Prevalence of Toxoplasmosis Infection in Iraqi Women with Different Types of Cancer

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Abstract

Background: *Toxoplasma gondii* is an obligatory intracellular parasite that is consider a major invasive parasite in immunocompromised individuals.
Objective: To determined the prevalence of *Toxoplasmosis* in patients with different types of cancer in Iraq.
Patients and Methods: Samples of blood were gathered from 258 women who included 112 healthy controls samples and 146 samples with different types of cancer. They were attended Oncology Teaching Hospital in the Medical City Hospital in Baghdad province from October, 2016 to February, 2017. Then the sera were tested to determine the anti- T. *gondii* antibodies (IgG and IgM) using enzyme linked immunosorbent assay.
Results: The highest seropositive rate of T. *gondii* IgG were noted in patients with lymph node cancer followed by breast, colorectal, liver, pancreas, lung, ovary, prostate cancer which was (100%, 77.50%, 77.42%, 75.00%, 66.67%, 66.67%, 54.55%, 28.57%) respectively with significant differences (P<0.01). This study focused on breast and colorectal cancer. According to the age groups, the seroprevalence of anti- T. gondii IgG was the highest in the age group (26-35) years in patients with colorectal and breast cancer which was (378.309 IU/ml, 374.561 IU/ml respectively) compared with control group (148.917 IU/ml). In regard to the anti- tumor dosage, the highest mean titer of IgG observed in dosage (0), the mean titer of IgG in patients with colorectal and breast cancer whose were seropositive to anti-T. gondii IgG were (242.016 IU/ml and 227.275 IU/ml) respectively, while in seronegative patients to anti-T. *gondii* IgG were (8.594 IU/ml and 6.011 IU/ml) respectively.
Conclusion: These finding suggest that incidental rate of toxoplasmosis is higher in cancer patients. Thus, the incidental rate of toxoplasmosis could be considered as an indication to the high risk of cancer. In addition, anti- T. *gondii* IgG test has to be taken into consideration as markers for staging cancer disease.
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Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite. It is a coccidian parasite of cats with all warm blooded animals, humans consider as intermediate hosts [1]. The seropositivity level varies in different regions of the world, measuring between 30% and 60% in most countries [2]. T. *gondii* infection in healthy hosts is rarely symptomatic, but

toxoplasmosis occurred in immunocompromised individuals may result in a high risk of illness [3]. T. *gondii* triggers an innate immune response through neutrophils recruitment to the site of infection, followed by production of proinflammatory cytokines from Th1 [4]. Cell-mediated immunity plays a major role against parasitic infection caused by T. gondii. It is accompanied by the transformation of tachyzoites into tissue cysts (bradyzoites) which cause the chronic infection [5]. Two organelles, rhoptries and micronemes, are important organelle that secreting proteins during the invasion process [6]. After the cellular invasion, T. *gondii* resides within a vacuole imitative from the plasma membrane of the host cells. T. *gondii* multiply asexually [7]. Around three weeks post infection, resistance of individual develops and tissue cysts may form in numerous organs, primarily in brain and muscles. These quiescent cysts permit the parasite to evade the adaptive host immune. When the tissue cysts rupture, the released quiescent cysts are killed by the host immune system. If immune system becomes compromised, such as due to chemotherapy in cancer or AIDS, the bradyzoites develop into tachyzoites, causing active toxoplasmosis [8].

Cancer affecting both developing and developed countries [9]. The prevalence of cancer is rising due to the aging, smoking, and overweight of the population [10]. There are noticeable increase in the prevalence level of younger ages in Eastern Mediterranean regions [11]. However, little is known about the epidemiology of T. gondii infection in patients who are immunocompromised that undergoes neoplastic disease or immunosuppressive therapy and has received little attention in Iraq [12].

Patients and Methods

Two hundred-fifty eight women (112 healthy control samples, 146 samples with different kinds of cancer) were enrolled in this study. They were attended to Oncology Teaching Hospital in the Medical City Hospital in Baghdad province from October, 2016 to February, 2017. Samples of blood of 5ml were taken from vein of all women. The sample was collected in sterilized (Gel Clot activator vacuum tubes) and left for 30 minutes at room temperature for clotting. Then, the samples were centrifuged at 3000 round per minute (rpm) for 10 minutes for serum aspiration then dispensed into 3 eppendroff- tubes by using micropipette and stored at -20 ºC for future immunological analysis. ELISA kits (Acon Toxoplasma IgG EIA (I231-1091) and IgM EIA (I231-1101) was used to determine the anti- T. *gondii* antibodies (IgG and IgM) in cancer patients.

Statistical Analysis

Chi-square test was used to significant compare between percentages using the Statistical Analysis System- SAS (2012) [13]. In addition, least significant difference –LSD test was used to study the significant compare the means in this study.

Results

The incidental rates of anti T. *gondii* antibodies in diverse types of cancer disease.

The results showed that the highest seropositivity rates of anti-T. gondii IgG was observed in lymph node cancer (100%), followed by breast cancer patients (CRC) (77.50 %), colorectal cancer patients (77.42%), liver cancer patients (75.00%), lung and pancreas cancer (66.67%), ovary cancer (54.55%) whereas the lowest seropositivity rates observed in prostate cancer (28.57%). All groups had a significantly higher seroprevalence compare with the control group ($P < 0.05$, $P < 0.01$).

Table (1): The serological examination of anti T. gondii antibodies in diverse types of cancer.

