



Etiotropic and Pathogenetic Therapy of Autism Spectrum Disorder: Case Series of 6 Children

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Abstract

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that reveals itself by social communication problems, restrictive/repetitive behavior, and language impairment. ASD is a growing problem in the USA and in the world with no commonly-accepted etiology resulting in the absence of effective methods of treatment. Based on more than 80 scientific publications we are proposing the following understanding of ASD: it is a genetic disorder, in which some changes in DNA are resulting from a congenital mother to fetus transmitted infection and maternal immune activation. The infections and maternal immune activation result in oxidative stress and production of pro-inflammatory cytokines and other mediators. Based on this understanding, we developed a method of long-term etiotropic and pathogenetic therapy tailored to major chronic/latent infections, inflammation and immune system aberration. We present six cases of ASD treatment, which included the antiviral medication Valacyclovir and five nutritional supplements. The presented results are based on five cycles of treatment continued for 5 months. In all six cases the treatment resulted in social communication skills and behavioral improvements well as positive changes in the physical and psychological conditions. These improvements covaried with a tendency to normalization of blood and immune parameters. Social communication skills, behavioral, physical and psychological improvements also positively affected parents whose subjected quality of life increased over course of the treatment. According to parents of these children, the proposed treatment had superior efficacy compared to other types of treatment that their children underwent before.

Keywords: Autistic Disorder; Behavior; Immune System; Infection; Inflammation; Nutritional supplements; Treatment; Valacyclovir

Introduction

Based on more than 80 scientific publications we are proposing the etiopathogenesis of ASD. Our path to determining this etiopathogenesis is as follows: There are several studies indicating that ASD is a genetic disorder [1,2] that is the result of a congenital, mother-to-fetus transmitted infection and maternal immune activation [3-8]. The infections that have been shown to be associated with ASD include but are not limited to: rubella virus [7,9], human herpes virus 1/2 or HSV [10,11], human herpes virus-4 or EBV [12], human herpes virus-5 or CMV [13,14], human herpes virus-6 or HHV-6 [15], Chlamydia and Mycoplasma spp. [15]. In addition, the infection and maternal immune activation result in the production of pro-inflammatory cytokines and other mediators of inflammation [4,16-19]. Persistent infection and inflammation induce oxidative stress and the expression/suppression/methylation of susceptible genes that participate in the brain development [1,2,20-24]. As a result of these genetic alterations, the fetus's brain can develop atypically [25-29], and fetal brains can be additionally challenged by neuroinflammation, which is a common feature of ASD children [30-33]. Moreover, according to [31] this neuroinflammation can be classified as chronic encephalitis. Furthermore, chronic inflammation, genetic alterations, and immune system aberration contribute to gastrointestinal tract diseases, folate deficiency and neurohormone alterations, which are all common in ASD children [34-42]. All these factors affect a child's brain development and lead to an autistic phenotype, which may be noticed in the early period of the child's life [43-47], even though ASD is not typically officially diagnosed until the child is 3 to 4 years old [48]. Infection,

inflammation, aberrant immune system, genetic changes, GI tract diseases, folate deficiency and altered neurohormone levels comprise the signs and symptoms typical of ASD [1,49-53].

In this case series, we present 6 cases of ASD treatment according to a regimen tailored to chronic/latent infections, inflammation, and immune system aberration. The cases presented represent the first 6 cases that have been treated in accordance to the regimen including the antiviral medication Valacyclovir, and five nutritional supplements. The supplements were: olive leaf, elderberry, and astragalus root extracts, amino acid L-lysine, and probiotic BC-30.

Valacyclovir is an Acyclovir prodrug. Compared to Acyclovir, it has a higher bioavailability-55% which is 2-4 times higher than the bioavailability of Acyclovir [54]. Valacyclovir shows the highest stability profile compared to other Acyclovir prodrugs [55].

Valacyclovir, as well as Acyclovir, was reported to be effective in the inhibition of herpes viruses: HHV-1 [56], HHV-2, HHV-3/VZV [57,58], and HHV-4/EBV [59,60], HHV-5/CMV [61]. In addition to effectiveness higher than Acyclovir, Valacyclovir is not more toxic than Acyclovir. In a 2002 clinical trial, Valacyclovir was both well tolerated by children and effective against Epstein-Barr virus, which cannot be inhibited by Acyclovir. The only side effects, which appeared in a small number of children, were headache in 8%, and gastrointestinal disturbances in 16% [62].

Valacyclovir is recommended for the treatment of viral infections in children by regulatory agencies in the countries where the treatments were performed [63,64]. Many studies demonstrated that Valacyclovir is well-tolerated by children [65] and rare side reactions included only headache and diarrhea [66] with no serious adverse effects (stated in the annotation to the drug) described in the meta-analysis, analyzing the existing studies on the usage of Valacyclovir for children.

L-lysine is an amino acid that is essential for the human organism. Usually L-lysine is obtained from the diet-from casein, phosphoprotein which is contained in dairy products [67]. L-lysine is essential for human organisms because it participates in the processes of proper growth, muscle protein building and metabolism [67]. L-lysine can inhibit herpes simplex virus by reducing the cyto-pathogenicity of the HHV-1[68-70]. The study by [68] demonstrated that patients who took L-lysine for 6 months had 2.4 times fewer cases of HHV-1 infection than patients in the placebo-control group. In another study by [70] it was shown that recurrence rate of HHV-1 was also lower within those who took L-lysine in comparison with the placebo group. It was also reported that L-lysine did not cause any toxic or side effects [68]. Additionally, L-lysine was shown to be effective against such conditions as anxiety, mood disturbances, and headaches [67], which are very common for children with ASD.

