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Serologic and Genetic Markers of Gluten Intolerance in Autism Spectrum Disorders.

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ABSTRACT

The paper discusses digestive disorders which are often accompanied by alterations in the psychic sphere. The authors present the study investigating serologic and genetic markers of gluten intolerance in children and adolescents with autism spectrum disorders. The participants of the study included 33 children, aged 3-15, suffering from autism spectrum disorder. All patients were tested to gliadin antibodies IgG, deamidated gliadin peptide antibodies IgA, immunoglobulin A, haplotypes DQ2 and DQ8. As it has been stated, gliadin antibodies IgG were revealed in 12.1%-13.8%, haplotypes DQ2 and DQ8 had 41.9% incidence in children with autism. No deamidated gliadin peptide antibodies IgA were revealed in patients. Prevailing form of gluten intolerance in children is considered to be gluten sensitivity that can be manifested in 40-50% of patients. Autoimmune form of gluten intolerance (celiacia) can be observed in isolated cases, though 41.9% of children are predisposed to it. Prior to gluten-free dietary intervention all patients have to undergo laboratory tests to reveal various forms of gluten intolerance and conclude on further application of gluten-free diet.

Keywords: gluten intolerance, gluten sensitivity, autism spectrum disorders, gluten-free diet.

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INTRODUCTION

Currently there is fragmented data that gluten intolerance can be accompanied not only by gastrointestinal, but various extraintestinal disorders, including psycho-neurological disorders. Modern approaches distinguish three forms of gluten intolerance. Celiacia, a form of chronic immune-mediated enteropathy caused by gluten consumption, is considered to be the most thoroughly studied.Globally its incidence in genetically susceptible populationamounts to 0.3-1-2% by various estimates. Apart from celiacia, there is also allergy to gluten andgluten sensitivity (GS) – non-autoimmune non-allergic gluten intolerance [1-3]. Currently a strict gluten-free diet that arrests symptoms of the disease appears to be the only effective therapy for all three forms of gluten intolerance [4].

Celiacia is called a "great mime" for a wide variety of clinical courses. Symptoms and course peculiarities of its typical (gastroenterological) form are reported to be the most investigated today. However, its atypical form can be manifested by psycho-neurological disorders which include cerebellar ataxia, epilepsy with encephalic calcification, neuropathy [5-7].

Gluten sensitivity accompanied by a variety of intestinal and extraintestinal manifestations, is also a "great mime". It is noteworthy, that the 3rd International Expert Meeting on Gluten Related Disorders (Salerno, 2014) included psycho neurological manifestations, namely, sleep disorders, impaired sensitivity, mood swings, hallucinations, autism, schizophrenia in the list of rare GS manifestations.

Clinical practice confirms that digestive disorders are often accompanied by disordersin the psychic sphere. There is some evidence that socially significant psychic disorders, such as autism spectrum disorders (ASD), are based on autoimmune processes mediating them [8, 9, 10]. A systemic autoimmune response (ANCA and ASCA*)* occurs in children with ASD and gluten intolerance [11]. Special eliminating diets are sometimes applied as ASD therapy [9]. P.Whiteley et al. (2010) presented data of the «ScanBrit» study of gluten-free (and casein-free (GF and CFD)) diets in treating children with ASD. The study demonstrated positive dynamics of autism course under the condition of the adherence to the diet.However,many researchers highlight the fact thatnumerous studies have reported no evidence of obvious effect of gluten-free diet intervention [12]. There are a number of research studies reflecting ASD association withGS [13] (genetically determined form of gluten intolerance) rather than with celiacia. However, currently there is no reliable and consistentevidencesupporting this issue, which makes diagnostics of various forms of gluten intolerance in patients with ASD an acute clinical challenge. The investigation of the impact of various forms of gluten intolerance will allow to develop clinically based recommendations on including GFD in the medical and social support for children with ASD and Down syndrome (DS), which can result in increased learning abilities and stabilization of psycho-neurological status.

Aim of study: to investigate serologic and genetic markers of gluten intolerance in children and adolescents with autism spectrum disorders.

