Case Series of 57 Autism Spectrum Disorder Children from Central Asia and Eastern Europe

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Abstract

Published evidence suggests that congenital infections caused by a variety of infectious agents can result in an infant’s inflammation and immune system disruptions, which have potential to cause neurodevelopmental changes leading to autism spectrum disorder (ASD). We conducted the assessment of 57 ASD children through web-mediated communication, questionnaires, and laboratory analyses of blood parameters. The results show that 57 children from Central Asia and Eastern Europe diagnosed with ASD had multiple signs of infections, inflammation, immune system disruption, and folate deficiency. Our collected information gives us an assumption that initial negative changes resulting in the autistic phenotypes in these children were initiated during the mother’s pregnancy producing a number of negative etiopathogenic changes resulting in the disorder.

Keywords: Autistic disorder; Blood Test; Infection; Inflammation; Immune system; Pregnancy

Introduction

Autism Spectrum Disorder (ASD) is neurodevelopmental pathology with social communication problems, repetitive/restricted behavior, and language impairment [1]. It is a rapidly growing problem and it is estimated that in the USA in 2018, 1 of 59 children were born with ASD [2,3], while 50 years ago, the incidence of ASD was almost 40 times lower—only 1 in 2500 [4].

Autistic phenotype is a result of genetic changes in the brain development [5-7]. These changes, according to a number of publications, are the result of DNA damage due to oxidative stress [8-10]. Oxidative stress, being a part of inflammation process, not only damages DNA, but also inhibits DNA’s repair mechanisms [9,10]. In addition to the production of reactive oxygen species, the inflammation process is characterized by induction of a variety of inflammatory and pleotropic cytokines [11], which additionally contribute to DNA damage. Signs of inflammation, both in the brain [12-14] and systemic [13,15] as well as signs of immune system disruption [16] are identified in ASD children and according to some studies. Also perinatal inflammation in the brain could lead to the appearance of autistic symptoms in a child [17-20].

The available publications show that inflammation could be a result of a wide variety of infections that may play a role in the initiation and promotion of ASD. There are a variety of viruses that infect neurons, such as Herpes Simplex Virus 1, 2 (HSV), Varicella Zoster Virus (VZV), Epstein-Barr Virus (EBV), Cytomegalovirus (CMV), Human Herpes Virus types 6 and 8 (HHV 6 and HHV 8), arboviruses, rabies, polyomaviruses and enteroviruses [21]. There are also publications that show an association between ASD and some of these viruses. Particularly: HSV [22,23], EBV [24], CMV [25,26], HHV 6 [27], rubella virus [28], Chlamydia spp., and Mycoplasma spp. [29]. Moreover, the chronic/latent course of the infection disrupts the immune system and this affects brain development. Additionally, it was reported that inflammation in the brain, caused by the attempts of the host to combat a viral infection could be the major cause of different neurodegenerative and neural dysfunction processes [21].
Inflammation disrupts immune system function. A 1998 study cited mounting evidence for the role of immune dysfunction along with an environmental pathogen as contributing factors in ASD development [30]. It has been observed that the disruption of the immune system is characterized by a disbalance of natural killer cells (NK cells), monocytes, T cells, and by decreased regulatory cytokines TGFβ1 and IL-10 indicating the inflammatory shift [8]. Immune system disruptions are also common in ASD children. It has been shown that inflammatory factors TNFα, IL-1β and IL-6 are increased [31], IR1 (CD4:CD8) is dysregulated [32], and T-helper cell count decreased [33] in ASD children. Additionally, some studies have shown that maternal immunity malfunction during pregnancy could explain abnormal fetal brain development [34,35]. The disrupted immune system cannot effectively perform its function, and this results in a chronic mode of the infection and inflammation.

Folate deficiency is also common in ASD children [36]. It was shown to induce DNA abnormalities [37-39] and inflammation [40], to enhance the production of the pro-inflammatory cytokines [41], and to inhibit CD8+ T lymphocytes [42].

