Correlation of Toxoplasmosis Seroprevalence and Serum Level of Interleukin-10 in Iraqi Breast Cancer Women

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Abstract

Background: Toxoplasmosis is regarded as one of the most important global life-threatening diseases in immune-compromised people. The intracellular protozoon *Toxoplasma gondii* is the causative pathogen of toxoplasmosis. Aim of this study is to investigate the possible association between *T. gondii* infection and breast cancer (BC) in Iraqi women, also to assess the effect of *T. gondii* on interleukin 10 (IL-10) of the immune response. By ELISA method, blood samples from 81 women with breast cancer and 60 apparently healthy women have been examined for presence of anti-*toxoplasma* antibodies, also the levels of serum IL-10 were estimated in these subjects. Results showed that women with BC had the highest prevalence rate of toxoplasmosis. The anti- *T.gondii* antibodies was 38.27% for IgG and 4.93% for IgM, while in the control group it was 26.66% and 0.0% respectively, with no significant difference for IgG (P =0.148) and IgM (P = 0.081). A highly significant difference was found in the level of IL-10 between BC women with toxoplasmosis (10.24%) and BC women without toxoplasmosis (3.6%). These findings suggest that incidence rate of *T. gondii* infection is higher in breast cancer women and higher level of IL-10 than their cross-matched control subjects. Therefore, we recommend the inclusion of a screening test for toxoplasmosis and the level of IL-10 in BC patients as in their routine workup.

***Keywords**: Breast cancer, ELISA, interleukine-10, Iraq, Toxoplasma gondii*

Introduction

Toxoplasma gondii is an obligate intracellular coccidian parasite widely distributed in the world [(1)]. In immunocompetent individuals, the infection with *T. gondii* is generally controlled by the immune system and often pass unnoticed, however, it is life threatening in the immunocompromised individuals [(2,3)] . Symptoms of the infection often involve cancer patients [(3)] .

The parasites are linked to various cancers, which may interfere with the host cell's genetic machinery and may result in immune system impairment and chronic inflammations [(4,5)] .

Toxoplasmosis is regarded to play an important role in malignancy induction and must be further investigated as a probable oncogenic pathogenic agent to human-beings [(6)].

Many studies indicated that seroprevalence of toxoplasmosis is significantly higher in cancer patients having chemotherapy including breast cancer, HIV/ AIDS and organ-transplant recipients than non-cancer patients [(7- 9)].

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The most common malignancy in women is breast cancer [10,11] . Breast cancer is one of the most warning to females health issues in Iraq, as it is the main cause of women's mortality after cardiovascular diseases, with a mortality rate of 23% [12]. Despite new diagnostic and treatment options, 30% of women diagnosed with invasive breast cancer will develop metastatic disease [13] ; therefore, early detection is critical for patient survival. Development of new therapeutic strategies and prognostic methods could be assisted by measuring and interpreting Th2 cytokine levels in BC patients [14].

The role of IL-10 in breast cancer is controversial as a result of various IL-10 studies in breast cancer [15,16] . It has been shown to exhibit both tumor promoting and -inhibiting properties [17], it inhibits the pro-inflammatory functions of antigen-presenting cells (APCs) through expression of antagonizing costimulatory molecules. Its low expression is associated with poor survival outcome [18].

The prevalence of toxoplasmosis among Iraqi cancer women has not been adequately studied. So, in our work, we aimed to find the possible correlation between *toxoplasmosis* and breast cancer, also to assess the effect of *T. gondii* on the levels of IL-10.

Materials and Methods

The current cross-sectional study was performed over the period from February 2019 to November 2019 on blood samples collected from a total of 141 subjects.

The all patients were females recruited from the oncology teaching hospital in the medical city hospital and Emamayn Al-Kadhimayn medical city in Baghdad, after approval of University of Baghdad/ College of Pharmacy ethical committee and with their consent in participating in the study. The patients and healthy (control) women have been of different geographic residencies and different age groups, ranged between 19 -70 years old. The mean ages were 44.5y. and 43.2y. respectively..

From all the study groups (patients and controls), 5 ml of venous blood were collected in plain tubes, and centrifuged for 5 minutes at 3000 rpm to obtain serum which was placed in two Eppendorf tubes and stored at -20º C.

