Assessment of Soluble PD-1 and PD-L1 in Iraqi Women Patients with Breast Cancer with Toxoplasmosis

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Abstract

Toxoplasma gondii is an obligate apicomplexan intracellular protozoan parasite and considered the most common global parasite which infects a wide range of warm-blooded animals and is the etiological agent of one of the most common parasitic infections in humans. Breast cancer is the most common cancer in women worldwide; nearly 1.7 million new cases were diagnosed in 2012, making it the second most common type of cancer. The main objective of the present study was to investigate the sero-prevalence of the anti-*Toxoplasma gondii* IgG antibodies in Iraqi breast cancer patients and to clarify the role of soluble programmed death-1 (sPD-1) and (sPD-L1) in Iraqi Iraqi breast cancer patients with toxoplasmosis. Enzyme Linked Immunosorbent Assay (ELISA) was used to detect anti- *T. gondii* IgG antibodies in the sera of 108 patients with breast cancer and 50 apparently healthy controls. The results showed that 26(26%) samples of sera patients have been founded breast cancer with toxoplasmosis, 80(74%) samples have breast cancer, 10(20%) cases have control toxoplasmosis (those patients were had toxoplasmosis but showing no symptoms) and 40 (80%) cases samples were considered as a control group without any infections. Sera (sPD-1 and sPDL-1) levels were determined by ELISA using a quantitative sandwich enzyme immunoassay technique. The results showed that levels of sPD-1 and sPDL-1 levels were significantly higher in patients group than healthy subjects (P<0.01).

Keywords: *Toxoplasma gondii, Toxoplasmosis, Breast cancer, sPD-1 and sPDL-1*

Introduction

Toxoplasma gondii is an obligate intracellular coccidian parasite distributed widely throughout the world. The most common form of infection in humans is asymptomatic, but it causes opportunistic infections in immune compromised individuals. Symptomatic infection is usually characterized by lymphadenopathy and reticular cell hyperplasia [(1)].

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Cancer constitutes an enormous burden on society affecting both developing and developed countries and based on GLOBOCAN estimates [(2)], about 14.1 million new cancer cases and 8.2 million deaths occurred in 2012 worldwide. The occurrence of cancer is increasing because of the growth and aging of the population, as well as an increasing prevalence of established risk factors such as smoking, overweight, and physical inactivity [(3)].

Breast cancer is abnormal growth of cells and ducts lining the breast branches. The growth of these cells is random and uncontrollable and has the ability to spread to tissues, cells and other organs

of the body. Both men and women can have breast cancer. Studies have shown that breast cancer is rare in men. Breast cancer is the most common cancer in women worldwide; nearly 1.7 million new cases were diagnosed in 2012, making it the second most common type of cancer[4].

Toxoplasmosis is also considered to have a role in cancer induction. For instance, a study reported that the risk of brain cancer in human's increases in patients with *T. gondii* infection, and another study showed that the mortality rates positively correlated with the seroprevalence of *T. gondii* [5]. The latter study suggested that *T. gondii* should be further investigated as a possible oncogenic pathogen to humans [5].However, there are several studies indicating that the seroprevalence of toxoplasmosis is significantly higher in patients with cancer than non-cancer patients, including breast cancer [6-7].

Toxoplasma gondii is seen in some malignancies like lymphoma, acute and chronic myeloma. Recent studies show that *Toxoplasma gondii* antibodies can be seen in women with breast and ovarian cancer. Although the mechanism behind this is unknown, but there is no doubt that Toxoplasmosis paves the way for tumor development [8].

In Iraq, a recent study [9] showed that the proportion of breast cancer in females was 33.81%. In comparison with the other Arabic countries, the rate reported by Al-Hashimi and Wang [9] was very similar to that reported in Lebanon but lower than that observed in several Arab neighboring countries such as Turkey and Iran [10].

PD-1 is a trans membrane glycoprotein type I with 50~55 kDa molecular weight and composed of 288 amino acids [11]. Human PD-1 proteins have 60% homology with murine PD-1 (mPD-1) [12]. This cell surface monomer protein is an inhibitory receptor [13] and belongs to the Ig superfamily [14], specifically the CD28 cytotoxic T lymphocyte antigen-4 (CTLA-4) family [15].

