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6 ASSESSMENT OF MARKERS OF INFLAMMATION AND NEURONAL DAMAGE IN PATIENTS WITH AUTISM SPECTRUM DISORDERS ASSOCIATED WITH GENETIC DEFICIENCY OF THE FOLATE CYCLE

JUSTIFICATION

Autism spectrum disorders (ASD) currently occur in at least 1 % of children in the modern population, and the trend towards an increase in the frequency of this psychiatric pathology among the pediatric population continues [6]. Although more than 100 genetic causes of the ASD phenotype in children have been proposed, most of them are rare and do not significantly affect either the high prevalence of the disease or the trend towards an increase in the frequency of these disorders in the population. Genetic deficiency of the folate cycle (GDFC) is a fairly common pathology of the human genome, which may explain the current epidemiological features of ASD. The evidence base for the association of GDFC and ASD in children is based on the results of 5 meta-analyses of randomized controlled clinical trials conducted from 2013 to 2020 and covering the results of 8 to 25 trials involving 1361 to more than 3000 children with ASD and 6591 to 7257 healthy children [13, 17, 20, 21, 22]. It has been established that GDFC leads to the development of a number of pathological biochemical changes in the body [14, 25, 26], including hyperhomocysteinemia [11], which predispose to the development of oxidative stress [6, 8]. The results of a meta-analysis of randomized controlled clinical trials by Guo B.Q. et al. 2020, which included 31 trials involving 3304 children, including 1641 patients with ASD without specifying the genetic nature of the disease, demonstrated that hyperhomocysteinemia is closely associated with ASD in children, being a typical feature of this heterogeneous cohort of patients as a whole (Hedges's g = 0.56; 95 % CI = 0.36–0.76, P < 0.001) [11]. Since hyperhomocysteinemia is a specific biochemical phenomenon for GDFC, the results of this meta-analysis indicate that GDFC, among other genetic abnormalities, is currently the leading factor in the genetic predisposition to the development of ASD in children. The evidence for the development of oxidative stress with an excess of prooxidant and a deficiency of antioxidant molecules in ASD is based on the results of two meta-analyses and systematic reviews of randomized controlled clinical trials, covering 87 trials involving 9109 patients [6, 8]. It is the pathological changes in the biochemical profile and the associated oxidative stress that are considered to be the cause of impaired immune system development in children with ASD associated with GDFC [16, 19]. Such patients develop a special form of immunodeficiency [1], which leads to the development of a number of immune-dependent complications, in particular encephalopathy, which, in fact, leads to the clinical picture of ASD [3], an infectious syndrome with a predominance of intracellular opportunistic and conditionally pathogenic microbes [4, 18], an immunoinflammatory syndrome, including persistent immune-mediated enterocolitis [10, 24], an allergic syndrome [12], an autoimmune syndrome [5, 9], and an increased tendency to develop neoplasia [7]. Actually, encephalopathy in GDFC, in addition to the direct metabolic mechanism of development, mediated, in particular, by the neurotoxic effect of homocysteine [3], is mostly due to the influence of immune-dependent mechanisms – infectious factors [4, 18], autoimmune reactions to neurons and myelin of the cerebral hemispheres [5, 9], systemic/intracerebral inflammation [15, 23].

Evidence for the development of a persistent systemic inflammatory response in children with ASD is based on the results of 2 meta-analyses of randomized controlled clinical trials. In particular, data from the first systematic review and meta-analysis of randomized controlled clinical trials show increased serum concentrations of the pro-inflammatory mediators interleukin-1beta (IL-1beta) ($p < 0.001$), interleukin-6 (IL-6) ($p = 0.03$), interleukin-8 ($p = 0.04$), interferon-gamma (IFN-gamma) ($p = 0.02$), eotaxin ($p = 0.01$), and monocyte chemotactic factor 1 ($p < 0.05$) and decreased levels of the anti-inflammatory cytokine transforming growth factor beta 1 ($p < 0.001$) in children with ASD ($n = 743$) compared to healthy subjects ($n = 592$) [15]. The results of a meta-analysis of randomized controlled clinical trials prepared by Saghazadeh A. et al., which includes 38 trials involving 2487 children, show a significant increase in serum concentrations of tumor necrosis factor alpha (TNF-alpha), IFN-gamma, IL-1beta, and IL-6 in children with ASD compared to healthy individuals [23].

