

Correlation analysis of expression of CC and CXC chemokines in children with autism spectrum disorder

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Abstract

To investigate the relationship between the expression of CC and CXC chemokines and autism spectrum disorder (ASD).

A total of 62 children with ASD (ASD group) and 60 gender- and age-matched normal children (control group) admitted to our hospital from January 2019 to January 2020 were included in the study. Monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 α (MIP-1 α), macrophage inflammatory protein-1 β (MIP-1 β), regulated upon activation, normal T-cell expressed and secreted (RANTES), interleukin-8 (IL-8), monokine induced by interferon (IFN)- γ (MIG), and purified human interferon- γ -induced protein-10 (IP-10) were detected in the ASD group. The correlation between the above indexes and the severity of the ASD group was analyzed.

Significantly increased MCP-1 levels ($P < .01$) along with the markedly decreased MIP-1 α and MIP-1 β levels ($P < .01$) were detected in the venous blood of the ASD group compared with the control group. In addition, they exhibited no significant difference (yet a downward trend) in the level of RANTES ($P > .05$). Children in the ASD group showed significantly decreased IP-10 levels ($P < .01$); however, they had no noticeable change (yet a decreasing trend) in the levels of IL-8 and MIG ($P > .05$). MCP-1 level was positively related to the Module 1 scores of Autism Diagnostic Observation Schedule-second edition (ADOS-2), whereas the levels of Childhood Autism Rating Scale MIP-1 α , MIP-1 β , IL-8, IP-10, and MIG were negatively correlated with the ADOS-2 Module 1 scores ($P < .01$). However, no significant correlation was found between RANTES and the ADOS-2 Module 1 scores ($P > .05$).

The levels of CC chemokines (MCP-1, MIP-1 α , MIP-1 β , and RANTES) and CXC chemokines (IL-8, IP-10, and MIG) are positively correlated with the pathogenesis of ASD. Inflammation is an important contributing factor to ASD.

Abbreviations: ABCS = Autism Behavior Checklist Scale, ADOS-2 = Autism Diagnostic Observation Schedule-second edition, ASD = autism spectrum disorder, CARS = Childhood Autism Rating Scale, IFN = interferon, IL-8 = interleukin-8, IP-10 = interferon- γ -induced protein-10, MCP-1 = monocyte chemoattractant protein-1, MIG = monokine induced by interferon (IFN)- γ , MIP-1 α = macrophage inflammatory protein-1 α , MIP-1 β = macrophage inflammatory protein-1 β , RANTES = regulated upon activation, normal T-cell expressed and secreted.

Keywords: autism spectrum disorders, CC chemokines, chemokines, CXC chemokines, RANTES

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This research was approved by the Medical Ethics Committee of Huanggang Pingan and Rehabilitation Hospital. Parents of all children understood the purpose, significance, and procedure of this research and signed the informed consent before admitting children to the study.

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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1. Introduction

Autism spectrum disorder (ASD), a group of neurodevelopmental disorders, is characterized by difficulties with social communication, a narrow range of interests or activities, and repetitive, stereotyped behaviors in children.^[1] Its incidence has been increasing rapidly worldwide. The World Health Organization stated ASD as one of the most rapidly increasing severe disorders in the world and has become a major public health concern that adversely undermines the health and quality of life.^[2] Apart from genetic factors, other factors such as immune system disorders, viral infections, and inflammation have been implicated in the occurrence and progression of ASD.^[3–5] Tsilioni et al found that IL-38 inactivated human microglia and linked to the pathogenesis of ASD.^[6] Similarly, Tonhajzerova et al demonstrated an increased expression of IL-8 in patients with ASD.^[7] Therefore, it is necessary to understand the relationship between some inflammatory factors and ASD fully.

Inflammatory chemokines, a group of low molecular weight bioactive peptides, which are mainly synthesized and secreted by inflammatory cells and histocytes, bind to homologous G protein-coupled receptors in vivo and trigger a series of metabolic reactions of G proteins in cells, thereby inducing directional cell migration.^[7] They play vital roles in inflammatory responses.

According to different positions of N-terminal cysteine residues, chemokines can be classified into the C, CC, CXC, and CX3C families. Currently, the typical factors of the CC family (including CCL2, CCL3, CCL4, and CCL5) and CXC family (including CCL8, CCL9, and CCL10) are primary inflammatory chemokines. Eftekharian et al profiled the cytokines in the serum of 30 Iranian ASD patients and found the dysregulation of IL-2, IL-6, CXCL8, and IL-17 in ADS patients.^[8] However, a tight association between the expression of some types of inflammatory chemokines and the occurrence of ASD in Chinese patients could not be established.

