

## The Chemotherapy Effect on Toxoplasmosis and Cytokines Status in Women with Breast Cancer

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### Abstract

*Toxoplasma gondii* is an opportunistic protozoan in immunocompromised patients and the reactivation of a latent infection could cause severe influence. This study aimed to investigate the chemotherapy effect on the levels of *T. gondii* immunoglobulins IgG, Interleukins (IL) IL-6, IL-12 and IL-23 in women with breast cancer. In this study, 190 women were enrolled (100 samples were taken from outpatient clinics as control groups and 90 samples of women with breast cancer who attended to Oncology Teaching Hospital in Medical City Hospital in Baghdad from different Governorates in Iraq). All serum samples were tested for *T. gondii* immunoglobulins IgG antibodies, IL-6, IL-12, and IL-23 levels using ELISA technique. The results showed that the highest mean titers of IgG and IL-23 were in untreated women with breast cancer infected with Toxoplasmosis ( $168.15 \pm 13.53$  IU/mL,  $258.67 \pm 17.05$  pg/mL) respectively, while the highest mean titers of IL-12 were in treated women with breast cancer infected with Toxoplasmosis ( $23.84 \pm 0.05$  pg/mL), while the highest mean titers of IL-6 were in untreated women with breast cancer infected with Toxoplasmosis ( $6.551 \pm 0.35$  pg/mL). The results of this study reveal that the Toxoplasmosis antibody and Cytokines status of women with breast cancer could be affected by the chemotherapy thus, the Toxoplasmosis antibody and Cytokines status should be known before, during and after chemotherapy.

**Keywords:** Chemotherapy, Cytokines, Toxoplasmosis, Breast cancer.

تأثير العلاج الكيميائي على الإصابة بداء المقوسات ومستويات السيتوكينات في النساء المصابات بسرطان الثدي  
انتصار جبار صاحب، داليا فالح و مها مصطفى

### الخلاصة

تعد المقوسات الكوندية من الطفيليات الانتهازية في المرضى الذين يعانون من نقص المناعة ، ويمكن أن يسبب إعادة تنشيط العدوى الكامنة والتي يكون لها تأثيراً شديداً. هدفت هذه الدراسة إلى تحديد تأثير العلاج الكيميائي على مستويات الغلوبولين المناعي *T.gondii* IgG ، IL-6 ، IL-12 و IL-23 في النساء المصابات بسرطان الثدي. شملت الدراسة تسجيل (190) امرأة (تم أخذ 100 عينة من العيادات الخارجية كمجموعة سيطرة و 90 عينة من النساء المصابات بسرطان الثدي اللواتي حضرن إلى مستشفى الأورام التعليمي في مستشفى مدينة الطب في بغداد من مختلف المحافظات في العراق). تم اختبار جميع عينات المصل من أجل الأجسام المضادة لـ *T. gondii* IgG ، IL-6 ، IL-12 ، و IL-23 باستخدام تقنية ELISA. أظهرت النتائج أن أعلى تراكيز IgG و IL-23 كان في النساء المصابات بسرطان الثدي المصاب بداء المقوسات وغير معالجات ( $168.15 \pm 13.53$  وحدة دولية / مل ،  $258.67 \pm 17.05$  بيكوغرام / مل) على التوالي. في حين أن أعلى مستوى تراكيز من IL-12 كان في النساء اللواتي يعانين من سرطان الثدي المصاب بداء المقوسات ( $23.84 \pm 0.05$  بيكوغرام / مل). في حين أن أعلى تراكيز من IL-6 كانت في النساء غير المعالجات مع سرطان الثدي المصاب بداء المقوسات ( $6.551 \pm 0.35$  بيكوغرام / مل). تكشف نتائج هذه الدراسة أن الأجسام المضادة لداء المقوسات والسيتوكينات للنساء المصابات بسرطان الثدي يمكن أن تتأثر بالعلاج الكيميائي.

الكلمات المفتاحية: العلاج الكيميائي ; السيتوكينات; داء المقوسات; سرطان الثدي.

## 1. Introduction

*Toxoplasma gondii* stimulates production of four types of antibodies (IgG, IgM, IgA and IgE) against both membrane and excretory antigens. In the presence of complement specific antibody lyses extracellular tachyzoites [1]. IL-5 consider the major cytokine responsible for the increase in the eosinophil production in parasitoses, whereas IL-6 stimulates production of antibodies and exerts a proinflammatory effect by stimulating the generation of acute phase proteins [2]. The Cytokines are initiated and maintenance the protective against *T. gondii* [3]. They act on numerous cells. In the event of Toxoplasmosis, they can be divided into 2 main types- protective and regulatory Cytokines [4]. The production of Cytokines leads to increase the secretion of superoxide (free radicals) and hydrogen peroxide ( $H_2O_2$ ) which pavimentar mechanical  $O_2$ -independent killing of *T. gondii* [5]. Resistance to early *T. gondii* infection is a delicate balance between the

production of proinflammatory Cytokines, which control parasite growth, and regulatory Cytokines, which limit host pathology [6].

