Pro-inflammatory cytokines in autistic children in central Saudi Arabia

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ABSTRACT

Objective: Abnormal inflammatory immune response might contribute to autism. Pro-inflammatory cytokines could induce some of the symptoms and signs of autism. Such as, social withdrawal eating and sleep disturbance. The aim of the current study was to examine whether autism spectrum disorders in Riyadh area are accompanied by activation of the pro-inflammatory response system.

Methods: The study was conducted in the Riyadh area between September 2003 and April 2004. Seventy-seven autistic child from the Riyadh area participated in the study, with confirmed diagnosis according to E-2 diagnostic criteria for autistic spectrum disorders. The parents/guardians filled a simple related questionnaire, then serum concentrations of tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) were measured in 65 autistic, 8 attention deficit disorder, 2 children with Rett's syndrome and 2 children with Asperger syndrome. The results were compared to age, and sex matched control children.

Results: This study showed a significantly increased production of TNF-α, IL-1 and IL-6 from the sera of autistic, attention deficit disorder, Rett's syndrome and Asperger syndrome children. There was no correlation between TNF-α, IL-1 or IL-6 and the degree of autism or the age of the affected child. Significant higher incidence of social withdrawal, sleeping and eating disorders were found among autism spectrum disorders compared to control.

Conclusions: These results suggest that autism may be accompanied by an activation of the macrophages. It is hypothesized that increased production of pro-inflammatory cytokines could play a role in the pathophysiology of autism spectrum disorders, such as social withdrawal, eating and sleeping disorders.

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Autism is a childhood disorder with age of onset 1½-3 years. During childhood, autistic children may fall behind their same aged peers in areas of communication, social skills and cognition.[1] In addition, dysfunction behavior may start to appear, such as self-injury, sleeping disorders, eating disorders and poor eye contact.[1,2] Autism is as common as one in 2500 live births worldwide and Saudi Arabia is no exception.[3] Although there is no known unique cause of autism, there is growing evidence that autism can be caused by a variety of disorders.[1,2] New evidence is emerging suggesting involvement of the inflammatory response system.[2,3] Proinflammatory cytokines, may induce some of the behavioral symptoms of autism, such as social withdrawal, resistance to novelty and sleep disturbances.[2,3] Researchers demonstrated increase interleukin-1 (IL-1) receptors antagonist (IL-1RA) and interferon-γ (IFN-γ). Those findings favor monocytes activation.[4,5] Jyonouchi and his workers in 2001[6] demonstrated more than two-folds increase in tumor necrosis factor-α (TNF-α), IL-1β, and IL-6 production from stimulated peripheral blood mononuclear cells of autistic children. In addition, they demonstrated higher levels of pro-inflammatory/counter-regulatory cytokine production from peripheral blood mononuclear cells. The results indicate excessive innate immune

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responses in several autism spectrum disorders children that may be most evident in TNF-α production.[6] Immune factors such as autoimmunity have been implicated in the genesis of autism, a neurodevelopmental disorder. Since autoimmune response involves immune activation, the plasma levels of interferon-α (IFN-α), interferon-γ (IFN-γ), interleukin-12 (IL-12), interleukin-6 (IL-6), TNF-α, and soluble intercellular adhesion molecule-1 (sICAM-1) were measured in autistic patients and age-matched normal controls. The levels of IL-12 and IFN-γ were significantly higher in patients as compared to controls. However, IFN-α, IL-6, TNF-α, and sICAM-1 levels did not significantly differ between autistics and control. Because macrophage-derived IL-12 is known to selectively induce IFN-γ in T helper type-1 (Th-1) cells, it is suggested that IL-12 and IFN-γ increases may indicate antigenic stimulation of Th-1 cells pathogenetically linked to autoimmunity in autism.[7] Based on these findings, the aim of the current study is to assess plasma levels of TNF-α and IL-1 and IL-6 in autistic children in the Riyadh area, Saudi Arabia. In addition, to correlate plasma levels of these cytokines to the degree of autism and age of the affected child.

Methods. ***Patient's and control selection.*** The study was conducted in Riyadh area between September 2003 and April 2004. Seventy-seven child, age ranging from 3.5-14 years old, with confirmed professional diagnoses, with one of the following disorders were selected: early childhood autism, attention deficit disorder (ADD), Rett's syndrome, and Asperger syndrome. The diagnosis was carried out either by a qualified psychologist, psychiatrist of neurologist, according to diagnostic criteria E-2 for autism. Written consent was obtained from the parents prior to the start of the study, and then the parents were asked to complete a questionnaire regarding the child's medical and behavioral history. In addition, 77 healthy age and sex matched controls, were recruited from King Abdul-Aziz University Hospital. All subjects were free from any infections, inflammatory or allergic reaction for at least 2 weeks prior to blood sampling. Exclusion criteria for autistic patients and healthy volunteers were subjects suffering from a neurological, inflammatory, endocrine or clinically significant chronic disease; immunocompromised subjects; subjects with tuberous sclerosis, and subjects receiving medication with known or potential interaction with immune and endocrine function. Blood samples were collected in plane tubes, allowed to clot, then centrifuged, and serum was collected and stored at -80ºC until assay time. TNF-α, IL-1 and IL-6 were measured by commercially available ELISA Kits (R&D Pharmaceutical, USA). The tests were carried out in duplicate.

