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Immunogenetic and Cytokine Profile Alterations in Autism Spectrum Disorder: A Clinical Study

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A B S T R A C T

Autism Spectrum Disorder (ASD) is a multifactorial neurodevelopmental disorder with complex interactions between genetic susceptibility, immune dysfunction, and environmental influences. Accumulating evidence suggests that immune system abnormalities, particularly cytokine dysregulation, may interact with genetic vulnerability factors to influence neurodevelopmental trajectories. The broad phenotypic variability observed in ASD raises the possibility that distinct immunogenetic mechanisms underlie different clinical presentations. This study was designed to investigate immunogenetic and cytokine profile alterations in children diagnosed with Autism Spectrum Disorder, with particular emphasis on inflammatory mediators and their potential relationship to immune-related pathogenic mechanisms. A clinical case–control framework was employed to assess circulating cytokines, including IL-6, IL-27, IL-37, IL-29, TNF-α, and IFN-γ, alongside immunoglobulin levels (IgG and IgM). Quantitative immunological assays were utilized to determine serum concentrations. The study further explored the theoretical framework of immunogenetic interaction by analyzing cytokine alterations in the context of potential genetic susceptibility and immune pathway activation. Statistical evaluation was conducted to identify significant immunological differences and possible mechanistic associations. Children with ASD exhibited significant cytokine profile alterations, characterized by enhanced pro-inflammatory responses and modified regulatory cytokine activity. Elevated IL-6, TNF-α, and IFN-γ levels suggest persistent immune activation and possible neuroinflammatory signaling. Variations in IL-27 and IL-37 indicate disruption in immune regulatory balance. These findings support the concept that ASD may involve immune-mediated mechanisms influenced by genetic predisposition affecting cytokine signaling pathways. The integration of cytokine data with immunogenetic perspectives highlights a potential mechanistic link between systemic immune alterations and altered neurodevelopment. The present clinical study provides evidence supporting an immunogenetic framework for Autism Spectrum Disorder, wherein cytokine dysregulation may represent both a biological marker and a mechanistic contributor to disease development. Understanding the interplay between immune signaling molecules and genetic vulnerability may facilitate the identification of biologically defined ASD subtypes and open avenues for targeted immunomodulatory interventions

INTRODUCTION

Autism Spectrum Disorder (ASD) represents a group of complex neurodevelopmental disorders marked by deficits in social communication, repetitive behaviors, and variable cognitive outcomes. Historical accounts trace the term "autism" to Eugen Bleuler (1911), with further clinical elaboration by Leo Kanner (1943) and Hans Asperger (1943), highlighting the early recognition of diverse phenotypes and the spectrum nature of the condition. Early descriptions of mutism and social withdrawal have since evolved into a nuanced understanding of ASD as a heterogeneous condition with both clinical and biological subtypes. The heterogeneity in ASD manifests not only in behavioral and cognitive domains but also at molecular and genetic levels, suggesting multiple interacting etiological mechanisms.

Emerging evidence indicates that immune system dysregulation interacts with genetic susceptibility to influence neurodevelopment in ASD. Immune-related genes, when altered, can modulate cytokine expression, humoral immunity, and inflammatory signaling pathways, thereby impacting brain development. Several studies have reported abnormal cytokine profiles, including elevated levels of Interleukin-6 (IL-6), Tumor Necrosis Factor-α (TNF-α), Interferon-γ (IFN-γ), and altered regulatory cytokines such as IL-27 and IL-37, in children with ASD. Dysregulation in immunoglobulins (IgG, IgM, IgA) has also been documented, suggesting that adaptive immune pathways are implicated in ASD pathophysiology. These findings support the concept of an immunogenetic framework, wherein genetic predisposition interacts with immune system alterations to influence the severity and presentation of ASD.

LITERATUTE RIVIEW

The clinical variability observed in ASD, ranging from severe verbal and social deficits to milder cognitive or behavioral impairments, underscores the need for precise biological characterization. Immunogenetic studies, combining cytokine profiling with gene analysis, can help delineate subtypes of ASD, identify potential biomarkers, and inform personalized intervention strategies. Such approaches also facilitate the understanding of neuroinflammatory mechanisms, gene-environment interactions, and immune-mediated contributions to neurodevelopment.

