GEORGIAN BIOMEDICAL NEWS

ISSN (Online): 2720-8796 ISSN (Print): 2720-7994 VOLUME 1. ISSUE 2. APRIL-JUNE 2023

# Heterogeneity in INF-γ Levels Among Children with Different Types of Epilepsy

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DOI: 10.52340/GBMN.2023.01.01.35

## ABSTRACT

Background: Epilepsy is the most frequent chronic neurologic disorder of childhood, with a reported higher incidence in underdeveloped countries. The pathogenesis of epilepsy involves complex mechanisms that are still not fully understood. In recent years, researchers have shown increasing interest in understanding the role of inflammation in epilepsy. One such component of the neuroinflammatory cascade is Interferon-Gamma (IFN-γ), a pro-inflammatory cytokine known for its role in immune responses. However, recent studies have revealed that IFN-γ possesses multifaceted properties, including potential neuroprotective and anticonvulsant effects. Multiple studies have reported elevated levels of IFN-γ in the serum, cerebrospinal fluid, and brain tissue of patients with epilepsy. The precise mechanisms through which IFN-γ contributes to epilepsy remain incompletely understood.

Objectives: The present study aimed to compare IFN-γ levels in drug-resistant epilepsy patients with those with well-controlled epilepsy and healthy controls.

Methods: This prospective cross-sectional study enrolled 56 children: 20 with drug-resistant epilepsy, 20 with well-controlled epilepsy, and 16 healthy controls matched in demographic characteristics. Venous blood samples were collected from all participants for cytokine (chemokines, PGE2, and interferon-gamma (IFN-γ) analysis.

Results: IFN-γ levels were not elevated in the study groups. The results indicate a considerable heterogeneity in IFN-γ levels among different types of epilepsy. The interquartile range (IQR) of targeted IFN-γ was lower in the drug-resistant epilepsy patients compared to well-controlled epilepsy and healthy control groups.

Conclusions: The role of IFN-γ in epilepsies is complex and context-dependent. The precise role of IFN-γ in epilepsy warrants exploration; further research is needed to explore the current understanding of the relationship between IFN-γ and childhood epilepsy. The findings from this study may contribute to a better understanding of the role of cytokines in the pathogenesis of epilepsy and could potentially support the development of novel therapeutic approaches targeting cytokine dysregulation in epilepsy.

Keywords: Blood-brain barrier, BBB; cytokine; drug-resistant epilepsy; interferon-gamma IFN-γ; neuroinflammation.

## INTRODUCTION

Epilepsy is the most frequent chronic neurologic disorder in childhood affecting 0.5-1% of children.[1] Regrettably, a higher incidence is reported in underdeveloped countries.[2] The pathogenesis of epilepsy involves complex mechanisms that are still not fully understood. In recent years, researchers have shown increasing interest in understanding the role of immune system components in epilepsy.[3-5] One such component is Interferon-Gamma (IFN-γ), a pro-inflammatory cytokine known for its role in immune responses. Studies have shown elevated levels of IFN-γ in the serum, cerebrospinal fluid, and brain tissue of children with epilepsy,[6-10] suggesting a role of immune system dysregulation and the potential involvement of IFN-γ in the disease process. IFN-γ plays a crucial role in activating microglia, the resident immune cells of the central nervous system, and promotes the release of additional pro-inflammatory molecules, which leads to the disruption of the blood-brain barrier (BBB), allowing the entry of immune cells and inflammatory mediators into the brain parenchyma.[8-10] IFN-γ has been implicated in disrupting the BBB by altering tight junction proteins and increasing BBB permeability,[11] which is crucial in maintaining a controlled brain environment. Thus, exacerbating neuroinflammation and the subsequent cascade of inflammatory responses may contribute to increased seizure susceptibility and disease progression in childhood epilepsy. IFN-γ has been shown to enhance excitotoxicity by modulating glutamate receptors and increasing glutamate release; excessive glutamate release, and subsequent neuronal damage can lead to neuronal hyperexcitability and contribute to the generation and propagation of seizures in childhood epilepsy.[12] The involvement of IFN-γ in childhood epilepsy also opens up potential therapeutic avenues. Modulating IFN-γ levels or its downstream signaling pathways could be explored to reduce neuroinflammation, inhibit excitotoxicity, and improve seizure control in affected children.[13] In conclusion, the dysregulation of IFN-γ in childhood epilepsy suggests its potential role in disease pathogenesis, neuroinflammation, excitotoxicity, and BBB dysfunction.

