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Serum Cytokine and Growth Factor Levels in Children with Autism Spectrum Disorder

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Background: The immune system may have a role in the pathogenesis of autism spectrum disorder (ASD), including typical and atypical autism. The aim of this study was to determine whether a cytokine and growth factor panel could be identified for the diagnosis and prognosis in children with ASD, including typical and atypical autism.

Material/Methods: This study included 26 children with ASD (typical or atypical) and 11 of their siblings who did not have ASD. A panel of ten serum cytokines and growth factors were investigated using addressable laser bead assay (ALBIA) and enzyme-linked immunosorbent assay (ELISA) kits. Results were correlated with scores using the Childhood Autism Rating Scale (CARS) and Autism Diagnostic Observation Schedule (ADOS) for the children with ASD and compared with the findings from their siblings without ASD.

Results: There were no statistically significant differences in serum cytokine and growth factor levels between children with ASD and their siblings. The scores using CARS and ADOS were significantly greater in children with typical autism compared with children with atypical autism as part of the ASD spectrum. Serum levels of cytokines and growth factors showed a positive correlation with CARS and ADOS scores but differed between children with typical and atypical autism and their siblings.

Conclusions: The findings of this study showed that serum measurement of appropriately selected panels of cytokines and growth factors might have a role in the diagnosis of ASD.

MeSH Keywords: Autistic Disorder • Clinical Laboratory Techniques • Cytokines

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Background

Autism is a neurodevelopmental condition that is characterized by difficulty with social interactions and social empathy, with limited and stereotypical interests, and atypical responses to sensory stimuli. The diagnosis of autism is usually made at an early age. Autism is now recognized to include developmental and behavioral changes that are varied in presentation and severity, which has resulted in the term, autism spectrum disorder (ASD) and includes typical and atypical autism.

ASD is now recognized to be a heterogeneous group of pervasive developmental disorders (PDDs), which also include Asperger's syndrome. In the current Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), ASD is categorized as a single condition, autism. In the International Classification of Diseases, 10th Edition (ICD-10), autism is subdivided into typical childhood autism (F84.0) and atypical autism (F84.1). However, in DSM-IV, atypical autism is classified as PDD, not otherwise specified (NOS).

The etiology of ASDs have not been established, and remain controversial. The occurrence of ASD may follow multiple pathways. Currently, ASD is thought to arise in genetically susceptible individuals in the prenatal period, possibly triggered by environmental factors [1]. The genetic predisposition to ASD is polygenic and associated with polymorphisms and mutations in genes located on several chromosomes. Examples of recently identified genes associated with ASD include *FOXP2*, which encodes for forkhead box protein P2 (FOXP2), a transcription factor expressed in the developing and adult brain that is key to speech and language development, and *RELN*, which encodes for reelin, an extracellular matrix glycoprotein involved in embryogenesis [1–7].

Epidemiological studies, and studies in animal models, have supported an association between ASD and factors affecting the fetus *in utero*, including maternal infections occurring during pregnancy, maternal use of certain drugs, such as valproic acid, and changes in the maternal microbiome, or the composition of the intestinal microflora [10,11–14]. In individuals with ASD, the organization and communication between nerve cells in the brain are affected, but the mechanisms of this abnormality remain to be determined. However, it is possible that ASDs are a manifestation of atypical development involving the nervous system, endocrine system, immune system, and the microbiome [2,8–14].

The findings of previously published studies have shown a role for the immune system in the pathogenesis of at least a subset of cases of ASD [15–19]. However, regarding the possibility of using the measurement of serum or plasma cytokines for diagnosis and prognosis in patients with ASD, some of the findings of these studies have been contradictory [8,20–23].

