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Plasma levels of serotonin, gastrointestinal symptoms, and sleep problems in children with autism

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Background/aim: Autism is a neurodevelopmental disorder identified with higher frequency of serotonin abnormalities and gastrointestinal (GI) and sleep problems. This study aimed to evaluate the plasma levels of serotonin, GI symptoms, and sleep problems, and their relationship with autism severity in children with autism.

Materials and methods: Thirty-five children with autism and 31 healthy subjects were studied. GI problems, sleep disorders, and severity of disorder were assessed. Plasma serotonin was determined using ELISA.

Results: There was no significant association between GI problems and autism severity, but a significant positive correlation was seen between different indicators of sleep disorder and severity of autism. Plasma levels of serotonin were significantly higher in autistic children and a significant negative correlation was observed between plasma levels of serotonin and autism severity ($r = -0.39$, $P = 0.02$).

Conclusion: Elevated plasma serotonin in autistic children and its negative correlation with disease severity may indicate involvement of the neurotransmitter in the neurophysiologic mechanism of autism.

Key words: Autism, serotonin, gastrointestinal symptoms, sleep problems, autism severity

1. Introduction

Autism is a neurodevelopmental and behaviorally defined disorder with multiple genetic and environmental risk factors that develops typically in the few first years of life (1). Several reports have suggested that gastrointestinal (GI) disorders and sleep disturbances are present in higher frequency among autistic children than in those typically developing (2–6). It has been described that 22.7%–84% of autistic children experience GI symptoms such as constipation, abdominal pain, diarrhea, and gastric irritation (7–9). In addition, it has been reported that 25%–80% of children with autism have sleep problems, including sleeping less, waking up more during the night, early morning awakening, shortened night's sleep, and parasomnia (10). Disturbed sleep potentially aggravates autism-related indicators, including over-activity, disruptive behavior, social difficulties, and disease severity (4,11).

Serotonin or 5-hydroxy tryptamine (5-HT) is a neurotransmitter derived from tryptophan and mainly found in the GI tract, blood platelets, and the central nervous system (CNS). It regulates a variety of behavioral, autonomic, and cognitive functions. Altered blood serotonin levels were the first biomarker found in autism studies (12,13). Rodent studies indicate a serotonin abnormality can occur during the development of autism. An animal study has reported that mice genetically depleted of brain serotonin demonstrate behavioral phenotypes that are greatly characteristic of autism, including social impairments, communication deficits, and repetitive behaviors (14). There is also evidence that reduction of placenta-derived serotonin levels during embryonic pregnancy leads to hypo-serotonergic situations in the forebrain of the fetus and causes autism (15). Clinical observations include both elevated and lowered levels of the neurotransmitter in subjects with autism compared to controls (16,17). Some investigations have reported that about one-third of autistic patients have elevated levels of whole blood or platelet serotonin (18,19).

According to previous studies, serotonin is indirectly involved in regulating the sleep–wake cycle through its metabolite melatonin (20,21). Moreover, it has been reported that supplementation with 5-hydroxy tryptophan (metabolic intermediate in the biosynthesis of serotonin

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and melatonin) regulated sleep and circadian sleep–wake rhythm in animals (22,23). Furthermore, changes in serotonin signaling have been demonstrated in the number of gastrointestinal diseases, including inflammatory bowel disease, irritable bowel syndrome, postinfectious irritable bowel syndrome, and idiopathic constipation (24,25). Taking these results together, it is postulated that serotonergic abnormalities are possibly associated with the clinical features of autism.

It appears that understanding any possible relation between plasma levels of serotonin with autism severity and both GI symptoms and sleep problems will be of help for better clarifying the pathogenesis and further management of these important two co-morbidities in autism. Therefore, this study aimed to evaluate plasma levels of serotonin, GI symptoms, and sleep problems, and their relationship with autism severity in children and adolescents diagnosed with autism.