Human PD-L1 belongs to the B7 family; the PD-L1 has an important role in immune evasion by tumor cells and can enhance tumor cell growth by promoting apoptosis among antigen-specific and tumor-reactive T cells [16]. PD-L1 also is necessary in maintaining immune homeostasis in normal physiological condition. This ligand down regulates cytotoxic T cell activity when it binds to specific receptors on T cells and protects normal cells from collateral damage [17]. Thus, tumor cells expressing PDL1 can hinder activation of new T cells [18]. The main objective of the present study was to investigate the sero- prevalence of the anti-*Toxoplasma gondii* IgG antibodies in Iraqi women patients with breast cancer with Toxoplasmosis and to clarify the role of soluble programmed death-1 (sPD-1) and (sPD-L1) Iraqi women patients with breast cancer with Toxoplasmosis.

Materials and Methods

Subjects and Samples

This study was included 108 samples of patients with breast cancer attending to Babylon Cancer Center affiliated to Marjan Teaching Hospital in Babil Governorate, Iraq. During the period from November 2019 to June 2019. Out of this sample, a group of 50 healthy subjects were considered as control group. The age of all patients and healthy subjects were ranged from 20 – 79 year. Five ml of venous blood were collected from each subjects (patients and control) and placed in gel tube, the serum was separated and divided in ependorff tubes then stored at -20C° until it is used.

Serological tests

1- ELISA *T. gondi* – IgG : The sera of all samples (Patients and control) were tested with the presence

of specific IgG antibodies of *Toxoplasma gondii*, via ELISA kits which had supported by (Bioactiva Company, Germany) and applied the test according to the manufacturer's instructions.

2-Serum Level of PD-1: Serum levels of **PD-1** was measured by using specific enzyme-linked immunosorbent assay) ELISA) kit (R&D Company, USA), according to the manufactures protocol.

3-Serum Level of PD-L1: Serum levels of **PD-L1** was measured by using specific enzyme-linked immunosorbent assay) ELISA) kit (R&D Company, USA), according to the manufactures protocol.

Statistical Analysis

The Statistical analyses were done by Statistical Package for the Social Sciences for Science (SPSS) version 2010. The statistical tests was included Descriptive statistical tables, Mean, Standard Deviation, under $P>0.05$ and $P<0.01$ to considered statistically significant.

Results

In the summarize examine results, the study samples showed that 28(26%) samples of sera patients have been founded breast cancer with toxoplasmosis , 80(74%) samples have breast cancer, 10(20%) cases have control toxoplasmosis (those patients were had toxoplasmosis but showing no symptoms) and 40 (80%) cases samples were considered as a control group without any infections (Table1).

The cut– off value of positive IgG (1 IU/ml) in all studied groups. The results recorded in the table 1 were shown higher results of levels of IgG in breast cancer with toxoplasmosis group as ±1.66 0.55 IU/ml, followed by positive control group 1.64 ± 0.35 IU/ml, and negative control group with value 0.48 ±0.24 IU/ml, while breast cancer group presented low results of this antibody 0.33± 0.25 IU/ml.

Table 1: Levels of IgG antibodies (IU/ml) for all study groups.

Groups	No. of Samples	%	Mean ± SD.	Lower value	Upper value
Breast cancer with toxoplasmosis	28/108	26	±1.66 0.55	1.12	2.61
Breast cancer	80/108	74	0.25 ± 0.33	0.12	0.72
Positive control	10/50	20	0.35 1.64 ±	1.31	1.73
Negative control	40/50	80	0.48 ±0.24	0.15	0.82

Table (2) shows the comparisons in the means of the IgG among all studied groups, highly significant differences ($P < 0.01$) were registered when comparing the values of IgG for the patient's breast cancer with toxoplasmosis and breast cancer only, and negative control .

Table 2: Multiple comparisons of the IgG concentrations (IU/ml) for potential couples between studied groups.