The results were analyzed by using SPSS for windows. Results were expressed as mean ± SEM. Statistical analysis for differences among the groups was assessed by Student's t-test. *P* values equal to or less than 0.05 were considered significant. Relationships between variables were assessed with Spearman test.

Results. Seventy-seven children participated in the study. Sixty-five autistics with confirmed diagnosis (61 males and 4 females), 8 males with ADD, 2 males with Aspergers, and 2 females with Rett's syndrome. The mean age for the total number of autistic children was 8.8±0.5 years. Seventy-one males (92.2%) and 6 females (7.8%) participated in the study, with a male to female ratio of 11.8:1. Eight-six percent (56) of the autistics suffered from social withdrawal, 47.6% (31) with sleeping disorders and 89% (58) with eating disorders. In the control group, only 8% (6) demonstrated social withdrawal, 17% (13) had sleeping disorders and 18% (14) had eating disorders. However, 87.5% (7), 37.5% (3) and 87.5%, demonstrated, social withdrawal, sleeping and eating disorders. As **Figure 1** demonstrates, TNF-α plasma concentration was significantly higher in autistic, ADD, Asperger syndrome and Rett's syndrome, as compared to the control. Further more, IL-1 plasma level was significantly higher in autistic children, ADD, Asperger syndrome and Rett's syndrome as compared to the control. In addition, IL-6 plasma level was significantly higher in autistic children, ADD, Asperger syndrome and Rett's syndrome as compared to the control. There were no significant correlations between the degree of autism or the age of autistic children and levels of TNF-α (r =- 0.21, *p* = 0.4), IL-1 (r = - 0.32, *p* = 0.6) or IL-6 (r =0.32, *p* = 0.39).

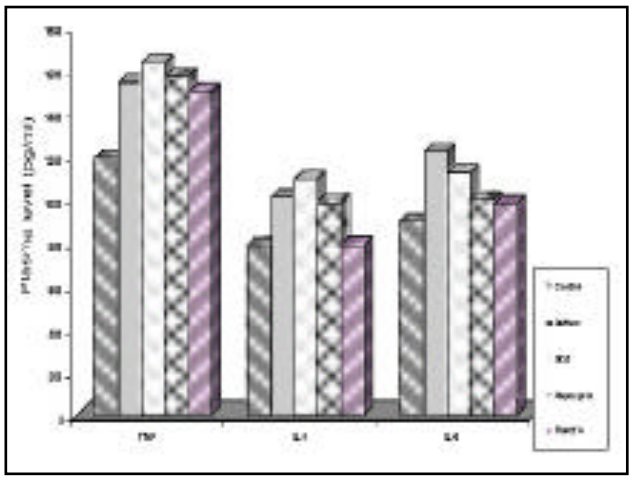

Figure 1 - Plasma levels of tumor necrosis factor-α (TNF), interleukin (IL)-1 and IL-6 (pg/ml), in early childhood autism, attention deficit disorder (ADD), Rett's syndrome, and Asperger syndrome, as compared to the control. Data presented as mean ± SD ($p<0.05$).

Discussion. Seventy-seven child with autistic spectrum disorders participated in the study. Sixty-five were autistic, with 61 females and 4 males. The male to female ratio was 15 to 1, more than the known international ratio of 4 to 1. This is probably due to more males having participated in the study than females. Furthermore, 2 males with Asperger syndrome, and 2 females with Rett's syndrome were included. The major findings of this study are that children with autistic spectrum disorders are characterized by significant increased production of all pro-inflammatory cytokines that is TNF-α, IL-1 and IL-6. Moreover, autistic spectrum disorders are associated with social withdrawal, and higher incidences of sleeping and eating disorders as compared to control children.

Innate immunity plays a key role in the development of subsequent adaptive immune responses by facilitating co-stimulatory/adhesion molecules expression. Dysregulation of the innate immune response could be harmful and may lead to broken tolerance and development of autoimmune disease.[8] Inflammatory and immune hyperactivity states have considerable mechanistic overlap, and evidence links inflammatory cytokine imbalance to autoimmunity, both of which appear to contribute to ASD.[9,10] Scientists have suggested a depressed immunity, autoimmunity, and inflammatory activation commonly seen in autism.