Because the pathogenesis of ASD is a complex process that may begin in the prenatal period, this is a time that the immune system may play an important role. A study was designed with the framework of an ongoing project at the University Hospital of Ostrava on the analysis of the immune profile of children with ASD. From these ongoing clinical studies and using data from the available published literature, a panel of cytokines and growth factors were identified for serum measurements in children with ASD and their siblings. The characteristics of the chosen cytokines and growth factors are described in Table 1.

The aim of this study was to determine whether a cytokine and growth factor panel could be identified for the diagnosis and prognosis in children with ASD, including typical (or classical childhood) autism, and atypical autism.

Material and Methods

This study was approved by the University Hospital of Ostrava Ethics Committee (Approval No. 320/2014) on April 17, 2014. For the study participants, parental informed consent was obtained.

Initially, thirty children with autism spectrum disorder (ASD), and fifteen of their siblings who were without ASD were recruited between June 2014 and May 2015. Based on a change in diagnosis following clinical review, or to scheduling difficulty, four children and their siblings exited the study. Therefore, the final study group consisted of 26 children with ASD and 11 of their siblings who were without ASD (Table 2).

All children who participated in the study underwent routine laboratory investigations, including serum biochemistry, and a complete blood count (CBC), including C-reactive protein (CRP). None of the children in this study were undergoing treatment with any medications for ASD. The characteristics of the chosen cytokines and growth factors, and their expected normal values are described in Table 1.

Serum cytokine and growth factor measurements were made using addressable laser bead assay (ALBIA) and enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Diagnostics). Serum measurements in both groups of study participants included brain-derived neurotrophic factor (BDNF), monocyte chemoattractant protein-1 (MCP-1), thymus- and activation-regulated chemokine (TARC) (or CCL17), interferon (IFN)-γ, transforming growth factor (TGF)-β, interleukin (IL)-6, IL-10, receptor for advanced glycation endproducts (RAGE), heat shock protein (HSP)-70, and tumor necrosis factor (TNF)-α (Table 1).

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Table 1. Characteristics of serum cytokines and growth factors evaluated in this study.

Cytokine/ growth factor	Producing cells	Receptors and receptors-bearing cells	Function	Expected serum concentration (pg/mL)
BDNF	Mainly brain, but also retina, motoric neurons, kidney, prostate, platelets and others	TrkB, LINGFR (p75)	Promotes survival of neurons in the subpopulation of the spinal root ganglia	6186–42580 mean 27793
TARC (CCL17)	Mainly in thymus, only transiently by stimulated peripheral blood mononuclear cells	CCR4 on T-cells	Chemoattractant for T-cells	71–848 mean 331
MCP1 (CCL2)	Mainly macrophages but also fibroblasts, endothelial and some tumor cells, neurons, astrocytes and microglia	T cells but also other cells	Chemoattractant for monocytes, lymphocytes and NK cells	200–722 mean 370
RAGE	RAGE expression by cells is stimulated during stress, e.g. in inflammation, as well as many other diseases including cancer	RAGE ligands: AGE, HMGB1, S100A12, S100A7, S100P, S100A8/A9 (calprotectin), MAC-1, amyloid-β-protein, phosphatidylserine etc.	Activation of pro-inflammatory genes after interaction with ligands	368–4354 mean 1794
HSP70	Produced by stressed cells mainly as intracellular substance	Many lectin receptors mainly on antigen presenting cells (APC), endothelial and epithelial cells	HSP70 protects cells against thermal and oxidative stress	0.67 ± 0.46
TGFβ1	Produced by many cells and platelets	Receptors are present on many cells	Regulates proliferation, differentiation and other functions of many cell types	18289–63416 mean 39592
TNFα	Mainly activated macrophages but also many other cell types as CD4+ T cells, NK cells, mast cells, eosinophils and neurons	TNF receptors are expressed by cells in many mammalian tissues, but especially by leukocytes	Primary role of TNF is the regulation of immune system cells. As an endogenous pyrogen, it is able to induce fever, apoptosis, cachexia, inflammation, and inhibit tumorigenesis, virus replication and cervical splicing through IL-1 and IL-6-producing cells.	<15.6
IL-6	Many cells including monocytes, fibroblasts and endothelial cells, after stimulation also by macrophages, T and B cells, mast cells, eosinophils, granulocytes, keratinocytes and glial cells	IL-6R (CD126) – common receptor	Strong pleiotropic cytokine regulating cell growth and differentiation with a significant role in immune response	<7.8
IL-10	Most of immune system cells, mainly Th2 cells	Tetrameric receptor consisting of 2 α and 2 β subunits; subunits are expressed on hematopoietic cells such as T, B, NK cells, mast and dendritic cells, while β subunit is expressed generally	Anti-inflammatory cytokine	<7.8
IFNγ	Activated T, B and NK cells	IFNGR1 and IFNGR2 present on T cells but also other cells (pleiotropic receptor)	Immunomodulatory effect	<15.6

