

## Pathological Correlation of Inflammatory Cytokines with Neurocognitive Impairment in Pediatric Epilepsy: A Clinical Cross-Sectional Study

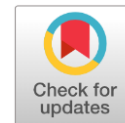
Zahra Anwar <sup>1\*</sup>, Aqsa Latif <sup>2</sup>, Saba Masud <sup>3</sup>

1- Consultant Paediatrician, THQ Hospital, Sangla Hill, Pakistan

2- Women Medical Officer, THQ Hospital, Sangla Hill, Pakistan

3- ST4 HST Emergency Medicine Trainee, Calderdale Royal Hospital, CHFT NHS Trust, Halifax, United Kingdom

\*Corresponding Author: Zahra Anwar Email: [zahra.anwar@gmail.com](mailto:zahra.anwar@gmail.com) Cell: +92 3362526669



### ABSTRACT

**Background:** Pediatric epilepsy is a common neurological condition characterized not only by recurrent seizures but also by notable disruptions in cognitive development during key stages of brain maturation. Recent studies have increasingly implicated neuroinflammatory processes particularly those involving pro-inflammatory cytokines as major contributors to both seizure activity and cognitive deterioration. Despite this, limited clinical evidence exists regarding the relationship between systemic inflammation and cognitive outcomes in epileptic children.

**Objective:** This study aimed to investigate the association between serum levels of key inflammatory cytokines (IL-6, TNF- $\alpha$ , and IL-1 $\beta$ ) and cognitive performance in children diagnosed with epilepsy.

**Methods:** Between January 2022 and March 2023, 100 pediatric epilepsy patients, ages 5 to 16, were selected from two tertiary healthcare institutions in Pakistan for a cross-sectional analysis. The Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV) was used to assess cognitive ability. TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 serum concentrations were measured by the enzyme-linked immunosorbent assay (ELISA). Multivariate linear regression and Pearson's correlation were used in the statistical analysis to find correlations between cytokine levels and cognitive scores.

**Results:** Serum levels of IL-6 ( $6.2 \pm 1.1$  pg/mL), TNF- $\alpha$  ( $7.9 \pm 1.5$  pg/mL), and IL-1 $\beta$  ( $5.0 \pm 1.3$  pg/mL) were substantially greater in participants with impaired cognitive function (Full Scale IQ < 85) than in those with normal cognitive function ( $p < 0.001$ ). IL-6 and Full-Scale IQ showed a considerable negative connection ( $r = -0.65$ ,  $p < 0.001$ ), with the Processing Speed Index being most affected. While IL-1 $\beta$  was particularly associated with working memory problems, multivariate regression revealed that IL-6 and TNF- $\alpha$  were independent predictors of worse cognitive scores.

**Conclusion:** Systemic inflammation, marked by elevated cytokine levels, is significantly associated with cognitive impairment in pediatric epilepsy, independent of seizure frequency or antiepileptic medication. These findings underscore the importance of inflammatory biomarkers in predicting neurocognitive outcomes and suggest potential benefits of integrating cytokine assessment into routine clinical evaluation to identify high-risk patients and explore anti-inflammatory treatment strategies.

**Keywords:** Pediatric epilepsy, cognitive impairment, cytokines, IL-6, TNF- $\alpha$ , IL-1 $\beta$ , neuroinflammation, WISC-IV



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third-party material in this article are included in the article's Creative Commons licence unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you must obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Received: 11/06/2025

Revised: 10/06/2025

Accepted: 11/06/2025

Published: 12/06/2025

## INTRODUCTION

Epilepsy is a chronic neurological condition marked by recurrent, unprovoked seizures resulting from abnormal electrical discharges in the brain. It is one of the most common pediatric neurological disorders, affecting a significant proportion of children globally. The burden of epilepsy in the pediatric population extends beyond the immediate clinical manifestations of seizures, encompassing a wide array of neurodevelopmental, behavioural, and cognitive complications [1]. Among these, neurocognitive impairment remains one of the most impactful and persistent challenges faced by affected children and their caregivers. The developing brain is particularly susceptible to disruptions caused by recurrent seizures, leading to impairments in intellectual function, memory, attention, executive processing, and learning capabilities. These deficits contribute to poor academic performance, reduced social integration, and diminished quality of life [2].

Multiple factors have been implicated in the cognitive deterioration observed in pediatric epilepsy. These include the age of seizure onset, seizure frequency and duration, seizure type, underlying etiology, and the use of antiepileptic drugs (AEDs). Early-onset epilepsy tends to have a more deleterious effect on neurocognitive outcomes due to interference with critical periods of brain maturation and synaptic organization [3]. Frequent or prolonged seizures further exacerbate neuronal injury and functional reorganization, thereby impairing higher-order cognitive processes. Moreover, while AEDs play a pivotal role in seizure control, some medications have been associated with adverse neurocognitive side effects, particularly when used long-term or in polytherapy. However, even in cases with optimal seizure control and careful pharmacologic management, many children continue to exhibit cognitive impairments, suggesting the presence of additional underlying mechanisms [4].

