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# Correlation Between Serum Level and Gene Expression of IFN-γ in Toxoplasmosis Patients and Its Association with Neurodevelopmental Disorders in Children

**Sarah Hassan Abed Fares[1*], Entsar Jabbar Saheb[2]**
[1]*Department of Biology, Collage of Science for Women, Baghdad University, Iraq*
[2]*Department of Biology, Collage of Science, Baghdad University, Iraq*

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**Abstract**

Toxoplasmosis, caused by the intracellular parasite *Toxoplasma gondii*, is linked to neurological and mental diseases. Interferon-gamma (IFN-γ) is a vital immune system cytokine that regulates immunological response. This study examines the relationship between the serum level and gene expression of IFN-γ in children with neurodevelopmental disorders infected with *T. gondii*. A total of 200 blood samples were collected from neurology and pediatric physicians at the Central Teaching Hospital of Paediatrics in Baghdad from October 2022 to May 2023. The serum levels of IFN-γ were measured using a competitive ELISA technique. A Quantitative Real-Time Polymerase Chain Reaction was performed for gene expression. The control group included 68children without toxoplasmosis and 32 who were infected with *Toxoplasma gondii*. In comparison, the patient group had 31 children with neurodevelopmental disorders and 69 without them, according to the ELISA (*Toxoplasma*-IgM and IgG) detection test. The results demonstrates that serum levels of IFN-γ are slightly higher in patients compared to the control group, and this difference is statistically significant and shows no significant variations in serum IFN-γ levels across the studied groups. The age interval (0-12) in children with neurodevelopmental disorders infected with *T. gondii* showed high serum levels compared to other groups. In gene expression studies, children with neurodevelopmental issues and toxoplasmosis had greater IFN- expression in gene expression .The study found that children with toxoplasmosis, especially those with neurodevelopmental problems, have increased gene expression of IFN-γ despite similar serum levels. IFN-γ may play a role in the development of neurological problems linked to congenital toxoplasmosis. Person correlation was used to evaluate the strong relationship between the serum level of IFN-y and its gene expression. The results of this study show a positive relation between IFN-y and its gene expression in the toxoplasmosis area.

Keywords: Toxoplasmosis, Children with neurodevelopmental disorders, Serum level of IFNγ, IFN-γ gene expression

*Email: Sarah.H@csw.uobaghdad.edu.iq

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# العلاقه بين المستوى المصلي والتعبير الجيني لانترفيرون كاما بالمرضى المصابين بداء المقوسات الكونديه وارتباطها بأضطرابات الجهاز العصبي لدى الاطفال

**ساره حسن عبد فارس*[1], إنتصار جبارصاحب[2]**

[1]قسم علوم الحياة ، كلية العلوم للبنات ، جامعة بغداد ، بغداد ، العراق

[2]قسم علوم الحياة ، كلية العلوم ، جامعة بغداد ، بغداد ، العراق

**الخلاصه**

داء المقوسات الكونديه ، هو مرض يسببه الطفيلي داخل الخلايا توكسوبلازما جوندي، مرتبط بالأمراض العصبية والنفسية. إنترفيرون-غاماهو سايتوكين حيوي في جهاز المناعة ينظم الاستجابة المناعية. تتناول هذه الدراسة العلاقة بين مستوى المصل والتعبير الجيني للانترفيرون -كاما وعلاقته بأضطرابات الجهاز العصبي لدى الأطفال المصابين بداء المقوسات الكونديه . فحصت الدراسة 200 عينة دم كاملة من أستشارية الجمله العصبيه واستشارية الأطفال في المستشفى التعليمي المركزي للأطفال في بغداد من أكتوبر 2022 إلى مايو 2023. تم قياس مستوياتالانترفيرون-غامافي المصل باستخدام تقنية ELISA التنافسية. تم إجراء تفاعل البوليميراز المتسلسل الكمي في الوقت الحقيقي لتعبير الجينات. كان لدى مجموعة التحكم 68 طفلًا بدون داء المقوسات و32 طفلًا مصابًا به، بينما كان لدى مجموعة المرضى 31 طفلًا مصابًا باضطرابات النمو العصبي و69 طفلًا بدونها، وفقًا لاختبار الكشف عن *Toxoplasma*- ELISA (IgM,IgG)وأظهرت النتائج أنه لم تكن هناك اختلافات كبيرة في مستوياتإنترفيرون-غامافي المصل بين المجموعات المدروسة، وأن الفئة العمرية (0-12) فيالاطفال المصابه باضطرابات النمو العصبي والمقوسات الكونديهأظهرت مستويات عالية من المصل مقارنة بالمجموعات الأخرى. الأطفال الذين يعانون من مشاكل في النمو العصبي والمقوسات الكونديه أظهروا مستويات أعلى من تعبير IFN-γ في دراسات التعبير الجيني. وجدت الدراسة أن الأطفال المصابين بداء المقوسات، وخاصة أولئك الذين يعانون من مشاكل في النمو العصبي، لديهم زيادة في تعبير الجينإنترفيرون-غاما، على الرغم من مستويات المصل المماثلة. قد يلعب الانترفيرون-غامادورًا في تطور المشاكل العصبية المرتبطة بداء المقوسات الكونديه الخلقية.