However, further research is necessary to unravel the precise mechanisms and therapeutic implications of IFN-γ in childhood epilepsy. Understanding the complex interplay between IFN-γ and childhood epilepsy may pave the way for developing targeted therapies to improve seizure control and long-term outcomes for affected children. In this study, Serum INF-gamma levels were measured in children with controlled and refractory epilepsy.

## METHODS

### Study design

A prospective case-control study was conducted to investigate the levels of specific cytokines in patients with different types of epilepsy compared to healthy controls. The study was approved by the Tbilisi State Medical University research ethics committee of the Medical Faculty (10.11.2019).

### Study population

A total of 56 participants were recruited from Zhvania Academic Clinic of Pediatrics included in the study, including patients in Study Group 1 (N=20) subjects who have a diagnosis of drug-resistant epilepsies, Group 2 (N=20) patients with controlled seizures, Group 3 (N=16) - afebrile nonepileptic controls without a history of neurological disorders. The inclusion criteria for patients with epilepsy included a confirmed diagnosis of epilepsy by a neurologist, no acute somatic illnesses, and no recent infectious or autoimmune diseases. In the controlled epilepsy group, we included only interictal symptomatic epilepsy children with proven epilepsy by EEG and clinical assessment who achieved seizure control for at least one year. In the refractory seizures group, according to the ILAE definition,[14] enrolled patients had recurrent seizures while treated with two first-line antiepileptic drugs with proven epileptic encephalopathies by EEG and clinical assessment. Controls were matched to cases based on age, gender, and ethnicity. The sum of the inclusion criteria is shown in Table 1.

TABLE 1. Exclusion and inclusion criteria for study groups

| | *Inclusion criteria* | | | *Exclusion criteria* |
|---|---|---|---|---|
| | **Controlled seizure group** | **Drug-resistant Seizure group** | **Healthy controls** | |
| Age, years | 0-16 | 0-16 | Randomly chosen neurotypical, seizure free (even never suffering from febrile seizures) children of matched age and sex and without underlying health conditions | **Acute somatic illnesses, recent infectious or autoimmune diseases** |
| Gender | No restrictions | No restrictions | | |
| Medical history | Previously diagnosed epilepsy | Recurrent seizures on the double first-line antiepileptic therapy | | |
| Geographic location | No restrictions | No restrictions | | |
| Disease severity | Mild to severe | Severe | | |
| Medication usage | Seizure free at least one year on treatment | Recurrent seizures despite treatment | | |
| Previous treatment | AEDs, not on the keto diet, vagal stimulator | Two first-line antiepileptic drugs | | |
| Comorbidities | Other neurological disorders | Other neurological disorders | | |

*Abbreviations: AEDs, triple antiepileptic drugs.*

### Data collection

All patients underwent systemic and neurological assessment. Medical records were reviewed to gather information on epilepsy subtype, disease duration, and antiepileptic medication usage.

### Blood sample collection

Venous blood samples were collected from all participants under sterile conditions. Samples were transported to the laboratory for further processing.

### Cytokine analysis

The levels of cytokines were measured in the collected blood samples. Enzyme-linked immunosorbent assay (ELISA) kits were used for quantifying various cytokines, such as chemokines, PGE2, and interferon-gamma (IFN-γ).

### Statistical analysis

Statistical analysis was performed using SPSS21; descriptive statistics, such as mean, standard deviation, median, and range, were calculated for continuous variables. The Shapiro-Wilk test was used to assess the normality of the data distribution. Independent Mann-Whitney U tests were conducted for non-normally distributed variables for group comparisons. Categorical variables were analyzed using chi-square or Fisher's exact tests. A p-value $< 0.05$ was considered statistically significant.

### Ethical considerations

The study was conducted following the principles of the Declaration of Helsinki. The privacy and confidentiality of the participants' information were strictly maintained throughout the study. Any identifiable data were anonymized and stored securely.