IL – interleukin; IFN – interferon; TGF – transforming growth factor; TNF – tumor necrosis factor; HSP – heat shock protein; RAGE – receptor for advanced glycation endproducts; TARC – thymus- and activation-regulated chemokine; MCP-1 – monocyte chemoattractant protein-1; BDNF – brain-derived neurotrophic factor; NK – natural killer.

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Table 2. Characteristics of the study participants with autism spectrum disorder (ASD), and their unaffected siblings.

Children with ASD						Unaffected siblings		
ID	Age (yrs)	Sex	CARS	ADOS	ASD	ID	Age (y)	Sex
D1	3.4	M	30.5	5	F84.1	K1	1.2	M
D2	4.5	M	31.5	4	F84.1	K2	1.4	M
D3	4.3	M	32.5	6	F84.0			
D4	3.2	M	38.5	10	F84.0	K4	5.6	F
D5	4.0	M	30.5	4	F84.1	K5	7.4	F
D6	5.6	M	45.0	8	F84.0	K6	7.1	F
D7	6.2	F	35.0	6	F84.0	K7	15.2	F
D8	5.4	M	36.0	6	F84.0	K8	4.1	F
D9	6.8	M	30.0	4	F84.1			
D10	3.8	M	35.0	6	F84.0			
D11	3.4	M	30.0	4	F84.1			
D12	6.1	M	43.5	9	F84.0	K12	4.6	F
D13	4.7	F	37.0	5	F84.1	K13	6.0	F
D14	4.9	F	44.5	9	F84.0			
D15	3.7	F	38.0	5	F84.1			
D16	3.8	M	37.5	6	F84.0			
D17	4.2	M	31.5	6	F84.1			
D19	5.7	M	41.5	8	F84.0			
D20	6.4	M	35.5	6	F84.0	K20	13.9	F
D21	3.8	M	35.0	6	F84.0			
D22	4.0	M	35.0	4	F84.0			
D23	5.1	M	39.5	6	F84.0	K23	14.7	F
D27	6.2	M	38.5	10	F84.0			
D28	3.5	M	44.5	10	F84.0			
D29	6.0	M	38.5	6	F84.0			
D30	3.2	F	31.5	6	F84.0			

ASD – autism spectrum disorder; M – male; F – female; CARS – Childhood Autism Rating Scale [37]; ADOS – Autism Diagnostic Observation Schedule (a comparative score is given) [38]; F84.0 – typical childhood ASD, according to the ICD-10; F84.1 – atypical ASD, according to ICD-10.

Serum measurements were made using a Human Magnetic Luminex screening kit (Ref. No. LXSAHM-8) (R&D Systems Diagnostics). Serum levels of TGF-β1 were determined using the screening kit (Ref. No. LTGM00) and a TGF-β base kit (Ref. No. LTGM100). Serum levels of HSP70 were measured also using the kit (Ref. No. DYC1663-2). Serum IFN-γ levels were measured using the ELISA-VIDITEST Interferon γ Kit (Ref. No. OD-326) and the Custom Quantibody® multiplex ELISA array kit (RayBiotech).