New scientific findings in the field have brought to focus the neuroinflammatory interplay and the key role it plays in not only the pathogenesis of epilepsy but also of the cognitive comorbidities that accompany epilepsy. Central nervous system (CNS) inflammation is no longer viewed as a merely a secondary effect of seizures but as a possible cause of epileptogenesis and dysfunction of neurons [5]. A set of proteins referred to as cytokines are some of the key mediators of this neuroinflammatory reaction. These soluble signalling molecules are small, soluble and they are produced by activated glial cells and infiltrating immune cells in response to a number of stimuli such as seizures, infections and trauma and they include interleukin-1 beta (IL-1  $\beta$ ), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-  $\alpha$ ). Their action produces long-lasting effects on neuronal excitability, synaptic transmission and plasticity, and their persistent increase can lead to a long-lasting shift in brain functioning [6].

IL-1 is the pro-inflammatory cytokine which has been demonstrated to disrupt hippocampal long-term potentiation (LTP) a cellular correlate of learning and memory and to increase neuronal excitability by modulating glutamatergic transmission. There is an increased amount of IL-1 in cerebrospinal fluid and serum of individuals with epilepsy and especially with pharmacoresistant seizures [5]. IL-6, another key cytokine, exhibits both pro- and anti-inflammatory properties depending on the signaling pathways involved, but its sustained elevation in epileptic tissue is commonly associated with increased seizure frequency and cognitive decline. TNF- $\alpha$  plays a critical role in modulating synaptic strength by influencing the expression of AMPA and GABA receptors and can contribute to neurotoxicity, BBB breakdown, and glial activation.

These cytokines, either individually or synergistically, are believed to disrupt the structural and functional integrity of neural networks essential for cognitive processing [6].

In pediatric patients, whose brains are in active stages of development, the consequences of sustained neuroinflammation can be particularly profound. Prolonged exposure to inflammatory mediators during sensitive periods of neurodevelopment may interfere with synaptogenesis, myelination, and cortical connectivity, thereby compromising cognitive trajectories [7]. Furthermore, children often present with coexisting conditions such as febrile seizures, systemic infections, or autoimmune disorders, which may further exacerbate the inflammatory burden and increase the risk of cognitive impairment. Studies in adult populations and animal models have consistently demonstrated the link between elevated cytokine levels and poor cognitive performance. However, evidence in children with epilepsy remains scarce, particularly in clinical settings within resource-limited regions [8].

The scarcity of research examining the pathological correlation between systemic inflammatory cytokines and cognitive function in pediatric epilepsy represents a critical gap in understanding the full spectrum of epilepsy-related morbidity [9]. Although studies have been performed on the inflammatory profile and the impact on seizure or drug resistant, there have been limited studies on the direct effect of inflammatory profile on the cognitive domains of intelligence, memory, processing speed and executive functions. Fewer still have tried to separate these associations with other clinical confounders such as seizure control, disease duration and AED regimen. Clinical studies that combine biochemical, neuropsychological, and clinical information are urgently required to achieve a comprehensive assessment of how inflammation can mediate cognitive results in children with epilepsy [12].

The purpose of this cross-sectional study is to explore the pathological correlation between the circulating levels of IL-1 beta, IL-6 and TNF-alpha and neurocognitive functioning of children with epilepsy. The research aims at explaining whether increased concentrations of these pro-inflammatory cytokines correlate with a poorer cognitive performance, and does not rely on other clinical factors like the type of seizures, their frequency, and history of seizure treatment [13]. Evaluation of these cytokines in relation to standardized test scores on cognitive functioning will be used to give an understanding of the inflammatory pathways that might be involved in cognitive deficit in epilepsy in children and the potential biomarkers that can be used in risk stratification and early intervention. The knowledge of such relationships might help in the formulation of new treatment methods that include anti-inflammatory interventions in the attempt to maintain and even improve the intellectual growth of children with epilepsy [14].

## **MATERIALS AND METHODS**

This research study was a cross-sectional analytical study, which was hospital-based clinical research. It was done to determine the pathological association between the systemic inflammatory cytokines and neurocognitive deficit among pediatric patients who have been diagnosed with epilepsy. The analysis was conducted in a 14-month period with no break between January 2023 and March 2025. The study was carried out in a tertiary care teaching hospital of Pakistan. The two hospitals have a wide and socioeconomically diverse pediatric population and are well geared to conduct clinical assessment and neuropsychological testing of children with chronic neurological diagnoses such as epilepsy.