Parameter	Group(1)	Group(j)	P-value	Sig.
IgG	Breast cancer with toxoplasmosis	Breast cancer	0.000	HS
		Positive control	0.748	NS
		Negative control	0.000	HS
	Breast cancer	Positive control	0.000	HS
		Negative control	0.657	NS
	Positive control	Negative control	0.000	HS
HS: Highly Significant at P< 0.01 ,; NS: No Significant at P> 0.05				

Table (3) showed the mean values of sPD-1 in all the groups, breast cancer patients has registered the highest value 111.39±27.36 pg/ml then breast cancer with toxoplasmosis patients 107.04±26.13 pg/ml, finally, positive and negative control groups has 106.46±10.82 pg/ml , 99.4±20.35 pg/ml respectively, also the table was referred to the highest and lowest response of sPD-1 levels.

Table 3 : Levels of sPD-1 (pg/ml) for all study groups.

Groups	No. of Samples	Mean± SD	Lower value	Upper value
Breast cancer with toxoplasmosis	28	107.04±26.13	85.41	141.54
Breast cancer	80	111.39±27.36	73.37	126.83
Positive control	10	106.46±10.82	81.42	122.31
Negative control	40	99.4±20.35	76.84	125.43

Table (4) referred to the differences of the means for sPD-1 among all studied groups, the results didn't record significant difference when comparing the level of sPD-1 in breast cancer patients with toxoplasmosis and the groups of breast cancer, positive and negative control respectively, while significant differences at probability of P<0.05 were recorded when comparing the breast cancer group with negative control.

Table 4: Multiple comparisons of the sPD-1 concentrations (pg/ml) for potential couples

Parameter	Group(1)	Group(j)	P-value	Sig.
sPD-1	Breast cancer with toxoplasmosis	Breast cancer	0.43	NS
		Positive control	0.95	NS
		Negative control	0.22	NS
	Breast cancer	Positive control	0.55	NS
		Negative control	0.02	S
	Positive control	Negative control	0.42	NS
HS: Highly Significant at P< 0.01; S: Significant at P< 0.05 ; NS: No Significant at P> 0.05				

between studied groups.

Table (5) showed high level of sPDL-1 in the positive control group 73.04 ±12.5 pg/ml compared to breast cancer group 71.48 ±13.32 pg/ml, negative control group 70.92 ±11.4 pg/ml and breast cancer with toxoplasmosis group 67.43±15.38 pg/ml respectively.

Table 5: Levels of sPDL-1 (pg/ml) for all study groups.

Groups	No. of Samples	Mean± SD	Lower value	Upper value
Breast cancer with toxoplasmosis	28	67.43 ±15.38	44.93	95.37
Breast cancer	80	71.48 ±13.32	45.37	105.23
Positive control	10	73.04 ±12.5	42.83	98.37
Negative control	40	70.92 ±11.4	41.28	108.63

Table (6) illustrates the differences between studied groups that found no significant differences (P>0.05) appear between a group of breast cancer with toxoplasmosis and breast cancer, positive, negative control.

Table 6: Multiple comparisons of the sPDL-1 concentrations (pg/ml) for potential couples

Parameter	Group(1)	Group(j)	P-value	Sig.
sPDL-1	Breast cancer with toxoplasmosis	Breast cancer	0.17	NS
		Positive control	0.25	NS
		Negative control	0.3	NS
	Breast cancer	Positive control	0.73	NS
		Negative control	0.83	NS
	Positive control	Negative control	0.65	NS
NS: No Significant at P> 0.05				

between studied groups.

Discussion

In people with normal immune system, the infection is usually self-limited, but the parasites can survive for years in the host body in the form of tissue cysts. During this phase, tissue cysts are controlled by humoral and cellular immune system, including T lymphocytes and macrophages [19]. People with immune compromised systems, especially those with higher chronic infection risk due to cellular immune deficiency, as well as patients with cancer, collagen tissue diseases, transplant recipients treated with immune suppressive drugs, or immune-deficient hemodialysis patients with chronic renal failure, are more susceptible to be infected with *T. gondii* [20].

The susceptibility to the infection with toxoplasmosis in immune compromised could be due to many reasons such as the geographical variation, customs, habits, difference in genetic susceptibility and the acquisition method of *Toxoplasma* infection [21, 22]. Persistent infections may promote cancer because long-term host defensive responses induce inflammation, which increases mutation rates [23].

IgG antibodies indicate chronic infection and an increased titer of IgG antibodies might show reactivation [24]. These chronic infections probably persist throughout the life and may remain undiagnosed until or unless it is reactivated as a result of severe immune suppression [25].