The integration of immunological and genetic assessments has become increasingly feasible with advancements in molecular and immunological techniques. High-sensitivity multiplex immunoassays allow for simultaneous quantification of multiple cytokines, providing comprehensive insight into systemic immune activity. Genetic studies targeting immune-related genes enable identification of variants that may confer susceptibility to immune dysregulation in ASD. Together, these tools offer a powerful framework to explore the mechanistic links between immunity, genetics, and ASD phenotypes.

Importance of the Study

This study addresses a critical gap in local research by investigating the immunogenetic and cytokine profiles of children with ASD in Baghdad. Understanding how immune dysregulation interacts with genetic predisposition may reveal specific disease mechanisms and potential biomarkers, thereby

advancing clinical management and therapeutic development for the local population.

Objectives

The study aims to:

- Quantify cytokine levels (IL-6, IL-27, IL-37, IL-29, TNF-α, IFN-γ) and immunoglobulin profiles (IgG, IgM, IgA).
- Explore potential links between cytokine alterations and genetic susceptibility in ASD.
- Assess biochemical and systemic markers contributing to immune-mediated neurodevelopmental changes.

By integrating immunological, biochemical, and genetic perspectives, the study seeks to clarify the complex immunogenetic landscape of ASD and provide a foundation for future personalized interventions targeting immune and genetic pathways.

METHODOLOGY

Participant Information and Data Forms

Parents or caregivers completed detailed sociodemographic questionnaires, which included child's age, gender, date of birth, familial background, education levels of parents, number of siblings, and monthly income. Psychiatric evaluations were performed by the research team, incorporating clinical observations and patient history from medical records. The Childhood Autism Rating Scale (CARS) was administered to quantify autism severity, ensuring accurate classification of participants for immunogenetic and cytokine analysis.

Autism Severity Assessment

The CARS tool was employed to evaluate the severity of autism in participating children. This scale consists of 15 items addressing behavioral, social, emotional, and sensory domains. Scores of 30–36.5 indicated mild-to-moderate ASD, while 37–60 indicated severe ASD. This stratification facilitated analysis of potential correlations between immune-cytokine profiles and autism severity.

Blood Collection and Immune Cell Isolation

Peripheral blood samples were drawn under sterile conditions from participants who fulfilled inclusion criteria. PBMCs were separated via Ficoll density gradient centrifugation for immunophenotyping, cytokine measurement, and NK cell activity assessment. Absolute lymphocyte counts and subset analysis were calculated for precise evaluation of immune cell populations.

Cytokine Profiling and Immunogenetic Considerations

PBMCs were stimulated in vitro with PMA/I to induce cytokine expression, followed by intracellular staining and flow cytometric analysis (FACSCalibur, Becton Dickinson, San Jose, CA). Cytokines measured included IL-6, IL-27, IL-37, IL-29, TNF-α, and IFN-γ. These measurements were interpreted in the context of potential genetic susceptibility, highlighting immunogenetic contributions to ASD phenotypes.

Serum Analysis of Cytokines and Biochemical Markers

Serum cytokine concentrations were quantified using ELISA kits (Quantikine, R&D Systems), employing a sandwich immunoenzymatic method. Humoral immunity markers (IgG, IgM, IgA) and biochemical indicators (SGOT, SGPT, serum creatinine, glutathione) were assessed to provide a comprehensive understanding of systemic immune function and metabolic status.

This materials framework allowed a detailed examination of immunogenetic influences and cytokine dysregulation in children with ASD, supporting robust clinical and mechanistic analyses while accounting for biochemical and environmental factors.

Participant Recruitment

The study included a total of 100 children diagnosed with ASD (ages 1–12 years) from Baghdad Medical College's Child Psychiatry Department. A control group of 30 age-matched children without developmental or psychiatric disorders was recruited. All participation was voluntary, and the control group was carefully matched for socio-economic and cultural background to minimize confounding variables.

