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Can the Proinflammatory Cytokines IL-6 and TNF-α Act as Biomarkers of Autism Spectrum Disorder? A Literature Review

¿Pueden las citoquinas proinflamatorias IL-6 y TNF-α actuar como biomarcadores del trastorno del espectro autista? Una revisión de la literatura

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SUMMARY

Background: *WHO reports that out of 160 children, one of them has autism spectrum disorder (ASD). Experts estimate that the cause of autism could be heavy metals. This review aims to assess the progress of research on the potential use of IL-6 and TNF-α cytokines as ASD biomarkers.* **Methods:** *This review uses sources from the PubMed and ScienceDirect databases. The keywords used were IL-6 AND TNF-α AND (Autism OR Autism Spectrum Disorder OR ASD). At the research identification stage, 63 studies were obtained, and after the screening, 12 studies were found that met the requirements for a review.* **Results:** *Some of the included studies showed differences in the expression levels of IL-6 and TNF-α in ASD children and controls, although not all. Proinflammatory cytokines such as IL-6 and TNF-α have been demonstrated to increase in ASD children's brains, although the mechanism is not yet understood.* **Conclusions:** *Despite inconsistent findings, evidence suggests that children with ASD show increased plasma expression levels of pro-inflammatory cytokines, including IL-6 and TNF-α. More in-depth and integrated research is needed to prove that IL-6 and TNF-α have the potential to be biomarkers of ASD.*

Keywords: *IL-6, TNF-α, autism spectrum disorder.*

RESUMEN

Antecedentes: *La OMS informa que, de 160 niños, uno de ellos presenta Trastornos del Espectro Autista (TEA). Los expertos estiman que la causa del autismo podrían ser los metales pesados. Esta revisión tiene como objetivo evaluar el progreso de la investigación sobre el uso potencial de las citocinas IL-6 y TNF-α como biomarcadores de TEA.* **Métodos:** *Esta revisión utiliza fuentes de las bases de datos PubMed y ScienceDirect. Las palabras clave utilizadas fueron IL-6 AND TNF-α AND (Autism OR Autism Spectrum Disorder OR ASD). En la etapa de identificación de la investigación se obtuvieron 63 estudios, luego del cribado se encontraron 12 estudios que cumplieron con los requisitos para su revisión.* **Resultados:** *Algunos de los estudios incluidos mostraron diferencias en*

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los niveles de expresión de IL-6 y TNF-α en niños con TEA y controles, aunque varios otros no. Se ha demostrado que las citocinas proinflamatorias como la IL-6 y el TNF-α aumentan en el cerebro de los niños con TEA, aunque aún no se comprende el mecanismo. **Conclusiones:** *A pesar de los hallazgos inconsistentes, alguna evidencia sugiere que en los niños con TEA ocurre un aumento de los niveles de expresión plasmática de citoquinas proinflamatorias, incluidas IL-6 y TNF-α. Se necesita una investigación más profunda e integrada para demostrar que la IL-6 y el TNF-α tienen el potencial de ser biomarcadores del TEA.*

Palabras clave: *IL-6, TNF-α, trastorno del espectro autista.*

INTRODUCTION

Autism Spectrum Disorder (ASD) is a pervasive developmental disorder characterized by persistent deficits in social communication and social interaction and repetitive and restricted patterns of behavior, interests, or activities (1). World Health Organization (WHO) reports the estimated global average prevalence of ASD is 62/10 000, or one in 160 children suffer from ASD (2).

The etiology of ASD is thought to involve an interaction between genetic (3,4), environmental (5), and immune (5,6) risk factors in the prenatal, perinatal, and postnatal periods. Changes in the immune system, such as cytokines, play a role in the incidence of ASD (7).

Cytokines function as messengers (8), linking innate and adaptive immune system cells. The immune system responds to infection, antigens, and associated immune challenges and involves many biological processes. Cytokines stimulate and activate the immune system and induce its synthesis and the synthesis of other cytokines. Cytokines can be grouped according to the type of response, namely proinflammatory and anti-inflammatory signaling and adaptive immunity (9).

