

The Role of Tumor Necrosis Factor Alpha in Adolescent Iraqi Children Infected with Toxoplasmosis

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DOI: <https://doi.org/10.55145/ajbms.2024.03.01.007>

Received August 2023; Accepted October 2023; Available online December 2024

ABSTRACT: Toxoplasmosis affects various biochemical markers, which has garnered attention recently. Toxoplasmosis biochemical marker values were the focus of this study. To evaluate the infection-related marker TNF alpha, TNF- α regulates inflammation, anti-tumor responses, and homeostasis via interacting with TNF-R1 and TNF-R2. TNF- α has a vital function in treating *T. gondii* by regulating hematopoietic stem and progenitor cell development. Despite therapeutic usage of anti-TNF- α medications, adverse effects such lymph proliferative disorders have been known to occur. This also suggests that one major mechanism by which cytokines operate is by inhibiting parasite invasion. TNF- α has the ability to directly influence the proliferation of intracellular parasites to different extents. The study included one hundred patients with toxoplasmosis, aged between eighteen and thirty years old. Among them, seventy had confirmed cases of infection, with forty samples from both male and female patients, while the remaining thirty samples were taken from healthy individuals serving as a control group. Baghdad Teaching Hospital, Al-Yarmouk Teaching Hospital, and many outpatient clinics will treat patients from different Iraqi governorates from November 2022 and October 2023. Additionally, we utilized the ELISA technique to determine the quantity of TNF-alpha present. With this study, there were no significant changes in blood levels of TNF- α between the control group and teens of different ages and genders, according to the results. The mean \pm SE values of the level in healthy teens (80.57 ± 5.27) and *T. gondii* patients (123.59 ± 4.35 , 158.85 ± 10.26) were not statistically different. The findings indicated that the mean values for healthy teenagers were 66.02 ± 5.89 and 87.84 ± 3.24 , respectively. There was no significant gender difference, with mean values of 149.65 ± 11.98 and 133.25 ± 3.96 , respectively. Patients with toxoplasmosis exhibited substantially higher TNF- α levels than the control group ($P \leq 0.01$). The average \pm SE TNF-alpha levels in teenagers infected with *T. gondii* were 136.86 ± 5.96 , whereas the control group had 87.84 ± 3.24 . Our research suggests that *Toxoplasma gondii* have the capability to exert effect on certain indicators, such as TNF-alpha. The findings indicate that adolescents are prone to a significant prevalence of toxoplasmosis diagnoses. Consequently, prioritizing health education for those with a heightened susceptibility to developing the disease is imperative.

Keywords: Toxoplasmosis, TNF- α , Tumor Necrosis



1. INTRODUCTION

Toxoplasmosis, a disease caused by protozoa, has a global impact, affecting both poor and developed nations. The recognized pathogen that has infected people is accidental ingestion of food, drink, soil, or raw meat tissues contaminated with oocysts, as well as organ transplant or eating of undercooked meat. The parasite is responsible for causing a range of autoimmune diseases [1, 2]. Toxoplasmosis is a globally prevalent parasitic condition that can be opportunistic. It can kill humans. Pathological impacts stress the immune system. Patients with impaired immune systems, such as cancer or AIDS patients [3, 4, 5], threaten public health and national finances. *Toxoplasma gondii* infections can induce retino-choroiditis, hydrocephaly, mental impairment, and foetal death, making them a public health problem. In addition, AIDS, organ transplant, and immunosuppressive patients might develop life-threatening encephalitis [6, 7]. Besides the eyes and neurological system, *Toxoplasma gondii* can infect the liver, pancreas, spleen,

heart, and lymph nodes [8]. Cytokines are mostly connected with CD4+ T cells in the cellular immune response. Th1-mediated macrophage activation, host resistance, and Toxoplasma parasite protection help manage the illness. Th2-mediated responses reduce macrophage activation and disease progression [9, 10]. TNF- α is crucial for regulating the immune system's response to pathogens. Activated mononuclear phagocytes are the primary producers of TNF- α , but NK, T, and mast cells can also contribute. The production and release of TNF- α facilitates the immune system-inflammatory relationship [11]. TNF- α has different effects on T, B, and DC cells, in addition to its pro-apoptotic role [12]. Both macrophages and lymphocytes emit TNF- α . This cytokine works with IFN- γ and IL-10 to increase oxygenated substance and nitric oxide production. These drugs kill Toxoplasma gondii and cause various inflammatory reactions and diseases in humans [13, 14]. Several immune cell types and organs release cytokines that exhibit a rather high solubility. Cytokines have a substantial impact on regulating both the adaptive and innate immune responses. Maintaining immune homeostasis requires a continuous effort to balance cytokines. Any excessive production or dysfunction of cytokines, together with a faulty immune response to these molecules, might contribute to the progression of immune-related disorders. The cytokine network is a carefully regulated mechanism, which allows for the restoration of cellular cytokine release to baseline levels after the stimulus is removed [15].