2. Materials and methods

2.1. Participants

In this case-control cross-sectional study, 35 children and adolescents with autism (24 boys, 11 girls) and 31 typically developing healthy children (18 boys, 13 girls) as a control group, aged 4–18 years, participated. The patients met the diagnostic criteria of autism according to the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) through diagnostic interview by a psychiatrist. The control group was healthy and normally developing children who attended the outpatient department of pediatric hospital for routine health care and matched for age and sex with the autistic group. Inclusion criteria for the autism group were no current use of any chelation treatment or prior diagnosis of autism, according to psychiatrist interviews and DSM-IV-TR criteria. Good mental and physical health according to the Strengths and Difficulties Questionnaire (SDQ) was the inclusion criterion for the control group. Subjects with neurological diseases such as cerebral palsy and tuberous sclerosis and metabolic disorders such as phenylketonuria were excluded.

The parents were asked to answer a self-constructed questionnaire about demographic information such as age, medicine consumption, age of parent and level of education, and presence or absence of epilepsy and other diseases. The study was approved by the Ethical Committee at Tabriz University of Medical Sciences, Tabriz, Iran. Informed consent was obtained from the subjects or their parents.

2.2. Blood collection

Venous blood samples were collected from both groups in test tubes containing sodium heparin as an anticoagulant. Samples were centrifuged at 3500 rpm at room temperature for 15 min. Poor platelet plasma was obtained and deep frozen at –70 °C until analysis time.

2.3. Assessment of autism severity

The Gilliam Autism Rating Scale (GARS) was used to confirm the diagnosis of autism and assess the severity of the disorder. The severity of autism was categorized as mild (score ≤52), moderate (score = 53–84), or severe (score ≥85), according to the GARS scale.

2.4. Assessment of gastrointestinal problems

The Rome III version (QPGS-III) questionnaire was used to evaluate gastrointestinal symptoms. The tool includes multiple sets of questions for evaluating functional dyspepsia, upper and lower abdominal pain associated with bowel symptoms, abdominal migraine, functional abdominal pain, constipation, nonretentive fecal incontinence, aerophagia, cyclic vomiting syndrome, and adolescent rumination syndrome.

2.5. Assessment of sleep problems

The Children's Sleep Habits Questionnaire-Abbreviated (CSHQ-A) was used as a screening tool to identify sleep disturbances such as bedtime resistance, sleep onset delay, and parasomnias. CSHQ-A is a 33-item questionnaire in which each item is rated on a three-point scale: 1 = rarely (0–1 time/week); 2 = sometimes (2–4 times/week); 3 = usually (5–7 times/week). Each question was asked in relation to the previous week.

2.6. Measurement of plasma serotonin

Plasma serotonin concentration was determined using a serotonin ELISA kit (LDN GmbH & Co. KG, Nordhorn, Germany). First, 25 µL of the acylated standards, controls, and samples were pipetted into the appropriate wells of the serotonin microtiter strips. Then 100 µL of the serotonin antiserum was added to all wells and they were incubated for 30 min at room temperature. All wells were washed 3 times thoroughly using wash buffer and incubated for 15 min after 100 µL of the conjugate was added. Finally, 100 µL of the substrate was pipetted into all wells and after 15 min incubation on a shaker 100 µL of the stop solution was added. The absorbance of the solution in the wells was read within 10 min using a microplate reader set to 450 nm.

2.7. Statistical analysis

The data were analyzed using SPSS, version 16.0. Demographic characteristics of the participants were summarized by either mean ± standard deviation (SD) for continuous data or percentage (frequency) for proportional data. Differences between the two groups were assessed by independent sample t-test for normally distributed data, Mann–Whitney U test for nonparametric variables, and chi-square test for qualitative data. Spearman's coefficient correlations were determined for associations between variables. P-values less than 0.05 were considered statistically significant.

3. Results

3.1. Participant Characteristics

Thirty-five children with autism and 31 healthy subjects, as a control group, completed the study. The mean ± SD of age for children with autism was 8.1 ± 4.0 years and for the control group was 7.3 ± 2.6. Demographics characteristics of all subjects are shown in Table 1. In the autism group, the percentage of mothers and fathers with high education was nonsignificantly greater than that in the control group. There were also no significant differences between the two groups in terms of demographic factors.

3.2. Gastrointestinal symptoms

In the autism group, 57.1% (n = 20) of the children had gastrointestinal problems, but only 3.2% (n = 1) of the control children were suffering from GI problems based on parent reports. The observed difference between the two groups was statistically significant ($P < 0.01$).