Inclusion Criteria

Children included in the case group met the following criteria:

- Age between 1–12 years
- Clinical diagnosis of Autistic Disorder, Atypical Autism (CTA-PDD), or Asperger's Disorder according to DSM-IV
- Parental agreement to participate

Exclusion Criteria

Children were excluded if they had:

- Chronic or systemic illnesses
- Acute or chronic inflammatory conditions
- Epilepsy or other neurological disorder
- Congenital metabolic diseases
- Recent psychotropic medication usage (past 2 months)
- Medications affecting the immune system, including corticosteroids and beta-blockers

Classification of Patients

The ASD group was subdivided into three age categories:

1. Under 5 years
2. 5–10 years
3. Over 10 years

This stratification allowed for age-specific immunogenetic analysis and evaluation of cytokine profiles across developmental stages.

Control Group

The control group underwent psychiatric evaluation and CARS scoring to confirm absence of ASD or other developmental delays. Blood samples were collected under sterile conditions for immunogenetic, cytokine, and biochemical analyses.

Immunoglobulin and Biochemical Assessment

IgG, IgM, and IgA were measured using radial immunodiffusion, and IgE using chemiluminescence methods, with age-adjusted reference ranges applied. Biochemical markers, including liver enzymes (SGOT, SGPT), glutathione (GSH), and serum creatinine (SCR), were evaluated to determine systemic biochemical status and oxidative stress levels.

Statistical Analysis

Data were analyzed using STATA v10. Descriptive statistics included counts and percentages for categorical data, and mean, standard deviation, median, minimum, and maximum for continuous data. Parametric comparisons were conducted using the Student t-test, while non-parametric data were analyzed using the Mann-Whitney U test. Correlations were assessed via Pearson (parametric) or Spearman (non-parametric) coefficients. Statistical significance was set at $p \leq 0.05$.

Ethics Approval

The study received formal ethics approval from Baghdad Medical University's Ethics Committee. Parents or guardians provided written informed consent after reviewing detailed study information. Confidentiality and voluntary participation were maintained rigorously.

RESULT AND DISCUSSION

Participants: 100 children with ASD (1–12 years) and 30 healthy controls (same age range). Age distribution similar:

- Group 1 (<5 years): 11 ASD children
- Group 2 (5–10 years): 22 ASD children
- Group 3 (>10 years): 67 ASD children

Gender: ASD group 84% male, 16% female; control group 60% male, 40% female; no significant difference (p=0.892).

Cytokine and Immune Findings:

- Intracellular cytokines (IFN-γ, TNF-α, IL-6, IL-27, IL-29, IL-37) significantly different between groups
- NK cytotoxicity significantly reduced in ASD
- ELISA results confirmed elevated TNF-α and IL-37 in ASD children
- IL-29 not significantly different (p=0.572)

Correlations with CARS-TF:

- IL-6: strong positive correlation with severity
- IFN-γ: moderate negative correlation

NK cytotoxicity: strong negative correlation

Regression analysis:

No significant effect on intracellular cytokine levels or NK cytotoxicity. Immunoglobulin findings:

- IgG and IgM significantly lower in ASD
- IgA and IgE not significantly different
- Suggests selective humoral immune deficiency in ASD

Study of Immunogenetic and Cytokine Profiles

This study investigated immunogenetic and cytokine profile alterations in children with Autism Spectrum Disorder (ASD) compared to healthy controls. A total of 100 children aged 1–12 years with ASD and 30 healthy children were included. The evaluation focused on immune cell markers, intracellular cytokine levels, NK cell cytotoxic activity, and genetic factors associated with immune responses. The study was analyzed in three aspects: (1) comparison of immune and genetic data between ASD and control groups, (2) association between autistic symptom severity (CARS score) and immunogenetic parameters in the ASD group, and (3) assessment of immune and cytokine profiles in children with ASD who developed autistic regression versus those who did not.

The ASD and control groups demonstrated significant differences in IL-6, IL-27, IL-29, IL-37, CD4+, IFN-γ+ CD4+, and NK cytotoxicity. CD markers, as part of the immune recognition system, serve as identifiers of cell types and their functional state. CD4+, the alpha chain of the IL-6 receptor, was significantly lower in ASD children ($p=0.023$). Previous research has reported reduced T and B lymphocyte counts and impaired lymphocyte responses in ASD (Stubbs, 1977; Plioplys, 1994).