In the present work, we explored the correlation between the expression of CC and CXC chemokines and the occurrence of ASD from the perspective of neuroimmunology to provide a reference for the selection of early screening and intervention methods to treat the disorder.

2. Materials and methods

2.1. General data

A total of 62 children with ASD admitted to our hospital between March 2019 and March 2020 were included as the ASD group. Each child in the ASD group was diagnosed by 2 pediatricians with 1 senior professional of associate chief physician and above grade.

2.1.1. Inclusion criteria.

- (1) children with Autism Behavior Checklist Scale (ABCS)^[9] score ≥ 57 points, and Childhood Autism Rating Scale (CARS)^[9] score ≥ 30 points, and diagnosed as ASD based on diagnostic criteria as per the Autism Diagnostic Observation Schedule-second edition (ADOS-2),^[10] the Autism Diagnostic Interview-Revised,^[11] and the Diagnostic and Statistical Manual of Mental Disorders-fifth edition^[12];
- (2) children whose parents were from Han nationality.

2.1.2. Exclusion criteria.

- (1) children suffering from genetic or metabolic diseases, such as Rett syndrome and fragile X syndrome;
- (2) children suffering from neurodevelopmental disorders other than ASD, such as simple language development disorders and intellectual disabilities;
- (3) children having a history of traumatic brain injury;
- (4) children complicated with serious physical diseases (including cardiac, pulmonary, hepatic, and renal insufficiency as well as allergic diseases such as allergic rhinitis and asthma), and severe neurological diseases (including uncontrolled seizures);
- (5) children diagnosed with other types of childhood mental disorders, such as somatopsychosis, schizophrenia, simple speech, or movement disorders;
- (6) children suffering from the upper or lower respiratory tract infections, food allergies, and severe trauma during 30 days before their inclusion in the study. In addition, 60 gender- and age-matched normal children and receiving physical examination in this hospital during the same period were included in the control group. However, premature babies, children with a family history of mental illness, and those with upper or lower respiratory tract infections, food allergies, or severe trauma 30 days before the physical examination were also excluded.

The ASD group included 52 males and 10 females aged 3.5 to 6 years with an average age of 3.82 ± 0.79 years. The control group

consisted of 43 males and 17 females aged 2 to 6 years with an average age of 3.90 ± 0.85 years (Fig. 1). No significant difference in gender composition and age distribution between the 2 groups ($P > .05$) was noticed. This research was approved by the Medical Ethics Committee of our hospital. Parents of all children understood the purpose, significance, and procedure of this research and signed the informed consent before admitting children to the study.

3. Methods

Venous blood (3 mL) of all children in both groups was collected in ethylenediaminetetraacetic acid *anticoagulant tube* in the early morning of the second day of enrollment. The specimens were kept at 4°C and centrifuged at 2000 rpm for 5 min. The supernatant was stored in a refrigerator at -80°C for subsequent use.

The following indicators were tested using enzyme-linked immunosorbent assay kits as instructed.

A. CC chemokines: This group of chemokines included the following:

- (1) CCL2 or monocyte chemoattractant protein-1 (MCP-1).
- (2) CCL3 or macrophage inflammatory protein-1 α (MIP-1 α).
- (3) CCL4 or macrophage inflammatory protein-1 β (MIP-1 β).
- (4) CCL5 or regulated upon activation, normal T-cell expressed, and secreted (RANTES) that regulates the expression and secretion of activated normal T cells.

B. CXC chemokines: This category included the following:

- (1) CXCL8 or interleukin-8 (IL-8).
- (2) CXCL9 or monokine induced by interferon (IFN)- γ (MIG) factor.
- (3) CXCL10 or interferon- γ -induced protein-10 (IP-10).

3.1. Observational indicators

- (1) The difference in the levels of serum MCP-1, MIP-1 α , MIP-1 β , RANTES, IL-8, IP-10, and MIG among children in the ASD and control groups was compared.
- (2) The correlation of serum MCP-1, MIP-1 α , MIP-1 β , RANTES, IL-8, IP-10, and MIG levels with the severity of the ASD group was analyzed.