## Introduction

Toxoplasmosis is one of the most common infections during pregnancy, transmitted from mother to child via the placenta. It causes morbidity and mortality prenatally. The number of pro- and anti-inflammatory cytokines is produced by placental and decidual tissues during a normal pregnancy[1]. Research shows that *T. gondii* has infected over one-third of the world's population, leading to congenital toxoplasmosis through its transplacental passage of the parasite to the fetus, fetal infection can lead to various health issues such as encephalitis, cerebral calcification, mental retardation, or psychomotor dysfunction [2].Infection can be transmitted through various means, including consuming undercooked meat, drinking contaminated water, or contacting cat feces. While many adults and children may not show symptoms, congenital transmission during pregnancy can lead to severe complications for the fetus, affecting the central nervous system and muscular systems[3]. These findings emphasize the importance of understanding the behavior and environmental factors that eventually increase the risk of such infection in pediatric populations, especially among those with a related neurological disease [4]. *T. gondii* tachyzoites possess the capability to infiltrate various categories of neuronal cells within the cerebellum, which subsequently regulates the signal transduction mechanisms and signaling pathways pertinent to a multitude of functions, including antimicrobial effector activities, immune cell differentiation, and cellular

apoptosis.[5]. Interferon-gamma (IFN-γ) serves as a vital cytokine in defending against illnesses by targeting specific cells or enhancing the immune system. IFN-γ was identified as the predominant cytokine crucial in the body's immune response against *T. gondii* infection[6]. IFN-γ release post-tissue damage caused by the disease leads to the degeneration of dopamine-producing neurons. In mouse models, type II IFN-γ plays a key role in infection management, as demonstrated by the deletion of the IFN-γ gene and specific antibody-mediated neutralization of IFN-γ. Interferon-gamma (IFN-γ) is a crucial pro-inflammatory cytokine influencing the innate immune response[7].

## 2. Materials and Methods

### *2.1. Study subject*

In the present study, 200 whole blood samples were collected from children in the age range interval (1-12 years) from Central Teaching Hospital from October 2022 to May 2023. The two primary groups, comprised of 100 patients and 100 controls, were further divided into four subgroups. The control group comprised 68 children without toxoplasmosis (CWOT) and 32 children with toxoplasmosis (CWT). The patient group included 100 individuals with neurodevelopmental disorders. Based on the screening for anti-*Toxoplasma* antibodies (IgG and IgM) via ELISA testing, the patient group was categorized into 31 samples of neurodevelopmental disorders children with toxoplasmosis (NDCWT) and 69 samples of neurodevelopmental disorders children without toxoplasmosis (NDCWOT).

### *2.2. Ethical approval*

Baghdad's Ministry of Health and Environment granted clearance. Local ethics committees of the Department of Biology, College of Science, University of Baghdad approved the study (CSEC/1121/0085).

### *2.3. Measurement of IFN-γ serum levels*

Children and controls were given five ml of venous blood by venipuncture. Three ml was placed in plain tubes, clotted, and centrifuged for 10 min at 3000 rpm. The serum was stored at -20 °C until use.

