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A Family History of Autoimmunity and Elevated Serum Serotonin Levels are Associated in Autistic Children

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ABSTRACT

Background: The increased frequency of family history of autoimmunity may be an outstanding feature among some patients with autism that points to their autoimmune background, with the target in this case being the developing brain. Serotonin has been shown to modulate immune responses. Hyperserotonemia, which is the most consistent biological finding in autism, may promote autoimmunity through reduction of Th1-type cytokines. We are the first to investigate the relationship between hyperserotonemia and the increased frequency of autoimmune diseases in families of patients with autism

Methods: A family history of autoimmune diseases in healthy controls and autistic children was ascertained. Serum levels of serotonin were measured, by ELISA, in 66 autistic patients, aged between 5 and 11 years, and 60 healthy-matched children.

Results: The frequency of autoimmune diseases among families of autistic children (48.4%) was significantly higher than normal children (6.6%), P <0.001. Autistic children had significantly higher serum serotonin levels than healthy children (P < 0.001). Increased serum serotonin levels were found in 50% of autistic patients. Autistic children with a family history of autoimmunity had significantly higher serum serotonin levels than children without such a history (P <0.001).

Conclusions: Hyperserotonemia may be one of the contributing factors to the immune system dysfunction that was reported in some autistic children. Inclusion of serum serotonin levels as a correlate may be useful in other future immune studies in autism to help unravel the long-standing mystery of hyperserotonemia and its possible role in the pathophysiology of this disorder.

KEYWORDS autism; autoimmunity; family history of autoimmunity; hyperserotonemia; serotonin.

Introduction

Some autistic children have an imbalance of T helper (Th)1/Th2 subsets toward Th2, which are responsible for allergic response and production of autoantibodies [1].

Autoimmunity to CNS may have a pathogenic role in autism [1]. This may be indicated by the presence of brain-specific auto-antibodies in some autistic children [2-8]. There is also an increase in the frequency of autoimmune disorders among autistic families [9-15].

Serotonin is a neurotransmitter that plays a role in brain development. It is formed by hydroxylation and decarboxylation of tryptophan. Disruption of serotonergic development can leave permanent alterations in brain function and behavior. This may be the case in autism [16,17]. Blood serotonin might serve as analogue marker for serotonergic function [18,19].

Serotonin, being well known for its role in depression, has been shown to modulate immune responses [20]. Hyperserotonemia may promote autoimmunity through reduction of Th1-type cytokines. This may result in an imbalance of T-helper (Th)1/Th2 subsets toward Th2. Hyperserotonemia may also promote autoimmunity through initiation of the delayed-type hypersensitivity responses, which has been proposed as a pathological mechanism leading to autism [21]. Accordingly, modifiers of the serotonin transmitter system such as compounds that affect the serotonin transporter, prejunctional serotonin receptors or postsynaptic serotonin receptors might represent a novel treatment of asthma and autoimmune disorders [22].

It was suggested that autism, without a discernible cause, may be a genetic disorder of serotonin metabolism. The interest in assessing serotonergic function in autism stems from its role in perception and filtering of sensory signals, social attachment and facilitation of formation of synapses which is crucial to acquire learning and memory [18].

We are the first to investigate the relationship between hyperserotonemia and the increased frequency of autoimmune diseases in families of patients a group with autism.

Methods

Study population:

This case-control study was conducted on 66 children who had classic-onset autism. The patients were fulfilling the criteria for the diagnosis of autism according to the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders [23].

The autistic group comprised 44 males and 22 females. They were recruited from the Pediatric Neuropsychiatric Clinic, Faculty of Medicine of Ain Shams University, Cairo, Egypt, during their follow up visits. Their ages ranged between 5 and 11 years (mean±SD = 8.7±1.8 years).

Inclusion criteria:

1- Patients who had no associated neurological diseases (such as cerebral palsy, tuberous sclerosis).

2- Patients who had no associated metabolic disorders (eg. Phenylketonuria) because these associated comorbidities with autism may influence the results of serum serotonin and anti-MBP levels.

3- Patients who were not receiving any medications.

The control group comprised 60 age- and sex-matched apparently healthy children. They included 40 males and 20 fe-

males. They were recruited from the Outpatients Clinic, Children's Hospital, Faculty of Medicine, Ain Shams University. They were the sibs of the children attending this clinic because of a minor illness (e.g common cold, tonsillitis and acute bronchitis). The control children were not related to the children with autism, and demonstrated no clinical findings suggestive of infections, allergic manifestations and immunological or neuropsychiatric disorders. Their ages ranged between 5 and 11 years (mean±SD = 8.6±1.9 years).