Elderberry extract in a nutritional supplement extracted from the black elder (*Sambucus nigra* L.). It has been used for many years in folk medicine because of its effectiveness against viruses, headache, and constipation [71]. It has antiviral (influenza A and B, RSV and parainfluenza), anti-inflammatory, immunomodulatory, and antioxidant activity. Several clinical trials showed that elderberry root extract was effective against influenza A and B virus infections [72-74], respiratory syncytial virus (RSV), and parainfluenza [72]. People with symptoms of influenza recovered on average 3 to 4 days faster than control groups in all the mentioned trials. In a placebo-controlled, double-blind study with 40 participants with influenza B virus infection, the full recovery of 90% of participants receiving the treatment was within 2 to 3 days, while the control group, which took a placebo showed complete recovery after 6 days [73]. In another study by [75], effectiveness of elderberry against HHV-1 was shown. In a clinical trial with 312 participants it was shown that elderberry extract positively influences both physical and mental health [76]. Furthermore, it was proven that there are no side or adverse effects to taking elderberry extract for all age groups including infants [74]. The effectiveness of the elderberry extract is due to cyanidin-3-glucoside and cyanidin-3-sambubioside flavonoids found in the plant [77,78], which modulate the immune system [74]. Flavonoids are also known to have antioxidant and prooxidant activities of low-density lipoprotein [77]. Anthocyanins which are a part of elderberry extract also possess anti-inflammatory properties [74].

Olive leaf extract has a long history of use in traditional medicine. Its hypoglycemic, antioxidant, antiviral and antimicrobial effects have been reported [79]. The polyphenols and flavonoids in olive leaves provides its spectrum of health benefits [79]. A double-blinded placebo-controlled trial [80] showed that olive leaf extract stimulates downregulation of genes that participate in the inflammatory process (EGR1, COX-2 and ID3). Another study by [81] demonstrated a significant reduction in the production of cytokines participating in the inflammatory processes. These results were supported by other studies [82,83]. Additionally, it was shown that olive leaf extract has antiviral and antioxidant activities [84,85]. For example, it decreased the viral infectivity of viral haemorrhagic septicaemia virus (VHSV) by 10-30% [85]. Antioxidant activity due to the presence of functional compounds found in olive leaves' flavonoid was

also reported [84]. Also, positive results were obtained by using olive leaf extract for Alzheimer's disease [86]. Among other plants, olive leaf extract, and particularly the biophenols contained in it, could provide the highest neuroprotection (74%) and strongest inhibitory activity against a number of enzymes participating in the development of Alzheimer's disease.

Astragalus root extract is extracted from *Astragalus membranaceus* Moench herb, and as well as the plants discussed above, was used for many years in traditional medicine because of its ability to stimulate the immune system [87]. It was observed that intake of astragalus root extract enhances the production of macrophages and lymphocytes [88,89]. These cells are key actors of the immune system. Similar to olive leaves and elderberry, astragalus contains flavonoids [87] that as previously stated, have a positive effect on the immune system. Moreover, astragalus extract can stimulate the growth of stem cells [88]. The antiviral, anti-inflammatory and antibacterial activities of the extract were shown by [87] and [90].

Bacillus coagulans probiotic contains a high concentration of spores ensuring its long-term stability and efficacy in the gut. This bacterium is reported to be non-pathogenic and an efficient producer of L(+) lactic acid [91]. It is capable of surviving in the acidic environment of the stomach, which explains its prolonged activity [92]. A main function of this probiotic is its ability to improve digestive health through activating more efficient digestive absorption [93] and modulation of gut microbiota [94]. It germinates in the small intestine and increases protein and carbohydrates absorption. A placebo-controlled clinical trial that included 141 children from 4-12 years old showed that *Bacillus coagulans* probiotic intake significantly improved the conditions of children with irritable bowel syndrome by relieving such symptoms as bloating, pain in the stomach, discomfort, urgency, etc. [95]. This property of probiotic is especially important for children with ASD since 70% were reported to have various problems of the gastrointestinal tract [40]. It was demonstrated that probiotic intake positively influences children with ASD because it targets two problems at once, microbiota-gut-brain axis, through balancing microbiota, and it inhibits the toxic effect of some compounds on the brain which leads to a decrease in ASD symptoms [96]. Moreover, several studies indicated that *Bacillus coagulans* probiotic intake increases immune response to viral agents. It was found that it increased production of T-cells as a response to viral infections [97]. It was also reported that *Bacillus coagulans* probiotic has the ability to inhibit 3 viruses of the herpes viruses' group: HHV-1, HHV-2 and HHV-3 [91]. The toxicological assessment of *Bacillus coagulans* probiotic showed that the supplement had no toxic or other side effects [92].

Methods

Participants

All children were self-referred by their parents. The study was not prospective and there were no exclusion criteria. The only inclusion criteria were, the request of parents of children with confirmed ASD diagnosis and the parents' written consent with a guaranteed non-disclosure of identities. The children were from different cities of two post-Soviet countries. All of the 6 children were males aged 2.5 to 5 years old.

Confirmation of Diagnoses

The study was conducted using written questionnaires and a telemedicine approach. The Skype® program was used for the web-mediated communication and consultations. All participants completed the questionnaire in accordance with DSM-5 via e-mail-based communication assessing the presence of the ASD symptoms and their severity to confirm the ASD diagnoses and prevalence of signs and symptoms. Additionally, the parents answered questions on the physiological and psychological condition of the children (presence of sleep disorders, anxiety, depression, hyperactivity, gastrointestinal tract issues and frequency of respiratory infections), on the history of mother's pregnancy, parturition, and the initial period of the child's life and on the types of treatments used prior to enrollment in this study.

It was explained to the parents that the study did not undertake the treatment of autism, but instead the study would be focused on the diagnosis and treatment of chronic/latent infections, reducing the inflammatory state and modulation of the child's immune system. Initially the parents were asked to have laboratory tests of their ASD-diagnosed child's immune status, presence of antibodies to viral and bacterial infections, and red and white blood cells counts. If the parents agreed, the tests were performed by local clinical diagnostic centers, which the parents were free to select.