Georgian Biomedical News
ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

## RESULTS

A total of 56 participants were included, comprising 40 patients with various types of epilepsy and 16 healthy controls. The seizure-controlled group had an average disease duration of 3.8 ± 1 years, while the refractory epilepsy group had a longer duration of 6.5 ± 0.5 years. It should be noted that none of the patients in the drug-resistant seizure group received treatment through a ketogenic diet or a vagus nerve stimulator. Most of these patients were prescribed triple antiepileptic drugs (AEDs) for their management.

Among the patients with epilepsy, two individuals had Landau-Kleffner syndrome, two with tuberous sclerosis or Lennox–Gastaut syndrome, one with Rett-like syndrome (STXBP1 epilepsy), two with Dravet syndrome, and one with West syndrome. The remaining participants had unclassified drug-resistant epilepsy. In the controlled epilepsy group, 5 had frontal lobe epilepsy, 3 had occipital lobe epilepsy, and 12 had generalized epilepsy.

Comparing the IFN-γ levels between the epilepsy groups, no significant differences were observed in the groups ($p > 0.5$). However, in the drug-resistant group, the mean IFN-γ level was 5.3 ±13.1 pg/ml, whereas, in the controlled seizure group, it was 1.8±4.27 pg/ml; in the healthy controls: 0.67±1.61 pg/ml. The interquartile range (IQR) of targeted IFN-γ was lower in the drug-resistant epilepsy patients compared to well-controlled epilepsy and healthy control groups (Tab.2).

TABLE 2. Descriptive analysis of INF gamma levels in different study groups

| | *Drug-resistant Epilepsy Group* | *Controlled Epilepsy Group* | *Healthy Controls* |
|---|---|---|---|
| Mean INF--γ | 5.29 | 1.81 | 6.695 |
| 95% CI for the mean INF--γ | -1.31, 11.9 | -0.25, 3.87 | -0.11, 1.45 |
| 5% trimmed mean | 3.01 | 1.11 | 0.48 |
| Mean | 0.000 | 0.000 | 0.000 |
| Median | 176.5 | 18.27 | 2.61 |
| Std. deviation | 13.28 | 4.27 | 1.62 |
| Minimum | 0.00 | 0.00 | 0.00 |
| Maximum | 51.70 | 16.20 | 4.76 |
| Interquartile range | 1.19 | .00 | .00 |
| Skewness | 2.988 | 2.699 | 2.180 |
| Kurtosis | 9.297 | 7.309 | 3.293 |

## DISCUSSION

In a recent study, we analyzed interictal quantitative levels of IFN-γ in the different clinical settings of childhood epilepsies. No significant differences were found among the groups. However, the results indicate a considerable heterogeneity in IFN-γ levels among different types of epilepsy. These findings could highlight the potential involvement of IFN-γ in the epileptogenesis and clinical characteristics of different clinical settings of epilepsy.

The dual nature of IFN- reveals its complex role in modulating seizure susceptibility and emphasizes its therapeutic potential. IFN-γ is typically considered a pro-inflammatory cytokine primarily associated with immune responses and neuroinflammation. In the developing brain, neuroinflammation can persist even after the initial trigger has resolved and can contribute to the development and progression of epilepsy.[15]

IFN-γ promotes neuroinflammation by activating microglia and astrocytes, producing additional pro-inflammatory cytokines and chemokines.[16] However, recent studies have revealed that IFN-γ possesses multifaceted properties, including potential neuroprotective and anticonvulsant effects.

IFN-γ has been shown to promote the clearance of cellular debris and apoptotic cells, which may limit neuronal damage following seizures.[17] Furthermore, IFN-γ has been implicated in regulating blood-brain barrier integrity, which could have implications for entering inflammatory cells and molecules into the brain.[18]

IFN-γ is typically pro-inflammatory and can exert anti-inflammatory effects in specific contexts. Several studies have reported alterations in IFN-γ levels in animal models and patients with epilepsy. Studies using animal models have shown increased IFN-γ expression in the brain following seizures, suggesting its association with epileptogenesis.[19] Furthermore, experimental manipulations that attenuate IFN-γ signaling pathways have reduced seizure frequency and severity, indicating a potential proconvulsant role for IFN-γ.[20]

In human studies, alterations in IFN-γ levels have been observed in different epilepsies. Some studies have reported elevated levels of IFN-γ in the serum or cerebrospinal fluid of patients with epilepsy compared to healthy controls.[21] These findings suggest that increased IFN-γ production may contribute to the inflammatory response in epilepsy. Moreover, higher IFN-γ levels have been associated with increased seizure frequency and disease severity in specific epilepsy subtypes,[22] indicating a potential correlation between IFN-γ dysregulation and disease progression.