One hundred children who had epilepsy were selected through the non-probability purposive sampling method. Children aged 5 to 16 years with a confirmed diagnosis of epilepsy, in accordance with the International League Against Epilepsy (ILAE) 2017 classification criteria, were included in the study. The sample size was determined using data from previous research that established a correlation between cytokine levels and cognitive outcomes in pediatric neurology, assuming a 95% confidence interval, 5% margin of error, and 80% statistical power. Participants were enrolled from pediatric neurology outpatient clinics and inpatient wards during routine follow-up appointments or hospital admissions for seizure management.

The inclusion criteria included both male and female children aged between 5 years and 16 years with a proven case of epilepsy of at least 6 months duration and the capability to conduct standardized neuropsychological testing with their age appropriate cognitive assessment measures. Children whose parents or other persons with legal rights to them gave informed written consent were included only. Strict exclusion criteria were also used and included children with known neurodevelopmental disorders that were not primarily related to epilepsy (e.g. autism spectrum disorder, Down syndrome), children with diagnosed psychiatric conditions, or those with a history of traumatic brain injury or children on immunosuppressive therapy including corticosteroids in the past three months. Also, patients who had recent or chronic systemic infections, autoimmune disease, or any other illness that was known to affect cytokine concentrations were excluded to provide biological uniformity and reduce possible confounders.

The Institutional Review Boards (IRBs) reviewed and approved the research protocol. Each of the parents or legal guardians of the children provided a written informed approval, and children aged more than 7 years gave a verbal approval. Confidentiality and anonymity of patients were maintained by use of distinct study identification codes. The Declaration of Helsinki and national ethical standards of research involving human subjects were all observed in all the procedures.

All the participants were also examined in detail by a pediatric neurologist that involved taking detailed history and physical examination. Data on the kind of epilepsy (generalized or focal), age of onset of the seizures, length of illness, how often the seizures occur (depending on whether the seizures are controlled (less than 1 per month) or uncontrolled (more than 4 per month)), and the number of antiepileptic drugs (AEDs) used were carefully documented. Such procedures as electroencephalography (EEG) and magnetic resonance imaging (MRI) were also reviewed where possible to evaluate the neurological substrate of epilepsy. Social-demographic factors including age, sex, level of education, nutritional status and family history of epilepsy were also recorded to allow adjustment of any possible confounders.

The Wechsler Intelligence Scale for Children - Fourth Edition (WISC-IV) a gold-standard psychometric tool that was used to assess cognitive skills in children was used to carry out the cognitive evaluation. The pediatric psychologist, who administered the test, was a trained professional in English and the native language of the subjects, and a quiet distraction-free place was used during the test. The evaluation gave scores of various cognitive areas such as Full Scale Intelligence Quotient (FSIQ), Working Memory Index (WMI), Verbal Comprehension Index (VCI), and Processing Speed Index (PSI). These scores were read with the help of age and culturally standardized norms. In this study, an IQ score of less than 85 in the Full Scale as an operational definition of cognitive impairment was used.

All the participants took part in venous blood collection (5 mL) between 8.00 a.m. and 10.00 a.m. after an overnight fast to eliminate circadian and metabolic fluctuations in cytokine production. The samples were taken in clear serum-separating tubes and taken to the lab in cold chain conditions. They were centrifuged at 3000 rpm, 10 minutes, and serum was separated, aliquoted to cryovials, and stored at -80 °C until analysis. The concentrations of the pro-inflammatory cytokines (IL-1-beta, IL-6 and TNF-alpha) were determined in serum with high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (BioLegend ®, USA). All biochemical assays were conducted in duplicate to ensure measurement consistency, and the average of the two readings was utilized for analysis. The ELISA kits used had the following analytical sensitivities: IL-1 $\beta$  at 1.2 pg/mL, IL-6 at 0.92 pg/mL, and TNF- $\alpha$  at 1.6 pg/mL. Each assay run incorporated internal controls and calibrators to validate procedural accuracy and ensure reproducibility.

Collected data were entered into a secure digital database and analyzed using IBM SPSS Statistics for Windows, Version 27. Continuous variables, including serum cytokine concentrations and cognitive test scores, were summarized as mean  $\pm$  standard deviation (SD). Categorical data, such as seizure classification and seizure control status, were reported as frequencies and percentages. The distribution of continuous variables was evaluated using the Shapiro-Wilk test for normality. Associations between cytokine levels and cognitive scores were analyzed using Pearson's correlation coefficient. For comparisons of mean cytokine levels across various clinical and cognitive subgroups, independent samples t-tests and one-way analysis of variance (ANOVA) were employed.

To determine whether cytokine levels independently predicted cognitive impairment after adjusting for potential confounders, multivariate linear regression analysis was conducted. Predictor variables included cytokine levels, age, sex, seizure type, seizure frequency, illness duration, and number of AEDs. A p-value of less than 0.05 was considered statistically significant in all analyses. The statistical plan was reviewed by an experienced biostatistician for accuracy and appropriateness of methodology.