The severity of ASD was evaluated using the ADOS-2 scale, which can be divided into 5 modules according to the language competence of children. Because the children included in this study were 3.5- to 6-year-old, Module 1 (pre-verbal/single words) was selected for evaluation which included social effect (communication and reciprocal social interaction) and restricted and repetitive behavior. Each item was scored as 0, 1, 2, and 3, in which 0 represented no abnormality, 1 represented mild abnormality, and 2 and 3 represented definite abnormality. A higher score indicates a more severe form of abnormality. Based on this, the correlation of expression of CC and CXC chemokines with the ADOS-2 Module 1 scores was analyzed.

3.2. Statistical methods

The SPSS version 22.0 statistical software was used to analyze the data. The measurement data conforming to the normal distribution are presented as mean \pm standard deviation. The comparison between the 2 groups was conducted by *t* test, and the count data were represented by the percentage. The comparison between

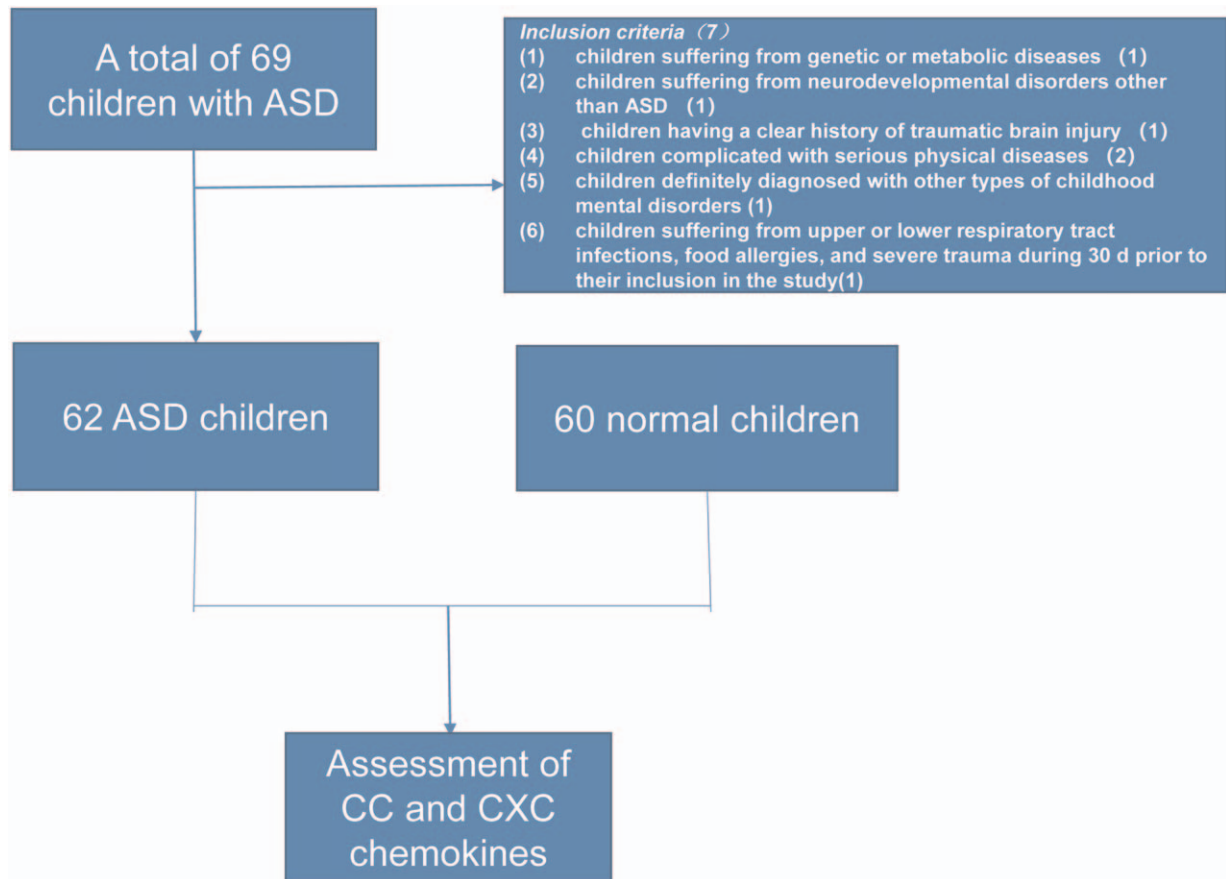


Figure 1. One patient flowchart.

multiple groups was conducted by chi-square test. Pearson correlation analysis was used to analyze the correlation between chemokine levels and the ADOS-2 Module 1 scores of the children. Value of $P < .05$ was considered statistically significant. Each experiment was repeated in triplicate at least 3 times.