### *2.4. IFN-γ gene expression*

Total RNA was extracted from blood samples using TransZol Up Plus RNA Kit according to the manufacturer's instructions. The RNA was stored at a temperature of -20°C. Relative quantification was used to quantify IFN-γ gene expression using Reverse transcriptase quantitative PCR(RT-qPCR). The primer used in this study was IFN-γ HRM Primers for rs2430561, forward primer: CATGTGCGAGTGTGTGTGT, and reverse primer: TCAGACATTCACAATTGATTTTATTC. Primers for RT-PCR were left Primer: TCATCCAAGTGATGGCTGAA and right Primer: ATATTGCAGGCAGGACAACC, with a melting temperature at 60.2°C and 59.9°C, respectively, and an amplicon size of 116 bp . Gene expression was normalized using the *GAPDH* gene as a reference and measured using ΔCt and the folding (2-ΔΔct) method. SYBR Green, a fluorescent dye that detects double-stranded DNA, including cDNA, was used to quantify real-time PCR in this work. The Ct value (cycle threshold) indicated amplification. The relative cycle threshold (2-ΔΔCt) methodology, first described by Livak and Schmittgen in 2001, was used to calculate changes in mature RNA expression. The study employed a Rotor Gene Q Real-Time CYTO PCR System (QIAGEN)

*2.5. Statistical analysis*

The outcomes study was conducted in San Diego, California, using GraphPad Prism 8.0.1. INF-γ concentration was shown as an average and standard deviation. A one-way ANOVA examined key differences. Statistically significant P-values were less than 0.05. RT-qPCR detected the INF-Y expression fold [7, 8].

## 3. Results

*3.1. Serum level of IFN-γ in the studied groups*

Results in Figure 1 demonstrate that the serum levels of IFN-γ are slightly higher in patients compared to the control group, and this difference is statistically significant. Table 1 shows that significant variability in IFN-γ concentrations is observed among various age demographics, particularly distinguishing between pediatric patients afflicted with toxoplasmosis and their counterparts who are not infected. The mean IFN-γ concentration was recorded at its peak in the NDCWT group of children aged 1-4 (160.6379), whereas the lowest mean was noted in the NDCWOT group aged 1–4 years, also diagnosed with toxoplasmosis (109.9188).The p-values for each age group comparison (0.1, 0.7, and 0.8) and overall comparisons (0.1, 0.8, 0.09, 0.6) suggest that the differences in INF-y levels are not statistically significant. This implies that, within the studied sample, age does not significantly affect INF-y levels.

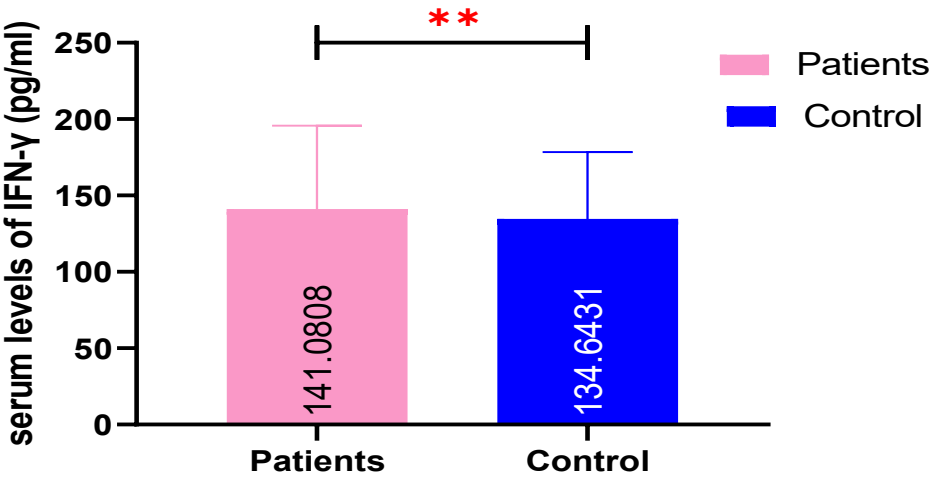


**Figure 1:**The serum level of IFN-γ comparison among the patients and control groups.

**Table 1:The mean level of INF-y in different ages according to toxoplasmosis.**

| Age | CWOT | CWT | NDCWOT | NDCWT | P-Value |
|---|---|---|---|---|---|
| **1-4** | 119.0450 | 146.1438 | 109.9188 | 160.6379 | 0.1 NS |
| **5-8** | 148.6883 | 135.0589 | 121.8100 | 156.9675 | 0.7 NS |
| **9-12** | 131.6333 | 125.4020 | 143.0875 | 129.5740 | 0.8 NS |
| **P-Value** | 0.1 NS | 0.8 NS | 0.09 NS | 0.6 NS | - |