All participants were assigned coded identifiers, and no personal identifiers were used in the data analysis process. Access to patient data was restricted to authorized study personnel. Laboratory results revealing abnormal cytokine levels or cognitive impairments were communicated confidentially to the clinical teams managing the participants, and necessary referrals were facilitated. Throughout the course of the study, patient safety, privacy, and ethical integrity were maintained without compromise.

## RESULTS

### General Overview of Study Population:

One hundred children with epilepsy, ages five to sixteen (mean age =  $10.74 \pm 3.4$  years), were enrolled in the research. With 58 males (58%) and 42 females (42%), there was a masculine preponderance. According to the age distribution, 28 participants (28%) were between the ages of 5 and 8; 39 participants (39%) were between the ages of 9 and 12; and 33 participants (33%) were teenagers between the ages of 13 and 16. 37% of individuals experienced focal seizures upon presentation, but the majority (63%) had generalized seizures. The average duration of epilepsy was  $2.8 \pm 1.6$  years, and the average age of seizure beginning was  $7.3 \pm 2.1$  years. Of the patients, 54% had controlled epilepsy ( $\leq 1$  month), while nearly half (46%) had uncontrolled seizures

( $\geq 4$ /month). EEG abnormalities were present in 91% of the patients, and MRI scans revealed structural abnormalities in 38%.

### Antiepileptic Drug Usage and Seizure Control:

Regarding treatment, 54 children (54%) were receiving monotherapy, 39 (39%) were on dual therapy, and 7 (7%) required polytherapy with three or more AEDs. Sodium valproate was the most frequently prescribed drug, followed by carbamazepine and levetiracetam. Among the patients with uncontrolled epilepsy, 68% were on two or more AEDs. Polytherapy was associated with higher serum cytokine levels and lower cognitive scores, though this relationship was confounded by seizure severity and frequency.

### Cognitive Performance and Impairment Distribution:

The cognitive performance of all participants was assessed using the Wechsler Intelligence Scale for Children – Fourth Edition (WISC-IV). The mean Full-Scale IQ (FSIQ) across the study population was  $85.2 \pm 14.8$ . Among the 100 children, 46 (46%) scored below 85, indicating mild to moderate cognitive impairment. The mean Working Memory Index (WMI) was  $81.3 \pm 12.5$ , Processing Speed Index (PSI) was  $77.6 \pm 13.2$ , and Verbal Comprehension Index (VCI) was  $86.1 \pm 14.3$ . Children with uncontrolled epilepsy had significantly lower WMI ( $75.6 \pm 9.4$  vs.  $87.8 \pm 11.6$ ,  $p < 0.001$ ), PSI ( $70.3 \pm 12.7$  vs.  $84.5 \pm 11.8$ ,  $p < 0.001$ ), and FSIQ ( $79.1 \pm 13.2$  vs.  $90.7 \pm 12.1$ ,  $p < 0.001$ ) than those with well-controlled seizures.

### Serum Cytokine Concentrations and Cognitive Correlation:

Serum cytokine analysis revealed a consistent pattern of elevation in pro-inflammatory markers among children with cognitive impairment. The mean serum levels of IL-6, TNF- $\alpha$ , and IL-1 $\beta$  were significantly higher in the impaired group (FSIQ  $< 85$ ) compared to children with normal cognitive scores (FSIQ  $\geq 85$ ). The mean IL-6 in cognitively impaired children was  $6.2 \pm 1.1$  pg/mL compared to  $3.6 \pm 0.9$  pg/mL in those with preserved cognition ( $p < 0.001$ ). Similarly, TNF- $\alpha$  levels were significantly higher in the impaired group ( $7.9 \pm 1.5$  pg/mL vs.  $5.1 \pm 1.2$  pg/mL;  $p < 0.001$ ). IL-1 $\beta$  also demonstrated a marked increase ( $5.0 \pm 1.3$  pg/mL vs.  $3.2 \pm 0.8$  pg/mL;  $p < 0.001$ ) as shown in table 1.

**Table 1:** Comparison of Cytokine Levels in Children with and Without Cognitive Impairment

Cognitive Group	IL-6 (pg/mL)	TNF- $\alpha$ (pg/mL)	IL-1 $\beta$ (pg/mL)
FSIQ $\geq 85$ (n=54)	$3.6 \pm 0.9$	$5.1 \pm 1.2$	$3.2 \pm 0.8$
FSIQ $< 85$ (n=46)	$6.2 \pm 1.1$	$7.9 \pm 1.5$	$5.0 \pm 1.3$
p-value	$< 0.001$	$< 0.001$	$< 0.001$

### Correlation Between Cytokines and Cognitive Indices:

Pearson correlation coefficients showed strong inverse relationships between cytokine levels and cognitive scores. IL-6 showed the strongest correlation with Full Scale IQ ( $r = -0.65$ ,  $p < 0.001$ ) and Processing Speed Index ( $r = -0.58$ ,  $p < 0.001$ ). TNF- $\alpha$  was also negatively correlated with Full Scale IQ ( $r = -0.61$ ,  $p < 0.001$ ) and Working Memory Index ( $r = -0.52$ ,  $p < 0.001$ ). IL-1 $\beta$  showed a moderate negative correlation with working memory ( $r = -0.49$ ,  $p < 0.01$ ) and overall IQ ( $r = -0.44$ ,  $p = 0.002$ ) as shown in table 2.

