Original Article

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Paediatrics Section

Altered Adaptive Cellular Immune Function in a Group of Egyptian Children with Autism

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ABSTRACT

Introduction: There is a growing evidence of immune system alteration in children with Autism Spectrum Disorder (ASD). These changes may include higher levels of pro-inflammatory cytokines in plasma, Cerebrospinal Fluid (CSF) and brain cells.

Aim: To evaluate the imbalance of the immune system in autism through correlating some immunological markers (C3, C4, CD4, IL12 and IL17) with severity of ASD.

Materials and Methods: The current case-control study included 120 subjects, 60 autistic children and 60 healthy control obtained from the Clinical Genetics Clinic, National Research Centre, Egypt from the period between December 2014 to December 2016. All candidates were subjected to full clinical evaluation in addition to ElectroEncephaloGraphy (EEG), hearing test and estimation of interleukins (IL12- IL17), C3-C4, CD4 levels in blood samples. Independent t-test, Chi-square test or McNemar test were used to analyse the data.

Results: The levels of IL12 (27.47 ±7.15) and IL17 (1630.46±310.42) were significantly higher, (p= 0.026, 0.005) respectively, while levels of CD4 were significantly lower (37.93± 3.25), (p<0.001) in autistic children compared to controls; however, there was no significant difference in C3 and C4 between the two groups. High statistically significant difference between autistic children with moderate and severe ASD in CD4 levels were seen, (p=0.018).

Conclusion: Autistic children may suffer from immunological dysfunction. Further efforts should be exerted to find out the relation between immune imbalance and the progression of ASD.

Keywords: Autism spectrum disorder, Complement system, Cytokines, Immunological disorders

INTRODUCTION

ASD is a neurodevelopmental disorder characterized by persistent deficits in social communication; social interaction and restricted, repetitive patterns of behaviour, interests, or activities [1]. Symptoms of ASD generally manifest in early childhood and persist into adulthood in most cases [2]. The role of genetic factors has been considered as a risk factor of ASD, however, its exact etiology is unknown, and no biomarkers have yet been discovered to be associated with ASD [3]. ASD according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) includes Autistic Disorder (AD), Asperger disorder, Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS) [4]. Growing evidence implicates imbalance of the immune system as a part of the pathophysiology of ASD [5].

Herein, we aimed to evaluate immune dysfunction in autistic children through measuring some immunological markers (C3, C4, CD4, IL12 and IL17) in blood samples and correlating the immune dysfunction in patients with severity of ASD and Gastrointestinal (GI) disorders (such as chronic constipation, diarrhea, abdominal distention and gastroesophageal reflux disease) which are considered the most common medical conditions associated with autism.

MATERIALS AND METHODS

This case-control study was conducted between December 2014 to December 2016. It included 60 autistic children aged from 6 to 12 years representing all children with ASD who were referred to the Clinical Genetics Clinic, National Research Centre, Egypt from both sexes through the period of the study and who met the diagnostic criteria of autism as defined in International Classification of Diseases, 10th edition (ICD-10) (qualitative abnormalities in reciprocal social interaction, qualitative abnormalities in communication and restricted, repetitive, and stereotyped patterns of behaviour, interests, and activities) [6]. Children with autoimmune disease such as (autoimmune blood disease, SLE, Rheumatoid Arthritis), allergic disorders such as (skin allergy- asthmatic patients- allergic rhinitis) were excluded from the study. Sixty age- and sex-matched healthy children served as control who had no history of delay in developmental milestones or any neurological or psychiatric disorders as reported by their parents.

Written informed consent was obtained from the parents after explanation of the aim of the study. All the patients' data were confidential, neither the data nor the collected samples were used in any other research. Approval was taken to conduct this research from the ethical committee of the Institute of Postgraduate Childhood Studies (Ain Shams University) and the ethical committee of the National Research Centre (NRC) in Egypt.

All autistic children were subjected to full history taking, pedigree construction up to three generations, complete clinical examination and anthropometric measurements. Electroencephalography (EEG), complete eye evaluation, hearing test and rating of the severity of autism using Childhood Autism Rating Scale (CARS) were also done to all autistic children. CARS were performed by trained clinician, to rate items indicative of ASD after direct observation of the child. It consists of 15 categories assessing autistic behaviours. Total scores range from 15 to 60; scores below 30 indicated that the individual is non-autistic, however, scores from 30 to 36.5 indicated mild to moderate ASD, while those from 37 to 60 indicated the severe form [7].

Laboratory Studies

Estimation of Interleukin-12 p70 (IL-12) and IL-17 levels using ELISA was performed for the quantitative detection of human interleukins [8].