It is important to further study the indicators of systemic inflammation in children with ASD to determine the most informative biomarkers of inflammation intensity that could be useful for clinical practice. The clinical significance of the phenomenon of persistent systemic inflammation in children with ASD needs to be clarified. It is advisable to study the relationship between increased serum concentrations of certain pro-inflammatory mediators and indicators of neuronal damage, which would provide additional evidence of the contribution of systemic inflammation to the development of encephalopathy and would allow us to propose new pharmacological methods of neuroprotection in children with ASD associated with GDFC for testing.

The aim of the research: to study serum concentrations of typical pro-inflammatory mediators TNF-alpha, IL-6, and tumor M2 pyruvate kinase (TM2PK) in children with ASD associated with GDFC, with clarification of their relationship with serum concentrations of marker molecules of CNS neuronal damage neuron-specific enolase (NSE) and S-100 protein to expand scientific ideas about the influence of the systemic inflammatory response on the development of encephalopathy in this pathology and to discover new points of application of neuroprotective treatment.

Materials and methods. The medical data of 225 children aged 2 to 9 years with GDFC, who were diagnosed with ASD, were analyzed. All of them were patients of the specialized neuroimmunological clinic Vivere (registration dossier dated 12/22/2018 No. 10/2212–M). Data for the study and processing of the material were carried out in accordance with contract No. 150221 dated 02/15/2021, and the conclusion of the bioethical examination commission (protocol No. 140 dated 12/21/2020 of the Bogomolets National Medical University). The clinical diagnosis of ASD was made by child psychiatrists according to the criteria of DSM–IV–TR (Diagnostic and Statistical Manual of mental disorders) and ICD–10 (The International Statistical Classification of Diseases and Related Health Problems). Pathogenic polymorphic variants of folate cycle genes were determined by PCR based on the detection of the nucleotide substitution MTHFR C677T in monoform, as well as – in combination with other nucleotide substitutions – MTHFR A1298C, MTRR A66G and/or MTR A2756G (111 individuals). These individuals constituted the study group (SG). The control group (CG) included 51 children (37 boys and 14 girls) of similar age distribution who did not suffer from GDFC.

In patients of both observation groups, the results of the study of serum concentrations of three typical pro-inflammatory mediators characterizing the intensity of systemic inflammation in the human body were analyzed, in particular – TNF-alpha (N up to 8.1 pg/ml), IL-6 (N up to 7 pg/ml) and TM2PC (N up to 20 U/ml). In parallel, in both observation groups, the data from measuring the concentration in the blood serum of two

characteristic indicators of neuronal damage were evaluated, namely – NSE (N up to 16.5 ng/ml) and S-100 protein (N up to 0.105 μg/l). At the time of laboratory studies, patients of both observation groups were not taking any medications that could affect the results of the tests.

Statistical processing of the obtained material was carried out by comparative and structural analyses. To determine the probability of differences between the studied laboratory parameters in the observation groups, the parametric Student's T-test with the confidence probability indicator p and the non-parametric criterion – the number of signs Z according to Urbach V.Yu. Differences were considered probable at $p<0.05$ and $Z<Z_{0.05}$. To study the associations between indicators of systemic inflammation and indicators of damage to CNS neurons, the odds ratio (OR) and 95 % confidence interval (95 % CI) were used. For the purpose of statistical calculations, Microsoft Excel was used.

This clinical study was performed as a fragment of research work commissioned by the Ministry of Health of Ukraine (state registration number 0121U107940).

Research results and their discussion. The structural analysis of the results of the study of laboratory indicators of systemic inflammation in the observation groups demonstrates that the most frequent disorder detected was an increase in the serum concentration of the pro-inflammatory mediator TM2PK, which was noted in 193 of 225 SG patients (86 %) and only in 11 of 51 CG children (22 % of cases). The serum concentration of the pro-inflammatory cytokine TNF-alpha was high in 139 of 225 SG children (62 %) and only in 7 of 51 CG children (14 % of cases). The serum concentration of another pro-inflammatory cytokine IL-6 was elevated in 69 of 225 SG children (31 %) and only in 3 of 51 CG children (6 % of cases) (**Fig. 6.1**).