CWOT: Children without toxoplasmosis; CWT: Children with toxoplasmosis; NDCWOT: Neurodevelopmental disorder children without toxoplasmosis; NDCWT: Neurodevelopmental disorder children with toxoplasmosis.

*3.2. Gene expression of IFN-γ*

Gene expression of IFN-γ was investigated in the studied groups by using qRT-PCR. The analysis of mRNA expression data after normalization with the *GAPDH* gene revealed that there is an elevation in the mRNA level of IFN-γ in the patient group compared to the healthy group (Table 2). The mean average of gene fold expression increased from 1 in healthy children to 1.37 in children who are suffering from toxoplasmosis, 1.29 in children who have a neurodevelopment disorder without toxoplasmosis, and to 1.74 in children who have a neurodevelopment disorder with toxoplasmosis.

**Table 2:** Fold of mRNA IFN-γ expression Depending on 2ΔCtMethod.

| Groups | Means Ct of INF-Y | Means Ct of GAPDH | ΔCt (Means Ct of *INF Y* Means Ct of GAPDH | $2^{-\Delta Ct}$ | ΔΔCt | $2^{-\Delta\Delta Ct}$ | Experimental group/ Control group | Fold of gene expression |
|---|---|---|---|---|---|---|---|---|
| **CWOT** | 23.641 | 16.71 | 6.931 | 0.0082 | -0.869 | 1.82639 | 1.82639/1.82639 | 1 |
| **CWT** | 23.191 | 16.7215 | 6.4695 | 0.0113 | -1.331 | 2.51489 | 2.51489/1.82639 | 1.37 |
| **NDCWOT** | 23.767 | 17.206 | 6.561 | 0.0106 | -1.239 | 2.36034 | 2.36034/1.82639 | 1.29 |
| **NDCWT** | 23.0785 | 16.9435 | 6.135 | 0.0142 | -1.665 | 3.17113 | 3.17113/1.82639 | 1.74 |

CWOT: Children without toxoplasmosis; CWT: Children with toxoplasmosis; NDCWOT: Neurodevelopmental disorder children without toxoplasmosis; NDCWT: Neurodevelopmental disorder children with toxoplasmosis; NDCWT.

Person correlation was used to evaluate the strong relationship between the serum level of IFN-y and its gene expression. The results of this study show a positive relation between IFN-y and its gene expression in the toxoplasmosis area (Figure 2).

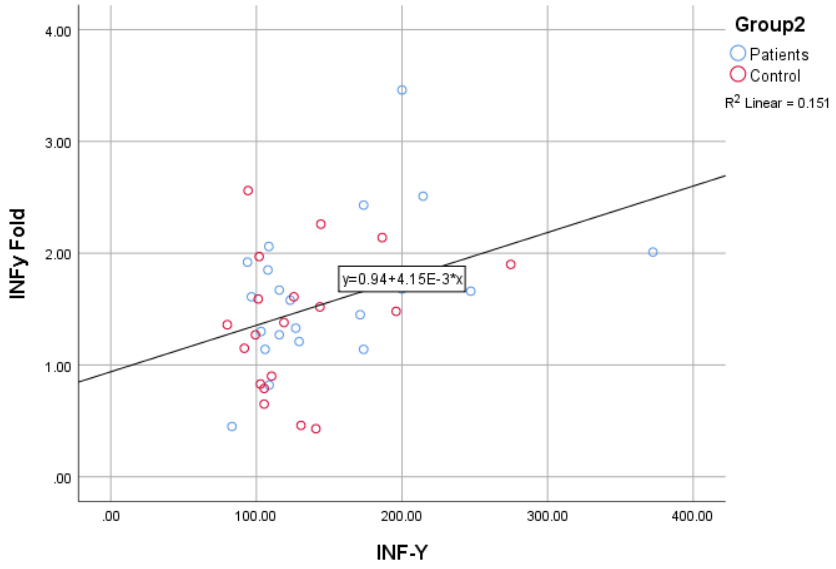


**Figure 2:** The correlation between IFN-γ and gene expression

## 4. Discussion

*4.1. Serum level of IFN-γ in the studied groups*

Studies have investigated the role of IFN-γ in *T. gondii* infection. It was found that in vivo blood levels of C-X-C motif chemokine ligand 9 (CXCL9), circulating CD4+CD25+ T-cells,