Cancer is a complex illness where it has relations between normal and neoplastic cells. Most of current cancer therapies depend on drugs or radiation that block cell division and kill dividing cells [7]. These types of treatment have serious side effects on normal proliferating cells. Immunologic approach is promising to provide a specific cancer therapy with less harm for normal body cells. Cancer immunotherapy works through enhancing the weak host immune response for developing tumors [8]. Pro- and anti-inflammatory Cytokines are considered to be a highly dynamic part of the inflammatory response. Cytokines are released at the site of inflammation (caused by an infectious pathogen or traumatic injury) and facilitate an influx of lymphocytes, neutrophils, monocytes, and other cells that participate in the clearance of the antigen and healing [9].

## 2. Materials and Methods

### 2.1. Subjects and Blood Collection

In this study, (190) women were enrolled from October/2017- February/ 2018. (100) samples were taken from outpatient clinics as control groups and (90) samples of women with breast cancer who attended to Oncology Teaching Hospital in Medical City Hospital in Baghdad from different Governorates in Iraq. Their ages ranged between (20-50) years.

Five milliliter of blood samples were collected from all patients. The sample was collected in sterilized Gel Clot activator vacuum tubes and left for (30) minutes at room temperature, and then the samples were centrifuged at (3000) round per minute for 10 minutes for serum aspiration and dispensed into Eppendorf- tubes and stored at -20 °C. ELISA kits (Acon *Toxoplasma* IgG ELISA (I231-1091), IgM ELISA (I231-1101) was used to determine the anti- *T. gondii* antibody (IgG). All serum samples then were divided into 4 groups: healthy women, women infected with Toxoplasmosis, women with Breast cancer infected with Toxoplasmosis, and women with Breast cancer not infected with Toxoplasmosis. As well as, samples were tested for serum mean titer of IL-6, IL-12 and IL-23 by using the cusabio human interleukin 6 kits (Cat. No: CBS-E04638h), the SHANGHAI human interleukin 12 kits (Cat. No: YHB1704Hu) and the cusabio human

interleukin 23 kits (Cat. No: CBS-E08461h). Chi-square test was used to significant compare between percentage and least significant difference–LSD test was used to significant compare between means in this study.

### 3. Results

#### The mean levels of IgG, IL-6, IL-12, IL-23 before and after therapy in Breast cancer patients according to Toxoplasmosis infection

The effect of chemotherapy on the level of IgG antibodies to *T. gondii* in women with Breast cancer was investigated. The result showed that the higher mean titer was in untreated women with Breast cancer infected with Toxoplasmosis ( $168.15 \pm 13.53$  IU/mL), while the mean titer in treated women with Breast cancer infected with Toxoplasmosis was ( $102.43 \pm 8.92$  IU/mL).

The mean titer of IL-6 in women with Breast cancer infected with Toxoplasmosis was ( $6.551 \pm 0.35$  pg/mL), while the mean titer of IL-6 in women with Breast cancer infected with Toxoplasmosis was ( $5.622 \pm 0.19$  pg/mL).

The mean titer of IL-12 in women with Breast cancer infected with Toxoplasmosis was ( $9.16 \pm 0.72$  pg/mL), while the mean titer of IL-12 in women with Breast cancer infected with Toxoplasmosis was ( $17.50 \pm 0.81$  pg/mL).

The higher mean titer of IL-23 in women with Breast cancer infected with Toxoplasmosis was ( $258.67 \pm 17.05$  pg/mL), while the mean titer of IL-23 in women with Breast cancer infected with Toxoplasmosis was ( $200.53 \pm 10.61$  pg/mL). All of these results have shown in Table (1).

Table (1): The mean levels of anti-*Toxoplasma* IgG, IL-6, IL-12, IL-23 before and after therapy in Breast cancer according to Toxoplasmosis infection.