The parasitic infection with *T. gondii* considers the most frequent protozoan causing opportunistic infections in immunocompromised individuals. However, little is known about the epidemiology of *T. gondii* infection in patients who are immunocompromised that having immunosuppressive therapy [26] . Chronic inflammation commonly stimulates carcinogenesis and may prompt an individual to cancer [27]. The present study displayed that (26 %) of breast cancer patient and (10 %) of the control group were confirmed to be positive for *Toxoplasma* IgG antibodies.

Recently, Yuan et al. [28] conducted study to determine *T. gondii* antibodies in 42 Chinese breast cancer patients by using ELISA and they found (9.53%) positivity rates of *T. gondii* IgG in cancer

patients than the control individuals. Cong et al. [29] collected blood from 67 Chinese breast cancer patients to detect anti-*T. gondii* antibodies by ELISA and reported that the prevalence of anti-*T. gondii* IgG in breast cancer patients (35.8%) was significantly higher than that in controls (17.4%). Molan and Rasheed [26] conducted study to determine *T. gondii* antibodies in 106 Iraqi breast cancer patients by using ELISA and they found (56.6%) positivity rates of *T. gondii* IgG in cancer. Ahmed and Saheb [30] detected a study to determine the serum levels of *T. gondii* IgG antibodies in 80 Iraqi breast cancer and their results showed that the overall sero positivity rate was 77.5%. Assim and Saheb [31] detected a study to determine the serum levels of *T. gondii* IgG antibodies in 90 Iraqi breast cancer and their results showed that the overall sero positivity rate was 72.22%.

T cell exhaustion is a state of cellular hyporesponsiveness that occurs in response to continued antigen stimulation or inflammation, wherein T cells produce fewer cytokines and cytotoxic molecules, lower expression levels of activating receptors, and increased expression levels of inhibitory receptors [32]. T cell exhaustion was first characterized in chronic viral infection models but is now widely studied in cancer [33], bacterial infection [34], and parasitic infection models [35, 36] and is in part programmed response to limit immune pathology in these settings.

PD-1/PD-L1 pathway is found to play a key role in escape of cancer from immune surveillance,

with PD-1 expression seen on effector T-cells and exhausted T-cells in tumor microenvironment (TME)

and PD-L1 expression seen on cell surface in several types of cancers including bladder, lung, colon, breast, kidney, ovary, cervix, melanoma, glioblastoma, multiple myeloma and T-cell lymphoma [37].

Furthermore, no study has reported the detection of serum PD-1 and PDL-1 in breast cancer patients with toxoplasmosis at less in Iraq. In this study, it was found that soluble PD-1 and PDL-1 levels was increased in both the breast cancer with toxoplasmosis patients and breast cancer compared to healthy controls, suggesting that both the breast cancer with toxoplasmosis and breast cancer patients had immune suppression.

In this respect, there are no available literatures about the role of sPD-1 and sPDl-1 in toxoplasmosis. However, some studies have evaluated the serum level of sPD-1 during other infectious disease. These studies observed significantly higher levels of sPD-1 among patients with chronic HCV [38], *Echinococcus granulosus* ([39], pulmonary tuberculosis and those whom having active pulmonary TB with co-incidental *Strongyloides stercoralis* [40] infection than control subjects free from these diseases.

High level of sPD-1 among toxoplasmosis patients may be explained based on the mechanism of soluble sPD- 1 formation by proteolytic cleavage of the membrane-bound form and nature of *Toxoplasma gondii* as intracellular pathogen that inhibition of host-cell apoptosis is one of its survival strategies [41]. Soluble PD-l inhibits the PD-1/PD-L signaling pathway primarily via interacting with the cell surface molecules, PD-1/PD-L1 binding creating a negative signal and lead to inhibit the activation and proliferation of T cells. Thus, increase in sPD-1 level inhibits the PD-1/PD-L1 signaling pathway in T cells through negative feedback. Consequently, it reduces the inhibition of T cell activation and increases the activity of the immune system for managing CL. This supports the findings of Wang *et al*. [42] who suggested that sPD-1 blocks the membrane PD-1 binding site on activated T-cells, thereby attenuating the PD-1 signaling pathway and increasing the immune response.

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

Conflict of Interest: None

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