Several cytokines showed an increase in the brains of ASD children, such as interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-α), transforming growth factor-β1 (TGF-β1) and macrophage chemo-attractant protein-1 (MCP-1). Additionally, the cerebrospinal fluid of autistic children has been shown to have significantly higher levels of MCP-1, IL-8, and other pro-inflammatory molecules (10,11). These findings suggest that the immune system and cytokines play an important role in the pathogenesis of autism.

Changes in cytokine expression levels can be used as biomarkers to identify ASD (7). Several cytokines are relevant for proinflammatory biomarkers, such as IL-6 and TNF-α. TNF-α is a central regulator of inflammation and is elevated in the cerebrospinal fluid of children with ASD (12). Evidence is now available to accept the concept that the brain recognizes cytokines, among them IL-6 as molecular signals of sickness. Interleukin 6 (IL-6), promptly and transiently produced in response to infections and tissue injuries, contributes to host defense by stimulating acute phase responses, hematopoiesis, and immune reactions. Although its expression is strictly controlled by transcriptional and posttranscriptional mechanisms, dysregulated continual synthesis of IL-6 plays a pathological effect on chronic inflammation and autoimmunity. In the nervous system, the classical proinflammatory cytokine interleukin-6 (IL-6) plays essential roles in the development, differentiation, regeneration, and degeneration of neurons but acts as a molecule with both beneficial and destructive potentials (13).

This literature review aims to assess the potential use of IL-6 and TNF-α cytokines as ASD biomarkers.

METHOD

This study included the literature related to the expression of IL-6 and TNF-α in children with ASD. Articles were then selected with pre-determined inclusion and exclusion criteria. Articles on IL-6 and TNF-α expression differences in children with ASD were sourced from the PubMed and ScienceDirect databases. The keywords used were IL-6 AND TNF-α AND (Autism OR Autism Spectrum Disorder OR ASD). At the research identification stage, 63 studies were obtained. The criteria for inclusion

of research in this review were published from 2017 to the present. After screening, 12 studies were found that met the requirements for review.

RESULTS

A review of 12 selected studies showed differences in IL-6 and TNF-α expression levels in ASD (Table 1).

Interleukin-6

Soon after its discovery, it was demonstrated that some astrocytoma and glioma lines expressed the classical proinflammatory cytokine IL-6 when stimulated with IL-1β, which prompted speculation that IL-6 could have a role in the central nervous system as molecular signals of sickness, where IL-6 play an essential role in the development, differentiation, regeneration, and degeneration of neurons (13). IL-6 is an important driver of acute and chronic inflammation and has been reported to act as a T-cell survival factor (26).

Table 1. Research on IL-6 and TNF-α expression levels in ASD

Biomarkers	Author	NcaselNcontrol Age	Conclusion
IL-6 and TNF-α	Hughes et al., 2022 (14).	25|20 5-6 y	There is a difference in the level of IL-6 expression after lipopolysaccharide stimulation
IL-6 and TNF-α	Ahmad et al., 2019 (15).	53|28 3-12 y	Increased expression of TNF-α and IL-6 in children with ASD
IL-6 and TNF-α	Kutuk et al., 2020 (16).	195|162 ± 7 y	IL-6 levels were higher in children with ASD than controls, while TNF- was no difference.
IL-6 and TNF-α	Guloksuz et al., 2017 (17)	75|35 3-7 y	The pro-inflammatory cytokine TNF-α is increased
IL-6 and TNF-α	Shen et al., 2019 (18)	30|30 2-6 y	There are differences in the inflammatory response of peripheral blood mononuclear cells (PBMC), especially TNF-α and IL-6, in autistic children compared to healthy controls
IL-6 and TNF-α	Cao X et al., 2021 (19)	45|41 2-19 y	There is a potential mechanistic relationship between dysbiotic levels of specific butyrate-producing gut microbiota and overexpression of the cytokines TNF-α and IL-6 in children with autism
IL-6	Ning J et al., (2018) (20)	102|41 2-8 y	IL-6 levels in the combination of macrophage migration inhibitory factor (MIF) in children with autism are higher than in healthy controls
IL-6 and TNF-α	Ferencova et al., 2023(21)	20|20 ± 12 y	There was an increase in IL-6 levels in ASD adolescents compared to controls, but there was no increase in TNF-α levels
TNF-α	Naglaa et al., 2020 (22)	30|35 2-10 y	TNF-α concentrations were significantly higher in children with ASD compared with controls
IL-6 and TNF-α	Nadeem et al., 2022 (23)	30|30 2-8 y	The inflammatory cytokines IL-6 and TNF-α were increased in B cells of ASD subjects, when compared with a group of normal children
IL-6 and TNF-α	Ben Othman et al., 2021(24).	24|0 4-10 y	TNF-α plasma level is high (>5pg/mL), plasma IL-6 level is relatively normal
IL-6 and TNF-α	Prosperi et al., 2019 (25).	85|0 3-6 y	The pro-inflammatory cytokines TNF-α and IL-6 are lower than reported