To find the possible correlation between toxoplasmosis and BC by examining the seropositivity rate of the anti-*T. gondii* antibodies, participants were divided into two groups:

- Group [1]: includes 81 BC women (treated with chemotherapy).
- Group [2]: composed of 60 healthy subjects who have no malignancy (according to clinical examinations).

The enzyme immunoassay technique was used to detect IgG and IgM levels with the Biorex diagnostics LTD (Antrim technology park, UK) kit.

To estimate the role of IL-10 in Iraqi breast cancer women, participants were further divided into four groups:

- Group A: patients having breast cancer and have *T.gondii* infection.
- Group B: patients having breast cancer and not have *T.gondii* infection.
- Group C: apparently healthy individuals not known to have malignancy and have *T.gondii* infection.
- Group D: apparently healthy individuals not known to have malignancy and not have *T.gondii* infection (referred to as control group).

The serum IL-10 levels were measured by using the Enzyme–linked immunosorbent assay (ELISA) Kits from Elabscience, Chinese company.

Statistical Analysis

In this study, the Chi-square was used to compare between percentages, and the least significant difference (LSD) test was used to compare between means. The comparison of significance (P-value) in any test was: S= Significant ($P<0.005$). HS= Highly Significant ($P<0.001$). NS= Non Significant ($P>0.005$).

Results

IgG & IgM Toxoplasma antibodies determination

The results showed that the positivity rate of *T. gondii* IgM and IgG antibodies was higher in patients

in comparison with the control group women. The prevalence rate of anti- *T.gondii* antibodies was 38.27% for IgG and 4.93% for IgM in BC women included in our study. The positive cases for IgM were positive for IgG antibodies concurrently. The control group showed 26.66 % and 0.0 % in the same order of anti- *T.gondii* antibodies (Table 1), with no statistically significant difference for IgG (P =0.148) and IgM (P = 0.081).

Table 1: Distribution of anti-*T. gondii* antibodies using ELISA method in breast cancer patients and healthy females.

anti-*Toxoplasma* antibodies	Breast cancer women (n=81)		Healthy women (n=60)		Chi-square	P-value
	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)		
IgG	31 (38.27)	50 (61.72)	16 (26.66)	44 (73.33)	2.089	0.148
IgM	4 (4.93)	77 (95.06)	0 (0.0)	60 (100.0)	3.049	0.081
IgG+ IgM	4 (4.93)	77 (95.06)	0 (0.0)	60 (100.0)	3.049	0.081

The mean difference is significant at the 0.05 level.

The mean titers of anti-*T. gondii* IgG antibody in groups 1 and 2 were 32.6 IU/mL and 22.5IU/mL, respectively, with no significant differences in mean titers between the studied cases. The current study recorded a higher seroprevalencerate of toxoplasmosis in women with BC.

Moreover, in the 4 positive women for *T. gondii*, cancer was metastasized to liver and bone. The IgG titers ranged between 19.4 and 44.6 IU/mL, with one of the cases being positive for IgM and IgG antibodies, simultaneously, and her cancer was metastasized to lung in addition to liver and bone.

Estimation of the level of IL-10

Considering serum IL-10 concentrations for BC patients infected with toxoplasmosis, the mean value was higher than other groups (35.03 pg/ml), as shown in Fig.1.

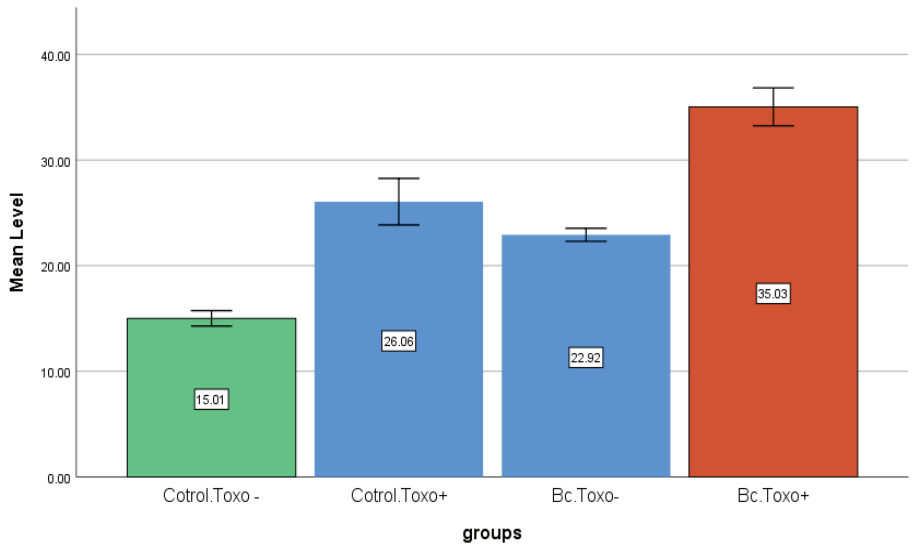

Figure (1): serum levels of IL-10 in studying groups according to *T.gondii* infection