Development of some brain alteration in ASD children starts in the fetus [8], and infection, maternal inflammation and immune system activation during pregnancy may produce autistic changes in a child [43,44]. Based on the published information on the role of infections, inflammation and immune system disruption, this case series was focused on these elements of etiopathogenesis in the group of 57 children with ASD.

**Methods**

**Participants**

There were 57 children from 14 cities of Central Asia and East Europe with ASD diagnoses that were independently established at local diagnostic centers, whose parents were self-referred to us. There were no preselection, exclusion, or inclusion criteria other than a confirmed ASD diagnosis and written consent to participate in the study supported by verbal consent. There were 8 children ages 18 months to 3 years, 16 children ages 3 to 5, 13 children ages 5 to 7, and 20 children ages 7 to 16. There were 49 boys and 8 girls (G/B ratio=1:6.1). Initial information collection.

This study was not prospective and was conducted using email questionnaires and a telemedicine approach. It focused on the assessment of ASD symptomatology and on specific changes of blood parameters. The method of telemedicine was shown in several studies to be as effective as in-persons communication with ASD patients and parents [45-47] as well as being comfortable and cost-effective [48]. The Skype® program was used for the web-mediated consultation based on its cost-free availability.

The initial information on the signs and symptoms of ASD were collected from parents of ASD children using an email questionnaire developed in accordance with the Diagnostic and Statistical Manual of mental disorders DSM-5. It included the questions related to social communication, behavior, and language problems. The parents provided written detailed information with examples of the current problems, their children had in these categories.

The symptoms and signs related to these problems were assessed in accordance with the scoring used in ADI-R (Autism Diagnostic Interview-Revised) [49], in which 3 is severe, 2 is moderate, 1 is mild, and 0 indicates no problem. The questionnaire had additional questions grouped into two categories, which included: (a) neurological and psychological disorders, including sleep deprivation, hyperactivity, anxiety, epilepsy, and depression, and (b) gastrointestinal tracts disorders, and the frequency and severity of acute and chronic respiratory diseases. The severity of these conditions was grouped into the same category with scoring in which 3 is severe, 2 is moderate, 1 is mild, and 0 indicates no problem. Additionally, the mothers were requested to complete an additional questionnaire related to pregnancy peculiarities, newborn delivery, and the early period of the child’s life prior to the diagnosis of ASD.

After collecting the initial information from parents, further communication was conducted through web-mediated consultations using Skype® program.

**Laboratory Analyses**

All parents agreed to perform blood analyses to detect the presence of antibodies to viral and bacterial infections, the status of cellular immunity, and changes in the white and red blood cell counts. Local independent diagnostic specialists performed all the analyses and the results were recorded by them and presented to us for assessment.

The list of specific analyses included:

- Red and white blood cell counts*.
All 57 children in the study had social communication problems, signs of restricted and repetitive behavior, and language impairment.

- **Social communication:** Out of 57, 56 children had communication problems of severe and moderate extent with the only one having mild problems.

- **Restrictive and repetitive behavior:** Out of 57 children, 48 had severe and moderate problems, while 9 had mild problems.

- **Language impairment:** There were 44 children who had severe and moderate impairment, while 13 had mild impairment (severe impairment is defined as nonverbal state, moderate impairment as having a vocabulary of a few words, and mild impairment as the ability to speak, but with a limited vocabulary and using short sentences and a tendency to ramble).

**Neurological and psychological disorders:** There were 50 of 57 children who had some form of neurological and psychological disorders such as epilepsy, depression, hyperactivity, anxiety, and sleep disorders.

**Respiratory and GI tract disorders:** There were 40 of 57 children who had respiratory and GI tract disorders. Respiratory tract disorders included frequent and long-duration respiratory infections, and severe forms of respiratory infections. GI disorders included nausea induced by food smells, food aversions, irregular stool, frequent constipation or diarrhea, and fungal infections in the GI tract.