The data from the comparative analyses indicate that there was a significant difference in the studied indicators of systemic inflammation in the observation groups due to a significantly greater proportion of increased serum concentrations of all three studied laboratory indicators of systemic inflammation among children with ASD associated with GDFC, compared with healthy children in the control group ($p<0,05$; $Z<Z_{0,05}$).

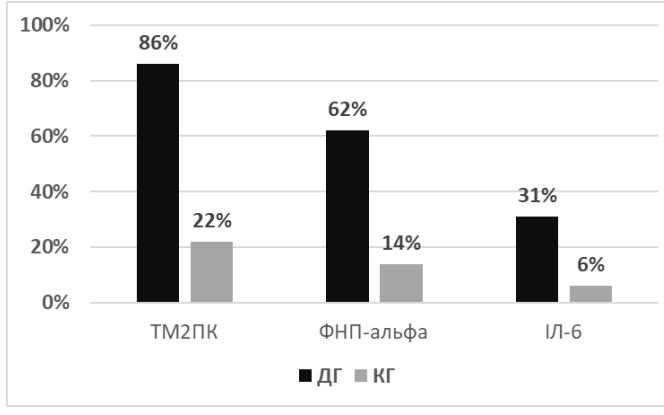

Fig. 6.1. Results of studying the structure of SG (n = 225) and CG (n = 51) in cases of elevated serum concentrations of the studied pro-inflammatory mediators

TM2PK was the most representative biomarker of systemic inflammation in the CG, since an increase in the serum concentration of this pro-inflammatory mediator was noted in almost all patients. However, a relatively large number of false-positive results (22 % of cases among healthy children) somewhat reduces the informativeness of this indicator in assessing the intensity of systemic inflammation in children with ASD associated with GDFC. Probably, in controversial cases, this indicator should be taken into account only with additional confirmation of the results of studying other indicators of inflammation with a smaller number of false-positive results. The concentration of TNF-alpha was increased in most children in the CG, but the number of positive results was almost a third less than in TM2PK. The small number of false-positive results (14 %) allows us to consider TNF-alpha as an informative indicator of the inflammatory reaction, which, however, does not characterize the group as a whole due to a relatively large number of negative results (38 % of cases). Serum IL-6 concentration was elevated only in one third of cases among children of the CG, which does not allow us to consider this biomarker representative of all patients with ASD associated with GDFC, for whom the development of persistent systemic inflammation in the body is characteristic. However, the smallest number of pseudo-negative results of this indicator allows us to consider it the most reliable among other indicators studied in this study. In particular, serum concentrations of TNF-alpha and IL-6 can be used to verify the results of measuring the concentration of TM2PC, for which pseudo-positive results are characteristic in every fifth patient.

The mean serum concentration of TM2PC in SG was high and equaled 63.1±3.74 U/ml, significantly exceeding the similar indicator in CG (23.2±0.91 U/ml) ($p<0.05$; $Z<Z_{0.05}$) (**Fig. 6.2**). The level of the mean serum concentration of TM2PC exceeded the upper limit of reference values three times, which indicates a large range of fluctuation of its measurement results. In contrast, the mean concentration of TNF-alpha in SG was 13.7±0.65 pg/ml, only 60 % exceeding the upper limit of the normal range, demonstrating a smaller range of fluctuation of measurement results. Comparison with similar data from the CG ($X = 5.3±0.38$ pg/ml) indicates a significant increase in serum TNF-alpha concentration in children with ASD associated with GDFC compared to healthy children ($p<0.05$; $Z<Z_{0.05}$). The average serum concentration of IL-6 in SG was elevated and was 8.7±0.57 pg/ml, which was only 20 % higher than the upper reference level, indicating a narrow range of fluctuation in the measurement results of this indicator. There was a significant difference in this indicator in SG and CG ($X = 3.6±0.27$ pg/ml) ($p<0.05$; $Z<Z_{0.05}$) **(Fig. 6.3)**. The greater the range of fluctuation in the measurement results of the indicator, the easier it is for the clinician to assess the intensity of inflammation and compare the results of studies in different patients. Therefore, TM2PC better than TNF-alpha and IL-6 characterized the intensity of inflammation, due to the large range of fluctuation of the results more fully demonstrating the gradation of the intensity of the systemic inflammatory reaction in different patients.

