values outside the normal limits and in the group of children and young people with ASD who do not they take medication, a percentage of 13.33%, i.e. 2 participants have values outside the normal limits. The medication given by the psychiatrist can have the unwanted consequence of an imbalance in the *Bifidobacterium* fiber level.

Regarding the statistical interpretation of the results obtained with *Ruminococcus* by subjects with ASD and those with neurotypicals, we note that in the Mann-WhitneyU test we have $p = 0.048 < 0.05$, which shows us that we have significant differences regarding the distribution of values in children and young people diagnosed with ASD and in neurotypical subjects from the control group. The median test indicates the same thing, because $p = 0.041 < 0.05$, so we have statistically significant differences between the median values of the two groups.

After comparing the values, we notice that in the group formed by children and young people with ASD we have a percentage of 23.33%, i.e. 7 participants

who have values outside the normal limits and in the group of neurotypical children and young people we have a percentage of 6.67 %, i.e. 2 participants who have values outside the normal limits.

We notice that in the group of children and young people with ASD who take medication we have a percentage of 33.33%, i.e. 5 participants who have values outside the normal limits and in the group of children and young people with ASD who do not take medication a percentage of 13.33%, i.e. 2 participants have values outside the normal limits. Participants from the group of children and young people with ASD present higher microbiome imbalances than participants from the control group. This can be influenced by the diagnosis of autism but also by inadequate nutrition and further research should be carried out in which emphasis is placed on the connection between nutrition and the microbiome. Due to an inadequate diet, specific nutrients important for making learning more efficient 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 individuals. Modulation of the gut microbiota in ASD individuals with gastrointestinal disorders appears to be a promising target for future medical research.

Diet plays an important role in determining gut microbial composition and function; therefore, a selective diet can influence the gut microbial community. Probiotics have recently been used in several clinical trials as an adjunctive treatment to conventional therapy in patients with autism spectrum disorders.

Children with autism spectrum disorder and gastrointestinal symptoms showed many imbalances in the gut microbiome. A probiotic approach should act as a means to restore a healthy microbiota. To confirm the efficacy of probiotic therapy in children with autism spectrum disorders, larger studies with laboratory analysis of the microbiota are needed for better direction.

Further research on the brain-gut-microbiome axis may lead to new methods of identifying gastrointestinal disorders in children with autism spectrum disorders and new treatments to reduce symptomatology 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 correlation between the variables, because the value of the probability coefficient p was higher 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 a possible increase in sodium is associated with an increase of iron. We also have the value of the coefficient of determination R2Linear = 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 us that we have a weak to moderate negative correlation indicating that a possible decrease of phosphorus is associated with a decrease in magnesium. We also have the value of the coefficient of determination R2Linear = 0.111.

The questionnaire addressed to parents

After analyzing and interpreting the answers provided by the parents, we notice that a percentage of 71.1% of them mentioned that the specialist doctor recommended them to carry out some detailed biochemical analyses. To discover the cause of a problem, you must analyze all the possibilities that exist. If more complex investigations are carried out, certain biochemical imbalances can be identified that can be the basis of the aggravation of symptoms in autism.

We notice that in the case of vitamin D, approximately half of those who took the dosage of this biochemical marker had low values and we believe that more investigations should be carried out in this regard. A suitable diet with products rich in vitamin D can help its absorption more than drugs, the latter being chemical substances that implicitly have adverse reactions. Proper nutrition can help children with autism much more.

Another important element that emerges from the interpretation of the responses is that 80% of those who had the microbiome analysis had negative results, but after receiving treatment from the specialist their condition improved, which is a good thing. It was observed that a percentage of 42.2% of children with autism also have food intolerances, a fact that is worrying and measures must be taken in this regard.

A percentage of 71.1% of parents believe that an inadequate diet can lead to the worsening of symptoms in autism. This fact is also confirmed by the research carried out in recent years. Regarding the biochemical analysis of glutathione, a percentage of 73.3% of the respondents declared that they did not perform this analysis, which is not good because glutathione plays an important role in the proper functioning of the body.

What we wanted to highlight following the creation 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 state of health depends can improve through teamwork.

All partners involved in the recovery of children with ASD must have the good of the children as their common interest. Children with autism usually have a deficit in social functioning. Society should get more involved in helping these families because they too are members who should become useful to society.

CONCLUSIONS

The participants from the group of children and young people with ASD present imbalances both at the level of the analyzed biochemical markers and at the level of microbes of the intestinal human greater than the participants in the control group. Diet plays an important role in determining gut microbial composition and function; therefore, a selective diet can influence the gut microbial community. Probiotics have recently been used in several clinical trials as an adjunctive treatment to conventional therapy for patients with autism spectrum disorders. To confirm the efficacy of probiotic therapy in children with autism spectrum disorders, larger studies with laboratory analyzes of the microbiota are needed for better direction. Due to improper nutrition, specific nutrients important for harmonious development 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 individuals.

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.

Possible treatments for autism spectrum disorders include medications that have been developed to treat other conditions that secondarily interfere with some of the symptoms of autism.

Microbiome analysis may shed light on autism. Gastrointestinal disorders can affect the health of a child diagnosed with ASD, making certain symptoms worse. Because the causes of autism are unknown, the disease cannot be prevented. The consequences of autism can only be reduced through the timely recognition of specific symptoms as well as through quick and effective treatment. Genetic testing can be helpful for a couple who want to have children and have or have had a family history of autism, as there are several determinants that are thought to play a role in the development of the condition. Carrying out detailed biochemical analyzes during pregnancy are extremely important because a metabolic imbalance can favor the appearance of autism in the child. It is desirable that family doctors are aware of the importance they have in the proper development of the pregnancy and must guide the mother at all times so that the result is the desired one. Children with autism are unable to realize what is

happening around them, and this thing will accompany them throughout their lives. As a result, they face difficulties in communicating with others and adapting to the environment.

In conclusion, it can be said that autism spectrum disorders appear more and more frequently in recent years. They appear in childhood and persist throughout life, so early diagnosis is recommended. Thus, a multidisciplinary team consisting of pediatricians, psychiatrists and psychologists (specializing in autism) is needed to confirm the diagnosis and its severity. Potential treatments for the manifestations of autism spectrum disorders include medications 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.

We note that our research contributes to expanding the current knowledge base regarding biochemical imbalances in autism and provides new motivations for further investigations. The identification of blood markers with a determining role in autism spectrum disorders could be a significant advance in the identification of new methods of therapeutic intervention.

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