The local Ethical Committee of the Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia, approved this study. In addition, an informed written consent of participation in the study was signed by the parents or the legal guardians of the studied subjects.

Study measurements:
Clinical evaluation of autistic patients: This was based on clinical history taking from the caregivers, clinical examination and neuropsychiatric assessment. In addition, the degree of the severity of autism was assessed by using the Childhood Autism Rating Scale (CARS) [24] which rates the child on a scale from one to four in each of fifteen areas (relating to people; emotional response; imitation; body use; object use; listening response; fear or nervousness; verbal communication; non-verbal communication; activity level; level and consistency of intellectual response; adaptation to change; visual response; taste, smell and touch response and general impressions). According to the scale, children who have scored 30-36 have mild to moderate autism, while those with scores ranging between 37 and 60 points have severe autism.

In addition, a family history of autoimmune diseases in controls and children with autism was ascertained in an identical fashion, but not in a blinded manner, by an expert rheumatologist. Parents were asked to fill out a questionnaire regarding which first- (parents and sibs) or second-degree relatives (grandparents, uncles and

aunts) had received a diagnosis of specified autoimmune disorders. A list of autoimmune diseases with descriptions was provided. There was a verification of the diagnosis of autoimmune diseases via medical record review. The disorders inquired about in the questionnaire included rheumatoid arthritis, juvenile rheumatoid arthritis,

systemic lupus erythematosus, insulin-dependent diabetes mellitus, rheumatic fever, vasculitis, ankylosing spondylitis, dermatomyositis, polymyositis, scleroderma, uveitis, Sjogren's syndrome, polyarteritis nodosa, Wegener's granulomatosus, Takayasu's arteritis, psoriasis, multiple sclerosis, vitiligo, myasthenia gravis, amyotrophic lateral sclerosis, Crohn's disease, ulcerative colitis, autoimmune

thyroiditis, idiopathic thrombocytopenic purpura, Addison's disease, pemphigus, and Guillain-Barre syndrome. These disorders were chosen because all have a

known or suspected autoimmune cause.
Assessment of cognitive function (memory, attention, language, concept formation, problem solving, executive and visuospatial functions) with age-appropriate, translated and validated psychometric instruments that were administered by well-trained psychologists using a set of Arabic norms [25] for a translated Wechsler Intelligence Scale for Children, 3rd edition (WISC-III) [26]. This scale is the most commonly used test to assess cognitive function in the children. Three global measures were examined in the present study. The verbal intelligence quotient (IQ) is derived from different subtests including information, similarities, arithmetic, comprehension, vocabulary and digit span. The performance IQ is derived from different subtests including picture completion, block design, picture arrangement, object assembly and digit symbol.The full-scale IQ is the sum of the verbal and performance IQ. The individual subtests may be particularly useful because each depends on a variety of capabilities and dysfunction of any one could result in a low score on one of the global measures. Cognitive dysfunction is diagnosed when the difference between verbal and performance IQ is more than 15 and / or the result of one or more of the individual subtests is below 7 and / or the full scale IQ is below 70.

Assessment of serum serotonin levels: Serum serotonin levels were assayed by using serotonin EIA kit (Biosource Europe S.A. rue de l'industrie 8 1400 Nivelles Belgium). Principally, the competitive serotonin EIA kit uses the microtiter plate format. Serotonin is bound to the solid phase of the microtiter plate. Acylated serotonin and solid phase bound serotonin compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase serotonin is detected by anti-rabbit peroxidase. The substrate TMB/peroxidase reaction is read at 450 nm with a filter wave length 620 nm. The corresponding serotonin concentrations are determined from the standard curve by matching their mean absorbance readings with the corresponding serotonin concentrations in ng/ml. To increase accuracy, all samples were analyzed twice in two independent experiments to assess inter-assay variations and to ensure reproducibility of the observed results ($P > 0.05$).

Statistical analysis:
The results were analyzed by commercially available software package (Statview, Abacus concepts, inc., Berkley, CA, USA). The data were non-parametric, thus they were presented as median and interquartile range (IQR), which are between the 25th and 75th percentiles. Mann-Whitney test was used for comparison between these data. Chi-square test was used for comparison between qualitative variables of the studied groups. Spearman's rho correlation coefficient "r" was used to determine the relationship between different variables. For all tests, a probability (P) of less than 0.05 was considered significant. Patients were considered to have elevated serum serotonin if their levels were above the highest cut-off values (275 ng/ml) which were the 95th percentiles of serum serotonin levels of healthy controls as the distribution of the data was non-parametric.