NK Cell Cytotoxic Activity: Although NK cell counts were similar between ASD and control groups, NK cytotoxic activity (NKCC) was significantly reduced in ASD ($p<0.001$). These results align with prior findings (Warren et al., 1987; Vojdani et al., 2008; Enstrom et al., 2009), suggesting that functional NK impairment occurs without a decrease in cell number. Reduced NKCC may predispose ASD children to viral infections. Chronic stress also reduces NKCC activity (Esterling, 1996).

Cytokine Profiles: IL-6+ CD4+ levels were elevated in ASD ($p<0.001$), while IFN-γ+ CD4+ levels were decreased ($p=0.008$), indicating a Th2-biased immune response. Gupta et al. reported similar findings with decreased Th1 and IFN-γ+ cytotoxic cells and increased Th2 and IL-4+ cytotoxic cells in ASD. No significant differences were found in IL-27+ or IL-29+ CD4+ levels ($p=0.800$), consistent with previous studies. IL-6 elevation promotes Th2 differentiation and inhibits IFN-γ-mediated activation. Napolioni et al. (2013) found significantly higher IL-6 in nonverbal ASD children compared to healthy siblings.

TNF-α and IFN-γ levels varied across studies. Saresella et al. (2009) reported increased TNF-α and IFN-γ-producing CD4+ and CD8+ T cells in ASD compared to siblings and controls. Singh et al. (1996) found elevated plasma IL-12 and IFN-γ in ASD. Ashwood et al. (2011) observed increased GM-CSF, TNF-α, and IL-13 production after PHA stimulation, with IL-8 elevated in unstimulated cultures. Th2 cytokines (IL-4, IL-10, IL-13) inhibit macrophage activation and suppress Th1-mediated immunity. Overall, data indicate a Th2-skewed immune response in ASD.

Few studies examined IL-29 as a diagnostic or prognostic marker. Some trials reported higher IL-29 in controls than in patients, though not statistically significant (Erturk, 2015). In this study, ASD patients without regression had higher IL-29 levels than those with regression ($p=0.01$), while baseline IL-29 did not differ significantly from controls ($p=0.622$). These results suggest IL-29 may have immunoregulatory and anti-proliferative roles.

Serum TNF-α ranged from 14.60 to 140.62 pg/mL (mean 36.94), and IL-6 ranged from 4.35 to 9.61 pg/mL (mean 6.61 ± 1.28). Elevated IL-6 may promote acute-phase protein production (Maggio, 2006). IL-27 regulates Th1, Th17, and innate immune responses and may be therapeutic in autoimmune conditions by inducing regulatory T cells that secrete IL-10 (Villarino, 2004; Sun, 2015; Liu, 2014). IL-37 has emerged as a key anti-inflammatory cytokine, with elevated levels in various autoimmune, inflammatory, and cardiovascular disorders and significant correlations with CRP and ESR, supporting its potential as a biomarker (Li, 2014; Lupia, 2014).

This study provides the first comprehensive assessment of IL-37 in serum in Iraq, based on 30 healthy subjects.

CONCLUSION AND RECOMMENDATION

Early immunogenetic and cytokine alterations may influence brain development and contribute to ASD pathophysiology. Significant differences between patients and controls were found in IL-6, IL-27, IL-29, IL-37, TNF-α, IFN-γ, CD4+, and NK cytotoxicity. Correlations between these parameters and CARS scores suggest immune dysregulation may impair neurodevelopment. It remains unclear whether these alterations are causal or secondary to chronic stress associated with ASD. Further research is needed to clarify the role of immune dysfunction in autism and explore potential therapeutic targets.

Limitations and Advantages

Sociodemographic factors were similar between groups. Participants with medication use, epilepsy, metabolic disorders, infections, or chronic diseases were excluded to reduce confounding. Despite this, the immune system's complexity and susceptibility to multiple interactions limit interpretation. The relatively small sample size reduces statistical power, but larger studies may provide more definitive results.

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