Then for a more accurate assessment of the problems we used a questionnaire on pediatric gastrointestinal symptoms, Rome III version (QPGS- III). Based on this questionnaire, 44.1% of autistic children had GI problems as shown in Table 2. The most common gastrointestinal symptom was constipation (n = 10, 15.2%).

3.3. Sleep problems

A comparison of CSHQ scale scores for the autism and control groups is shown in Table 3. There were significant differences in bedtime resistance, sleep anxiety, parasomnias, daytime sleepiness, and total scale score ($P < 0.05$) between the two groups. There were no significant differences with sleep duration, sleep onset delay, or night waking subscales ($P > 0.05$).

3.4. Plasma level of serotonin and its association with GI and sleep dysfunction and demographic factors

As shown in the Figure, the autism group had significantly higher plasma serotonin levels (2.73 ± 0.61 ng/mL) than the healthy children (2.35 ± 0.31 ng/mL) ($P = 0.002$). No significant correlation was found between plasma levels of serotonin and all medications used.

Table 1. Sociodemographic characteristics of autistic and control groups.

Characteristics	Autism (n = 35)	Control (n = 31)	P
Sex *			
Male	68.6 (24)	58.1 (18)	0.38
Female	31.4 (11)	41.9 (13)	
Age (year)**	8.1 ± 4.0	7.3 ± 2.6	0.34
Area of residence*			
Urban	82.9 (29)	77.4 (24)	0.57
Rural	17.1 (6)	22.6 (7)	
Education of mother*			
<High school	51.4 (18)	67.7 (21)	
High school only	28.6 (10)	32.3 (10)	0.07
College/postgraduate degree	20.0 (7)	0 (0)	
Education of father*			
<High school	42.9 (15)	61.3 (19)	
High school only	34.3 (12)	29.0 (9)	0.09
College/postgraduate degree	22.9 (8)	9.7 (3)	
Occupation of mother*			
Working	14.3 (5)	3.2 (1)	0.25
Housewife/retired	85.7 (30)	96.8 (30)	
Ordinal position of child*			
First born	54.3 (19)	58.1 (18)	0.78
Second born or later	45.7 (16)	41.9 (13)	
Medications*			
Yes	97.1 (34)	0 (0)	<0.001
No	2.9 (1)	100 (31)	

* expressed as percent (number); ** expressed as mean ± SD.

Table 2. Symptoms of gastrointestinal problems.

Symptoms	% (n) with problems
Functional constipation	15.2 (10)
Cyclic vomiting syndrome	6.1 (4)
Nonretentive fecal incontinence	6.1 (4)
Irritable bowel syndrome	3.0 (2)
Functional abdominal pain	6.1 (4)
Abdominal migraine	7.6 (5)
Aerophagia	0.0 (0)
Adolescent rumination syndrome	0.0 (0)

Table 3. Comparison of CSHQ[a] scale scores between autism and control groups.

	Case (n = 35)	Control (n = 31)	P
Subscale item			
Bedtime resistance	9.23 ± 4.63	6.35 ± 2.19	0.0001*
Sleep onset delay	2.23 ± 0.97	2.57 ± 1.07	0.18
Sleep duration	5.46 ± 1.03	5.52 ± 1.06	0.82
Sleep anxiety	6.06 ± 3.40	4.06 ± 3.18	0.01*
Night wakening	4.00 ± 2.63	3.81 ± 2.40	0.75
Parasomnias	8.89 ± 6.38	3.16 ± 2.05	0.0001*
Sleep-disordered breathing	3.11 ± 2.04	3.87 ± 2.32	0.16
Daytime sleepiness	13.29 ± 4.96	9.97 ± 2.47	0.0001*
Total scores	54.54 ± 18.85	39.29 ± 5.86	0.0001*

[a] Children's Sleep Habits Questionnaire-Abbreviated
Data are presented as mean ± SD
*P values less than 0.05 were considered statistically significant.