IL-6 has been related to the prevalence of autism spectrum disorder. In effect, Hughes et al. demonstrated an increased innate inflammatory cytokine, such as IL-6, in ASD; however, the origin of excess IL-6 in ASD has not been identified. This author explored the specific responses of circulating monocytes from autistic children, where isolated $CD14^+$ monocytes from whole blood were stimulated for 24 h under three conditions: media alone, lipoteichoic acid to activate TLR2, and lipopolysaccharide to activate TLR4, finding that after TLR4 activation, $CD14^+$ monocytes from autistic children produce increased IL-6 compared to monocytes from children with typical development. IL-6 concentration is also correlated with worsening restrictive and repetitive behaviours. These findings suggest dysfunctional activation of myeloid cells and may indicate that other cells of this lineage, including macrophages and microglia in the brain, might have a similar dysfunction (14). Likewise, Ahmad et al. assessed whether IL-16 expression is associated with immune dysfunction in children with ASD, examining IL-16 expression in $CD4^+$, $CD8^+$, $CD14^+$, $CCR3^+$, and $CXCR7^+$ cells in typically developing (TD) controls and children with ASD using flow cytometry in peripheral blood mononuclear cells, showing that IL-6 expression is increased significantly in children with ASD compared with TD control, suggesting that IL-16 expression could play an essential role in immune alteration in children with ASD (15). Furthermore, Kutuk et al. (16) determined the expression levels of several interleukins in peripheral blood mononuclear cells of children with ASD and healthy controls to determine the contributions of cytokines to ASD. The study included 195 children with ASDs (80.5 % male) and 162 controls (73.6 % male) in Türkiye, showing that most ASD children with severe autistic symptoms had higher levels of IL-6 (16). Shen et al. study on peripheral blood mononuclear cell (PBMC) protein expression in children with autism compared with healthy controls using isobaric tags for relative and absolute quantitation proteomic approaches, showed differences in IL-6 levels (18). Similar results showing overexpression of the cytokine IL-6 in children with autism compared to controls in the dysbiotic gut microbiota and dysregulation of cytokine profile study (19). In China, a study on 102 children with autism, showed that IL-6 levels in the combination of macrophage migration inhibitory factor (MIF) were higher than healthy control children (20). Likewise, Nadeem et al. research regarding the imbalance of inflammatory cytokines showed that IL-6 increased in B cells of ASD subjects when compared with a group of normal children (23).

On the contrary, Ben Othman et al. studied 24 paediatric outpatients with ASD at the Tunisian Military Hospital and showed that IL-6 blood levels were relatively normal (24). In addition, in a study by Prosperi et al. in 85 ASD preschool children, the proinflammatory cytokine IL-6 was lower than that reported in a previous study in children with systemic inflammatory conditions (25). However, these two studies did not have a control of healthy children.