**Table 2:** Pearson's Correlation Between Cytokine Levels and Cognitive Scores

Cytokine	FSIQ (r)	WMI (r)	PSI (r)	VCI (r)
IL-6	-0.65***	-0.54***	-0.58***	-0.42**
TNF- $\alpha$	-0.61***	-0.52***	-0.46**	-0.38**

IL-1 $\beta$	-0.44**	-0.49**	-0.41**	-0.31*
--------------	---------	---------	---------	--------

*\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001*

### Multivariate Regression Analysis

FSIQ was used as the dependent variable in a multivariate regression analysis to ascertain the independent prognostic potential of cytokines for cognitive deterioration. Low IQ was significantly predicted by IL-6 ( $\beta = -0.48$ ,  $p < 0.001$ ) and TNF- $\alpha$  ( $\beta = -0.36$ ,  $p = 0.002$ ), regardless of age, sex, seizure frequency, or number of AEDs. As seen in table 3, IL-1 $\beta$  was a significant predictor of WMI ( $\beta = -0.41$ ,  $p = 0.001$ ), confirming its involvement in working memory failure.

**Table 3:** Multivariate Linear Regression for Predicting FSIQ

Variable	$\beta$ Coefficient	95% Confidence Interval	p-value
IL-6 (pg/mL)	-0.48	-0.72 to -0.26	<0.001
TNF- $\alpha$ (pg/mL)	-0.36	-0.58 to -0.15	0.002
IL-1 $\beta$ (pg/mL)	-0.22	-0.51 to -0.03	0.061
Seizure Frequency	-0.18	-0.35 to -0.02	0.034
Age	0.06	-0.08 to 0.19	0.412
Number of AEDs	-0.11	-0.27 to 0.04	0.143

### Subgroup Findings:

Among children with focal epilepsy, inflammatory markers were slightly lower compared to those with generalized epilepsy. Children on polytherapy ( $\geq 3$  AEDs) had higher levels of IL-6 and TNF- $\alpha$  compared to those on monotherapy, although these findings were not statistically significant after adjustment for seizure frequency. Nutritional status and parental education also influenced cognitive scores indirectly, as undernourished children and those from less educated families had lower IQ scores and higher cytokine levels, indicating a possible socio-environmental interaction with biological pathways.

The results indicate a strong and statistically significant association between elevated serum levels of IL-6, TNF- $\alpha$ , and IL-1 $\beta$  and decreased neurocognitive function in children with epilepsy. IL-6 was found to be the most robust predictor of impaired cognitive performance, particularly affecting IQ and processing speed. TNF- $\alpha$  and IL-1 $\beta$  were more closely associated with working memory deficits. These findings support the hypothesis that systemic inflammation contributes significantly to cognitive deterioration in

pediatric epilepsy, independently of traditional clinical parameters.

### DISCUSSION

These results of this clinical cross-sectional study provide attractive evidence that systemic inflammation, especially inflammation with elevated levels of IL-6, TNF- $\alpha$  and IL-1 $\beta$ , is related strongly with neurocognitive impairment in childhood epilepsy. This research study is not only a confirmation of earlier postulated hypotheses about the role of neuroinflammation in epileptogenesis but it also widens our knowledge base as it directly links certain inflammatory biomarkers to the cognitive performance in children [15]. This study sample consisted of a representational range of the cases of pediatric epilepsy with controlled and uncontrolled seizure patterns, different drugs regimes, and a wide age distribution. These features made the results more widely applicable and gave a detailed understanding of the possible disparities in the influence of inflammation on neurodevelopment [16].

The most remarkable discovery was the high serum concentration IL-6 among the

children with cognitive impairment, especially those with lower Full-Scale IQ and those with lower processing speed. Crossing the blood brain barrier, IL-6 has been widely reported in the pathology of the central nervous system (CNS) such as multiple sclerosis, Alzheimer disease and epilepsy. Its persistently high level in the setting of seizures can lead to the dysfunction of the hippocampus, loss of synaptic plasticity as well as activation of glia which all can interfere with cognitive processing. Our data revealed that IL-6 was the strongest independent predictor of low Full-Scale IQ, even after adjusting for confounding variables such as age, sex, seizure frequency, and number of antiepileptic drugs [10, 11].