Treatment Groups and Regimens

When the presence of viral/bacterial infection was confirmed by blood test results, the parents received the following recommendations for treatment:

All children were divided into two groups by age and treatment regimens:

Ages up to 3 years: 3 cycles of Valacyclovir treatment (125 mg., twice a day). Each cycle continues for 3 weeks with a 9-day break, along with the continuous intake of 125 mg of each supplement and 0.5 bln CFUs of probiotic per day without breaks.

Ages 3 to 7: 3 cycles of Valacyclovir treatment (250 mg., twice a day). Each cycle continues for 3 weeks with a 9-day break, along with the continuous intake of 250 mg of each supplement and 1 bln CFUs of probiotic per day without breaks.

The parents were recommended not to administer any other drugs or supplements apart from cases when there were conditions which required the intake of other medications, for example, appropriate painkillers for headaches or antibiotics in case of bacterial infections, etc.

Assessment of the Results

The assessments were based on the results of monthly or bi-monthly tests for detection of antibody levels to infections in blood samples, red and white blood cell count tests, and cellular immunity tests, as well as on the parents' observation of their children in terms of ASD symptoms and ASD-related disorders. Analyzing the changes in blood parameters, we were mainly focusing on six parameters of red and white blood cell counts and six parameters of cellular immunity in relation to the reference ranges established at the local diagnostic centers. These are: erythrocyte sedimentation rate (ESD), neutrophils count, lymphocytes count, erythrocytes count, mean corpuscular volume (MCV), red blood cell distribution by width, T-lymphocytes, T-suppressors, T-helpers, B-lymphocytes, NK cells, and immunoregulatory index (IRI) in relation to reference levels accepted in the local diagnostic centers. Selection of these parameters was based on the fact that they show the presence/absence of infection, inflammation, and any immune system aberrant changes. For example, erythrocytosis is a sign of hypoxia [98], which may be caused by oxidative stress [99] and of folate-deficiency anemia [100]. Lymphocytosis may be a sign of chronic herpetic infection [101,102]. Neutropenia usually indicates a viral infection [103], inflammation and a disrupted immune system [104]. Increased or decreased MCV, as well as increased RDW, point to a folate deficiency [105-107] and increased ESD is a sign of infection [108], and inflammation [109]. Reduced CD4-CD8 ratio (T-helpers/T-suppressors ratio) indicates chronic infection, anemia, or negative nervous system condition. Having low or high numbers of B cells, T cells, or NK cells indicates immune system aberration [110].

The assessment of the treatment results was conducted during videoconferences after a cycle of Valacyclovir (initiated by the parents). Before videoconferences, the parents were asked to prepare a written description of the observed changes in their children. To rule out potential subjectivity, we discouraged the parents from reporting assumptions and requested that they only report obvious changes and compare any changes with previous periods before treatment (if there were any in previous periods). Additionally, the parents were asked to fill out three questionnaires.

1) CARS-2 (Childhood Autism Rating Scale-2) [111], which is the upgraded version of the CARS, one of the instruments for the assessment of the severity of the autistic symptoms in children. The questionnaire consisted of 15 questions about autism symptoms, which were rated on a 4-point scale with an option of choosing 0.5 interval points. The sum of the points was interpreted in the following way: 0-30 - no autism; 30-36 - mild to moderate autism; 36 to 60 - severe autism. This questionnaire was not intended for diagnostic purposes. In this study it was used only for the evaluation of the dynamics of the treatment results.

2) PedsQL (Pediatric Quality of Life Inventory) [112]. This questionnaire is used to assess health-related quality of life in children. It can be used for the evaluation in both healthy children and in children with acute and chronic health conditions. It consists of 21 questions, where the severity of physiological, psychological, emotional, and school problems are rated by parents on a 5-point scale, where 0 - is the absence of problem and 4 - always a problem. The 0-4 scale items on a 0-100 scale are as follows: 0=100, 1=75, 2=50, 3=25, 4=0. The results were calculated for physical, psychological, and overall functionality. The scores were between 0 to 100, and 0 indicates the lowest functionality and 100 - the highest functionality.

3) Caregiver Strain Questionnaire [113] is used to assess the quality of life of parents who bring up children with emotional disturbances. Parents' quality of life reflects the severity of a child's disorder, so in order to additionally assess all the dynamics this questionnaire was also used. It consists of 21 questions, each assessing strains in terms of the extent to which certain occurrences or events (e.g., missed work, financial strain, trouble with neighbors) or feelings (e.g., sadness, guilt, worry) have been a problem as a result of the child's emotional or behavioral problems.

The response options range from one (not at all a problem) to five (very much a problem) on a Peabody Treatment Likert-type scale. With the purpose of clarity in the results section the scores were recalculated for a 0-100 score diapason, where 0 indicated the lowest possible quality of life (subjective) and 100 indicated the highest possible quality of life (subjective).

Results

The section is divided into 4 parts: Summary of CARS Scores, Summary of PedsQL Scores, Summary of Caregiver Strain Questionnaire Scores, and Description of Individual Cases.

Summary of CARS Scores

According to the responses to CARS, all children had positive changes after each cycle of the treatment. Although, by the 5th cycle, some of the children’s results may be interpreted as a complete recovery, it should not be considered this way, because parental bias may take place in these cases. The scores were used in order to assess the positive dynamics and to make a quantitative assessment in addition to the qualitative assessment provided in the d) part of this section. The calculated scores for each child are represented in Figure 1. It was seen that parents noticed positive changes after almost every cycle of treatment. Nevertheless, it was seen that after the third cycle the dynamics tended to slow, while the most noticeable changes occurred during the first three cycles.