1. Participants underwent laboratory pre-diagnosis prior to ELISA colorimetric measurement of tumor necrosis factor alpha.
2. Examining if toxoplasmosis infection levels of the cytokine TNF alpha vary between male and female teenagers.
3. The study also demonstrated that TNF- α increased during infection but returned to a healthy level following therapy.

2. MARERIALS AND METHOD

From November 2022 to the end of October 2023, researchers from Baghdad Teaching Hospital's Haematology Department in Baghdad Medical City carried out the study. People whose ages varied from eight years old to thirty years old were a part of the present study. The individuals that made up the control group ranged in age from eighteen to thirty and were all carefully chosen for their health.

2.1 Collection and Preparation of Blood Samples

Verified the accuracy of the recorded data, such as gender and age .Subsequently Preserved blood samples stored in cryogenic conditions A 5 ml intravenous blood sample will be collected from each patient. Each participant in the study had their blood drawn using a sterile syringe and subsequently transferred to an EDTA-free test tube. A 10-minute 3000 rpm centrifugation followed 20 minutes of cooling at room temperature. Using an absorbent micropipette, the serum was removed and stored in testing containers at 20–16 degrees Celsius. So they split into two groups: Toxoplasmosis affects 40 people, 30 male and 30 female [16].

2.2 T. gondii infection detection ELISA kits

The German company Demeditec Diagnostics GmbH's products were utilized in accordance with the guidelines provided by the maker. Through the use of an immunochromatography-based fast test kit and an enzyme-linked immunosorbent assay (ELISA), we were able to identify specific IgM and IgG antibodies against Toxoplasma gondii. Tumor necrosis factor alpha detection by ELISA procedure.

We followed the manufacturer's instructions to determine biochemical and immunological parameters using it. We measured TNF-alpha levels using ELISA kits from the immuno lab CUSABIO (USA).

2.3 Calculation of the results

The purpose of measuring the TNF- α level in infected patients' sera using ready-to-use ELISA kits was to identify any changes in its levels caused by toxoplasmosis and to comprehend the function of TNF- α in protecting against this infection. The results for both concentrations of serum TNF- α were identical.

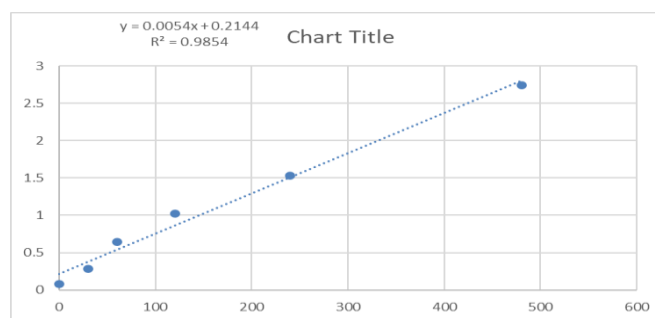


FIGURE 1. - The TNF- α Standard Curve

Table 1. - TNF- α Standards OD and concentrations

	O D reading	Conc (pg/ml)
S	2.745	480
S	1.535	240
S	1.027	120
S	0.643	60
S	0.279	30
S	0.078	0

3. STATISTICAL ANALYSIS

Using SAS (2018), researchers compared study parameters across patient and control groups. A T-test allowed us to compare means statistically. This study used a chi-square test to compare 0.05 with 0.01, which was significant.

3.1 Results and Discussion

The distribution of studied groups according to some parameters.

Age ratio:

You can see the breakdown of the participants in this study by age group in table (2). Across all categories, the most common age ratio was. Additionally, 17(42.50%) of the patients analyzed were teenagers, defined as those between the ages of 8 and 15. The age group revealed to be between 16 and 30 years old had the greatest ratio at 23 (57.50%), while the control group had the lowest ratio at 12 (40.00%) and 18 (60.00%). The statistical study found no age differences among respondents.

Table 2. - Non-Significant

Factor	Patients (No=40)	Control (No= 30)	P-value
Age groups: 8-15 yr.	17 (42.50%)	12 (40.00%)	0.347 NS
No (%) 16-30 yr.	23 (57.50%)	18 (60.00%)	0.315 NS
P-value	0.074 NS	0.108 NS	---

Table 3 and Figure 2 show the impact of age groups on TNF- α . Age significantly affected the distribution of results in each group ($P \leq 0.01$). TNF- of infected teenagers (8-15 years) and (16-30 years) was the most common age mean in all groups. Patients infected with toxoplasmosis had the highest TNF- α levels (158.85 \pm 10.26). Age 16-30 years (123.59 \pm 4.35) had substantially higher TNF- α levels compared to control group (79.09 \pm 4.57) and adolescent group (80.57 \pm 5.27). Statistical analysis revealed no significant ($P < 0.01$) differences between *T. gondii* patients and control groups.