4. Results

4.1. Comparison of CC and CXC chemokine levels between 2 groups of children

For CC chemokines, the ASD group showed significantly increased MCP-1 levels ($P < .01$) and markedly decreased MIP-

1α and MIP- 1β levels ($P < .01$) compared with the control group. However, it showed no significant difference (yet a downward trend) in the level of RANTES ($P > .05$). For CXC chemokines, the ASD group showed significantly decreased IP-10 levels ($P < .01$) but had no noticeable change (yet a decreasing trend) in the levels of IL-8 and MIG ($P > .05$), as shown in Table 1.

4.2. Correlation between the levels of CC and CXC chemokines and the severity of ASD

ADOS-2 total score was positively correlated with MCP-1 levels and was negatively correlated with MIP- 1α , MIP- 1β , IL-8, IP-10,

Table 1
Comparison of CC and CXC chemokine levels between 2 groups of children (pg/mL).

Indicators	ASD group (n=62)	Control group (n=60)	t	P
MCP-1	184.21 ± 4.53	133.95 ± 4.30	62.831	0
MIP- 1α	181.94 ± 5.67	301.50 ± 6.79	105.773	0
MIP- 1β	42.62 ± 4.08	65.29 ± 3.82	31.67	0
RANTES	2983.37 ± 362.47	3026.30 ± 21.16	0.916	.362
IL-8	73.98 ± 5.81	74.79 ± 2.67	0.986	.326
IP-10	297.46 ± 64.04	432.05 ± 8.27	16.146	0
MIG	970.96 ± 37.68	980.56 ± 12.05	1.881	.062

Venous blood (3 mL) of all the children in both groups was collected in the early morning of the second day of enrollment. enzyme-linked immunosorbent assay quantitation was performed to assess the levels of these below indicators.

IL-8 = interleukin-8, IP-10 = interferon- γ -induced protein-10, MCP-1 = monocyte chemoattractant protein-1, MIG = interferon (IFN)- γ , MIP- 1α = macrophage inflammatory protein-1 α , MIP- 1β = macrophage inflammatory protein-1 β , RANTES = normal T-cell expressed and secreted.

Table 2
The correlation between the levels of CC and CXC chemokines and the severity of ASD on ADOS-2 score.

Indicators (chemokines)	ADOS-2 total score	
	<i>r</i>	<i>P</i>
MCP-1	0.766	.000
MIP-1 α	-0.913	.000
MIP-1 β	-0.898	.000
RANTES	-0.114	.377
IL-8	-0.842	.000
IP-10	-0.851	.000
ME	-0.807	.000

The severity of ASD children was evaluated by the ADOS-2 scale. Pearson correlation analysis was used to analyze the correlation between chemokine levels and the ADOS-2 Module 1 scores of the children.

IL-8 = interleukin-8, IP-10 = interferon- γ -induced protein-10, MCP-1 = monocyte chemoattractant protein-1, MIG = interferon (IFN)- γ , MIP-1 α = macrophage inflammatory protein-1 α , MIP-1 β = macrophage inflammatory protein-1 β , RANTES = normal T-cell expressed and secreted.

and MIG levels ($P < .01$). However, it was not significantly correlated with the RANTES level ($P > .05$), as shown in Table 2.

5. Discussion

ASD is a group of neurodevelopmental disorders. At present, the cause of ASD is not yet fully understood. Neuro-immuno-inflammatory interactions begin in early neurodevelopment and continue throughout life. The immune-inflammatory system plays an important role in many aspects of neuronal function.^[10] Many studies have shown that immunoinflammatory disorders play a central role in autism.^[11] There are persistent immunoinflammatory disorders in ASD patients and animal models, including autoimmunity, infection, and fetal reactive antibodies, which are all related to ASD.^[12] Some scholars have discovered that several molecular signaling pathways, including cytokine downstream pathways, receptor MET, major histocompatibility complex molecules, microglia, and complement factors link immune inflammation with the ASD phenotype.^[13] Even changes in the prenatal immune environment may increase the risk of ASD in children.^[14] It is reported that acute immune inflammation activation in pregnant women during an infection can also increase the incidence of ASD, which is closely related to the levels of maternal cytokines such as IL-2, IL-6, and IL-10.^[15] Accumulating evidence by several studies demonstrated immune dysfunction and inflammatory response in children with ASD and supported that ASD is associated with both acquired and innate immune response abnormalities.^[16,17] Han et al demonstrated that maternal inflammatory states were linked with neurodevelopmental disorders, including ASD.^[18] Therefore, dysfunctional immune defenses and inflammatory reactions in children with ASD could be important precipitating factors for triggering this disorder.