Collection of ASD anamneses: signs of deviation in pregnancy, parturition, and in early life

In order to understand possible roots of this disorder, the medical history of pregnancy, parturition, and a child’s initial period of life were collected into the anamneses of the disorder.

Out of 57 cases, the parents of 35 children answered questions regarding pregnancy, newborn delivery, and first few months of the child’s life, while 22 did not provide this information.

**Pregnancy period:** There were 30 mothers who experienced various complications during the pregnancy period. Some of the mothers had more than one complication. There were 14 cases of unidentified respiratory infection, 3 cases of herpes infections, 3 cases of bacterial infection (ureaplasma and mycoplasma), 1 rubella virus infection, and 1 unidentified gastrointestinal infection. There were 7 cases of increased risk of miscarriage, 4 cases of partial placenta detachment, and 2 cases of vaginal bleeding.
Parturition (mothers): There were 10 women who had stimulated parturition and 9 had cesarean sections. There were 3 cases of premature birth and 3 cases of prolonged periods of anhydramnios before birth.

Parturition (children): There were 15 children born with perinatal hypoxia, 13 children had neonatal asphyxia and hypoxia, 5 had newborn jaundice, and 8 were diagnosed with CNS damage (mostly hydrocephaly).

Initial period of life: There were 25 parents who reported the common patterns of a child who was unusually quiet and characterized by the parents as “causing no inconvenience”. Communication with the parents revealed that these children had little social interest, decreased engagement of attention, and marked passivity. 9 other children, according to their parents were, “very problematic”, “hypersensitive”, “irritable”, and “suffered from sleeplessness”. Only 1 child’s state was described as “usual”.

Additionally, 13 children were reported as having frequent respiratory and gastrointestinal infections starting mostly in the immediate perinatal period. In all cases the parents reported no connection between the described patterns and any vaccination.

The summary of these patterns is presented in Figure 2.

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Figure 1: Severity and Types of ASD Symptoms and ASD-related Disorders
It demonstrates the percentage of children (y-axis) who have symptoms and disorders typical for ASD (x-axis). Severity of symptoms is divided into 4 degrees of severity from 0 no symptoms to 3 severe

Figure 2: The Prevalence of Complications in Pregnancy, at Birth, and in the Early Months
The figure shows the percentage of complications during pregnancy, at birth, and in the early months of the children’s lives. Total percentage in a column may exceed 100%, because some mothers and newborns had more than one complication.
As seen in Figure 2, only 14.3% of mothers did not report any complications. The most common pregnancy complication was infection, with 57% of the respondents reporting it. No mothers’ parturition problems were reported in 28.5% of cases. More than half of the mothers had unnatural deliveries, including stimulation and cesarean section. At birth only 11.4% of children did not have any problems, while about 80% had both prenatal and perinatal hypoxia and asphyxia. During the first months practically all children had unusual behavior, presented by marked passivity and absence of social engagement or by increased sensitivity and irritability.

**Alterations in blood tests’ results in relation to reference ranges**

**Red blood cells count:** Red blood cell count tests showed that out of 57 cases, there were 27 cases of erythrocytosis, 14 cases of changes in red blood cell mean corpuscular volume (9 increased and 5 decreased), 17 cases of increased red cell distribution width, and 14 of increased erythrocyte sedimentation rate.

**White blood cells count:** There were 20 cases of lymphocytosis and 16 cases of neutropenia.

The number of children having deviations from reference ranges is presented in percentages in Figure 3. In Figure 4, the number of deviations in the blood count is shown.

![Figure 3: Changes in Blood Analysis of the Study Subjects](image)

**Figure 3:** Changes in Blood Analysis of the Study Subjects

RBC – red blood cells, NEU – neutrophils, LYM – lymphocytes, MCV – mean corpuscular volume, RDW – red cells distribution by width, ESD – erythrocytes sedimentation rate

The results are presented as a percentage of children with increased/decreased indices in relation to all children. The increases are presented in red and the decreases in blue colors.