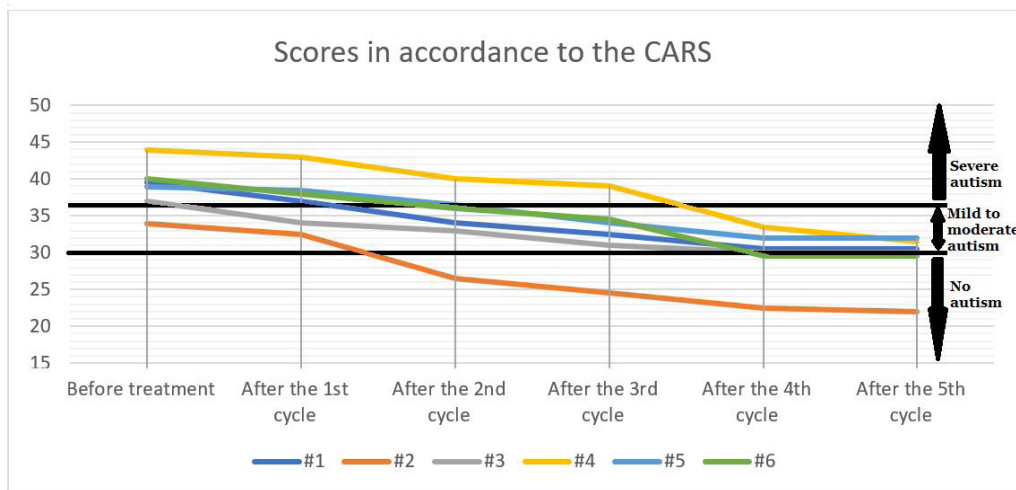


Figure 1: The graphical representation of the scores of the CARS given by parents of each child. The maximum possible score is 60, indicating the most severe form of autism (in parents’ perception) and the minimal score is 15, indicating the complete absence of autistic symptoms. Although in cases #2 and #6 the results by the 5th cycle may be interpreted as the absence of autism, it should not be perceived this way. Nevertheless, it was seen that the parents of each child noticed improvements after almost every cycle of the treatment. The tendency of the lines to decrease indicates the improvements of the children’s autism-like behavior and the shift from a severe form of autism to a milder one

The mean scores of the CARS questionnaire were calculated after each course to find the common trend. The mean scores are represented graphically in Figure 2. According to the mean scores, the most pronounced effect was observed between the 1st and the 2nd cycles, slowing down by the 5th cycle. The mean score decreased from 39 to 29.

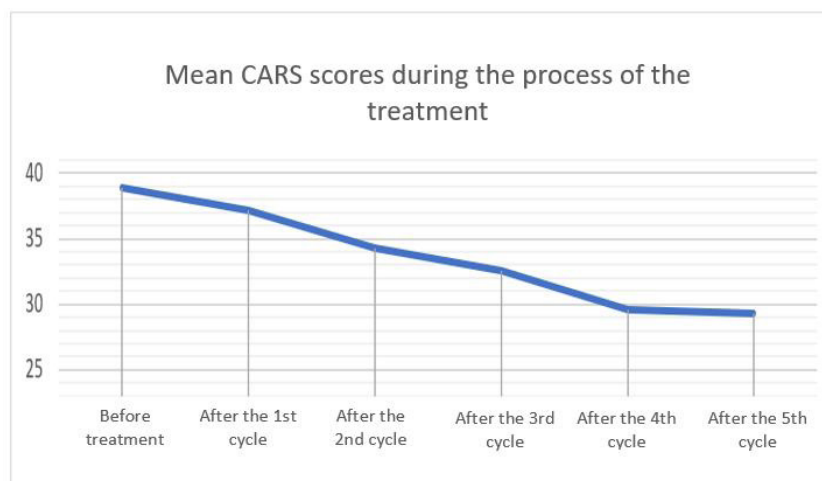


Figure 2: The graphical representation of the mean scores of the CARS questionnaire after each of the cycles. It was seen that the mean score was decreasing over the cycles of the treatment indicating improvements of children’s condition

Summary of PedsQL Scores

PedsQL was not created for the assessment of the autism symptoms, but rather created to assess the overall quality of a child’s life (both healthy and with chronic or acute illness). The well-being is assessed based on two types of functionality-physical and psychological. Table 1 represents the individual scores in each group as well as the overall well-being of the child after each of the treatment cycles and before treatment.

| Category | Case # | Before treatment | After the 1 st cycle | After the 2 nd cycle | After the 3 rd cycle | After the 4 th cycle | After the 5 th cycle |
|------------------------------------|--------|------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Physical condition | 1 | 75 | 75 | 75 | 75 | 75 | 75 |
| | 2 | 100 | 100 | 100 | 100 | 100 | 100 |
| | 3 | 72.2 | 77.8 | 83.3 | 100 | 100 | 100 |
| | 4 | 77.8 | 83.3 | 83.3 | 83.3 | 83.3 | 83.3 |
| | 5 | 55.5 | 66.7 | 83.3 | 94.4 | 94.4 | 94.4 |
| | 6 | 100 | 100 | 100 | 100 | 100 | 100 |
| Psychological condition | 1 | 55.8 | 55.8 | 59.6 | 59.6 | 61.5 | 61.5 |
| | 2 | 71.8 | 69.2 | 66.7 | 69.2 | 74.4 | 71.8 |
| | 3 | 66.7 | 69.2 | 76.9 | 82 | 84.6 | 82 |
| | 4 | 43.6 | 46.2 | 58.9 | 64.1 | 64.1 | 60 |
| | 5 | 50 | 50 | 53.3 | 60 | 60 | 60 |
| | 6 | 59.7 | 58.9 | 76.9 | 82 | 82 | 82 |
| Overall (physical + psychological) | 1 | 61.8 | 61.8 | 64.5 | 64.5 | 65.8 | 65.8 |
| | 2 | 80.7 | 78.9 | 77.1 | 78.9 | 82.5 | 80.7 |
| | 3 | 68.4 | 71.9 | 78.9 | 87.7 | 89.4 | 87.7 |
| | 4 | 54.4 | 57.9 | 66.6 | 70.1 | 70.1 | 66.7 |
| | 5 | 52.1 | 56.3 | 64.5 | 72.9 | 72.9 | 72.9 |
| | 6 | 71.9 | 71.8 | 83.8 | 87.7 | 87.7 | 87.7 |

Table 1: The summary of responses to the PedsQL (Pediatric quality of life inventory). The questions in the questionnaire were divided into two categories-physical functioning and psychological functioning. The maximum possible result in each category as well as the overall was 100, indicating the greatest functioning, while the lowest possible result was 0, indicating the poorest functioning. The increase in the scores over the courses indicates the increase of the children’s quality of life through the improvement of physical and psychological functioning

While physical functionality was quite low only in one case and by the end of the 5th cycle was relatively high in all children, psychological functionality was low at the beginning of the treatment in all cases. By the end of the 5th cycle it significantly increased in all children. Consequently, overall well-being also increased in all children. Based on this, it can be concluded that the treatment positively affected the quality of life for the children with autism.