Analysis and evaluation of measured data were performed using Excel and R statistical software and data analysis tools.

Results

In this study, 26 children with autism spectrum disorder (ASD) (typical or atypical) and 11 of their siblings who did not have ASD underwent serum measurements for the following

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Table 3. Results of selected serum levels of cytokine and growth factors in study participants with autism spectrum disorder (ASD), and their unaffected siblings.

Parameter	N	Age (yrs)	Serum concentration (pg/mL)									
			BDNF	HSP70	TGFβ1	RAGE	MCP1	TARC	TNFα	IL-6	IFNγ	IL-10
ASD, mean	26	4.69	35331	217	134115	12668	1590	2546	21.08	15.79	138.54	20.3
ASD, median	26	4.38	35612	184	132788	11178	1465	1737	11.28	<0.30	<4	<1.2
Siblings, mean	11	7.38	34413	233	131637	13312	1338	2611	7.14	0.33	<4	1.4
Siblings, median	11	6.01	36028	159	123607	12298	1295	2427	2.90	<0.30	<4	<1.2
ASD, % decrease			0	33.33	0	0	0	0	0	50		
ASD, % increase			0	0	100	100	100	84.61	76.92	23.07	30.77	46.15
Siblings,% decrease			0	90.91	0	0	0	0	0	0		
Siblings,% increase			0	9.09	100	100	100	100	54.45	0	0	0
ASD/siblings unpaired t-test (p)	26/11	0.110	0.750	0.851	0.835	0.612	0.162	0.919	0.031	0.055	0.058	0.027
ASD/siblings paired t-test (p)	11		0.621	0.4797	0.881	0.696	0.297	0.655	0.096	0.195	0.247	0.173

ASD – autism spectrum disorder; IL – interleukin; IFN – interferon; TGF – transforming growth factor; TNF – tumor necrosis factor; HSP – heat shock protein; RAGE – receptor for advanced glycation endproducts; TARC – thymus- and activation-regulated chemokine; MCP-1 – monocyte chemoattractant protein-1; BDNF – brain-derived neurotrophic factor.

cytokines and growth factors: brain-derived neurotrophic factor (BDNF), monocyte chemoattractant protein-1 (MCP-1), thymus- and activation-regulated chemokine (TARC), interferon (IFN)-γ, transforming growth factor (TGF)-β, interleukin (IL)-6, IL-10, receptor for advanced glycation endproducts (RAGE), heat shock protein (HSP)-70, and tumor necrosis factor (TNF)-α (Table 1).

Table 3 shows that in the study population, the expected normal values obtained from the literature and supplied by the manufacturers' kits were different (Table 1). Table 3 shows the serum and growth factor measurements of the participating children with ASD and their siblings.

Table 4 shows the comparison of values and cross-correlation of the Childhood Autism Rating Scale (CARS) and Autism Diagnostic Observation Schedule (ADOS) in children with typical and atypical autism in ASD.

Figure 1 shows how concentrations of IFNγ can vary by different diagnostic methods using three different kits. Figure 2 shows the correlation of cytokine and growth factor levels using the CARS and the ADOS evaluation methods in children with ASD and their normal siblings.

Discussion

The results of this study in children with autism spectrum disorder (ASD) and their unaffected siblings, showed that serum levels of cytokines and growth factors using the Childhood Autism Rating Scale (CARS) and Autism Diagnostic Observation Schedule (ADOS) scoring systems correlated relatively well in this study. However, the correlation of the CARS and ADOS scores in children with typical autism were greater when compared with those in children with atypical autism, in ASD. CARS and ADOS values for the typical autism group were also greater compared with those of the atypical autism group.