TNF- $\alpha$  also demonstrated a significant inverse relationship with cognitive scores, particularly with working memory and IQ. This aligns with its established role in promoting excitotoxicity and altering synaptic receptor expression [12]. TNF- $\alpha$  can destabilize the excitatory-inhibitory balance in neural networks by increasing AMPA receptor expression and reducing GABAergic inhibition, thereby contributing to both seizure propagation and cognitive deficits. Elevated TNF- $\alpha$  levels have been associated with drug-resistant epilepsy and prolonged seizure duration, conditions which were also common among our study participants with cognitive decline [13].

IL-1 $\beta$ , while moderately elevated, was specifically associated with lower Working Memory Index scores. This cytokine has a well-documented impact on hippocampal LTP and memory formation. Inflammatory modulation of hippocampal microcircuits by IL-1 $\beta$  can lead to deficits in memory encoding and retrieval, as reflected in our findings. Although IL-1 $\beta$  did not independently predict IQ after adjustment, its strong association with working memory underscores its role in domain-specific cognitive impairment [14].

Another key observation from this study was the exacerbation of cognitive impairment among children with poorly controlled seizures. These children not only had significantly higher cytokine levels but also scored lower across all cognitive domains [15]. This finding supports the concept that seizure frequency acts synergistically with systemic inflammation to impair brain function. Moreover, children on polytherapy regimens exhibited higher cytokine levels and worse cognitive outcomes compared to those on monotherapy. While this may reflect the underlying severity of epilepsy, it also raises concerns about drug-inflammation interactions and their cumulative neurotoxic effects [16].

Our results also point toward important socio-demographic modifiers. Children from undernourished backgrounds or with low parental education levels demonstrated higher cytokine levels and worse cognitive performance, suggesting that environmental and systemic stressors may interact with biological inflammation pathways. Although not the primary focus of the study, these findings highlight the need for comprehensive epilepsy care that addresses nutritional, educational, and psychosocial factors alongside biomedical interventions [17].

The cross-sectional nature of this study limits our ability to draw causal conclusions. Longitudinal studies would be valuable in determining whether cytokine levels can predict long-term cognitive outcomes or response to anti-inflammatory interventions. Furthermore, cytokine levels were assessed only peripherally, and future studies incorporating cerebrospinal fluid analysis or neuroimaging correlates of inflammation could provide deeper insights into CNS-specific processes [18].

Despite these limitations, the study provides valuable clinical evidence supporting the role of inflammation in pediatric epilepsy-



related cognitive decline. It offers potential avenues for early identification of at-risk children and emphasizes the need to develop targeted anti-inflammatory strategies as adjuncts to traditional epilepsy treatment. The integration of cytokine screening in routine care may help tailor individualized treatment plans, particularly in resource-limited settings where access to advanced neuroimaging and long-term neuropsychological support is limited [19, 20].

## CONCLUSION

This study **demonstrates** a significant pathological correlation between elevated inflammatory cytokines specifically IL-6, TNF- $\alpha$ , and IL-1 $\beta$  and neurocognitive impairment in children with epilepsy. Among these, IL-6 emerged as the most consistent and independent predictor of lower Full-Scale IQ and impaired processing speed, while TNF- $\alpha$  and IL-1 $\beta$  were strongly associated with working memory deficits. These findings suggest that systemic inflammation plays a critical role in mediating cognitive dysfunction beyond seizure burden and medication use. Children with uncontrolled epilepsy and those on multiple antiepileptic drugs exhibited higher cytokine levels and more severe cognitive deficits, underscoring the compounded effect of inflammation and disease severity. Recognizing and addressing the inflammatory component of epilepsy may provide an opportunity to mitigate long-term neurodevelopmental harm. Future research should focus on longitudinal tracking of inflammatory markers, evaluating the efficacy of anti-inflammatory therapies, and integrating multi-domain care approaches to optimize cognitive outcomes in pediatric epilepsy. Incorporating cytokine profiling in clinical settings may serve as a valuable biomarker strategy for identifying high-risk children, guiding treatment, and improving quality of life in this vulnerable population.

## Conflict of Interest:

ZA, AL, and SM declare that they have no conflicts of interest related to this study. No financial, personal, or professional affiliations influenced the conduct or reporting of this research.

## Funding:

No external funding was received for this study. All research-related costs were covered by the authors and associated institutions.

## Acknowledgements:

The authors express their sincere gratitude to the staff of the pediatric neurology and laboratory departments for their technical assistance. Special thanks to all children and families who participated in this study.

## Authors' Contributions:

**ZA:** Study conceptualization, patient recruitment, clinical data collection, and manuscript drafting.

**AL:** Laboratory coordination, cytokine analysis, data entry, and critical review of the manuscript.

**SM:** Statistical analysis, literature review, interpretation of results, and final manuscript editing.

All authors read and approved the final version of the manuscript.