MATERIALS AND METHODS

The participants of the study included 33 children, aged 3-15, suffering from autism spectrum disorder (median line – 8 years of age, 25 quartile – 6 years of age, 75 quartile – 11 years of age, average age: 6.7 years), residents of the Voronezh region, and their parents. There were 27 male patients and 6 female patients. Most of the patients were on the usual diet, and only 4 children were on gluten-free diet, as well as casein-free diet, for 1-4 years. Their parents were surveyed with the specially developed questionnaire including questions on parents' awareness about GFD as a means of therapy, child's condition before and after dietary intervention, complications of GFD application in the complex of therapeutical procedures.

All stages of study conform to legislation of the Russian Federation, international ethic norms and reference documents of research organizations. The performed study was approved by the Ethic Committee, Federal State Budget Educational Institution of Higher Education "Voronezh N.N.Burdenko State Medical University", Ministry of Healthcare of the Russian Federation. Parents gave a written informed consent for participation of their children in the study.

All patients were examined to reveal currently known markers applied in the diagnostics of various forms of gluten intolerance. Polymerase chain reaction technique was applied to specify haplotypes DQ2 (DQA1*501 DQB1*201) and DQ8 (DQA1*301 DQB1*302) of the HLA-system indicating at genetic predisposition to celiacia. OOO "DNA-Technology" packages were applied in this testing. 31 out of 33 children were examined due to technical reasons. This marker is characterized by the negative predictive value, i.e. lack of the given haplotypes gives high chances to exclude celiacia. Enzyme immunoassay (EIA) for serological markers was also performed to specify:

1. deamidated gliadin peptide antibodies IgA characterized by high specificity (86-93%) and sensitivity (81-95%) for celiacia diagnostics, a reference range is 0-10 units/ml;
2. gliadin IgG antibodies, that are not considered to be diagnostically relevant for celiacia according to present-day views, but are revealed in 50% of patients with GS (Salerno, 2014), a reference range is 0-25 units/ml.

Patients' venous blood taken from the median cubital vein served as the study material. All patients were also examined to reveal the immunoglobulin A level to exclude selective IgA deficiency – a sufficiently widespread condition in children (1:100) resulting in false-negative serological tests. Packages produced by ZAO "Vektor-Best-Yug" company were used for EIA.

Statistical analysis was performed using Statistica 6.0 program. Descriptive statistic methods (relative values expressed in percentage, median line and inter-quartile range specification – 25-75%) were used.

RESEARCH RESULTS AND DISCUSSION

The level of deamidated gliadin peptide antibodies IgA did not exceed a reference rangein all patients participated in the study. This level allowed to rule out celiacia in 28 patients, while in 5 patients it might be determined by different factors.As it has been mentioned above, 4 children were on the prolonged GFD; the level of all specific antibodies decreases to normal values in all patients with celiacia in case of their adherence to gluten-free diet. Selective IgA deficiency was revealed in one child (an absolute value was 0.01 mg/ml; a reference range was 0.7-4.5 mg/ml).It is important to note that no patients were examined for serological markers to gluten intolerance prior to the diet therapy. This complicated diagnosing celiacia based on serological tests in 5 children; while 3 children with revealed propensity score markers had such a potential (one child with each haplotypes DQ2/ DQ8, DQ8 and DQ2).

The increased level of deamidated anti-gliadin peptide antibodies IgG was revealed in 12.1% (4 patients out of 33) in general. In patients, who were not on GFD, the increased level of deamidated anti-gliadin peptide antibodies IgG was revealed in 13.8% (4 out of 29 patients); this proved the presence of gluten sensitivity. Normal concentration of the abovementioned antibodies in the blood serum of four children being on the prolonged GFD is obviously determined by adherence to a strict and long-term the diet. Therefore, in spite of the negative results of the examination GS may be diagnosed in these children based on the clinical efficiency of the diet therapy reported by the parents (for this reason they adhere to the diet). It should be noted that it was the clinical approach which was recommended by the International Expert Meeting on Gluten Related Disorders (Salerno, 2014). This approach includes assessment of the dynamics of clinical symptoms in a patient when changing his usual diet into a gluten-free diet and then applying gluten challenge. The issue on gluten challenge to patients with psycho-neurological disorders is debatable, and it needs further discussion and consensus. Thus, GS was diagnosed in 24.2 % (8 patients out of 33) based on serological and clinical values. However, it should be pointed out that true frequency of GS is higher in children with ASD. There are two points worth consideration. First, gliadin IgG antibodies in GS aredetected only in 50% of patients; and, second, the majority of patients with autism included in the study (80-85%) were not on experimental gluten-free diet. With the view of the latter we can expect GS presence in 40-50% children with ASD.