$CD4^+$ T cells were detected and counted using Flow cytometry. The monoclonal antibody was purchased from Dako, Denmark. Flow cytometry analysis operation was started by Laser alignment using immune-check beads and then the single and double color immunofluorescence protocol was defined. Isotopic control was used to provide a baseline for determining the minimum fluorescence above which positive cells were identified. Data analysis was done to determine percentage positivity for the antibody [9].

Complement C3 and C4 were measured using minineph human C3 and C4 kits. For determination of complement (soluble antigen concentration) by nephelometric methods which involve a reaction with specific antiserum to form insoluble complexes. The procedure was carried out on a minineph analyser (Turbid metric method) [10].

STATISTICAL ANALYSIS

Quantitative data were analysed using SPSS version 16.0, with mean values for continuous variables compared using Independent t-test, and differences between proportions assessed using either the chi-square test or McNemar test. The level of statistical significance for all tests was set ≤0.05.

RESULTS

The current study included 56 autistic males (93.3%) and 4 autistic females (6.7%). The control subjects were 46 males (76.7%) and 14 females (23.3%), their ages were ranged from 6-12 years with mean age of (8.7±1.3) for autistic children and (7.9±1.6) for controls. There was no significant difference between cases and control groups regarding sex or age distribution.

Regarding EEG abnormalities shown in [Table/Fig-1], autistic children with abnormal EEG were 26(43.3%). However, 54(90%) had normal MRI.

[Table/Fig-2] shows that the levels of IL12 (27.47 ±7.15) and IL17 (1630.46±310.42) were significantly higher in autistic children than in healthy controls (p=0.026, 0.005) respectively. However, there was no significant difference in levels of C3 and C4 between autistic children and control group. On the other hand, CD4 levels were decreased significantly in autistic children compared to healthy controls (p<0.001).

Variables	Number of case & percentage (N =60)
EEG	
Normal	34 (56.7%)
Positive	26 (43.3%)
MRI	
Normal	54 (90%)
Prominent cortical sulci	2 (3.3%)
Prominent temporal horn of Lateral vent	2 (3.3%)
Retrocerebellar cyst	2 (3.3%)
GIT Manifestations	
Negative	46 (76.7%)
Positive	14 (23.3%)
CARS	
Mild/ Moderate	46 (76.7%)
Severe	14 (23.3%)

[Table/Fig-1]: EEG, magnetic resonance imaging (MRI) & gastro-intestinal (GIT) findings in autistic children.

Variable	Autistic children (N=60) Mean ± SD	Control (N=60) Mean ± SD	p-value
IL-12(pg/ml)	27.47±7.15	14.43±5.35	0.026*
IL-17(pg/ml)	1630.46±310.42	1489.88±226.04	0.005*
C3 (mg/L)	1.95±0.83	2.33±0.79	0.077
C4 (mg/L)	0.37±0.14	0.43±0.09	0.066
CD4 (%)	37.93±3.25	40.82±2.08	<0.001*

[Table/Fig-2]: Comparison between autistic children and the control group regarding immunological profile.
*p≤0.05 is significant

On evaluating the immune dysfunction in autistic children with GI disorders [Table/Fig-3], revealed that IL-12 level (21.43±4.41) was significantly lower in autistic children with GIT signs (p=0.017). There was no statistically significant difference between the autistic children with positive and negative GI signs regarding all other studied variables.

There was no statistically significant difference regarding degree of severity of autism and values of IL-12, IL-17, C3, C4, However, there was high statistically significant difference between autistic children with moderate and severe ASD in CD4 levels as shown in [Table/Fig-4].

Variables	Positive GIT signs (N=14) Mean ± SD	Negative GIT signs (N=46) Mean ± SD	p-value
Age	5.71±1.60	6.68±2.73	0.835
IL-12 (pg/ml)	21.43±4.41	30.45±8.29	0.017*
IL-17 (pg/ml)	1633.21±445.59	1675.72±415.39	0.916
C3 (mg/L)	1.73±0.68	2.02±0.89	0.432
C4 (mg/L)	0.34±0.16	0.37±0.14	0.577
CD4 (%)	38.04±4.15	37.78±3.05	0.855

[Table/Fig-3]: Comparison between autistic children with positive and negative GIT signs in the studied variables.
*p≤0.05 is significant

Variables	Mild/moderate (N=46) Mean ± SD	Severe (N=14) Mean ± SD	p-value
IL-12 (pg/ml)	27.3±8.11	28±5.8	0.954
IL-17 (pg/ml)	1626.75±348.52	1642.64±340.88	0.969
C3 (mg/L)	1.98±0.92	1.86±0.46	0.733
C4 (mg/L)	0.36±0.14	0.42±0.14	0.345
CD4 (%)	38.69±3.16	35.44±2.23	0.018*

[Table/Fig-4]: Comparison between autistic children regarding the severity using CARS.
*p≤0.05 is significant

DISCUSSION

Previous research results have shown that autistic children suffer from immunological dysfunction [11,12]. Moreover, much evidence indicates a strong link between imbalance in cytokines level and abnormalities in T helper cells in neuropsychiatric disorders and autism [12,13].