## Availability of Data and Materials:

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## REFERENCES

1. Bartolini L, Moran MP, Norato G, Thomas B, Dick AD, Wells E, et al. Differential activation of neuroinflammatory pathways in children with seizures: A cross-sectional study. *Seizure*. 2021;91:150-8.doi:10.1016/j.seizure.2021.05.022
2. Arend J, Kegler A, Caprara ALF, Almeida C, Gabbi P, Pascotini ET, et al. Depressive, inflammatory, and metabolic factors associated with cognitive impairment in patients with

- epilepsy. *Epilepsy & Behavior*. 2018;86:49-57.doi: 10.1016/j.yebeh.2018.07.007
3. Zhao K, Chen P, Alexander-Bloch A, Wei Y, Dyrba M, Yang F, et al. A neuroimaging biomarker for Individual Brain-Related Abnormalities In Neurodegeneration (IBRAIN): a cross-sectional study. *eClinicalMedicine*. 2023;65.doi: 10.1016/j.eclinm.2023.102276
  4. Zervides KA, Jern A, Nystedt J, Gullstrand B, Nilsson PC, Sundgren PC, et al. Serum S100A8/A9 concentrations are associated with neuropsychiatric involvement in systemic lupus erythematosus: a cross-sectional study. *BMC Rheumatology*. 2022;6(1):38.doi: 10.1186/s41927-022-00268-w
  5. McGuire JL, Gill AJ, Douglas SD, Kolson DL, group CHA-RTER. Central and peripheral markers of neurodegeneration and monocyte activation in HIV-associated neurocognitive disorders. *Journal of NeuroVirology*. 2015;21(4):439-48.doi: 10.1007/s13365-015-0333-3
  6. Fagan AM, Henson RL, Li Y, Boerwinkle AH, Xiong C, Bateman RJ, et al. Comparison of CSF biomarkers in Down syndrome and autosomal dominant Alzheimer's disease: a cross-sectional study. *The Lancet Neurology*. 2021;20(8):615-26.doi: 10.1016/S1474-4422(21)00139-3
  7. Zheng Y, Cai X, Wang D, Chen X, Wang T, Xie Y, et al. Exploring the relationship between lipid metabolism and cognition in individuals living with stable-phase Schizophrenia: a small cross-sectional study using Olink proteomics analysis. *BMC Psychiatry*. 2024;24(1):593.doi: 10.1186/s12888-024-06054-x
  8. Colgecen E, Celikbilek A, Keskin DT. Cognitive Impairment in Patients with Psoriasis: A Cross-Sectional Study Using the Montreal Cognitive Assessment. *American Journal of Clinical Dermatology*. 2016;17(4):413-9.doi: 10.1007/s40257-016-0187-3
  9. Parisi GF, Diaferio L, Brindisi G, Indolfi C, Umamo GR, Klain A, et al. Cross-Sectional Survey on Long Term Sequelae of Pediatric COVID-19 among Italian Pediatricians. *Children* [Internet]. 2021; 8(9).doi: 10.3390/children8090769
  10. Liu X, Ma Y, Ouyang R, Zeng Z, Zhan Z, Lu H, et al. The relationship between inflammation and neurocognitive dysfunction in obstructive sleep apnea syndrome. *Journal of Neuroinflammation*. 2020;17(1):229.doi: 10.1186/s12974-020-01905-2
  11. Soares JZ, Valeur J, Šaltytė Benth J, Knapskog A-B, Selbæk G, Bogdanovic N, et al. Associations Between Intrathecal Levels of Vitamin D, Cytokines, and Core Biomarkers of Alzheimer's Disease: A Cross-Sectional Study. *Journal of Alzheimer's Disease*. 2022;89(3):825-34.doi: 10.3233/jad-220407
  12. Checkouri T, Missamou F, Pion SDS, Bikita P, Hemilembolo MC, Boussinesq M, et al. Association between altered cognition and Loa loa microfilaremia: First evidence from a cross-sectional study in a rural area of the Republic of Congo. *PLOS Neglected Tropical Diseases*. 2023;17(6):e0011430.doi: 10.1371/journal.pntd.0011430
  13. Chen Y, Le D, Xu J, Jin P, Zhang Y, Liao Z. Gut Microbiota Dysbiosis and Inflammation Dysfunction in Late-Life Depression: An Observational Cross-Sectional Analysis. *Neuropsychiatric Disease and Treatment*. 2024;20(null):399-414.doi: 10.2147/NDT.S449224
  14. Ruthirakuhan M, Herrmann N, Andreazza AC, Verhoeff NPLG, Gallagher D, Black SE, et al. Agitation, Oxidative Stress, and Cytokines in Alzheimer Disease: Biomarker Analyses From a Clinical Trial With Nabilone for Agitation. *Journal of Geriatric Psychiatry and Neurology*. 2020;33(4):175-84.doi: 10.1177/0891988719874118
  15. Alfaki DA, Hussein M, Hassan M, Eloraish AG, Elbasheir MM. Inflammatory immune mediators and Plasmodium falciparum infection: a cross-sectional study among Sudanese patients with severe and uncomplicated malaria. *Exploration of Immunology*. 2023;3(4):406-15.doi: 10.37349/ei.2023.00109
  16. Williams ME, Stein DJ, Joska JA, Naudé PJW. Cerebrospinal fluid immune markers and HIV-associated neurocognitive impairments: A systematic review. *Journal of*