The exact mechanisms through which IFN-γ exerts its effects in epilepsies are not fully understood. However, several hypotheses have been proposed. One possibility is that IFN-γ promotes neuroinflammation by activating microglia and astrocytes, producing additional pro-inflammatory cytokines and chemokines. This cascade of inflammatory events may contribute to the maintenance and propagation of seizures.[22] Additionally, IFN-γ has been shown to modulate the excitability of neurons by altering ion channel expression and synaptic plasticity, potentially enhancing seizure susceptibility. IFN-γ can modulate immune responses by promoting the production of anti-inflammatory cytokines, such as interleukin-10 (IL-10), which may dampen neuroinflammatory processes


ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

associated with seizures. IFN-γ may indirectly contribute to seizure resistance by mitigating the pro-convulsive effects of neuroinflammation.[10]

IFN-γ has been shown to influence neuronal excitability, potentially affecting seizure susceptibility directly. Studies suggest that IFN-γ can modulate the expression and function of ion channels and neurotransmitter receptors, including GABA receptors.[23] GABA is the primary inhibitory neurotransmitter in the brain, and enhancing its inhibitory effects can reduce seizure activity. IFN-γ may promote inhibitory neurotransmission and neuroprotective properties in various neurological conditions. It can promote neuronal survival, enhance synaptic plasticity, and exert antioxidant effects. These neuroprotective mechanisms may help maintain the integrity of neuronal networks and prevent excessive excitability. Thus, low IFN-γ levels could contribute to seizure resistance.[24]

The potential role of IFN-γ in promoting seizure resistance opens up new avenues for therapeutic interventions.[25] Harnessing the protective effects of IFN-γ or developing strategies to enhance its activity could represent novel therapeutic approaches for epilepsy. Modulating IFN-γ levels or its downstream signaling pathways may offer neuroprotective benefits and reduce seizure frequency and severity. Targeting neuroinflammation and immune pathways has emerged as a potential approach for managing drug-resistant epilepsy in preclinical and clinical studies as adjunctive treatments to improve seizure control and overcome drug resistance.[26]

Despite the growing evidence implicating IFN-γ in epilepsies, several questions and challenges remain. The precise cellular sources of IFN-γ and the specific signaling pathways involved in its actions within the epileptic brain require further investigation. Moreover, the factors contributing to the dysregulation of IFN-γ in epilepsy, such as genetic predisposition or environmental triggers, need to be elucidated. While the precise role of IFN-γ in childhood epilepsy still warrants exploration, further research is needed to explore the current understanding of the relationship between IFN-γ and childhood epilepsy. The findings from this study may contribute to a better understanding of the role of cytokines in the pathogenesis of epilepsy. They could potentially support the development of novel therapeutic approaches targeting cytokine dysregulation in epilepsy.

Several limitations were acknowledged in this study. Firstly, the sample size was relatively small, which may limit the generalizability of the findings. Additionally, the study design was cross-sectional, restricting the ability to establish a causal relationship between cytokines and epilepsy. Further longitudinal studies with larger sample sizes are needed to confirm and expand upon these findings.

## CONCLUSIONS

The role of IFN-γ in epilepsies is complex and context-dependent. The modulation of immune responses, anti-inflammatory effects, and direct influences on neuronal excitability highlight the potential role of IFN-γ in promoting epileptogenesis. Further research is needed to fully understand the mechanisms underlying these effects and explore the therapeutic potential of IFN-γ-based interventions in epilepsy. The exploration of IFN-γ in seizures may pave the way for developing innovative treatments that could improve the management of epilepsy and enhance the quality of life for affected individuals.

## AUTHOR AFFILIATION

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## ACKNOWLEDGEMENTS

This research was funded by the Shota Rustaveli National Science Foundation of Georgia Fundamental Research Foundation grant FR-18-16052.

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ISSN (Online): 2720-8796 ISSN (Print): 2720-7994

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ISSN (Online): 2720-8796 ISSN (Print): 2720-7994