and *T. gondii* specific IFN-γ+CD4+ T-cells that were measured 30-45 days after birth were highly accurate in identifying[9]. *T. gondii* infection-specific IL5+CD4+ T-cells and IFN-γ+Natural killer cells in vitro provided an accurate early prognosis of ocular abnormalities in babies with congenital toxoplasmosis. In agreement with Ad'hiah *et al.,* IFN-γ levels were greatest (mean = 39.4 pg./ml)[9]. Research indicates that in rats, dendritic cells (DCs) recognize T. *gondii* via TLR11/12, leading to Interleukin-12 (IL-12) production and NK cell activation for early parasite clearance. Research indicates that aborted pregnant women who were seropositive for *T. gondii* had higher levels of IFN-γ in their blood (P= 0.0193)[10]. Studies show that IFN-γ is crucial for suppressing *T. gondii* infection in the central nervous system (CNS), as shown by antibody ablation treatments that reactivate chronic infection and severe encephalitis in animals. Immune-related GTPases (IRGs) are upregulated in vitro and in animals lacking key pathway components to eliminate intracellular *T. gondii* by interferon-activated gene expression. IFN-γ treatment in vitro aids astrocyte parasite elimination. In vitro, microglial cells react to Tumor necrosis factor (TNF), IL-6, or IFN-γ to inhibit parasite replication. Neurons stimulated with IFN-γ may not remove parasites in vitro due to altered transcriptional responses. In mice, type I interferon controls chronic CNS infection and type II immunity after acute infection. Acute parasitemia was greater in animals lacking type I interferon signaling by bioluminescent imaging and flow cytometry. The infection-induced immune response can harm neuronal function despite its beneficial role in infection management. IFN-γ can disrupt neurogenesis, increase nitrogen monoxide (NO) production from microglia, cause dementia, and inhibit dendritic processes[11]. According to a study that aimed to analyze the immunological characteristics of Korean children with ASD, it was reported that the IFN-γ levels showed a significant decrease in ASD children compared to the healthy children, which does not match the results revealed from the present study[1]. The same results were conducted in the study that was carried out by Barzinij [12]; they revealed no differences in IFN-γ levels in all subject groups involving healthy children's samples and patient samples with different age intervals.

*4.2. Gene expression of IFN-γ*

The NK cells, dendritic cells, and neutrophils in the innate immune system and CD4+ and CD8+ T cells in the acquired immune system produce IFN-γ during intracellular pathogen infection. The release of IFN-γ causes innate immune cells to express effector proteins that kill *T. gondii* PV and impede growth [13]. The "YETI" mouseline was previously used to analyze IFN-γ production after *T. gondii* infection. The bicistronic IFN-γ/*eYFP* gene, linked to an Encephalomyocarditis (EMCV) IRES, replaces the native *Ifng* gene in this line[14].A significant finding is the role of IFN-γ, a cytokine produced by NK cells and T cells, in enhancing the immune response against *T. gondii*. IFN-γ activates various immune mechanisms, including the expression of inducible nitric oxide synthase (iNOS) and indoleamine 2,3-dioxygenase (IDO), which help suppress the growth of the parasite[15]. In a study by Nishiyama and colleagues discovered that T cell-derived IFN-γ is crucial for anti-*T. gondii* host defense[16].