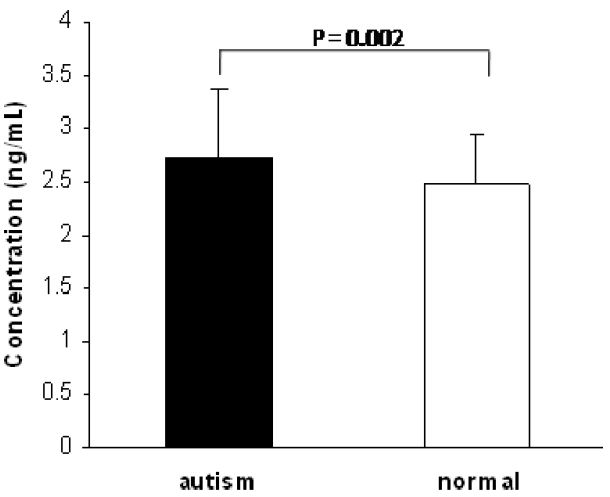

Figure. Plasma levels of serotonin in autistic (n = 35) and healthy children (n = 31). The data were generated by the translog and expressed as mean ± SD.

Among the problems related to sleep, only sleep-disordered breathing problem was negatively correlated with plasma levels of serotonin (r = –0.22, P = 0.05). There was no significant relationship between all indicators of GI and other sleep disturbances with plasma levels of serotonin (data not shown).

3.5. Severity of autism and its association with clinical and laboratory factors

As shown in Table 4, 17.1% (n = 6) of the autistic children were recognized with mild, 48.6% (n = 17) with moderate, and 34.3% (n = 12) with severe disorder level. There was a significant negative correlation between plasma levels of serotonin and autism severity (r = –0.39, P = 0.02). In autistic children, there was no significant correlation between child's age and birth rank with the severity of disease (r = –0.19, P = 0.17), (r = 0.48, P = 0.75).

As shown in Table 5, there were significant correlations between autism severity and bedtime resistance (r = 0.56,

Table 4: Severity of autism by sex.

Severity	Boys	Girls	Total
Mild	12.1 (4)	5 (2)	17.1 (6)
Moderate	37.14 (13)	11.46 (4)	48.6 (17)
High	20.1 (7)	14.2 (5)	34.3 (12)

Expressed as a percent (number)

Table 5. Correlation between autism severity and CSHQ[a] scores.

Subscale item	R	P
Bedtime resistance	0.56	0.0001*
Sleep onset delay	–0.10	0.56
Sleep duration	0.75	0.61
Sleep anxiety	0.55	0.0001*
Night wakening	0.30	0. 07
Parasomnias	0.83	0.0001*
Sleep-disordered breathing	0.50	0.0001*
Daytime sleepiness	0.64	0.0001*
Total scores	0.81	0.0001*

[a] Children's Sleep Habits Questionnaire-Abbreviated
*P values less than 0.05 were considered statistically significant.

P < 0.01), sleep anxiety (r = 0.55, P < 0.01), parasomnias (r = 0.83, P < 0.01), sleep-disordered breathing (r = 0.50, P < 0.01), daytime sleepiness (r = 0.64, P< 0.01), and total scores (r = 0.81, P < 0.01). There was no significant association between any indicators of GI symptoms and severity of autism**.**

4. Discussion

In the present study, more than half of the children with autism were found to have GI problems. Numerous investigations have indicated the presence of GI problems in autistic children (2–8). These problems can lead to other difficulties such as feeding problems, social difficulties, and autism severity scores (9,26). Finding of the source of these problems and providing therapy can be helpful in reducing the severity of disease. The exact cause of the high rate of GI problems in autistic children is unknown, but a series of assumptions about the mechanism of these problems has been proposed: these symptoms may be due to disruption of endogenous gut microflora promoting overgrowth of pathogenic microorganisms suspected to produce neurotoxins (27). GI symptoms may also be related to a disruption in the mucosal lining of the gut, causing malabsorption of large proteins such as gliadin and casein. Peptides derived from gluten and casein fail to become amino acids and increased gut permeability then allows the peptides to leak into the blood stream, where they circulate and eventually cross the brain–blood barrier, which could ultimately lead to inflammation and alter neurologic functions (28). A number of studies have investigated potential changes in mucosal serotonin signaling in pathologic conditions of the gut, because the predominant site of serotonin synthesis, storage, and release is the enterochromaffin cells of the intestinal mucosa. Changes in serotonin signaling have been demonstrated in a number of gastrointestinal diseases (24,25). Another hypothesis is related to the stereotyped behaviors. Stereotyped diets may result in an inadequate intake of fiber and fluids or high intakes of processed food, which can cause gastrointestinal problems (29). Medication administered to children with autism can influence gut function; for example, stimulants can cause abdominal pain (30,31). Finally, in some reports, decreased activity of digestive enzymes has contributed to GI problems in

children with autism. Horvath's study showed that 49% of children with autism who underwent endoscopy had deficiencies in one or more disaccharidase enzymes, especially lactase and maltase (32). This deficiency can lead to malabsorption and GI problems.