![Figure 4: Number of Children having N Changes in Blood Test](image)

**Figure 4:** Number of Children having N Changes in Blood Test

The x-axis indicates the number of changes in blood counts (the indices that were out of reference ranges), while the y-axis represents the number of children in whom X amount of changes in blood count were found. Most children (12 in the two group and 13 in the four group) had 2 and 4 blood counts out of reference ranges.

Notes:

There were other changes such as increased thrombocyte and abnormal hemoglobin level, but these changes were in less than 10% of the children. Moreover, since the tests were conducted in different laboratories in accordance with the preferences of the parents and availability of tests in their cities, not all the same parameters were tested. The abovementioned parameters were tested in all 57 children. The other additional parameters were not considered here because they are either not a common feature for ASD children or they were not tested for in all the children. However, when the number of changes in every child was counted, all changes were taken into consideration.
At the same time, it was shown [62], that in Russia, which is located in the region of Central Asia and Eastern Europe, the average meanings of all these blood parameters among neurotypical children fall within the reference ranges.

**Alterations in cellular immunity tests in relation to reference ranges**

Out of the 57 children, 51 had cellular immunity tests done, while 6 children did not have the tests. There was immunity changes in 39 of the 51 children tested. Some of the children had multiple cellular immunity parameters out of reference ranges. The results of the immune analyses are shown below:

1. Decrease in T-lymphocytes count in 7 children,
2. Decrease in T-helpers count in 15 children,
3. Increase in T-suppressors count in 15 children,
4. Decrease in B-lymphocytes count in 9 children,
5. Decrease in NK cells count in 11 children,
6. Reduced immune regulatory index (IRI) in 18 children.

The prevalence and number of changes in cellular immunity are graphically displayed in Figures 5 and 6.

![Figure 5: The Changes in the Cellular Immunity of the Study Subjects](image)

This figure shows the percentage of children (y-axis) in whom each of the immune cells (x-axis) were out of reference ranges. The decrease of the indices is shown in red and the decrease in blue.

As seen in Figure 5, the most common changes were an increase in T-suppressors count in 35% of the children, and a decrease in T-helpers count and in the immune regulatory index (IRI) in 30% of the children each. The levels of B-lymphocytes and NK cells were either above or below reference ranges.

As it is seen in Figure 6, the great majority of ASD children had signs of a disrupted immune system. Only 12 children did not have measurable changes in cellular immunity, while another 39 had alterations in the immune system that revealed themselves by either increase or decrease of parameters beyond corresponding reference ranges.

At the same time, it was shown [63], that in Russia, which is located in the region of Central Asia and Eastern Europe, the average meanings of all these immune parameters among neurotypical children fall within the reference ranges.

**Presence of antibodies to chronic infections**

The blood tests showed that all 57 children had increased levels of antibodies to viral infections and some of them had increased levels of antibodies to bacterial infections. The most prevalent viruses detected were: CMV and EBV, which were present in the blood samples of 42 and 41 children respectively. Congenital rubella virus was detected in the blood of 35 children. HSV was detected in the blood of 14 children. Bacterial infections were detected in the

Notes:

*As with the red and white blood cells counts, there were parameters tested only by some children, or there were parameters that changed only in a small minority of the children. So, only the parameters that are the most common, and were tested in all children are included. However, when the number of changes in every child was counted, all changes were taken into consideration.*
following numbers: Mycoplasma spp. in 11 children, H. pylori in 9 children and Chlamydia spp. in 2. The percentages of detected viral and bacterial agents are shown in the Figure 7.

Out of 57 children, there were 47 children who had only IgG antibodies to the detected viruses. In 5 children IgM antibodies to CMV, EBV and HSV were detected and in 5 other children the presences of EBV and CMV were detected by using PCR analysis.