In autism, there are several clues that molecular mimicry and other autoimmune processes are operative. Among these are elevated urinary neopterin and biopterin, most likely resulting from TNF-α stimulation of immune cells.[11] Serum autoantibodies to myelin basic protein were found in 58% of autistic children.[12] In a related study, anti-brain autoantibodies reached 27% for IgG-type and 36% for IgM-type.[13]

Tumor necrosis factor-α is one of the most pluripotent cytokines known, and is consistently pro-inflammatory.[14] It is produced systemically, including by microglia and astrocytes of the brain, and is a suspected major contributor to the inflammatory and autoimmune brain pathologies.[15] Also, TNF-α is considered an agent involved in several neurodegenerative diseases. Interleukin-1 is a significant pro-inflammatory cytokine of a particular importance in systemic response to inflammation. It synergizes with TNF-α in the pathogenesis of many diseases, such as rheumatoid arthritis, inflammatory bowel disease, septic shock and several autoimmune diseases. They are the primary pro-inflammatory cytokines, released from macrophages and can start a cascade of inflammatory reactions.[16] Production of IL-6 is increased in many clinical situations characterized by tissue injury, such as trauma, ischemia, burns, malignant conditions, exposure to toxins and aseptic irritants, infections, immune hypersensitivity reactions, and autoimmune diseases. Interleukin-6 is a cytokine that plays an essential role in immune and acute phase reactions.[17] It is mainly produced by cells of the monocytic lineage, but T and B lymphocytes may produce this cytokine. Increased concentration of IL-6 in peripheral blood is observed in many autoimmune disorders, such as systemic lupus erythematosus, rheumatoid arthritis and type 1 diabetes.[18] In addition, it is considered that IL-6 may constitute one factor in the development of the autoimmune status. In summary, IL-6 seems to be a major mediator of the host response to tissue injury in many autoimmune and inflammatory diseases and plays an important role in regulating the immune, hepatic, hematopoietic, skeletal, and neuroendocrine systems. Abnormalities that lead to persistent oversecretion or undersecretion of IL-6 or excessive or blunted effects of IL-6 may be involved in various disease states, including autoimmune and inflammatory diseases.[18]

However, it is still not known whether inflammatory changes are responsible for active nerve cell death or whether they play a protective role in neurodegeneration. In Parkinson's disease (a neurodegenerative disease), these changes are related to neuronal loss and suggests that anti-inflammatory treatment for disease such as parkinsonian patients could have beneficial effects in the progression of the disease by slowing down the process of neuronal loss,[19] and this might be the case in autism.

Microorganisms or allergens in food or the environment frequently initiate autoimmune diseases, and it is thought that viral infections play a role in the initiation of an immune response against brain antigens. As a result, a neurodegenerative disease might start, such as autism spectrum disorders.[12] An association between virus serology and brain antibodies has been demonstrated by Singh et al.[13] Some scientists hypothesized that the increased production of pro-inflammatory cytokines may play a role in the etiology of some autistic symptoms typical for autism. Such as social withdrawal, suppression of exploratory behavior, sleep disturbance and mood alteration.[5]

Jyonouchi et al[6] demonstrated inflammatory imbalance in ASD subjects by using measures of TNF-1, IL-1 and IL-6. The antioxidant vitamins C and E, and reduced glutathione may help lower these in vivo, but currently the most promising approach is generous dosing with long-chain, omega-3 fatty acids.[20] Ongoing work with clinical and animal models of inflammatory over-activation has established that inflammation can be inhibited by loading the cell membranes with long-chain, omega-3 fatty acids. The omega-6 to omega-3 fatty acids balance in cell membranes helps determine the pro- to anti-inflammatory balance of the cell membranes. Given that many, if not most, autistic children appear to produce excessive amounts of TNF-α and other pro-inflammatory cytokines,[21] it is reasonable to expect the anti-inflammatory long-chain, omega-3 fatty acids should offer benefit.

The rationale for long-chain, omega-3 fatty acid benefits, namely, resetting the balance of prostaglandins and cytokines, can be extended to the management of pro-inflammatory coagulation states.[22,23]

Results from the current study demonstrated significantly higher levels of pro-inflammatory cytokines TNF-α, IL-1 and IL-6 in autistic spectrum disorders, as compared to normal. This might be related to some of the main characteristics of autistics such as, abnormal social behavior, abnormal sleeping and eating disorders in autistic spectrum disorder children. Furthers studies of this might help to elucidate a role for the immune system in the pathogenesis of the disorder, and might lead to possible intervention measures in the future.