Table 3. - Effect of Age groups in TNF- α of patients and control groups

	Mean \pm SE of TNF- α		T-test
	8-15 yr.	16-30 yr.	
Patients	123.59 \pm 4.35	158.85 \pm 10.26	35.611 NS
Control	79.09 \pm 4.57	80.57 \pm 5.27	18.76 NS
T-test	36.481 **	45.062 **	---

** ($P \leq 0.01$).

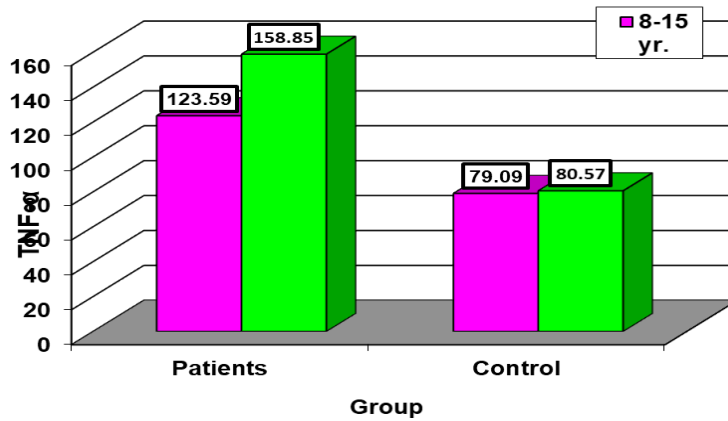


FIGURE 2. – Effect of Age groups in TNF-α of patients and control groups

Gender ratio:

The relative distribution of each group according to the gender is shown in table (4). The most frequent gender ratio was found in all studied groups were revealed. The majority of studied subjects for the adolescents which represent in the female group and accounted for about 22(55.00%) for toxoplasmosis co-infected, the male group 18(45.00%) adolescents for toxoplasmosis infected.

Table 4. - Non-Significant

Factor	Patients (No=40)	Control (No= 30)	P-value
Male	18 (45.00%)	12 (40.00%)	0.166 NS
Female	22 (55.00%)	18 (60.00%)	0.107 NS
P-value	0.107 NS	0.166 NS	---

Results also revealed that the in present study according to the TNF-α level increased significantly (P<0.01) is shown in (Figure 3). Findings revealed in TNF-α levels between the female group and the male group. Adolescents who Concentration for had the highest mean TNF-α levels (149.65 ±1.98), (133.25 ±3.96) versus lower (66.02 ±5.89), (87.84 ±3.24) in the control group.

Table 5. - Effect of Gender in TNF-α of patients and control groups

Group	Mean ± SE of TNF-α		T-test
	Male	Female	
Patients	149.65 ±11.98	133.25 ±3.96	27.84 NS
Control	66.02 ±5.89	87.84 ±3.24	22.07 NS
T-test	31.95 **	26.72 **	---

** (P<0.01).

The distribution of TNF- α with toxoplasmosis infected patients.

The study found that the T.gondii group had considerably greater blood TNF- α levels than the control group. TNF- α levels in T.gondii teenagers were significantly higher ($P \leq 0.01$) compared to the control group, with lower levels displayed in Figure 3 and Table 6 (136.86 \pm 5.96 and 76.54 \pm 3.66).

Table 6. - TNF- α level of Toxoplasma gondii positive group and the control group

Group	Mean \pm SE of TNF- α
Patients	136.86 \pm 5.96
Control	76.54 \pm 3.66
T-test	15.147 **
P-value	0.0001

** ($P \leq 0.01$).

The mean value of TNF- α level in the sera of studied groups were exhibited in figure (3) The elevated level of TNF- α had been observed in patient adolescents which was significantly ($P \leq 0.01$), while it was recorded the lowest level in the sera of control adolescents group which represented (in comparison to the patient's groups. Highest significant ($P \leq 0.01$) difference was observed in levels of TNF- α between infected and healthy control groups.