Thus, the mean serum concentrations of all three studied parameters were significantly increased in the SG compared with the CG, indicating the presence of laboratory signs of systemic inflammation in children with ASD associated with GDFC, and is consistent with the results of relevant meta-analyses of randomized clinical trials [15, 23]. However, significant differences were noted both in the proportion of cases with increased serum concentrations of a particular studied parameter, and in the levels of mean serum concentrations and the range of fluctuation of measurement results for each indicator.

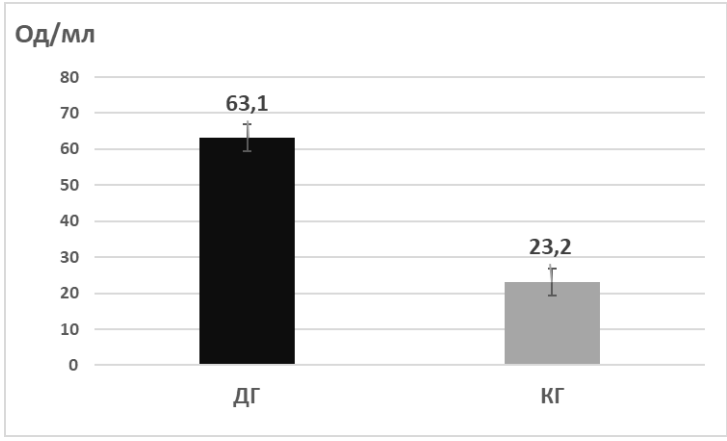

Fig. 6.2. Mean values (<<Eqn005.eps>>±m) of serum TM2PC concentrations in SG (n = 225) and CG (n = 51)

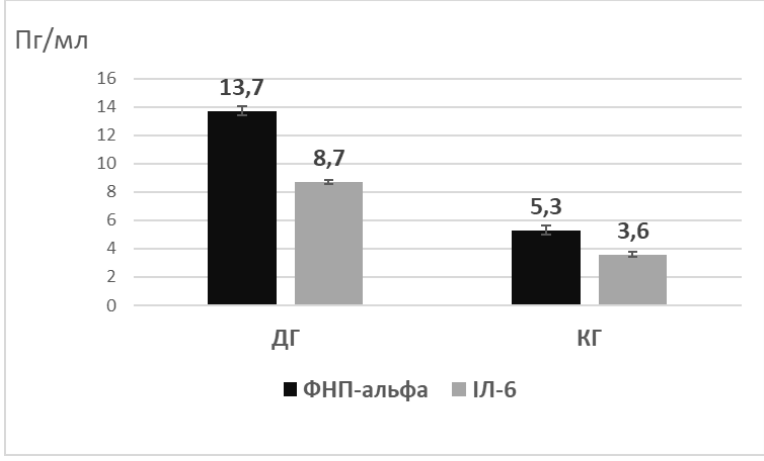

Fig. 6.3. Mean values (<<Eqn006.eps>>±m) of serum concentrations of TNF-alpha and IL-6 in SG (n = 225) and CG (n = 51)

TM2PC was the most sensitive and labile laboratory indicator of systemic inflammation among other studied indicators in children with ASD associated with GDFC, which, however, had the lowest specificity, giving false-positive results in at least every fifth patient. In contrast, IL-6 was the least sensitive and labile

indicator of systemic inflammation in SG, which, however, was characterized by high specificity, giving false-positive results only in every twentieth patient, i.e. at least 4 times less often than TM2PC. TNF-alpha was characterized by average sensitivity and lability with a relatively small number of false-positive results, therefore, this indicator among the three studied laboratory indicators can be considered the most informative for assessing the intensity of inflammation in children with ASD associated with GDFC. A comprehensive analysis of the results of measuring all three studied indicators of systemic inflammation is important, since each of them has certain advantages and disadvantages compared to the others.