Tumor Necrosis Factor-α

TNF-α is a proinflammatory cytokine produced by monocytes, macrophages, and other immune cells such as B cells and activated T cells (27). TNF-α is a central regulator of inflammation and is elevated in the cerebrospinal fluid of children with ASD (12).

Research conducted by Ahmad et al. in 53 children with ASD and 28 control children in Saudi Arabia showed an increase in TNF-α expression in children with ASD compared to controls (15). The same study in ASD children in Tunis showed high plasma levels of TNF-α (>5pg/mL) (24). The increase in the proinflammatory cytokine TNF-α was also seen in the research report of Guloksuz et al. in 75 children with autism in Istanbul, Turkiye (17). Likewise, research conducted on 35 children with autism in Egypt showed that TNF-α concentrations were significantly higher in children with ASD compared to controls (22).

A study of peripheral blood mononuclear cell (PBMC) protein expression in 30 children with autism at the Maternal and Child Health Hospital of Baoan, Shenzhen, China showed differences in levels of TNF-α (18). Similarly, Cao et al. on 45 children with autism at the Second Affiliated Hospital of Kunming Medical University, Kunming, China, showed overexpression of the

cytokine TNF-α (19), and Nadeem demonstrated that inflammatory cytokine TNF-α increased in B cells of ASD subjects (23).

TNF-α can also be used as a marker for autism in adolescents. The research findings found a significant difference in the concentration of TNF-α compared to healthy adolescent controls (19,21). However, studies showed no difference in TNF-α expression levels in children with ASD compared to controls (14,16). In a study by Prosperi et al. in 85 ASD preschool children, the proinflammatory cytokine TNF-α was lower than that reported in a previous study in children with systemic inflammatory conditions (25).

DISCUSSION

Previous studies have shown that cytokines are elevated in the blood, brain, and cerebrospinal fluid of ASD subjects (28). Proinflammatory cytokines such as IL-6 and TNF-α have been demonstrated to increase in the brains of ASD children, although the mechanism is not yet understood. The ASD child shows symptoms of autism such as impaired communication and social interaction and restricted and repetitive behavior.

Proinflammatory cytokine IL-6 is a component of the first immune response. It causes an inflammatory reaction that triggers subsequent adaptive immunological reactions, such as the proliferation and activation of T cells (26).

TNF-α is a proinflammatory cytokine produced by monocytes, macrophages, and other immune cells such as B cells and activated T-cells (27). TNF-α is a central regulator of inflammation and is elevated in the cerebrospinal fluid of children with ASD (12).

Antigens that enter the body will carry out an immune response, and macrophages carry out phagocytosis of foreign antigens. Foreign antigens present in macrophage cells will immediately be recognized by the major histocompatibility complex (MHC) gene (29). Interleukin-2 activates Th-cells (T helper), which are then differentiated into Th1-cells and Th2-cells (5). Th2 cells will stimulate B cells by secreting IL-6 cytokines. B cells then produce plasma cells and memory cells. Plasma cells produce immunoglobulin A (Ig-A) antibodies. Ig-A is a glycoprotein molecule attached to the α chain. Ig-A then binds to the antigen (7,30,31).

Immune responses that do not function appropriately against antigens, such as a decrease in IFN-γ, IL-6, TNF-α, and Ig-A, will cause neurodevelopmental disorders through their effects on epigenetic modifications in the brain (32).

A postmortem study conducted on ASD found two brain abnormalities in the limbic system, namely the amygdala and hippocampus, which regulate emotions, aggressiveness, sensory input, and learning processes (33). The level of the disorder is then manifested in disturbances in the development of communication, social interaction, and observable behavior (3,34).

The general evidence suggests increased plasma levels of pro-inflammatory cytokine expression, including IL-6 and TNF-α. The inconsistency found may be due to phenotypic and neurobiological heterogeneity in children with ASD (17).

CONCLUSION

Research examining the relationship between the expression levels of IL-6 and TNF-α and ASD includes a variety of complex factors. This literature review examines the potential use of IL-6 and TNF-cytokines as ASD biomarkers. The authors browse the literature related to this, and although they try to find a study free from bias, they cannot avoid it altogether.