Results
Acording to CARS, 34 patients had mild to moderate autism, while 32 patients had severe autism. In addition, 34 autistic children had subnormal intellectual function (intelligence quotient below 70); 19 had mild mental retardation (intelligence quotient = 50-69), and 15 had moderate mental retardation (intelligence quotient = 35-49. None of the healthy control children had subnormal intellectual function (table 1).

Serum serotonin levels in autistic children and healthy controls
Autistic children had significantly higher serum serotonin levels [median (IQR) = 300 (574) ng/ml] than healthy controls [median (IQR) = 40.5 (11) ng/ml], $P < 0.001$ (figure 1). Increased serum serotonin levels were found in 50% (33/66) of autistic patients.

Patients with severe autism had significantly higher serum serotonin levels than children with mild to moderate autism, $P < 0.001$ (table 2). Also, the frequency of hyperserotonemia was significantly higher in children with severe autism (90.6%) than patients with mild to moderate autism (11.8%), $P < 0.001$. Furthermore, there were significant positive correlations between serum levels of serotonin and the values of CARS in autistic patients ($P = 0.001$).

Patients with cognitive dysfunction had significantly higher serum serotonin levels than children with normal cognitive function, $P < 0.001$ (table 2). In addition, the frequency of hyperserotonemia was significantly higher in children cognitive dysfunction (79.4%) than patients with normal cognitive function (18.8%), $P < 0.001$. Moreover, there were significant positive correlations between serum levels of serotonin and the values of the full scale IQ in autistic patients ($P = 0.01$).

Male and female autistic children had comparable values of serum serotonin levels ($P > 0.005$). In addition, serum seroto-

nin levels had no significant correlations with the age of the children with autism (P > 0.005).

A Family history of autoimmune diseases in patients with autism

Thirty two children with autism (48.4%) had a first or a second-degree relative with an autoimmune disease [rheumatoid arthritis in 20 patients, insulin-dependent diabetes mellitus in 6 patients, autoimmune thyroiditis in 3 patients , systemic lupus erythematosus (SLE) in 2 patients, and rheumatic fever in one patient], table 1. Seven out of the 32 autistic children with a family history of autoimmune disease (21.9%) had a mother with an autoimmune disease (4 had rheumatoid arthritis, 2 had autoimmune thyroiditis and one had insulin-dependent diabetes mellitus).

On the other hand, a family history of autoimmune diseases was found in 4 of the studied 60 (6.6%) healthy children (rheumatoid arthritis in 2 children, autoimmune thyroiditis in one child and insulin-dependent diabetes mellitus in one child), table 1. None of the healthy children with a family history of autoimmune disease had a mother with such diseases.

The frequency of autoimmune diseases among families of children with autism was significantly higher than normal children (P < 0.001).

Relationship between a family history of autoimmune diseases and hyperserotonemia in autistic children

Autistic children with a family history of autoimmunity had significantly higher serum serotonin levels [median (IQR) = 670 (112) ng/ml] than children without such a history [median (IQR) = 98.5 (82) ng/ml], P < 0.001 (figure 2).

Also, the frequency of hyperserotonemia was significantly higher in children with a family history of autoimmunity (93.7%) than patients without such a history (8.8%), P < 0.001 (table 3).

Discussion

Autism without a discernible cause may be a genetic disorder of serotonin metabolism [18]. Immune abnormalities are also commonly observed in this disorder. Immune system dysfunction may represent novel targets for treatment in autism [1].

In our series, autistic children had significantly higher serum serotonin levels than healthy controls (P < 0.001). These results conform with other studies which reported hyperserotonemia [27-31] and increased platelet serotonin [32,33] in autistic patients.

One study reported a significant increase of the level of serotonin mRNA in the platelets of autistic patients which could suggest serotonin system dysregulation in autism [34]. Hyperserotonemia, is a common biomarker in autism. The integrin β3 receptor subunit gene is a quantitative trait locus for the whole blood serotonin levels. Integrin β3 interacts with the serotonin transporter (SERT) in both the platelets and the midbrain in autism. Furthermore, multiple studies have now reported gene-gene interaction between the integrin β3 and SERT genes in association with autism [35]. Elevated serotonin level in platelets, whole blood and serum is the most consistent biochemical abnormality found in autism [36].

Research which investigated the mechanism of hyperserotonemia in autism reported that it might result from the increased serotonin uptake by platelets or decreased serotonin receptor binding sites on platelets. Also, parents of autistic children, who themselves had elevated blood serotonin levels, recorded significantly higher scores of depression and obsessive compulsive disorders than parents with normal serotonin levels [37]. Therefore, serotonin may represent a marker for familial autism [38].

