BAFF as a Potential Marker for Toxoplasma Gondii Seroprevalence Among Cancer Patients on Chemotherapy

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BAFF as a Potential, Toxoplasma, gondii Seroprevalence

ABSTRACT
Background: Toxoplasmosis is a global zoonotic disease, that is mainly asymptomatic in healthy persons, but critical and life-threatening in immunocompromised patients. The infection is caused by an opportunistic obligate intracellular parasite protozoon "Toxoplasma gondii" (T. gondii) that can cause fatal complications in immunosuppressed patients. Cancer patients are being immunocompromised from the cancer itself and on the other hand cancer therapy they receive may reactive a latent T. gondii infection that leaves them at high risk. The B-cell activating Factor (BAFF) is a cytokine that plays an essential role in the activation, maturation, and survival of B cells. It is vital for the appropriate functioning of the immune system. Monitoring BAFF levels in cancer patients undergoing chemotherapy could be important for preventing or controlling infections in these vulnerable patients. Objectives: This study aimed to detect seroprevalence of T. gondii in cancer patients receiving chemotherapy and assess the levels of BAFF in those patients. Materials and Methods: 150 serum samples were collected from oncology patients having different types of cancer and receiving chemotherapy, from different ages. And 30 samples from healthy people for control. In this cross-sectional study, samples were collected from the 1st of October 2023 to the 1st of March 2024, at Kirkuk Oncology and Hematology center. The method used was the Sandwich Enzyme-Linked Immunosorbent Assay (Sandwich-ELISA) technique. Results: the overall anti-T. gondii IgG and IgM seropositive samples were found to be 67 (37.2%) in the total study population and 67(44.7%) in cancer patients. BAFF levels were elevated in cancer patients infected with T. gondii compared with the cancer patients with no infection and the healthy control group. A significant difference was indicated with (p-value 0.0004). Conclusions: A high rate of T. gondii seroprevalence was revealed among oncology patients receiving chemotherapy with elevated levels of BAFF associated with T. gondii infection in those patients.

1. Introduction

About one-third of human beings are infected chronically with T. gondii. The disease is mainly transmitted by ingestion of tissue cysts that may present in raw or undercooked meat of the animals infected by the parasite, also by the consumption of uncooked vegetables or water contaminated with cat feces containing the oocysts of T. gondii, and can be transmitted transplacentally (1). T. gondii's lifecycle is complex and highly regulated, interchanging between sexual developing stages in felines and several asexual forms in the intermediate hosts(2). T.gondii is a successful parasite that can invade and multiply inside any nucleated cell of a wide range of warm-blooded hosts including humans(3). This major zoonotic pathogen has a substantial phenotypic variation and genetic diversity that have been suggested to be responsible for the difference in clinical outcomes, mostly associated with reproductive failure and neurological and ocular signs (4). Even though toxoplasmosis was identified 70 years ago, yet, there is no appropriate solution effective to treat this disease(5). Reactivation of a chronic infection in immunocompromised persons may lead to severe symptoms and death (6).

Cancer is mainly caused by mutations or changes in the DNA of cells and it is one of the main causes of death worldwide (7). Chemotherapy causes immunosuppressive effects in cancer patients that make them more vulnerable to opportunistic infections including toxoplasmosis. Latent infection reactivation may also occur in those patients emphasizing the importance of screening, early detection, and treatment of toxoplasmosis to limit morbidity in this population (8)

T. gondii IgM antibodies are key indicators of recent or current infection with toxoplasmosis. Studies

revealed that IgM antibodies are detected in the duration of primary infection (9). IgG antibodies in Toxoplasmosis reveal recent or chronic phases (10)

The B-cell activating Factor (BAFF), also named B-Lymphocyte Stimulator (BLyS) is a cytokine from the tumor necrosis factor (TNF) family, it is produced mainly by hematopoietic cells, BAFF is a survivor factor for B-cell that functions in activation, supporting, differentiation, proliferation and preventing the deletion of these cells (11). High levels of BAFF are associated with cancer severity and response to therapy (12). Regarding toxoplasmosis, BAFF signaling may influence the pathogenesis of the disease by stimulating cell persistence and dropping apoptotic rates, possibly leading to unusual biological actions in cells (13).

2. Methodology

Study population

A total of 180 serum samples were collected for the study, 150 serum samples from oncology patients having different types of cancer and receiving chemotherapy, from different ages. And 30 samples from healthy people not having cancer for control. Samples were collected from the 1st of October 2023 to the 1st of March 2024, at Kirkuk Oncology and Hematology center.