Although the findings are inconsistent, several research results show increased expression levels of pro-inflammatory cytokines. More in-depth and integrated research is needed to prove that IL-6 and TNF-α are potential biomarkers of ASD and to determine the diagnosis of ASD, no longer just based on clinical symptoms.

REFERENCES

1. American Psychiatric Association A. DSM-5-TR: Diagnostic and statistical manual of mental disorders. Fifth Edit. Textbook of Psychiatry for Intellectual Disability and Autism Spectrum Disorder. 2022:951-952.
2. WHO. Autism spectrum disorders. 2021. Available from: https://www.who.int/news-room/fact-sheets/detail/autism-spectrum-disorders
3. Taylor MJ, Rosenqvist MA, Larsson H, Gillberg C, D'Onofrio BM, Lichtenstein P, et al. Etiology of autism spectrum disorders and autistic traits over time. JAMA Psychiatry. 2020;77(9):936-943.
4. Johnson CR, Brown K, Hyman SL, Brooks MM, Aponte C, Levato L, et al. Parent training for feeding problems in children with autism spectrum disorder: Initial randomized trial. J Pediatr Psychol. 2019;44(2):164-175.
5. Hughes HK, Mills Ko E, Rose D, Ashwood P. Immune dysfunction and autoimmunity as pathological mechanisms in autism spectrum disorders. Front Cell Neurosci. 2018;12:1-26.
6. Ravaccia D, Ghafourian T. Critical role of the maternal immune system in the pathogenesis of autism spectrum disorder. Biomedicines. 2020;8:1-21.
7. Masi A, Glozier N, Dale R, Guastella AJ. The Immune System, Cytokines, and Biomarkers in Autism Spectrum Disorder. Neuroscience Bull. 2017;33:194204.
8. Goines PE, Ashwood P. Cytokine dysregulation in autism spectrum disorders (ASD): Possible role of the environment. Neurotoxicol Teratol. 2013;36: 67-81.
9. Masi A, Quintana DS, Glozier N, Lloyd AR, Hickie IB, Guastella AJ. Cytokine aberrations in autism spectrum disorder: A systematic review and meta-analysis. Mol Psychiatry. 2015;20(4):440-446.
10. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol. 2005;57(1):67-81.
11. Capano L, Dupuis A, Brian J, Mankad D, Genore L, Hastie Adams R, et al. A pilot dose finding study of pioglitazone in autistic children. Mol Autism. 2018;9(1):1-14.
12. Chez MG, Dowling T, Patel PB, Khanna P, Kominsky M. Elevation of Tumor Necrosis Factor-Alpha in Cerebrospinal Fluid of Autistic Children. Pediatr Neurol. 2007;36(6):361-365.
13. Dantzer R. Cytokine, Sickness Behavior, and Depression. Immunol Allergy Clin North Am. 2009;29:247-264.
14. Hughes HK, Onore CE, Careaga M, Rogers SJ, Ashwood P. Increased Monocyte Production of IL-6 after Toll-like Receptor Activation in Children with Autism Spectrum Disorder (ASD) Is Associated with Repetitive and Restricted Behaviors. Brain Sci. 2022;12(2):1-13.
15. Ahmad SF, Ansari MA, Nadeem A, Bakheet SA, AL-Ayadhi LY, Attia SM. Elevated IL-16 expression is associated with development of immune dysfunction in children with autism. Psychopharmacology (Berl). 2019;236(2):831-838.
16. Kutuk MO, Tufan E, Gokcen C, Kilicaslan F, Karadag M, Mutluer T, et al. Cytokine expression profiles in Autism spectrum disorder: A multi-center study from Turkey. Cytokine. 2020;133:1-8.
17. Guloksuz SA, Abali O, Cetin EA, Gazioglu SB, Deniz G, Yildirim A, et al. Elevated plasma concentrations of S100 calcium-binding protein B and tumor necrosis factor-alpha in children with autism spectrum disorders. Rev Bras Psiquiatr. 2017;39(3):195-200.
18. Shen L, Feng C, Zhang K, Chen Y, Gao Y, Ke J, et al. Proteomics study of peripheral blood mononuclear cells (PBMCs) in autistic children. Front Cell Neurosci. 2019;13:1-16.
19. Cao X, Liu K, Liu J, Liu YW, Xu L, Wang H, et al. Dysbiotic Gut Microbiota and Dysregulation of Cytokine Profile in Children and Teens With Autism Spectrum Disorder. Front Neurosci. 2021;15:1-14.
20. Ning J, Xu L, Shen CQ, Zhang YY, Zhao Q. Increased serum levels of macrophage migration inhibitory factor in autism spectrum disorders. Neurotoxicology. 2019;71:7-5.
21. Ferencova N, Visnovcova Z, Ondrejka I, Hrtanek I, Bujnakova I, Kovacova V, et al. Peripheral Inflammatory Markers in Autism Spectrum Disorder and Attention Deficit/Hyperactivity Disorder at Adolescent Age. Int J Mol Sci. 2023;24(14):1-27.
22. Abd-Allah NA, Ibrahim OM, Elmalt HA, Shehata MA, Hamed RA, Elsaadouni NM, et al. Thioredoxin level and inflammatory markers in children with autism spectrum disorders. Middle East Curr Psychiatry. 2020;27(1):1-7.
23. Nadeem A, Ahmad SF, Al-Harbi NO, AL-Ayadhi LY, Sarawi W, Attia SM, et al. Imbalance in pro-inflammatory and anti-inflammatory cytokines milieu in B cells of children with autism. Mol Immunol. 2022;141.
24. Ben Othman A, Slama H, Cherif E, Azaiez M, Gharsallah H. Inflammatory cytokines dysfunction in autism spectrum disorder. Eur Psychiatry. 2021;64(S1).