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References

1. Fein D, Joy S, Green L, Waterhouse L. Autism and pervasive developmental disorders. In: Fogel B, Schiffer R, Rao S, editors. Neuropsychiatry. Baltimore (MD): Williams & Wilkins; 1996.
2. Trottier G, Srivastava L, Walker CD. Etiology of infantile autism. A review of recent advances in genetic and neurological research. ***J Psychiatry Neurosci*** 1999; 24: 103-115.
3. Cobi J, Heijren P, Philip DA. Autism and immune system. ***J Child Psych*** 1997; 38: 337-349.
4. Fiumara A, Sciotto A, Barone R, D'Asero G, Munda S, Pavone L. Peripheral lymphocyte subsets and other immune aspects in Rett syndrome. ***Pediatr Neurol*** 1999; 21: 619-621.
5. Croonenberghs J, Bosmans E, Deboutte D, Kenis G, Maes M. Activation of the inflammatory response system in autism. ***Neuropsychobiology*** 2002; 45: 1-6.
6. Jyonouchi H, Sun S, Le H. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. ***J Neuroimmunol*** 2001; 120: 170-179.
7. Singh VK. Plasma increase of interleukin-12 and interferon-gamma. Pathological significance in autism. ***J Neuroimmunol*** 1996; 66: 143-145.
8. Medzhitov R, Janeway Jr C. Innate immunity. ***N Engl J Med*** 2000; 343: 338-344.
9. Sabatelli M, Madia F, Mignogna T, Lippi G, Quaranta L, Tonali P. Pure motor chronic inflammatory demyelinating polyneuropathy. ***J Neurol*** 2001; 248: 772-777.
10. Jyonouchi H, Sun S, Itokazu N. Innate immunity associated with inflammatory responses and cytokine production against common dietary proteins in patients with autism spectrum disorder. ***Neuropsychobiology*** 2002; 46: 76-84.
11. Messahel S, Pheasant AE, Pall H, Ahmed-Choudhery J, Sungum-Paliwal RS, Vostanis P. Urinary levels of neoptrin and biopterin in autism. ***Neurosci Lett*** 1998; 241: 17-20.
12. Singh VK, Warrene RP, Odell JD, Warren WL, Cole P. Antibodies to myelin basic protein in children with autistic behavior. ***Brain Behav Immun*** 1993; 7: 97-103.
13. Singh VK, Lin SX, Yang VC. Serological association of measles viruses and human herpes viruses-6 with brain autoantibodies in autism. ***Clin Immunol Immunopath*** 1998; 89: 105-108.
14. Stubbs EG, Crawford ML. Depressed lymphocyte responsiveness in autistic children. ***J Autism Child Schizophr*** 1977; 7: 49-55.
15. Warrren RP, Margaretten NC, Pace NC, Foster A. Immuno abnormalities in patients with autism. ***J Autism Dev Disord*** 1986; 16: 189-197.
16. Barkhudaryan N, Dunn AJ. Molecular mechanisms of action of interleukin-6 on brain, with special reference to serotonin and the hypothalamo-pitutary-adrenaocortical axis. ***Neurochem Res*** 1999; 24: 1169-1180.
17. Maes M, Scharpe S, Meltzer HY, Bosmans E, Suy E, Calabrese J et al. Relationships between interleukin-6 activity, acute phase proteins, and function of the hypothalamic-pitutary-adrenal axis in severe depression. ***Psychiatry Res*** 1993; 49: 11-27.
18. Kishimoto T, Akira S, Taga T. Interleukin-6 and its receptors: A paradigm for cytokines. ***Science*** 1992; 258: 593-597.
19. Barcia C, Fernandez Barreiro A, Poza M, Herrero MT. Parkinson's disease and inflammatory changes. ***Neurotox Res*** 2003; 5: 411-418.
20. Jyonouvhi H, Sun S, Le H. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune response in children with autism spectrum disorders and developmental regression. ***J Neuroimmunol*** 2001; 120: 170-179.
21. Cohen HJ, Pieper CF, Harris T, Rao KM, Currie MS. The association of plasma IL-6 levels with functional disability in community-dwelling elderly. ***J Gerontol A Biol Sci Med Sci*** 1997; 52: M201-M208.
22. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. ***N Engl J Med*** 1997; 336: 973-979.
23. Bataille R, Klein B. C-reactive protein levels as a direct indicator of interleukin-6 levels in humans in vivo. ***Arthritis Rheum*** 1992; 35: 982-984.

Dedication

This work is dedicated to my colleague the late Dr. Ahmad Al-Jarallah (Consultant Neuropediatrician, King Khaled University Hospital, Riyadh), who gave me all the support I needed when conducting my research on Autism, but did not live to see it completed. "May Allah grant him Jannah (Amen)."

From the Editors: The Editors and Staff of Neurosciences extend their heartfelt condolences to the family, friends and colleagues of Dr. Ahmad Al-JarAllah, a distinguished and respected member of the Neurosciences Community.