The question of the clinical significance of systemic inflammation observed in children with ASD associated with GDFC is fundamental. In particular, it is necessary to clarify the contribution of systemic inflammation to the development of encephalopathy in such children and to find the most informative biomarkers for assessing the inflammatory mechanism of cerebral damage. To do this, we analyzed the associations of the studied indicators of systemic inflammation with serum concentrations of indicators of CNS neuronal damage NSE and S-100 protein (Table 1). The choice of the latter is not accidental, since their informativeness for assessing the severity of encephalopathy in children with ASD has been demonstrated in relevant clinical studies. Zheng Z. et al. conducted a meta-analysis of randomized clinical trials to study the informativeness of the use of serum concentrations of neurotropic calcium-dependent protein S-100 in children with ASD. The results of 10 trials involving 822 participants were analyzed. It has been shown that the concentration of S-100 protein in serum is significantly higher in children with ASD compared to healthy individuals and can be used as a biomarker of neuronal damage in such cases (standardized mean difference (SMD) = 0.97, 95 % CI = 0.41–1.53; p < 0.001) [27]. Accordingly, Lv M.N. et al. conducted a specially designed controlled clinical study involving 80 patients with ASD, demonstrating significantly increased concentration of NSE in serum in children with this mental disorder compared to healthy individuals [14].

● **Table 6.1.** Results of the association study (OR; 95 % CI) of the studied indicators of systemic inflammation and indicators of neuronal damage among SG patients (n = 225)

Indicator	TM2PC	TNF-alpha	IL-6
NSE	6,667; 1,668– 26,639	11,667; 2,064– 65,945	26,667; 1,843– 385,793
S–100 protein	7,570; 1,888– 30,351	10,000; 1,784– 56,060	15,200; 1,157– 199,642

As can be seen from the data in **Table 6.1**, all three studied indicators of systemic inflammation are associated with an increase in serum concentrations of laboratory indicators of neuronal damage NSE and protein S–100, which indicates a connection between the systemic inflammatory response and the development of encephalopathy in children with ASD associated with GDFC. The determination of increased serum concentrations of the studied indicators of systemic inflammation in the results of laboratory examinations of the patient sharply and reliably increases the risk of identifying high levels in the blood serum of such markers of CNS neuronal damage as NSE and protein S–100, which can be used in clinical practice when planning and organizing paraclinical examinations of the patient.

The strongest association was found for IL-6 and markers of neuronal damage, but the clinical application of this phenomenon may be limited by the low sensitivity of serum IL-6 concentration as an indicator of systemic inflammatory response in the defined group of patients. TM2PK demonstrated the weakest association with serum NSE and S-100 protein concentrations among the other studied indicators, which can be explained by the relatively large number of false-positive results when measuring this laboratory indicator. TNF-alpha occupied an average position in terms of the closeness of association with indicators of neuronal damage compared to TM2PK and IL-6. In general, the rule was true: the more specific the laboratory indicator was in assessing the intensity of the systemic inflammatory response in children with ASD associated with GDFC, the closer the association it demonstrated with the studied indicators of neuronal damage and better characterized the neurotoxic effect of systemic inflammation on the brain of patients.

The data revealed in this study not only expand the current understanding of the development of systemic inflammation and its impact on the formation of encephalopathy in children with ASD associated with GDFC, but also open the way to testing new therapeutic anti-inflammatory strategies for neuroprotection by targeting neutralizing pro-inflammatory molecules. The success of infliximab, a monoclonal antibody drug to the TNF-alpha molecule, in children with ASD could prompt more decisive action to plan and implement further dedicated clinical trials to study the efficacy and safety of modern anti-inflammatory treatment approaches in children with ASD associated with GDFC.

Conclusions. In patients with ASD associated with GDFC, serum concentrations of proinflammatory markers such as TM2PC, TNF-alpha, and IL-6 are elevated, indicating a state of systemic inflammation in the body of these children. If we talk about the clinical significance of the studied indicators of systemic inflammation, the latter differ significantly in sensitivity, lability, and specificity. TM2PC is the most sensitive and labile indicator with a relatively large number of false-positive results, while TNF-alpha occupies an intermediate position, and IL-6 is characterized by the lowest sensitivity and lability, but the highest specificity. None of the studied indicators can be considered ideal for assessing the state of systemic inflammation in children with ASD associated with GDFC, which implies the need for comprehensive data analysis. All studied indicators of systemic inflammation are associated with an increase in serum levels of neuronal damage indicators NSE and S-100 protein, which confirms the established notions about the role of systemic inflammation in the induction of encephalopathy in children with ASD associated with GDFC, and opens the way to testing new therapeutic strategies for anti-inflammatory neuroprotective therapy.