25. Prosperi M, Guiducci L, Peroni DG, Narducci C, Gaggini M, Calderoni S, et al. Inflammatory biomarkers are correlated with some forms of regressive autism spectrum disorder. Brain Sci. 2019;9(12):1-14.
26. Li B, Jones LL, Geiger TL. IL-6 Promotes T-cell Proliferation and Expansion under Inflammatory Conditions in Association with Low-Level RORγt Expression. J Immunol. 2018;201(10):2934-2946.
27. Jung MK, Lee JS, Kwak JE, Shin EC. Tumor necrosis factor and regulatory T cells. Yonsei Medical Journal. Yonsei University College of Medicine. 2019;60:126-131.
28. Wei H, Chadman KK, McCloskey DP, Sheikh AM, Malik M, Brown WT, et al. Brain IL-6 elevation causes neuronal circuitry imbalances and mediates autism-like behaviors. Biochim Biophys Acta - Mol Basis Dis. 2012;1822(6):831-842.
29. Nabgha-e-Amen, Eqani SAMAS, Khuram F, Alamdar A, Tahir A, Shah STA, et al. Environmental exposure pathway analysis of trace elements and autism risk in Pakistani children population. Sci Total Environ. 2020;712.
30. Eftekharian MM, Ghafouri-Fard S, Noroozi R, Omrani MD, Arsang-jang S, Ganji M, et al. Cytokine profile in autistic patients. Cytokine. 2018;108:120-126.
31. Gong W, Qiao Y, Li B, Zheng X, Xu R, Wang M, et al. The Alteration of Salivary Immunoglobulin A in Autism Spectrum Disorders. Front Psychiatry. 2021;12.
32. Loke YJ, Hannan AJ, Craig JM. The Role of Epigenetic Change in Autism Spectrum Disorders. Front Neurol. 2015;26;6:107.
33. Lord C, Elsabbagh M, Baird G, Veenstra-Vanderweele J. Autism spectrum disorder. Lancet. 2018;392(10146):508-520.
34. Nardone S, Elliott E. The interaction between the immune system and epigenetics in the etiology of autism spectrum disorders. Frontiers in Neuroscience. 2016;20:1-9.