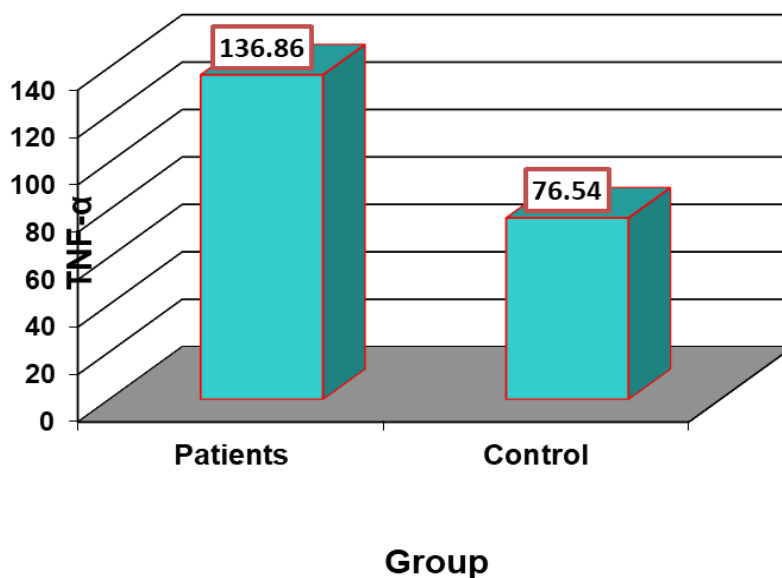


FIGURE 3. – Comparison between patients and control groups in TNF- α

The secretion of tiny proteins called cytokines and chemokines regulates several facets of cell differentiation, activation, and development. Their impact on immune system development, cell trafficking, and immunological tissues and organs is a notable feature of these compounds. The potential culprits behind TNF- α 's actions in toxoplasmosis include its proinflammatory properties and its effects on T cells, B cells, and plasmacytoid dendritic cells [17,18]. When a parasite infects a host, it changes the host's behavior via acting on the host's endocrine system. The gender has a key role in the host changes caused by Toxoplasma gondii infection [19]. Salih et al. (2020) found no age-related differences in Toxoplasma TNF alpha rates [20]. When comparing the sexes, the TNF-alpha rate was much greater in men. Other surveys [21, 22] also found similar outcomes. However, according to another study, the TNF-alpha rate is higher in male patients compared to female patients [23]. Females are more likely to get toxoplasmosis from a T.gondii infection, and this is probably because of the role that sexual hormones play in this illness [24]. Because of differences in immune response and personal hygiene practices, which make males more likely to contract Toxoplasma infections, men with greater testosterone levels are at a higher risk of contracting this disease [25]. There are a lot of potential explanations for the observed variation in T.gondii rates among teenagers. These include variations in sample size, geographical location, diagnostic method, housing, socioeconomic position, and immunological state [26]. Their results demonstrated that increasing levels of serum TNF- α indicate disease activity, whereas decreasing levels indicate a successful response to therapy. The rise in cytokines is consistent with earlier findings [27]. Chang et al. (1990) [28]

found that IFN- γ stimulates TNF- α , which may be necessary for its anti-parasitic effects. The latest discovery contradicts this. Research suggests that TNF- α and IL-1 may play a significant role in modulating the immune response to parasite infection. Recent data suggest no link between rising TNF- α levels and IFN- γ levels. In 1993, Sher et al. [230 ψ 9] Blood levels of Th1 (TNF- α , IL-12, and IFN- γ) and Treg (IL-10) cytokines can measure the host immunological response to toxoplasmosis, as previously shown [31]. Except for IL-12, all of these cytokines increased significantly in patients [32]. Biomarkers also help understand therapy response, reducing recurrence. Identifying frequent infections in asymptomatic individuals in places where the illness is prevalent or in people with weakened immune systems requires toxoplasmosis biomarkers [33]. Their research on adherent cells, which release less TNF- α in response to the parasite, supports the latest discovery. TNF- α concentrations rose dramatically with age, suggesting that it alone is inadequate to induce NK cells to eradicate parasites Zhang et al. (2015) observed results similar to our study [35 34]. Earlier lab studies in infected female mice showed increased interleukins. In contrast, immune cell reactivity, evaluated by nitric oxide generation and spleen cell proliferation in the MLC test, decreases significantly compared to uninfected controls [36]. Therefore, these studies reveal that cytokine production fluctuates non-linearly with age.

4. CONCLUSION

It can be concluded that this cytokine gives an insight as possible biomarkers of *T. gondii* infection in the studied groups. Although there is little known about this concept, more studies are needed to investigate TNF- α level and more parameters in *T. gondii* patients and completely cured individuals as markers for disease progression. adolescents should be screened for *Toxoplasma* routinely. Clinicians should be more careful with this patients group to prevent the possibility of severe toxoplasmosis The low levels of Tumor necrosis factor alpha protein, age progress related factors can be changed in association to *T. gondii* infection among adolescents.

5. RECOMMENDATIONS

1. Study the effects of other parasites on Tumor necrosis factor.
2. Investigate *T. gondii*-related metabolic problems in autistic children.

Funding

None

ACKNOWLEDGEMENT

The authors would like to thank the anonymous reviewers for their efforts.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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