In the present work, we analyzed the most representative indicators of CC and CXC chemokines. Among them, MCP-1 is an important pro-inflammatory cytokine in CC chemokines. When the body parts undergo inflammation, a variety of cells, including monocytes, macrophages, and fibroblasts secrete MCP-1 under the induction by cytokines or viruses, resulting in increased MCP-1 levels in the blood. MIP-1 α , MIP-1 β , and RANTES are key anti-inflammatory factors among the CC chemokines and can resist and eliminate the invasion of

pathogens and build an innate immune barrier for the body.^[19,20] IL-8, IP-10, and MIG belong to the category of CXC chemokines. IL-8 and MIG are important pro-inflammatory factors. However, IP-10 is an anti-inflammatory factor that can activate lymphocyte receptors and recognize and adhere to the inflammation sites of lymphocytes. Under normal physiological conditions, IL-8 and IP-10 are in a dynamic balance. When this balance is disrupted, it may lead to an inflammatory response and cause a series of nerve injuries.^[21] Zerbo et al demonstrated differential expression of MCP-1, RANTES, and MIP-1 α in ASD.^[22] In congruence with previous reports, our findings showed that compared with healthy children, those with ASD showed significantly increased MCP-1 levels ($P < .01$) and significantly decreased MIP-1 α and MIP-1 β levels ($P < .01$). However, they exhibited no significant difference (except some downward trend) in the level of RANTES ($P > .05$). Furthermore, autistic children showed significantly decreased IP-10 levels ($P < .01$) but had no noticeable change (except decreasing trend) in the levels of IL-8 and MIG ($P > .05$). These observations indicate that the expression of pro-inflammatory factors in children with ASD increases, whereas that of anti-inflammatory factors decreases, causing an imbalance between anti-inflammatory factors and pro-inflammatory factors. Such imbalance decreases the body's immunity or defense mechanism against adverse stimuli and may induce a series of inflammatory reactions. In addition, the imbalance between pro-inflammatory factors and anti-inflammatory factors can severely damage nerve cells and brain tissues by stimulating oxidative stress response and endocrine cytokines. The abnormal expression of certain cytokines following a brain injury can aggravate nerve damage and even permanent loss, which is highly unfavorable for children with immature/underdeveloped nervous systems and can affect their normal neurodevelopment, leading to neurodevelopmental disorders. Therefore, inflammation is a primary driving factor for ASD, and the aforementioned CC and CXC chemokines may become potential biomarkers in the screening and diagnosis of ASD. The findings in this study are consistent with those reported by Zerbo et al,^[22] who showed that compared with healthy children, children with ASD showed increased serum MCP-1 levels and decreased RANTES levels. In addition, Han et al^[18] documented that the levels of MCP-1 and RANTES in children with ASD were significantly higher than those in normally developing children, whereas the levels of IL-8, IP-10, and MIG are significantly lower in autistic children than in normal children. The variations in IL-8, IP-10, and MIG levels are consistent with the results of our study; however, the result of the RANTES level is contrary to our findings. Such a difference could be attributed to different inclusion criteria, sample sizes, and detection methods of control group children between the 2 studies.

6. Conclusion

We analyzed the correlation of certain CC and CXC chemokines with the severity of the ASD group. We observed that the levels of MCP-1 were positively correlated with the total ADOS-2 score, MIP-1 α , MIP-1 β , IL-8, and IP-10. The MIG levels were negatively correlated with ADOS-2 total scores; RANTES levels were not significantly correlated with ADOS-2 scores. Collectively, the changes in the levels of CC chemokines, such as MCP-1, MIP-1 α , MIP-1 β , and RANTES, as well as CXC chemokines, such as IL-8, IP-10, and MIG, could be associated with the

pathogenesis of ASD. Our findings suggest that these factors might be potential diagnostic markers and therapeutic targets for the diagnosis and treatment of ASD.

Author contributions

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