REFERENCES

1. Maltsev, D. V. (2018). Evaluation of the immune status in children with autism spectrum disorders associated with genetic folate cycle deficiency. Likarska Sprava, 1-2, 11–23. https://doi.org/10.31640/jvd.1-2.2018(02)
2. Maltcev, D. V., Natrus, L. V. (2020). The Effectiveness of Infliximab in Autism Spectrum Disorders Associated with Folate Cycle Genetic Deficiency. Psychiatry, Psychotherapy and Clinical Psychology, 3, 583–594. https://doi.org/10.34883/pi.2020.11.3.015

3. Bhatia, P., Singh, N. (2015). Homocysteine excess: delineating the possible mechanism of neurotoxicity and depression. Fundamental & Clinical Pharmacology, 29 (6), 522–528. https://doi.org/10.1111/fcp.12145
4. Binstock, T. (2001). Intra-monocyte pathogens delineate autism subgroups. Medical Hypotheses, 56 (4), 523–531. https://doi.org/10.1054/mehy.2000.1247
5. Cabanlit, M., Wills, S., Goines, P., Ashwood, P., Van de Water, J. (2007). Brain-Specific Autoantibodies in the Plasma of Subjects with Autistic Spectrum Disorder. Annals of the New York Academy of Sciences, 1107 (1), 92–103. https://doi.org/10.1196/annals.1381.010
6. Chen, L., Shi, X.-J., Liu, H., Mao, X., Gui, L.-N., Wang, H., Cheng, Y. (2021). Oxidative stress marker aberrations in children with autism spectrum disorder: a systematic review and meta-analysis of 87 studies (N = 9109). Translational Psychiatry, 11 (1). https://doi.org/10.1038/s41398-020-01135-3
7. Crawley, J. N., Heyer, W.-D., LaSalle, J. M. (2016). Autism and Cancer Share Risk Genes, Pathways, and Drug Targets. Trends in Genetics, 32 (3), 139–146. https://doi.org/10.1016/j.tig.2016.01.001
8. Frustaci, A., Neri, M., Cesario, A., Adams, J. B., Domenici, E., Dalla Bernardina, B., Bonassi, S. (2012). Oxidative stress-related biomarkers in autism: Systematic review and meta-analyses. Free Radical Biology and Medicine, 52 (10), 2128–2141. https://doi.org/10.1016/j.freeradbiomed.2012.03.011
9. Frye, R. E., Sequeira, J. M., Quadros, E. V., James, S. J., Rossignol, D. A. (2012). Cerebral folate receptor autoantibodies in autism spectrum disorder. Molecular Psychiatry, 18 (3), 369–381. https://doi.org/10.1038/mp.2011.175
10. Furlano, R. I., Anthony, A., Day, R., Brown, A., McGarvey, L., Thomson, M. A. et al. (2001). Colonic CD8 and γδ T-cell infiltration with epithelial damage in children with autism. The Journal of Pediatrics, 138 (3), 366–372. https://doi.org/10.1067/mpd.2001.111323
11. Guo, B.-Q., Li, H.-B., Ding, S.-B. (2020). Blood homocysteine levels in children with autism spectrum disorder: An updated systematic review and meta-analysis. Psychiatry Research, 291, 113283. https://doi.org/10.1016/j.psychres.2020.113283
12. Li, H., Liu, H., Chen, X., Zhang, J., Tong, G., Sun, Y. (2020). Association of food hypersensitivity in children with the risk of autism spectrum disorder: a meta-analysis. European Journal of Pediatrics, 180 (4), 999–1008. https://doi.org/10.1007/s00431-020-03826-x
13. Li, Y., Qiu, S., Shi, J., Guo, Y., Li, Z., Cheng, Y., Liu, Y. (2020). Association between MTHFR C677T/A1298C and susceptibility to autism spectrum disorders: a meta-analysis. BMC Pediatrics, 20 (1). https://doi.org/10.1186/s12887-020-02330-3
14. Lv, M., Zhang, H., Shu, Y., Chen, S., Hu, Y., Zhou, M. (2016). The neonatal levels of TSB, NSE and CK-BB in autism spectrum disorder from Southern China. Translational Neuroscience, 7 (1), 6–11. https://doi.org/10.1515/tnsci-2016-0002
15. Masi, A., Quintana, D. S., Glozier, N., Lloyd, A. R., Hickie, I. B., Guastella, A. J. (2014). Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis. Molecular Psychiatry, 20 (4), 440–446. https://doi.org/10.1038/mp.2014.59
16. Mead, J., Ashwood, P. (2015). Evidence supporting an altered immune response in ASD. Immunology Letters, 163 (1), 49–55. https://doi.org/10.1016/j.imlet.2014.11.006