The mean scores of the PedsQL questionnaire were calculated after each course and are shown graphically in Figure 3. It was seen that the mean physical functionality increased by 11%, psychological functionality increased by 12%, and overall well-being increased by 12%. As well as in the case with the CARS scores, the most significant improvements were observed between the 1st and the 2nd cycles in both the physiological and psychological categories. The same was observed with the slowing down trend by the 4th and 5th cycles.

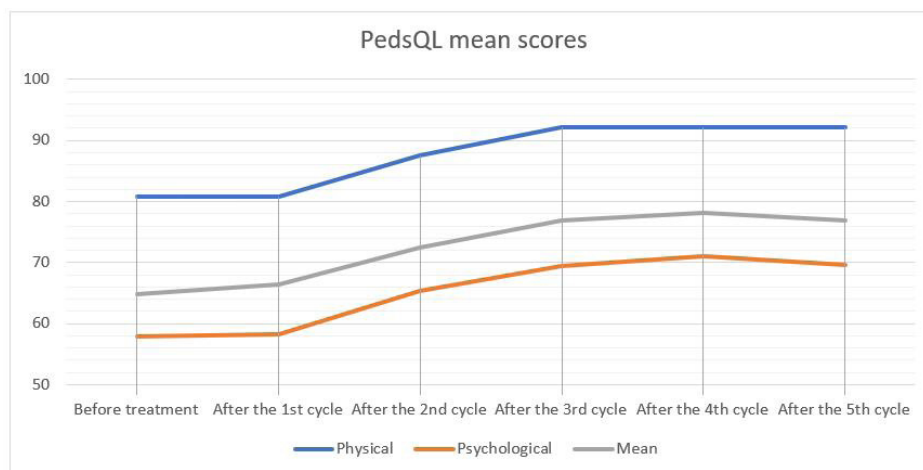


Figure 3: Mean scores of responses to the PedsQL (Pediatric quality of life inventory). The mean scores were increasing in all three categories. Both physical and psychological conditions were improving simultaneously with almost the same coefficient

Summary of Caregiver Strain Questionnaire Scores

Figure 4 represents the scores of the Caregiver Strain Questionnaire. The quality of life scores were increasing over the cycles of the treatment based on the subjective responses of the parents of all 6 children. The attempts to find common correlations between the changes in the CARS scores changes and changes in the scores of the Caregiver Strain Questionnaire did not give any positive result. For example, the most significant increases in the parents' quality of life were not in the same periods of time when their children had the most significant improvements. The percentage increase in the quality of the parents' lives did not correlate to the percentage increase of the CARS scores, meaning that the most significant increases of the quality of life were not observed in the parents whose children showed the most significant improvements. So, it seems to depend on individual perception, and the only conclusion that can be made based on this data is that the parents' quality of life was increasing over the cycles of their children's treatment.

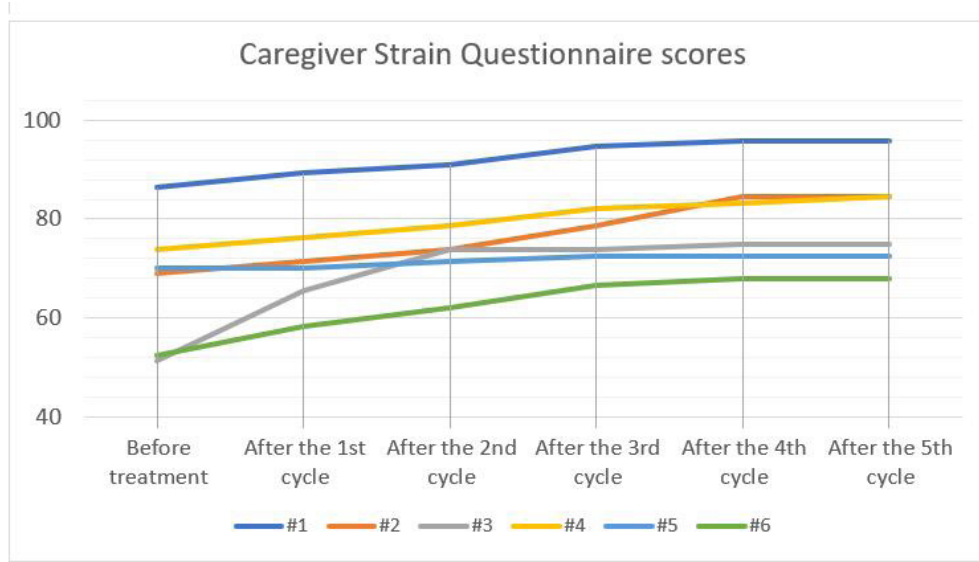


Figure 4: Summary of the responses to Caregiver Strain Questionnaire. The possible score range is 0-100. Each line represents the changes in the quality of life of every parent. The color of the line corresponds to the case of the study. All parents were experiencing a steady increase in their quality of life over the courses of treatment

The mean scores of the Caregiver Strain Questionnaire were calculated and are represented in Figure 5. It was seen that the mean scores were constantly increasing, but the form of the graph is different from the one which represents the changes in the CARS scores. In the case of the CARS there was a sharp change until the 4th cycle, and then a plateau. In this case, the scores were changing more consequently and more or less equally after each course, although with a plateau between the 4th and 5th cycles.