In the present work, patients with severe autism had significantly higher serum serotonin levels than patients with mild to moderate autism (P < 0.001). This may indicate that the extent of the elevation of serum serotonin levels was closely linked to the degree of the severity of autism. Previous research also reported a close association between serum serotonin levels and the severity of autism [29-31]. It is not easy to determine whether hyperserotonemia is a mere consequence of autism or has a pathogenic role in the disease.

Some studies reported that autistic patients with elevated blood serotonin levels had elevated serotonin transport into platelets [39]. However, the high serotonin levels in the platelets do not necessarily mean that this translates to high levels in the brain. In fact, there are reasons to think otherwise. First: levels of 5-hydroxyindole acetic acid, the end product of serotonin metabolism, have not been found to be elevated in cerebrospinal fluid of autistic children [40]. Second: serotonin reuptake inhibitors which increase brain serotonin levels resulted in improvement of behavioral disorders and language acquisition in 59% of autistic children. Also, the decrease in brain serotonin following acute tryptophan depletion resulted in worsening of sterotyped movements in autistic children [18]. Third: positron emission tomography neuroimaging using a serotonin precursor, revealed diminished serotonin synthesis in the left hemisphere in 5 out of 7 autistic children [41]. Fourth: it was suggested that autistic children may have an autoimmune disorder affecting brain serotonin receptors since 7 out of 13 autistic children had CSF antibodies against serotonin receptors [42]. So, reduced brain serotonin content, which is important for language production and sensory integration, may represent one mechanism underlying the pathophysiology of autism [41].

Autoimmunity to CNS may have a pathogenic role in autism [1]. This may be indicated by the presence of brain-specific auto-antibodies in some autistic children [2-8]. To further understand if autoimmunity could play a role in autism, we studied the frequency of autoimmune diseases in families of patients with autism in comparison to healthy children.

In our series, the frequency of autoimmune diseases among families of autistic children (48.4%) was significantly higher than normal children (6.6%), P <0.001. Previous research had also found an increased frequency of autoimmunity in families of autistic children [9-15]. This may be an outstanding feature among autistic patients that points to their autoimmune background; the target in their case being the developing brain. This implies that in some families, immune dysfunction, perhaps induced by certain environmental triggers, could express itself in the form of autism in one of its off springs. Despite of the fact that the origins of autoimmunity and the induction of the production of brain auto-antibodies in autism are unknown, the major histocompatibility complex genes and their products (e.g., HLA-DRB1 and C4B null alleles) might be involved [14,43].

In this work, we have tried to find a possible relation between the elevated serum levels of serotonin and a family history of autoimmunity in autism. Autistic children with a family history of autoimmunity had significantly higher serum serotonin levels than children without such a history (P <0.001). This is the first study that investigated such a relationship.

Serotonin has been shown to modulate some immune responses and hyperserotonemia may explain some of the abnormal cellular immune responses seen in autism. Serotonin has an important role in initiation of delayed-type hypersensitivity responses [44], which are important in autoimmunity, as serotonin initiates the activation of local endothelial cells to recruit effector T cells, and activates serotonin receptors on these recruited cells [45]. Some researchers found a correlation between serum serotonin levels and the presence of certain major histocompatibility complex genes (the extended haplotype B44-DR4 and the C4B null allele) [46] which had previously reported to be associated with autism and to play an important role in the development of autoimmunity [47,48].

It is possible that a common factor could account for both hyperserotonemia and immune abnormalities seen in autism. Such common factor may be the serotonin transporter (5-HTT)

which transports serotonin into platelets. 5-HTT is also found on immune cells where it can influence immune function [49]. Some studies found an association between autism and 5-HTT promotor gene polymorphisms [38]. However, the meta-analysis failed to find a significant overall association between either of the 5-HTT polymorphisms examined and autism [50]. In addition, hyperserotonemia may promote autoimmunity through reduction of Th1-type cytokines resulting in an imbalance of T-helper (Th)1/Th2 subsets toward Th2, which are responsible for the production of antibodies [21]. This imbalance was reported in some autistic patients [1].

To date, a definitive relationship between autism, serotonin and autoimmunity has not been fully established. On the basis of the preliminary results reported in this study, further research is warranted to determine the possible link between serotonin and brain auto-antibodies which are indicators of autoimmunity to brain in some autistic patients.