- Neuroimmunology. 2021;358:577649.doi: 10.1016/j.jneuroim.2021.577649
17. Algul FE, Kaplan Y. Increased Systemic Immune-Inflammation Index as a Novel Indicator of Alzheimer's Disease Severity: ratio and cognitive function in American older people: a cross-sectional national health and nutrition examination survey 2011–2014 (NHANES) study. *Frontiers in Public Health*. 2024;Volume 12 - 2024.doi: 10.3389/fpubh.2024.1375379
  19. Williams ME, Naudé PJW. The relationship between HIV-1 neuroinflammation, neurocognitive impairment and encephalitis pathology: A systematic review of studies investigating post-mortem brain tissue. *Reviews in Medical Virology*. 2024;34(1):e2519.doi: 10.1002/rmv.2519
  20. McGuire JL, Gill AJ, Douglas SD, Kolson DL, Group CHATER. The complement system, neuronal injury, and cognitive function in horizontally-acquired HIV-infected youth. *Journal of NeuroVirology*. 2016;22(6):823-30.doi: 10.1007/s13365-016-0460-5
  21. Tan S, Chen W, Kong G, Wei L, Xie Y. Peripheral inflammation and neurocognitive impairment: correlations, underlying mechanisms, and therapeutic implications. *Frontiers in Aging Neuroscience*. 2023;Volume 15 - 2023.doi: 10.3389/fnagi.2023.1305790
  22. Liang N, Nho K, Newman JW, Arnold M, Huynh K, Meikle PJ, et al. Peripheral inflammation is associated with brain atrophy and cognitive decline linked to mild cognitive impairment and Alzheimer's disease. *Scientific Reports*. 2024;14(1):17423.doi: 10.1038/s41598-024-67177-5
  - Journal of Geriatric Psychiatry and Neurology. 2025;38(3):214-22.doi: 10.1177/08919887241280880
  18. Yang H, Liao Z, Zhou Y, Gao Z, Mao Y. Non-linear relationship of serum albumin-to-globulin ratio and cognitive function in American older people: a cross-sectional national health and nutrition examination survey 2011–2014 (NHANES) study. *Frontiers in Public Health*. 2024;Volume 12 - 2024.doi: 10.3389/fpubh.2024.1375379
  23. Muscal E, Brey RL. Neurologic manifestations of systemic lupus erythematosus in children and adults. *Neurol Clin*. 2010;28(1):61-73.doi: 10.1016/j.ncl.2009.09.004
  24. Qiao S, Li H, Guo F, Cai G, Zhang Y. Research progress on cognitive impairment and the expression of serum inflammatory markers in patients with white matter hyperintensities: a narrative review. *Ann Transl Med*. 2022;10(7):421.doi: 10.21037/atm-22-1016
  25. Thompson LJ, Genovese J, Hong Z, Singh MV, Singh VB. HIV-Associated Neurocognitive Disorder: A Look into Cellular and Molecular Pathology. *International Journal of Molecular Sciences* [Internet]. 2024; 25(9).doi: 10.3390/ijms25094697
  26. Owens CD, Bonin Pinto C, Mukli P, Szarvas Z, Peterfi A, Detwiler S, et al. Vascular mechanisms leading to progression of mild cognitive impairment to dementia after COVID-19: Protocol and methodology of a prospective longitudinal observational study. *PLOS ONE*. 2023;18(8):e0289508.doi: 10.1371/journal.pone.0289508
  27. Li M, Li R, Lyu J-h, Chen J-h, Wang W, Gao M-l, et al. Relationship Between Alzheimer's Disease and Retinal Choroidal Thickness: A Cross-Sectional Study. *Journal of Alzheimer's Disease*. 2021;80(1):407-19.doi: 10.3233/jad-201142

**This Article May be cited As:** Anwar Z, Latif A, Masud S. Pathological Correlation of Inflammatory Cytokines with Neurocognitive Impairment in Pediatric Epilepsy. A Clinical Cross-Sectional Study: Inflammatory Cytokines and Cognitive Decline in Pediatric Epilepsy. *DEVELOPMENTAL MEDICO-LIFE-SCIENCES*. 2025;2(4):39–49. doi: 10.69750/dmls.02.04.0123

#### Publisher's Note:

Developmental Medico-Life-Sciences remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Developmental Medico-Life-Sciences  
Research and Publications Pvt Ltd.