Sample collection:

Serum samples were collected from each oncology patient and the healthy individuals for control. By using sterile syringes 7 ml of venous blood was obtained and placed into sterile gel tubes for the coagulation of blood samples so the tubes were put in rack at room temperature for about 20 minutes. After that, the blood tubes were centrifuged for 5–10 minutes at 3000 rpm to separate the blood serum. then the serum of each sample was divided and placed into 5 Eppendorf tubes and kept in a deep freezer (-20 C) in the laboratory up to the time of work.

Serological Tests:

All the tests included in the study (anti-*T.gondii* IgM, anti-*T.gondii* IgG, BAFF) were performed by ELISA technique, according to the manufacturer's direction (human ELISA, Sunlog Biotech Itd).

Statistical analysis:

The data were analyzed using the Minitab program system version 17, and an ANOVA test was applied. The means were compared by Duncun's multiple ranges at a significance level of (0.05).

Ethical approval:

The research was carried out according to the ethical principles outlined in the Helsinki Declaration. The patient's approval was obtained before taking the sample. To obtain this approval, a local ethics committee reviewed and approved the study protocol, subject information, and consent form using document number 604 (including the number and dated September 20, 2023)

3. Results and discussion

180) blood samples were included in the study (150) of the samples from oncology patients receiving chemotherapy, collected at Kirkuk Oncology and Hematology Center, and 30 blood samples from (apparently) healthy individuals served as control. Using the ELISA technique, 12 (6.7%) of samples were IgM positive, while 41(22.8%) of them were IgG positive, and 14 (7.8%) of samples had both IgM and IgG positive. Were as, the overall *T.gondii* seropositivity was 67 (37.2%), and 113 (62.8%) of samples were negative. In cancer patients, 67(44.7%) of the samples were anti-*T. gondii* seropositive and 83(55.3%) were negative. All samples of the control group were negative. A very significant difference with (P-Value 0.0007) was indicated.

Table (1) Anti-*T. gondii* antibodies seroprevalence using ELISA Technique in Total Samples Under Study

Groups	Patient number	IgM +ve No(%)	IgG +ve No(%)	IgM & IgG +ve No(%)	*T. gondii* –ve No(%)
Cancer Patient	150	12 (8%)	41 (27.3%)	14 (9.3%)	83 (55.3%)
		67 (44.7%)			
Control	30	0 (0%)	0 (0%)	0 (0%)	30 (100%)
Total	180	12 (6.7%)	41 (22.8%)	14 (7.8%)	113 (62.8%)
Total	180	67 (37.2%)			113 (62.8%)
P-Value		**0.0007**			

Studies about the prevalence of toxoplasmosis in oncology patients have been conducted in Iraq, China, Iran, and other countries and have revealed variable rates of Toxoplasmosis among cancer patients. In Basrah province, Southern Iraq, the prevalence of Anti-*T.gondii* IgG was 31%, with less incidence of Anti-*T.gondii*IgM at 0.8% (14). A study from northern Iran revealed the toxoplasma IgG seropositivity among cancer patients undergoing chemotherapy at 75.4%, with 5.43% of samples containing *T. gondii* DNA(15). Furthermore, a study in China revealed a rate of 11.96% of toxoplasmosis in females with gynecological tumors, with specific risk factors identified, such as contact with cats and a history of chemotherapy(16). These findings highlight the significance of understanding, controlling, and preventing this parasitic infection in cancer patients to avoid possible complications associated with this disease.

Another study (17) on 120 cancer patients and 60 controls showed higher Toxoplasma (IgG and IgM) levels (66.7% and 9.2%) in the group of cancer patients compared to the control group (33.3% and 6.7%). Furthermore, a study from Ankara, Turkey, on 673 patient samples showed lower levels of T. gondii IgM positivity (8%) and IgG positivity (28.7% (18).

The presence of IgM antibodies indicates a recent or acute *T. gondii* infection. IgM antibodies appear early in the infection and mostly decline within a few months. IgG antibodies indicate a previous infection and usually provide some level of immunity. IgG antibodies can persist for a lifetime, proposing a historical record of exposure (19). In cancer patients, detecting IgM can suggest a new infection, which is critical as their immune systems are compromised by chemotherapy (20). For cancer patients, IgG can help differentiate between a past infection and a new acute infection (when IgM is present) (14). It is important to mention that seropositive results do not always accurately reveal latent infection in cancer patients. This is because these patients may have received multiple blood transfusions from donors infected with *T. gondii*, which can result in the presence of passively acquired antibodies instead of indicating an active or latent infection; therefore, confirmatory tests are often essential to establish a definitive diagnosis (21).