Lau N.M. et al. [13] reported that children with ASD manifested considerably higher levels of gliadin IgG antibodies comparing with their healthy brothers and sisters (control group) ($p<0.01$). It was also stated that the level of gliadin IgG antibodies was statistically higher ($p<0.01$) in children suffering from autism and having gastro-intestinal symptoms comparing with those who did not complain of gastro-intestinal disorders.

Moreover, as it was found out, deamidated gliadin peptide antibody values did not differ between patients and the control group.

Genetic predisposition markers HLA DQ2 / DQ8 to celiacia were identified in 31 children andthen proven in 41.9% (14 patients). This could be compared to the commonstatictics of their incidence: 30% of population in general, 50% of patients with GS, 95-98% of patients with celiacia [2, 5]. 64.3% (9 children) of patients had haplotype DQ2, 28.6% (4 children) had haplotype DQ8, 7.1% (1 child) had a combination of DQ2/ DQ8. The revealed haplotype incidence conforms to data on DQ2 prevalence in children with gluten intolerance but without ASD in Voronezh region and other Russian regions as well [14, 15]. However, it should be highlighted that HLA-molecules DQ2/DQ8 detection does not give evidence of celiacia in these children, but only proves the fact of their predisposition to the occurrence of the given disorder.

The performed studies and their findingshave contributed to the challenge of gluten-free diet administration to children with ASD the current attitudes to which areambiguous and controversial. The parents' survey has demonstrated that 8 patients (24.2%) out of 33 children with ASD applied the diet therapy in various periods, but only 4 of them were following the diet during the study. All 8 parents, whose children were administered the diet as a part of the complex autism therapy, reported the improvement of psycho-neurological state in their children during the first 2-6 months after the beginning of gluten-free diet. Thus, according to parents' assessment, their children started to take interest in their peers; followed teaching guidelines more easily; their sleep pattern improved, mental alertness increased. The symptoms of gastrointestinal disorders alleviated, i.e. a regular formed stool occurred. The study has revealed no parents who denied the diet therapy positive effects. Nevertheless, only 4 children continue to adhere to the gluten-free diet; the other patients have discontinued it. The reasons for discontinuation were as follows: contradictory information about the efficiency of gluten-free diet, additional psychological burden on a child and family members, high cost and restricted availability of gluten-free products. It should be noted that parents of the children with celiacia face the same problems when taking up a diet therapy [18]; however, for parents of the children with ASD these difficulties are even worse due to manifestations of the disease itself.

CONCLUSIONS

- Gluten sensitivity (GS), that may occur in 40-50% of patients, is considered to be a prevailing form of gluten intolerance in children with autism spectrum disorders. Celiacia may occur in isolated cases, though, 41.9% of patients are predisposed to it.
- No deamidated gliadin peptide antibodies class A were revealed in patients. In children with autism spectrum disorders gliadin IgG antibodies are revealed in 12.1-13.8% of patients, haplotypes DQ2/DQ8 are revealed in 41.9% of patients.
- Prior to gluten-free dietary intervention all patients have to undergo laboratory tests to reveal various forms of gluten intolerance and conclude on further application of gluten-free diet.
- Currently there are no sufficient evidence to include GFD in ASD protocols, however, aspects of pathogenic significance of gluten intolerance in the development of autism spectrum disorders need to be further investigated, specified and individualized.

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