In this study, the standard ranges of normal serum values for cytokine and growth factor levels, as described in the literature and the kit manufacturer data, could not be applied to the children with ASD and their siblings. The inclusion of healthy controls without a family history of ASD was a limitation of this study that could have been used to confirm the normal ranges for these serum factors. In the absence of these data, in this study, it was possible to compare only the children with ASD with their siblings, who were without apparent symptoms

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Table 4. Comparison of the Childhood Autism Rating Scale (CARS) and Autism Diagnostic Observation Schedule (ADOS) in children with typical and atypical autism in autism spectrum disorder (ASD).

	N	CARS index		ADOS index	
		Mean	Median	Mean	Median
Typical ASD	8	38.2	38.0	7.1	6.0
Atypical ASD	18	32.4	31.0	4.6	4.5
t-test typ/atyp (p)		0.00126		0.00005	
cc CARS/ADOS		0.77039			
cc CARS/ADOS typ		0.69608			
cc CARS/ADOS atyp		0.39424			

Significant values (*p*) for unpaired t-tests, two-tailed distribution, and two samples with different variance for the hypothesis that both values are identical. cc – correlation coefficients; ASD – autism spectrum disorder; CARS – Childhood Autism Rating Scale; ADOS – Autism Diagnostic Observation Schedule.

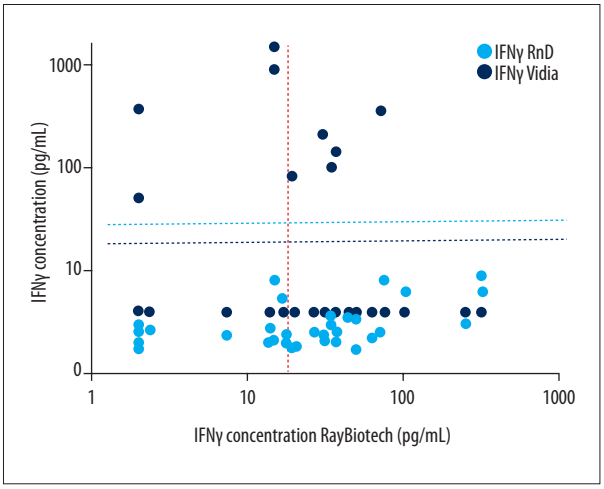

Figure 1. Comparison of interferon (IFN)-γ results using three different kits. Dashed lines show the upper limits of normal physiological values given for three applied kits for serum measurements by the kit manufacturers.

and signs of ASD. A further limitation of this study was that replication of the serum measurements was not performed.

Where the arithmetic mean and median values differ significantly, it is usual to infer a non-normal distribution of the values; an explanation for this was that some values might have been erroneous because of measurement or sampling handling error. Furthermore, the results obtained using the diagnostic kits from different manufacturers, using different methods, and in the absence of the use of universal standards may have resulted in different serum values, as shown in Figure 1 for measurement of the IFN-γ serum concentrations, which shows that the results for three methods serum IFN-γ varied. This variation is attributable to the fact that the immunoanalytical methods, based on the reaction of antigens with antibodies, have not yet been standardized [24,25]. However, in our laboratory, serum measurement outcomes were similar when total concentrations of selected cytokines were compared using the Custom Quantibody® multiplex ELISA array kit and the Luminex addressable laser bead assay (ALBIA) kits (*unpublished results*).

In this study, some interesting associations were identified between different serum cytokine and growth factor measurements and with CARS and ADOS scores (Figure 2). For example, serum levels of brain-derived neurotrophic factor (BDNF) showed a significant positive correlation in ASD cases of atypical autism, but a negative association with atypical autism. Also, serum levels of thymus- and activation-regulated chemokine (TARC) showed a significant negative correlation with CARS scores in cases with typical autism and a significant positive correlation with CARS scores in cases with atypical autism. However, there were no significant correlations between serum TARC levels and ADOS scores in cases with ASD, including cases of typical and atypical autism.