17. Shaik Mohammad, N., Sai Shruti, P., Bharathi, V., Krishna Prasad, C., Hussain, T., Alrokayan, S. A. et al. (2016). Clinical utility of folate pathway genetic polymorphisms in the diagnosis of autism spectrum disorders. Psychiatric Genetics, 26 (6), 281–286. https://doi.org/10.1097/ypg.0000000000000152
18. Nicolson, G. L., Gan, R., Nicolson, N. L., Haier, J. (2007). Evidence for Mycoplasma ssp., Chlamydia pneunomiae, and human herpes virus-6 coinfections in the blood of patients with autistic spectrum disorders. Journal of Neuroscience Research, 85 (5), 1143–1148. https://doi.org/10.1002/jnr.21203
19. Noriega, D. B., Savelkoul, H. F. J. (2013). Immune dysregulation in autism spectrum disorder. European Journal of Pediatrics, 173 (1), 33–43. https://doi.org/10.1007/s00431-013-2183-4
20. Pu, D., Shen, Y., Wu, J. (2013). Association between MTHFR Gene Polymorphisms and the Risk of Autism Spectrum Disorders: A Meta-Analysis. Autism Research, 6 (5), 384–392. https://doi.org/10.1002/aur.1300
21. Rai, V. (2016). Association of methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism with autism: evidence of genetic susceptibility. Metabolic Brain Disease, 31 (4), 727–735. https://doi.org/10.1007/s11011-016-9815-0
22. Sadeghiyeh, T., Dastgheib, S. A., Mirzaee-Khoramabadi, K., Morovati-Sharifabad, M., Akbarian-Bafghi, M. J., Poursharif, Z. et al. (2019). Association of MTHFR 677C > T and 1298A > C polymorphisms with susceptibility to autism: A systematic review and meta-analysis. Asian Journal of Psychiatry, 46, 54–61. https://doi.org/10.1016/j.ajp.2019.09.016
23. Saghazadeh, A., Ataeinia, B., Keynejad, K., Abdolalizadeh, A., Hirbod-Mobarakeh, A., Rezaei, N. (2019). A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders: Effects of age, gender, and latitude. Journal of Psychiatric Research, 115, 90–102. https://doi.org/10.1016/j.jpsychires.2019.05.019
24. Torrente, F., Ashwood, P., Day, R., Machado, N., Furlano, R. I., Anthony, A. et al. (2002). Small intestinal enteropathy with epithelial IgG and complement deposition in children with regressive autism. Molecular Psychiatry, 7 (4), 375–382. https://doi.org/10.1038/sj.mp.4001077
25. Wang, Z., Ding, R., Wang, J. (2020). The Association between Vitamin D Status and Autism Spectrum Disorder (ASD): A Systematic Review and Meta-Analysis. Nutrients, 13 (1), 86. https://doi.org/10.3390/nu13010086
26. Yektaş, Ç., Alpay, M., Tufan, A. E. (2019). Comparison of serum B12, folate and homocysteine concentrations in children with autism spectrum disorder or attention deficit hyperactivity disorder and healthy controls. Neuropsychiatric Disease and Treatment, 15, 2213–2219. https://doi.org/10.2147/ndt.s212361
27. Zheng, Z., Zheng, P., Zou, X. (2020). Peripheral Blood S100B Levels in Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Journal of Autism and Developmental Disorders, 51 (8), 2569–2577. https://doi.org/10.1007/s10803-020-04710-1