Therapy	IgG (IU/mL)		P-value
	Toxo(-ve)	Toxo (+ve)	
Before-therapy	$1.49 \pm 0.09$	$168.15 \pm 13.53$	0.0001 **
After-therapy	$1.20 \pm 0.06$	$102.43 \pm 8.92$	0.0001 **
P-value	0.1883 NS	0.0263 *	

IL-6 (pg/mL)			
	Toxo (-ve)	Toxo (+ve)	
Before-therapy	6.497 ± 0.54	6.551 ± 0.35	0.0061 **
After-therapy	4.96 ± 0.08	5.622 ± 0.19	0.0884 NS
P-value	0.0255 *	0.1443 NS	
IL-12 (pg/mL)			
	Toxo (-ve)	Toxo (+ve)	
Before-therapy	6.43 ± 0.59	9.16 ± 0.72	0.0317 *
After-therapy	7.60 ± 0.68	17.50 ± 0.81	0.0001 **
P-value	0.092 NS	0.0001 **	
IL-23 (pg/mL)			
	Toxo (-ve)	Toxo (+ve)	
Before-therapy	162.46 ± 12.75	258.67 ± 17.05	0.0271 *
After-therapy	140.42 ± 9.36	200.53 ± 10.61	0.0469 *
P-value	0.1492 NS	0.0882 NS	

\* (P<0.05), \*\* (P<0.01); NS: Non-Significant.

#### 4. Discussion

In cancer patients, immune function is declining and this is lead to increase the *Toxoplasma* infection which will cuase increasing in the chemotherapy duration [10]. The current result showed that the higher mean titer was in untreated women with Breast cancer infected with Toxoplasmosis compare with the mean titer of treated women with Breast cancer infected with Toxoplasmosis. Other previouses study showed the highest mean titer of women with Breast cancer infected with Toxoplasmosis was in dosage (0) followed by dosage (3) and (6) [11]. It was shown that the prevalence of anti-*T. gondii* antibodies in patients undergoing chemotherapy were found to be (24.4%). This means that the patients receiving immunosuppressant drugs have latent Toxoplasmosis, and there is a risk of reactivation of latent *Toxoplasma* can always occur in these patients [12]. Anti-cancer therapy is inhibited circulating anti-*Toxoplasma* antibodies (IgG + IgM) [13]. Other studies indicated that there was no a significant difference between anti-*Toxoplasma* seropositivity in the group of the patients on anti-cancer therapy compare with the group

which is off therapy [14, 15, 16, 17], this is could be due to the fact that the serum samples were taken within few days after the start of anti-cancer therapy before it exerts its immune suppression effect. Therefore, patients should be tested for anti-*Toxoplasma* antibodies at the time cancer diagnosis is established, then re-evaluate the serology tests within 2-4 weeks to detect the significant increasing of antibody index [18].

IL-12 is a perfect candidate for tumor immunotherapy due to its ability to stimulate both innate and adaptive immune responses against tumor cells [19]. The results show low serum level of IL-12 in untreated women with Breast cancer infected with Toxoplasmosis compare with treated women with Breast cancer infected with Toxoplasmosis with statistically significant differences ( $P < 0.01$ ). According to other study the differences on the serum levels of IL-12 was due to the effects of chemotherapy in breast cancer patients. There was no significant difference in the IL-12 at presentation and after chemotherapy [20], moreover, in order to elucidate the role of chemotherapy, we also evaluated the IL-23 serum levels after treatment. This study showed that the serum level of IL-23 in untreated women with Breast cancer infected with Toxoplasmosis was raised compare with treated women with Breast cancer infected with Toxoplasmosis. This finding agree with other study showed lightly decreased IL-23 levels after chemotherapy in corectal cancer patient [21]. IL-23 is defined as a cancer-associated cytokine because it induces tumor incidence and growth. Additionally, IL-23 not only stimulates macrophage and neutrophil infiltration, but also enhances the inflammatory mediators in the tumor microenvironment. IL-23 antagonizes IL-12 and IFN- $\gamma$ , which are crucial Cytokines for cytotoxic immune responses, and controls the inflow and activity of anti-tumor effector lymphocytes [22].

The immunocompromised patients (including HIV/AIDS patients, cancer patients, and transplant recipients) were associating with higher odds of *T. gondii* infection. Therefore, a routine serological screening test for *T. gondii* infection is recommended to be conducted in immunocompromised individuals in the endemic area [23]. Hence, it would be desirable that the antibody status of patients be known before, during and after chemotherapy [24].

**Conclusions**

Taking together, the results of this study suggest that the Toxoplasmosis antibody and Cytokines status of women with Breast cancer could be affected by the chemotherapy thus, the Toxoplasmosis antibody and Cytokines status should be known before, during and after chemotherapy. Hence, establishing a link between human Toxoplasmosis and tumors could give the cancer patients infected with Toxoplasmosis a chance to decrease morbidity and increased survival rates.

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