As described in Table 1, BDNF plays an important role and influences some functions, primarily in the brain, but is also produced outside the brain [26–28]. Peripheral sources of BDNF include platelets, in which about 90% of all blood BDNF is stored. Serum BDNF concentrations correlate well with BDNF concentrations in the brain [29]. This finding, in particular, provides a rationale for future studies to evaluate the rationale for a serum assay for BDNF in the diagnosis of ASD. However, previously published studies have shown that serum concentrations of BDNF in ASD, when compared with unaffected control subjects have not yet demonstrated an association with ASD [30–32].

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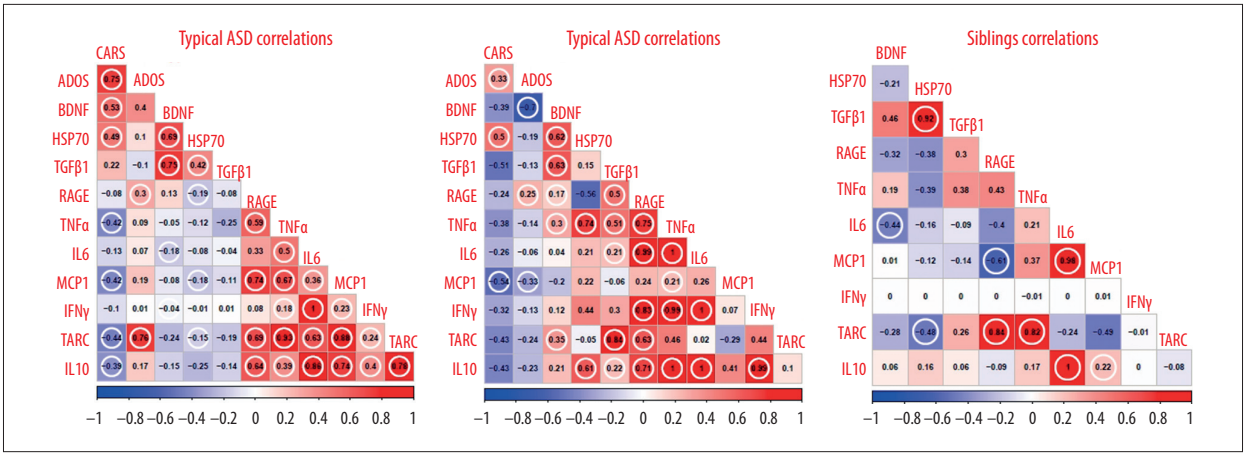

Figure 2. Correlation of cytokine and growth factor levels using the Childhood Autism Rating Scale (CARS) and the Autism Diagnostic Observation Schedule (ADOS) in children with autism spectrum disorder (ASD), and their normal siblings. Positive correlations are shown in red and negative in blue. The intensity of the color increases with the significance of correlations. Correlation coefficients are given inside the squares. Statistically significant correlations are highlighted by white circles.

Table 1 also shows that thymus- and activation-regulated chemokine (TARC) (CCL17) is a cytokine expressed primarily in the thymus, but is also produced by other tissues, including peripheral blood mononuclear cells (PBMCs) after phytohemagglutinin (PHA) stimulation [33–35]. PHA activates T-cells via the CCR4 receptor and plays an important role in the regulation of the inflammatory response. The hypothesis that drove this study was that the serum levels of inflammatory cytokines and growth factors in children with ASD would differ from those of their unaffected siblings [36]. However, the findings from the present study did not support this hypothesis.

Conclusions

In this study, serum levels of cytokine and growth factors differed among children with autism spectrum disorder (ASD) who had typical (or classical childhood) autism, atypical autism, and their unaffected siblings. However, the serum levels of individual cytokines and growth factors were not significantly different between these groups. However, the findings of this support the possibility of using an appropriate selection of serum cytokine and growth factor panels for the diagnosis ASD and emphasize the need to standardize quantitative methods for serum analysis.

Conflicts of interest

None.

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