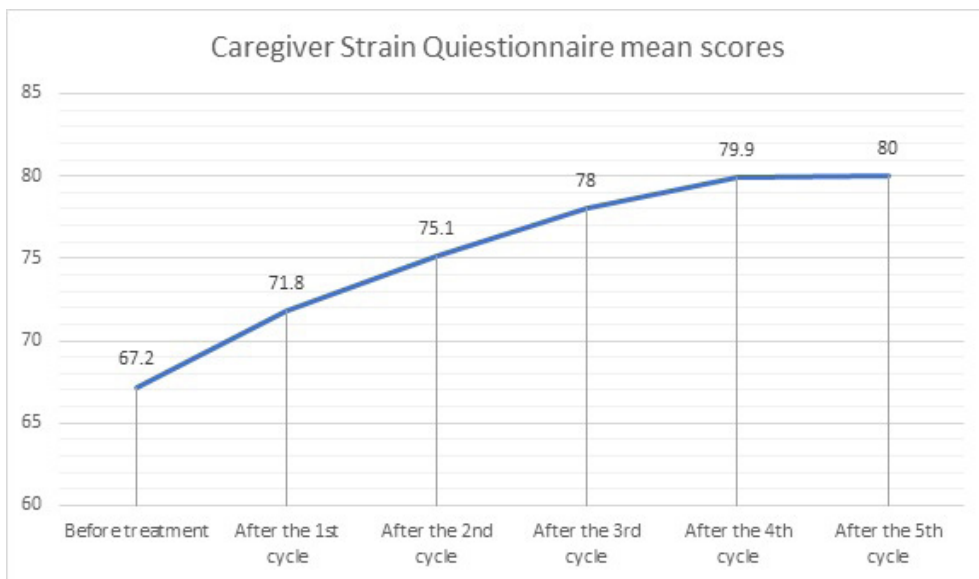


Figure 5: The mean scores of the responses to the Caregiver Strain Questionnaire. It was seen that the mean score was constantly increasing, with the difference between the 1st and the 5th cycle to be 12.8 points on the 0-100 scale

Description of the Individual Cases

Case 1. Male, 5 years old

Pregnancy and parturition: Pronounced toxicosis in the first trimester, prolonged labor of 7 hours. Long anhydrous period. High probability of hypoxia during pregnancy, ultrasound of the brain after birth confirmed hypoxia.

Early signs and symptoms: Until the age to 6 months, fell asleep poorly, Staphylococcus aureus infection, gastrointestinal tract disorders, and reduced emotional involvement. The child was diagnosed with ASD at age of 2 and did not understand the addressed speech, had absence of eye contact, did not play with children, and an absence of speech.

Signs and symptoms prior to treatment: Reduced vocabulary, could not build sentences, impaired eye contact, social communication problems, lack of understanding of how to play and communicate with others, reduced understanding, inability to explain own feelings, difficulties in participation in dialogues, restriction on the electrical supplies, hearing hypersensitivity, and hyperactivity.

Infections: Detected IgG to CMV, EBV and rubella virus were above reference ranges;

Red and white blood cells (in relation to the reference ranges established in the local diagnostic centers): Erythrocytosis, neutropenia, increased red blood cells distribution by width (RDW), increased erythrocytes sedimentation rate (ESD).

Immunity (in relation to the reference ranges established in the local diagnostic centers: Increased NK cells count, decreased B-lymphocytes count, reduced T-helpers/T-suppressors ratio.

Changes after the 1st cycle: If he is made busy, the hyperactivity goes away. After 2 weeks, he began to pay more attention to his siblings. At the end of the 1st course, when invited to the play with a brother and sister he expressed interest. Started trying to ask a question, but still does not realize that he would get an answer. In one sentence could describe how his day was. Speech has become clearer.

Changes after the 2nd cycle: The teachers in the kindergarten say that he has become more connected, found a friend, the speech has become clearer and was constantly improving, the concentration of attention increased, the perception to requests and questions increased, he became capable of making some decisions regarding his routines. Vocabulary was constantly increasing. Eye contact improved. Still there was a lack of perception (he did not understand the feelings of other people, there was no sympathy and no empathy). There was still an obsession with certain objects (especially household appliances). Decreased hypersensitivity to sounds, (for example, the work of electrical devices). The teachers said that he became more assiduous (he began to draw and sing better).

Changes after the 4th cycle: At the beginning of the 4th course he had chickenpox, but it was in a mild and short form. In comparison with the beginning, all teachers noted an improvement. He became even more assiduous and attentive. He made a friend in the kindergarten and took toys to his friend. Every morning he went to kindergarten with pleasure, because he had a friend there. Communication with other children improved, he started playing in the playground, riding a bicycle on his own, and then returns home by himself. Before this his mother was afraid to let him go somewhere on his own, and now she has started to give him more independence.

Changes after the 5th cycle: Periodically began to withdraw into himself. Since the beginning of this treatment only after this course, parents noted such features. But at the same time in kindergarten, the tutor noted that he had become more confident.

All blood and immune parameters are within reference ranges of the diagnostic center, apart from increased level of erythrocytes. IgG to EBV and rubella virus increased, IgG to CMV decreased.

Parents note that the treatment combined with corrective therapies was significantly more efficient, compared to corrective therapy alone and/or to other types of treatment that they underwent earlier.

Case 2, Male, 4 years old

Pregnancy and parturition: Respiratory infection in the 2nd trimester of pregnancy, stimulated parturition.

Early signs and symptoms: The child was restless, often cried, did not look into eyes, and had sleep deprivation, was looking for parents only when he was hungry.

Signs and symptoms prior to treatment: Moderate language impairment, reduced vocabulary, mild restricted and repetitive behavior, moderate difficulties with social communications, difficulties with understanding addressed speech, preference to stay alone, hyperactivity, anxiety, frequent hysterias.

Infections: Detected IgG to rubella virus and EBV (NA) above reference ranges.

Red and white blood cells (in relation to the reference ranges established in the local diagnostic centers): Neutropenia.

Immunity (in relation to the reference ranges established in the local diagnostic centers): Increased T-suppressor count, decreased NK cells count and T-helper/T-suppressor ratio.