The present study was designed to evaluate immune dysfunction in autistic children through evaluating cellular immunological markers CD4 and Cytokines (IL-12 and IL-17) and Complement markers (C3 & C4) in peripheral blood samples and to correlate the immune dysfunction in patients with the severity of disease regarding CARS results.

Sixty autistic children and the same number of apparently healthy, sex and age matched children were recruited for this study. Increased male predominance in our study was in agreement with Gauthier J et al., and Vincent JB et al., [14,15].

The current study revealed that 14 autistic children (23.3%) had GI disturbances such as constipation and abdominal distention. These results go in agreement with a study done by Penn AH et al., who found that children with (ASD) sometimes report gastrointestinal dysfunction such as constipation and abdominal distress that would indicate GI immunological disturbance [16]. The results of the present study were in accordance with the results done by Adams JB et al., who investigated gender differences regarding GIT symptoms and found that there were no statistically significant differences between males and females [17].

Our study showed that 34 autistic children (56.7%) had normal EEG and 26 children (43.3%) had seizures and/ or abnormal EEG, such observation is in concordance with Ramanujapuram RA [18], who found an increased prevalence of abnormal potentially epileptogenic activity in autistic children and about one in four patients develop seizures at puberty.

Among our studied group we found that only 10% of autistic children had abnormal MRI such as prominent cortical sulci, white matter dysmyelination, retrocerebellar cyst. Hardan AY et al., studied cortical thickness in light of the postmortem evidence of cortical abnormalities of the disorder and noticed increases in total cerebral sulcal and gyral thickness of both temporal and parietal lobes in autistic children compared to normal subjects [19]. These findings may contribute to the increased gray matter volume and subsequently total brain size, which is considered an important observation in autism and may also be related to anomalies in cortical connectivity. On the other hand, our results support the results of Zeglam AM et al., who reported that most of the autistic patients have normal MRI [20].

IL-12 is the prototype of a unique family of heterodimeric cytokines [21]. Toll-like receptor (TLR) signaling induces T cell synthesis of IL-12. Also, IL-12 production is positively regulated by IFN-8 which is induced by IL-12 itself [22].

In this study, IL-12 was significantly higher in autistic patients rather than control subjects. These results came in agreement with Enstrom AM et al., who found a clue of immune system imbalance in some children with ASD including higher levels of pro-inflammatory cytokines in serum, CSF and brain cells [23].

Our study also supports findings of Paunovic V et al., who proved that several cytokines are involved in autism and the mainly two major groups of cytokines, namely the IL-12 and IL-17 families have a prominent role in the pathogenesis of many autoimmune diseases such as systemic lupus and autism [24].

The current study showed that plasma levels of IL-17 were significantly elevated in autistic patients compared to control subjects; such results oppose those of Onore C et al., who found that the concentration of IL-23, but not IL-17, was significantly reduced in ASD compared to controls [25]. However, we agree with the study done by Al-Ayadhi LY and Mostafa GA which found an increase in serum IL-17 levels in a group of autistic children with significant correlation with the severity of autism [26].

To our knowledge, this study is the first study to measure serum level of IL-12 and IL-17 in relation to positive and negative GIT symptoms in autistic children. It was found that the IL-12 levels were significantly lower in autistic children with positive GI symptoms where p= 0.017. Whereas, no statistically significant difference in serum levels of IL-17, C3, C4 and CD4 between the two groups were observed.

We also found that most of the autistic children showed normal complement function. Moreover, a high significant difference between $CD4^+$ T cells level in autistic patients and controls was striking, the low serum level of $CD4^+$ T cells was related significantly to the severity of autistic symptoms using CARS. Also, our study goes in agreement with work done by Jyonouchi H et al., who proved that patients with autism had significantly lower levels of $CD4^+$ T cells than healthy controls [27]. These results are similar to those of Ashwood et al., who found that the levels of $CD3^+$ and $CD4^+$ T cells were significantly reduced in ASD which indicate that the adaptive cellular immune function is significantly altered in autistic patients [28].

LIMITATION

Although the research has reached to its aim, there were some limitations. Because of the time limit, the research was conducted only on relatively small number of autistic children who were attending the clinic. Therefore, to generalize the results, further studies should be performed on a large scale.

CONCLUSION

Our findings lead us to conclude that immunological dysregulation may be implicated in the pathophysiology of ASD that warrants further consideration. Further assessment of cellular immune function would find out the relationship between immune imbalance and the progression of behavioural and developmental changes throughout the course of autism including larger study group.

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