Conclusions

Hyperserotonemia may be one of the contributing factors to the immune system dysfunction that was reported in some autistic children. Inclusion of serum serotonin levels as a correlate may be useful in other future immune studies in autism to help unravel the long-standing mystery of hyperserotonemia and its possible role in the pathophysiology of this disorder.

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List of abbreviations

(CARS): Childhood Autism Rating Scale; (CNS): central nervous system; (IQR): interquartile range; (SERT): serotonin transporter; (Th): T helper cells.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Both authors designed, performed and wrote the research. In addition, both authors have read and approved the final manuscript.

Table 1. Demographic and laboratory data of children with autism and healthy control children

	Children with autism (n=66)	Control group (n=60)
Age (in years): Range Mean ± SD	5-11 8.7±1.8	5-11 8.6±1.9
Sex: (Male/Female)	44/22	40/20
Intelligence quotient: Above 70 50-69 35-49	32 19	60
CARS scores: Mild to moderate (30-36) Severe (37 -60)	15 34	
Family history of autoimmune diseases	32 (48.8%)	4 (6.6%)
Rheumatoid arthritis	20	2
Insulin-dependent diabetes mellitus	6	1
Autoimmune thyroiditis	3	1
systemic lupus erythematosus	2	
Rheumatic fever	1	
Serum serotonin (ng/dl): Range	50-782	32-320
Median (IQR)	300 (574)	40.5 (11)

CARS, Childhood Autism Rating Scale; IQR, interquartile ranges

Table 2. Serum levels of serotonin in relation to the degree of the severity of autism and cognitive dysfunction in autistic patients.

	Serum serotonin (ng/ml) Median (IQR)	Z	P
Mild to moderate autism Severe autism	99 (118) 670(133)	6.3	< 0.001
Patients with normal cognitive function Patients with cognitive dysfunction	93(60) 669 (290)	6.1	< 0.001

Table 3. Relationship between hyperserotonemia and the increased frequency of a family history of autoimmune diseases in autistic children.

	Normal serum serotonin (n=33)	Elevated serum serotonin (n=33)	x^2 (P)
Positive family history of autoimmunity (n=32)	2 (6.3%)	30 (93.7%)	
Negative family history of autoimmunity (n=34)	31 (91.2%)	3 (8.8%)	(0.6)

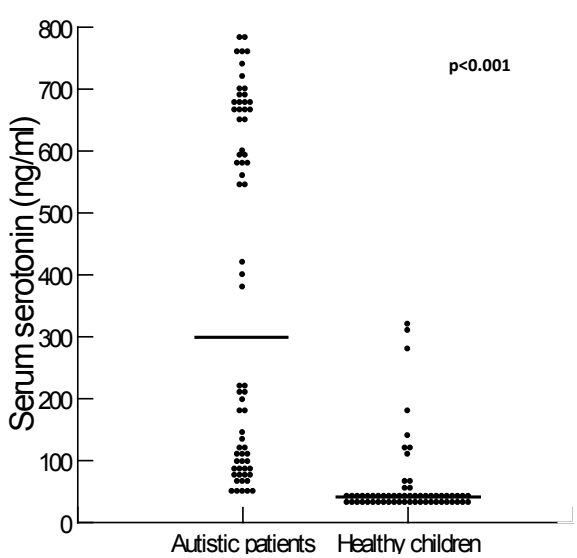

Figure 1. Serum serotonin levels in autistic patients and healthy children. Median value for each group is shown by a horizontal bar.

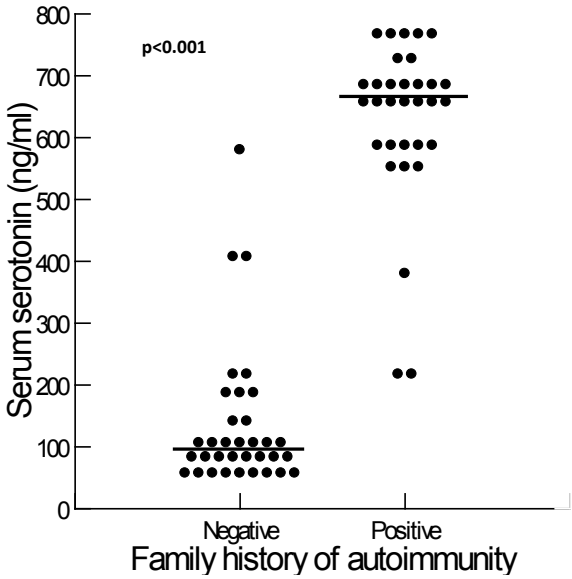

Figure 2. Serum serotonin levels in relation to a family history of autoimmune diseases in autistic children

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