Additionally, maternal immune stimulation increases neurodevelopmental risk. Increased cytokines, such as IFN-γ, are crucial for offspring brain development. IFN-γ activates an antiviral cellular response, blocking virus entry and multiplication. Additionally, IFN-γ is associated with brain growth. Neurite outgrowth was improved by transient IFN-γ stimulation of neural progenitors from human induced pluripotent stem cells. According to RNA sequencing research, IFN-γ-induced promyelocytic leukemia protein (PML) nuclear bodies maintained major histocompatibility complex class I (MHCI) gene upregulation during neuronal development[17]. To stimulate neurite outgrowth, IFN-γ needs both PML and MHCI[18]. In addition, IFN-γ dramatically altered gene expression in schizophrenia and

autism, suggesting a link between inherited and environmental risk factors[19]. Research indicates that IFN-γ signaling may have a role in the development of neurodevelopmental disorders[20]. Significantly, exposure of the embryo to high maternal levels of pro-inflammatory cytokines may cause human beings to get hallucinations Neurotropic affinity by *T. gondii* could change the physiology of CNS A lot of neurological diseases (ex: schizophrenia) are related to cytokines and inflammation within the body, the immune pathway was another possible mechanism because the pro-inflammatory cytokines led to *T.gondii*-induced apoptosis that resulted in neurodegenerative[21]. Many investigations have explored the relationship between *T. gondii* infection and many other conditions such as schizophrenia, epilepsy, depression, and bipolar disorder[22]. Infection with *T. gondii* alters the neurochemical composition of the host, thereby affecting the metabolic path of tryptophan, purine, and arginine. These are involved in the control of neurochemical concentrations, which are basic for cognitive functions and behavior[23]. Elevated serum interferon-gamma (IFN gamma) levels were found among children diagnosed with acute toxoplasmosis the study concludes that the imbalance in expression levels of host pro-inflammatory and anti-inflammatory cytokines also plays a critical role in determining the course of toxoplasmosis during pregnancy, the delicate balance of these cytokines may impact the likelihood of abortion or successful pregnancy progression[24]. The research has indicated that *T. gondii* can cause many changes to the brain through an inflammatory environment, particularly to the synaptic proteins that mediate interaction between neurons. This can affect cognition and behavioral patterns, blocking IFN-γ, which is a central immune-response cytokine. It was possible to reverse synaptic protein downregulation partially, showing that IFN-γ is an important component in the changes regarding inflammation found in the brain [3]. This new research will explore a possible association between maternal toxoplasmosis, an infection of the body caused by the *Toxoplasma parasite*, and the probability of developing autism in offspring. *Toxoplasmosis* is a common disease with severe serious risks, especially to pregnant women and individuals who are immunosuppressed [25]. Adding IFN-γ at the very beginning of neurogenic differentiation reshaped the neuronal network, the changes of which could be recapitulated during the development of neurodevelopmental conditions, such as but not limited to autism spectrum disorder (ASD) and schizophrenia[26]. The manuscript underscores the key role of interferons in mediating resistance to intracellular pathogens such as *T.gondii*, with emphasis placed on type II IFN, also known as IFN-γ. It is then further confirmed that both type I (IFN-α/β) and type III (IFN-λ) interferons play substantial roles following infection with *T. gondii*, hence signifying a complex interplay among the different types of interferons in host defense. The conversion of *T. gondii* tachyzoites to the bradyzoite form is suppressed in the Seasonal Malaria Chemopreventions (SMCs) and fibroblasts previously exposed to levels of interferon-gamma (IFN-γ), which once again confirms that IFN-γ plays a dual role in containing active infection as well as preventing chronic infection formation. The research findings have suggestive evidence that previous exposure to *T.gondii* in children could be associated with the development of ASD, in contrast to recent exposure. Biological autism research on broader outlooks and participants in combination with enhanced communication and resources could further support the Broader Autism Phenotype difficulties[27, 28].

## 5. Conclusion

The findings of the study suggest that while there is a notable seroprevalence of *T. gondii* antibodies among children with neurodevelopmental disorders, the direct impact on serum IFN-γ levels and the development of these disorders remains complex. The studies highlight increased gene expression of IFN-γ in children with neurodevelopmental disorders and toxoplasmosis, suggesting a potential role of this cytokine in the pathophysiology of these

conditions. However, the serum levels of IFN-γ do not show significant variations across different groups, indicating that the relationship between *T. gondii* infection and neurodevelopmental disorders may involve more intricate immunological mechanisms.

## Conflict of Interest

The authors declare that they have no conflict of interest."

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