Such difference was highly statistically significant (p value < 0.001) as shown in table 2. Non significant difference was appeared in the relation of control group with toxoplasmosis and BC group without toxoplasmosis (table 2).

Table (2): IL-10 serum levels in studying groups according to toxoplasmosis infection

			Multiple comparisons dependent variable LSD	
Groups	**Number**	**Mean ± S.E.(pg/ml) of IL-10**	**groups**	**p- value**
Control Toxo -	44	15.01 ± 0.72	Control Toxo +	0.000*
			BC Toxo -	0.000*
			BC Toxo +	0.000*
Control Toxo +	16	26.05 ± 2.20	Control Toxo -	0.000*
			BC Toxo -	0.105
			BC Toxo +	0.000*
BC Toxo -	50	22.92 ± 0.61	Control Toxo -	0.000*
			Control Toxo +	0.105
			BC Toxo +	0.000*
BC Toxo +	31	35.03 ± 1.79	Control Toxo -	0.000*
			Control Toxo +	0.000*
			BC Toxo -	0.000*

*. The mean difference is significant at the 0.05 level.

Discussion

It is assumed that when people previously get infected with chronic toxoplasmosis and are then infected with any type of cancer, there will be a high possibility of the reactivation of latent infections together with the opportunity of that cancer to become high and more aggressive [19,20] .

Current study investigated the seroprevalence rate of *T. gondii* in breast cancer women in order to find out any possible association between this infection and breast cancer. It have shown that women with BC have higher rate of both IgG and IgM antibodies for toxoplasmosis infection than do no BC women, but with non-significant difference, as shown in table 1. Although, *T.gondii* has been considered as an opportunistic infection, there was no association between a history of Toxoplasma infection and breast cancer..This finding was in line with previous studies in Iran [21,22] , but in contrast with the studies done in Egypt and China, which found a highly significant deference between the two groups [23,24] .

To control and clear up parasitic infections, human's immune system plays an essential roles [25]. The present study found that women with toxoplasmosis in case of presence or absence of BC, were secreted higher amounts of IL-10 than did the groups with no toxoplasmosis (table 2 and figure 1). However, the group of BC women

with toxoplasmosis showed higher level of IL-10 than all other groups with highly significant difference.

IL-10 has the ability for deactivate macrophage deactivation, IFN-γ induction by *T. gondii* as well as intracellular parasite survival facilitation. IL-10 can induce immune suppression during *Toxoplasma gondii* invasion, which is useful for the parasite and its host [26] . The elevated IL-10 levels in toxoplasmosis patients may be attributed to the parasite's ability to TH2 cytokine enhancement [27], or to induction of the products secreted at the stage of cell lysis by tachyzoites for the differentiation of naive B cells to IL-10-producing B regs [28].

Interleukins are confirmed to be able to contribute in BC proliferation, establishment and migration [29] . IL-10 acts as prognostic factor and even determines the survival rate of breast cancers [30]. Moreover, gene polymorphisms of IL-10 may determine the susceptibility for breast cancer [17].

This study has limitations, such as the small sample size, and no information was available at the time of first *T. gondii* infection and cancer induction among the studied cases. This study suggested that further studies should be performed to establish any association between *T. gondii* and breast cancer.

Conclusion:

Present study found higher rates of *T.gondii* seropositivity and highly significant difference in the IL-10 levels in BC women compared with healthy women. Therefore, we recommend the inclusion of a screening test for toxoplasmosis even it was non significant association and use the IL-10 as a laboratory tool which may serve as complementary tumor marker and contribute to the differential diagnosis in BC patients.

Acknowledgment: The authors would like to thank the oncology teaching hospital in the medical city hospital and Emamayn Al-Kadhimayn medical city for assistance to do specimens collection. Our gratitude also goes to Dr. Salma Sultan for her assistance in data statistical analysis.

Conflict of Interests: The authors declare that they have no competing interests. This research received no funding from any party.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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