Changes after the 2nd cycle: Active vocabulary is increasing, understanding and memory has improved. He still cannot immediately answer open-ended questions, but started to answer yes/no questions.

Changes after the 4th cycle: Significantly decreased hyperactivity and improved understanding. Speech is improving, vocabulary is increasing. Improved contact with other children. He began to speak louder, actively expresses dissatisfaction with something

Changes after the 5th cycle: Vocabulary continues to increase. Speech evolves. Now understanding and memory has improved. Began to answer open-ended questions. In the garden performs oral and written tasks. Now can explain how he felt in a particular situation. Sometimes comes up with stories (cheats). Now can make contact with children. Sometimes he offers toys himself or can exchange them. Before when he played with children, he would hurt or push someone. Now this is over. Became more patient, can stand and wait quietly in a line. The child had a respiratory infection during the 5th cycle.

Infections: IgG to EBV slightly decreased, IgG to rubella virus significantly decreased by 4 times.

Red and white blood cells (in relation to the reference ranges established in the local diagnostic centers): Increased RDW and increased ESD, neutrophils count was within reference ranges.

Immunity (in relation to the reference ranges established in the local diagnostic centers): Decreased T-helper/T-suppressor ratio, NK cells – within reference ranges.

Parents note that the treatment combined with corrective therapies was significantly more efficient, compared to corrective therapy alone and/or other types of treatment that they underwent earlier.

Case 3, Male, 3 years old

Pregnancy and parturition: Respiratory infection in the 2nd trimester of pregnancy.

Early signs and symptoms: The child was unusually quiet, rarely made any sounds, when he cried, he cried very quietly.

Signs and symptoms prior to treatment: Severe language impairment, absence of speech, only separate sounds, severe problems with social communication, misunderstanding of how to play and communicate with other children, reduced spectrum of emotions, preference to be separate from peers, impaired eye contact, mild repetitive and restricted behavior, stereotypical games, reduced sensitivity, frequent GI and respiratory tract disorders.

Infections: Detected IgG to EBV, CMV, HSV and Mycoplasma are above reference ranges.

Red and white blood cells (in relation to the reference ranges established in the local diagnostic centers): Erythrocytosis, neutropenia, lymphocytosis, increased RDW, decreased MCV.

Immunity (in relation to the reference ranges established in the local diagnostic centers): Decreased B-lymphocytes count.

Changes after the 1st cycle: It became possible to communicate with the child through gestures, before he could not do this. Falls asleep quicker. Started to repeat sounds after parents. Response and reaction to speech has appeared. Reduced excessive focusing. Improved eye contact. Ritualism became more pronounced.

Changes after the 4th cycle: During the 4th cycle he had acute bronchitis, but it did not affect his autistic symptoms. Verbality and understanding improved. He now combines syllables into words. No hysterias. Started playing with a younger brother. Began to express emotions. Began to answer questions.

After the 5th cycle: Neutrophils and lymphocytes counts are above reference ranges, increased ESD. All immune parameters are within reference ranges. IgG to EBV and CMV slightly decreased, IgG to HSV and Mycoplasma are within reference ranges.

Parents note that the treatment combined with corrective therapies was significantly more efficient, compared to corrective therapy alone and/or other types of treatment that they underwent earlier.

Case 4, Male, 4 years old

Pregnancy and parturition: Labor was stimulated and the child was born with hypoxia.

Early signs and symptoms: The child was very calm, could sit by himself for hours, did not need attention.

Signs and symptoms prior to treatment: Severe language impairment, absence of speech, understands only very simple speech, misunderstanding of how to communicate with other children, severe restricted and repetitive behavior, hyperactivity, reduced sensitivity.

Infections: Detected IgG to EBV, CMV, rubella virus and H. pylori were above reference ranges.

Red and white blood cells (in relation to the reference ranges established in the local diagnostic centers): Neutropenia.

Immunity (in relation to the reference ranges established in the local diagnostic centers): Decreased B-lymphocytes count and T-helpers/T-suppressors ratio, increased NK cells count.

Changes after the 1st cycle: During the first two weeks, vocalization worsened, began to take off panties and socks (does not want to wear them), nocturnal enuresis appeared, repetitive behavior intensified, behavior deteriorated. After two weeks his behavior normalized. Then during the second two weeks he said his first word, then repeated it several times.

Changes after the 2nd cycle: Several new words, new sounds and new syllables appeared.

Changes after the 3rd cycle: Began eating new types of food (eats almost everything, before was very selective). He began to repeat the vowels, began to pay attention to other children; he eats in the kindergarten along with everyone and does not jump as it was before; he learned to use the toilet by himself.

Changes after the 5th cycle: Positive dynamics in understanding, assiduity improved, began to slowly play with toys (cars), sounds appeared: yes; no; ta; di; na; ba; si; ma.

Blood and immune parameters did not change. IgG to EBV did not change, IgG to CMV decreased, IgG to rubella virus increased, IgG to H. pylori were within reference range.

Parents note that the treatment combined with corrective therapies was significantly more efficient, compared to corrective therapy alone and/or other types of treatment that they underwent earlier.

Case 5, Male, 2.5 years old

Pregnancy and parturition: Were not complicated

Early signs and symptoms: The child was very quiet, rarely cried, and had frequent respiratory infections.

Signs and symptoms prior to treatment: Significantly reduced speaking ability, few words in vocabulary, inappropriate usage of words, noticeable difficulties with social communication, impaired eye contact, preference to stay alone, ignores other people, mild repetitive behavior.

Infections: Detected IgG to rubella virus were above reference ranges.

Red and white blood cells (in relation to the reference ranges established in the local diagnostic centers): All blood parameters were within reference ranges.

Immunity (in relation to the reference ranges established in the local diagnostic centers): Decreased T-lymphocytes count, reduced T-helpers/T-suppressors ratio, increased B-lymphocytes count.