Cancer treatments, including chemotherapy, radiotherapy, and surgery, may reduce the morbidity and mortality related to malignancies; however, they may deteriorate immune resistance and cause stages of severe cytopenia. The immune suppression caused by cancer and its treatments paves the way for *T. gondii* to complicate the clinical course of cancer patients (22).

Chemotherapy may affect the immune system, potentially changing the typical antibody response (23). The presence of IgM might not be as prominent or might persist longer due to immunosuppression. In patients with positive IgG (indicating past exposure), there's a risk of reactivation of latent infection due to their weakened immune state. Regular serological testing for toxoplasmosis in oncology patients can help in the early detection and management of *T. gondii* infections (24).

In cancer patients, *T. gondii's* seropositivity rate of *37.2%* highlights a significant exposure risk to *T. gondii*. This increased rate of *T. gondii* seroprevalence in this population may be attributed to many reasons; the weak immunity of Cancer patients undergoing chemotherapy makes them more susceptible to opportunistic infections, including *T. gondii*. This immunosuppression may cause reactivation of latent infections or increased susceptibility to new infections. Also, the long-term nature of cancer and chemotherapy may increase the duration of exposure to potential sources of infection, such as contaminated food, water, or soil. On the other hand, cancer patients have to undergo various medical procedures, including hospital stays and interventions, which increases their exposure to infections and may expose them to contaminated surfaces and equipment (20) (23). Furthermore, cancer patients, due to side effects of chemotherapy like nausea or mouth lesions, might change their diet and consume more undercooked or raw foods if they can tolerate them better, possibly elevating the risk of toxoplasmosis infection. These factors highlight the importance of sensitive awareness and preventive measures to decrease the risk of acute toxoplasmosis in cancer patients, especially those undergoing immune suppressor treatments(25)

***T. gondii* seropositivity**	**Patients**	**BAFF pg/ml (Mean±S.D.)**
***T. gondii* IgM +ve cancer+**	12	5.090±1.650 a
***T. gondii* IgG +ve cancer+**	41	4.939±1.990 a
***T. gondii* IgM +ve IgG +ve cancer+**	14	3.570±1.607 ab
***T. gondii* IgM –ve IgG –ve cancer+**	83	2.398±0.695 bc
Healthy control	30	1.025±0.981 c
P-value		0.0004

Effect of *T. gondii* infection on the level of B-cell Activating Factor (BAFF) in cancer patients :

Table (2) Level of B-cell Activating Factor (BAFF) (Mean±S.D.) in the groups of the study concerning *T. gondii* seropositivity

Note: *The same letters denote no significant differences among them under the level of ($p<0.05$). **The different letters indicate significant differences among them under the level of ($p<0.05$).

The results show elevated BAFF levels in *T. gondii* IgM+ve patients mean±S.D. (5.090±1.650) followed by *T. gondii* IgG +ve patients mean±S.D. (4.939±1.990). All the groups of cancer patients had higher levels of BAFF when compared with the healthy control group with

mean±S.D. (1.025±0.981). A significant variation was revealed among the groups with (P-value 0.0004).

The cytokine BAFF is a member of the TNF superfamily, and its role is vital in organizing immune responses. This protein is expressed by macrophages, monocytes, neutrophils, dendritic cells, and subpopulations of B cells and T cells; BAFF is involved in infection, tissue homeostasis, and inflammation (26).

During acute toxoplasmosis, there is an immediate immune response with the activation of both innate and adaptive immunity. This results in the production and increasing levels of cytokines, including BAFF. BAFF is crucial for the survival, proliferation, and differentiation of B cells. Acute toxoplasmosis triggers an increase in BAFF production to support the expansion and differentiation of B-cells, which are crucial for the antibodies production against the parasite (27). The acute infection also induces pro-inflammatory cytokines production, comprising TNF-α and IFN-γ, that can stimulate the expression of BAFF by various cells, including dendritic cells and monocytes (28). On the other hand, during chronic toxoplasmosis, the immune system tries to maintain a persistent response to control latent infection. Hence, BAFF levels remain elevated to support the ongoing need for B-cell activity, as BAFF is essential for preserving memory B-cells that assist in long-term immunity. The elevated BAFF ensures a rapid response if the parasite reactivation occurs(29).

Cancer triggers an immune response, and BAFF acts in the activation and survival of B-cells to produce antibodies against tumor antigens. Tumors may create an inflammatory microenvironment that produces cytokines and growth factors that can contribute to increased BAFF levels (30).

Chemotherapy often results in the reduction of rapidly dividing cells, including immune cells such as B-cells. In response, the body upregulates BAFF to stimulate the recovery and proliferation of B-cells and other immune cells. Chemotherapy can also induce tissue damage and inflammation, leading to the release of BAFF as an attempt of the body to repair tissue and regenerate immune cells (31).

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