In the current study, children with autism had significantly more sleeping problems than the control children. The results show that the autism group had significantly higher scores in bedtime resistance, sleep anxiety, parasomnias, sleep-disordered breathing, daytime sleepiness, and CSHQ total score than the control group. Our results are in agreement with previous studies that reported a high prevalence of sleep disturbances in children with autism (5,6,8). The origin of sleep disorders such as GI problems is not known. It has been hypothesized that the sleep difficulties seen in autism are the result of the aberrant activation of neural circuitry involved in the control of both rapid eye movement (REM) and non-REM sleep. Another hypothesis may be related to production of melatonin. Serotonin is the precursor for melatonin and impaired plasma or brain levels of serotonin alter melatonin secretion in humans. It has been suggested that melatonin regulation may be abnormal in autism (33). In the present work, a significant correlation was observed between autism severity and bedtime resistance, sleep anxiety, parasomnias, sleep-disordered breathing, daytime sleepiness, and total scores. This result was similar to Tudor's study finding indicating a positive correlation between the severity of sleep problems and the severity of autism symptoms (34). In another study, it was demonstrated that fewer hours of sleep per night predict overall autism severity scores and social skills deficits (5).

In the present work, greater levels of plasma serotonin were observed in autistic children. Findings from previous investigations on this topic are inconsistent. Increased plasma or whole blood levels of serotonin have been shown in earlier studies. Hranilovic et al. showed that the platelet serotonin level was significantly higher in autistic adults than the control sample and hyperserotonemic subjects made up 32% of the patients (35). Conversely, Nagwa et al. reported a low level of plasma serotonin in autistic children compared to controls (36). Inconsistent findings in the studies evaluating peripheral 5-HT in autistic patients might be due to differences in sampling demographics (e.g., age range, sex, ethnicity), patient characteristics (e.g., hyperactivity grade), subjects used as controls, 5-HT measurement protocol, place of 5-HT measurement (serum, plasma or platelet), or biomaterials and analytical procedures used (17). The exact mechanism of the enhanced circulation serotonin in autistic patients has remained unclear. Different hypotheses have been expressed in relation to disturbance of serotonin metabolism in autism. Nakamura et al. in their study suggested that the hyperserotonemia is related to serotonin receptor (SERT) dysfunction that leads to lower serotonin entry into the cell and thus increases the accumulation of serotonin in the blood (37). Increased synthesis of 5-HT in the intestine, altered release from the enterochromaffin cells, and gene dysfunction (e.g., a mutation in the tryptophan 2,3-dioxygenase gene) have also been suggested as possible causes (38–41). In the present study, the neurotransmitter level was negatively correlated with disease severity and sleep-disordered breathing problems. An association of hyperserotonemia with the stereotyped behavior or severity of autism has been suggested in some studies (42,43). Moreover, research with animal models has suggested that brain serotonin level controls sleep–wake behavior. Monti in a study on animals demonstrated that 5-HT knock-out mice exhibit greater amounts of REM sleep than their wild-type counterparts (44). Serotonin receptor knock-out mice also showed a significant increase in wakefulness and a reduction in slow wave sleep (44). In agreement with Nakamura et al.'s suggestion, it is speculated that due to serotonin receptor signaling disturbance, serotonin is unable to enter the cells and perform its function and this subsequently leads to compensatory elevation of serotonin production. High serotonin levels may function as endogenous feedback to try to overcome the pathogenesis of autism. However, more studies are needed to clarify the issue.

The present study found that the incidence of GI and sleep disorders in autistic children was higher than in healthy children; on the other hand, a significant relationship was found between sleep problems and plasma serotonin level with the severity of the disorder. Screening of autistic children who suffer from this problem and application of appropriate treatment can reduce the severity of symptoms and also ameliorate feeding problems. Since autism is increasing and adverse effects are more extensive, studies with larger sample size are needed.

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