However, regardless of the type of infection, the majority of children had a polyinfection ranging from 2 to 5 infections. Figure 8 shows the burden of infections.

Figure 8 shows that only 8 children had been infected with a single infection: antibodies to EBV and rubella virus were detected in 3 children each, and antibodies to CMV in 2 children. In the majority of cases, the most frequent combinations of infections included CMV, EBV, and rubella with some cases of HSV, Mycoplasma, H. pylori, and Chlamydia being rarer infectious agents in a combination. It shows that infectious agents in ASD children are present mainly in combinations with some random distribution in a particular child, but with the prevalence of the herpesviridae group and rubella viruses.

At the same time, it was shown [64], that in Russia, which is as well located in the region of Central Asia and Eastern Europe, 48% of neurotypical children are diagnosed with chronic viral infections (including, but not limited to: herpetic infections, measles, mumps, rubella and gastrointestinal infections), while as it was observed that 100% of the ASD children participated in the study have chronic viral infection.
Discussion

The results of the study show that all children had persistent/latent infections primarily caused by EBV, CMV, and rubella virus. Majority of children had from 2 to 5 infections with only 8 children having one infection.

Almost all children had abnormalities in white and red blood cells counts. These changes show inflammation and folate deficiency and point at the presence of infection. Folate deficiency was confirmed by the analysis of the blood parameters. Erythrocytosis is a sign of hypoxia [52], which may be caused by oxidative stress [53] and of folate-deficiency anemia [54]. Lymphocytosis may be a sign of chronic herpetic infection [55]. Neutropenia may indicate viral infection [56] and weakened immune system [57]. Increased and decreased MCV, as well as increased RDW, point at a folate deficiency [58-60] and increased ESD is a sign of infection, anemia [61], and inflammation [12].

Most children had moderate to severe disruption to their immune system. Changes in their immune system show presence of infections, chronic inflammation, and the inability to fight infections.

It was shown, in the cases where it was possible to collect additional information, that the life and disease anamneses show a likelihood of the disorders’ congenital nature.

Additionally, the results of blood analyses show consistency with changes described in a number of previous studies, supporting conclusions that a congenital infection together with inflammation and immune system disruption could be a trigger and a promoter of ASD.

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) Because laboratory tests were conducted in different diagnostic centers we were unable to calculate statistical significance.

c) Parents were not asked whether the children received MMR vaccine. Thus, the levels of IgG to rubella virus could be affected by the vaccine (not necessarily meaning that vaccination could be an etiologic factor of ASD, because it has been proven by many studies that vaccination does not cause autism [65]).

d) Due to the small size of the group of the observed children, the results obtained may be applied only to children from that region.

Conclusions

1) The results of this case series show that the observed 57 children from Central Asia and Eastern Europe, and diagnosed with ASD in accordance to the DSM-5 had detectable signs of infections, inflammation, immune system disruption, and folate deficiency.

2) In the majority of these children, infections were represented by not a single infection, but by poly-infection.
3) In these children, there is a likelihood that the disorder started as a congenital infection, which most likely produced symptoms of chronic/latent infection, inflammation, and immune system disruption throughout the extended period of life after birth.

Compliance with Ethical Standards
The study was funded by FLAASK, LLC.

The authors declare that they have no conflict of interest.

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.

Authors’ Contributions
KA contributed to the conceptualization, conceived of the study, participated in its design and coordination, interpretation of the data, and drafted the manuscript; SF helped to secure funding for the study, participated in its design, coordination and interpretation of the data, and drafted the manuscript; AT participated in the coordination of the study, in data interpretation, drafting and revising the manuscript; AM participated in data interpretation, drafting and revising the manuscript; TI in the initial design of the study, assisted in data interpretation and revision the manuscript. All authors read and approved the final manuscript.

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