Changes after the 1st cycle: At the beginning of the treatment (first 12 days) headache. Refuses to take olive extract. The vocabulary increased, the efficiency of training with a speech therapist increased, the speech therapist praised him, he began to do everything that she said. Reduced repeatability of actions. Increased interest in drawing. Improved contact with other people and increased response to questions. Understanding has improved as well as communication skills.

Changes after the 2nd cycle: His understanding has improved significantly. Previously all the time he looked at his hands and now that behavior is almost gone. Expresses willingness to play with other children. The gesticulation improved and speech became more conscious, uses verbs in correct grammatical form. In the kindergarten, when all the children had respiratory infections, he was not sick.

Changes after the 5th cycle: Understanding is constantly improving, started speaking more, eye contact is not impaired anymore, “significant changes” in his emotional functionality.

Blood parameters did not change-all were within reference ranges. All immune parameters apart from decreased B-lymphocytes were within reference ranges. IgG to rubella increased.

Parents note that the treatment combined with corrective therapies was significantly more efficient, compared to corrective therapy alone and/or other types of treatment that they underwent earlier.

Case 6, Male, 4 years old

Pregnancy and parturition: At the first trimester there was a risk of miscarriage. Parturition was stimulated and the child was born with jaundice.

Early signs and symptoms: The child was very calm. He did not need any attention.

Signs and symptoms prior to treatment: Nonverbal, had severe social communication problems, repetitive movements and signs of restricted behavior, aggressive behavior and difficulties with communication, excessive focus on some objects, stereotypical running, frequent respiratory infections.

Infections: Detected IgG to EBV, CMV and rubella virus are above reference ranges.

Red and white blood cells (in relation to the reference ranges established in the local diagnostic centers: Erythrocytosis.

Immunity (in relation to the reference ranges established in the local diagnostic centers: Reduced T-helpers/T-suppressors ratio.

Changes after the 1st cycle: Immunity improved. He did not have respiratory infection during the first cycle, (he used to get sick 1-2 times a month and it was always difficult to endure). At the beginning of the first course, he became more tearful. He began to differentiate his parents, singling out each separately “mom, dad”, also began to formulate his wishes for the first time verbally “Mom, give me”. A new element of communicativeness appeared, sings songs with sister.

Changes after the 2nd cycle: Before the treatment, there was no speech at all, now he still does not initiate conversation, but 30 new words appeared in his vocabulary. Focusing remains, but the objects of the focus have changed. Now he has become more aware of other people, tries to make contact with them, takes their hands, looks into their eyes. There is an understanding of several complex tasks and before he did not understand tasks at all. Negative emotionality on the 2nd course was practically absent. Mutual understanding with his sister improved. Did not have respiratory infections.

Changes after the 3rd cycle: Did not have respiratory infections. Improved attention. Became more open to external factors and increased understanding of external events of the world. Some improvements in his communicative function, the appearance of a conscious reversed speech. Emerged induced speech. Began to cheat. Improving cognitive function. The bad behavior is preserved. He is very resistant. The mother thinks that it can still be a character trait. Changes for the better in terms of ritualism and restricted behavior. Restrictiveness in choosing the color of clothes remains.

Changes after the 4th cycle: Increased vocabulary. When he wants something he says it, before he did not. He used to watch cartoons without sound, but now he is interested in listening. Learned a verse, previously could not learn a verse at all.

After the 5th cycle: Erythrocytosis and increased ESD; decreased B-lymphocytes count, T-helpers/T-suppressors ratio were within reference range. IgG to EBV slightly increased, IgG to CMV slightly decreased, IgG to rubella did not change.

Parents note that the treatment was significantly more efficient, compared to other types of treatment that they underwent earlier.

Discussion

In all cases it was shown that the treatment resulted in behavioral improvements as well as changes in the physical and psychological conditions of the children as reported by the parents after each cycle of the treatment. This indicates that the treatment of autism comorbidities, which include chronic viral/bacterial infections, chronic inflammation, aberrant immune system, and gastrointestinal problems, significantly improves the quality of life for autistic children as well as leads to the decrease of autistic symptoms. There is a covariation between the normalization of the blood and immune parameters and these positive changes. Autistic symptoms such as impaired verbal function, social communication problems and repetitive behavior, to the best of our knowledge, were not reported to be affected by any other types of treatment. The presence of these positive effects indicates that etiological treatment aimed at the underlying conditions may help to improve the severity of the disorder in children.

Such changes also positively affect parents, whose subjective quality of life increased over the courses of the treatment. All parents noted the positive efficacy of treatment, compared with other types of treatment that their children underwent earlier.

This study has several limitations:

- a) The assessment of the children's changes was done by parents, who could be biased. Although, as was reported in the description of the individual cases, the changes in some children were also noticed by psychologists and teachers, there was no standardized system of assessment for all children.
- b) In future studies ADI-R (Autism Diagnostic Interview-Revised) needs to be used, because it is a "gold standard" of diagnostic instruments for ASD.
- c) Only males were assessed in this study. However, this was not because only males were included in the study, but because at the time of writing this manuscript, only males had undergone 5 cycles of the treatment.
- d) Because laboratory tests were conducted in different diagnostic centers we were unable to calculate statistical significance.
- e) Since the combination of the supplements and antiviral medication were used at once, it is not clear which product contributed to improvement, and a better designed study or studying the effect of one agent at a time is required for the detailed understanding of the mechanisms underlying the observed improvements.

Conclusions

- 1) The proposed treatment resulted in significant improvements in overall health conditions in all 6 cases.
- 2) According to the parents, the proposed treatment either alone or in combination with correctional therapies was superior to any treatment used before.
- 3) During the entire treatment period the health of the children was constantly improving.
- 4) The results of treatment show that (at least in some cases) there is a possibility of significant improvement leading to the children becoming neurotypical.
- 5) The proposed concept and treatment method should be studied further and applied and tested in larger groups of ASD children
- 6) In the course of treatment, positive changes in the children's quality of life led to the constant improvement of the quality of their parents' life too.

Compliance with Ethical Standards

Authors have no competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from parents of all individual participants included in the study.

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