Cancer kinds	Samples No	IgG(+)		IgG(-)		IgM(+)		IgM(-)	
		No	%	No	%	No	%	No	%
CA. Breast	80	62	77.50	18	22.50	0	0.00	80	100
CA. CRC	31	24	77.42	7	22.58	0	0.00	31	100
CA. Ovary	11	6	54.55	4	45.45	1	9.09	10	90.91
CA. liver	8	6	75.00	2	25.00	0	0.00	8	100
CA. Prostate	7	2	28.57	5	71.43	0	0.00	7	100
CA. Pancreas	3	2	66.67	1	33.33	0	0.00	3	100
CA. Lung	3	2	66.67	1	33.33	0	0.00	3	100
CA. Lymph node	3	3	100	0	0.00	0	0.00	3	100
Total	146	108	73.97	38	26.03	1	0.68	145	99.32
Healthy control	112	23	20.54	89	79.46	0	0.00	112	100
Chi-square	---	9.63 **		9.63 **		4.39 *		4.39 *	
* (P<0.05), ** (P<0.01).									

Compare IgG levels in different ages of studying groups: Table (2) according to the age groups, the seroprevalence of toxoplasmosis was the highest in the age group of (26-35) years in patients with colorectal cancer (CA.CRC) and breast cancer (CA. Breast) (378.309 IU/ml and 374.561 IU/ml) respectively, statistically were found significant differences (P<0.01) between patients and healthy control which was (148.917 IU/ml).

Table (2): Compare IgG levels in different ages of studying groups.

Age (year)	Healthy control IgG (IU/ml)		CA. Breast IgG (IU/ml)		CA.CRC IgG (IU/ml)		P-value
	Toxo(-)	Toxo(+)	Toxo(-)	Toxo(+)	Toxo(-)	Toxo(+)	
15-25	1.732	129.749	3.825	173.24	11.774	220.249	0.0001 **
26-35	1.283	148.917	3.939	374.561	4.961	378.309	0.0001 **
36-45	3.732	149.871	3.163	210.176	3.1444	330.521	0.0001 **
P-value	0.0267 *	0.099 NS	0.085 NS	0.0001 **	0.0052 **	0.0001 **	----
*(P<0.05), ** (P<0.01), NS: Non-significant.							

Compare IgG levels between different anti-cancer dosages in CA. Breast and CA. CRC: Table (3); in regard to the anti- tumor dosage, the highest mean titer of patients with CA. CRC and CA. Breast whose are seropositive to anti-T. gondii IgG was in dosage (0) which was (242.016IU/ml, 227.275IU/ml) respectively, followed by dosage (3) and (6). While the mean titers of patients with CA. CRC and CA. Breast

whose are seronegative to anti-T. gondii IgG in the dosage (0) was (8.594IU/ml and 6.011IU/ml) respectively. There were significant differences (P< 0.01). In comparison between CA. Breast and CA.CRC patients whose are seropositivite to anti-T. gondii IgG, the results shown the higher mean titer was in CA. CRC patients with dosage (0) followed by CA. Breast patients with dosage (0). There were significant differences (P< 0.01).

Table (3): Compare IgG levels between different anti-cancer dosages in CA. Breast and CA. CRC.

Dosage	CA. Breast IgG (IU/ml)		CA. CRC IgG (IU/ml)		P-value
	Toxo (-)	Toxo (+)	Toxo (-)	Toxo (+)	
Dosage0	6.011	227.275	8.594	242.016	0.0001 **
Dosage3	3.524	129.929	5.302	218.12	0.0001 **
Dosage6	3.371	96.211	3.485	101.477	0.0001 **
P-value	0.0057 **	0.0001 **	0.0062 **	0.0001 **	---
** (P<0.01).					

Discussion

This study highlighted a possible association between T. *gondii* infections with different types of cancer. The results showed that the highest seropositivity rates of anti-T. *gondii* IgG was observed in lymph node cancer (100%), followed by breast cancer patients (77.50 %), colorectal cancer (77.42%), liver cancer patients (75.00%), lung and pancreas cancer (66.67%), ovary cancer (54.55%) whereas the lowest seropositivity rates obsrerved in prostate cancer (28.57%). All groups had a significantly higher seroprevalence compare with the control group (P < 0.05, P < 0.01). The positivity rates of anti-T. *gondii* IgM were 1(0.68%) in cancer patients while there was no positivity rates for anti-T. gondii IgM among the control group. Several studies have showed that the incidence rate of anti-Toxoplasma IgG in patients with breast cancer was 60% and anti-Toxoplasma IgM 0% [14]. Patients under chemotherapeutical treatment affecting absence or delay of IgM antibody production in cancer patients [15]. High levels of IgG and the absence of IgM antibodies are correlated with chronic latent infection which acquired in the past. T. *gondii* was higher in a population with cancer compared with population without cancer infection. This finding agrees with other studies [16-18]. This results may be due to the fact that patients with malignant tumor are immunocompromised, this led to increases their susceptibility to this parasitic infection [12, 18-20]. The breast cancer and colorectal cancer groups showed higher seropositivity rate than other caner groups, this finding agrees with the findings of other study [12]. Chronic inflammation commonly stimulate carcinogenesis and may prompt an individual to cancer [21, 22].

This study demonstrated that the seroprevalence rate of toxoplasmosis increase in the limit age in cancer patients, other studies demonstrated that the seroprevalence rate of toxoplasmosis increase with age [23, 24]. Another study concluded that age is a critical factor for both breast cancer and toxoplasmosis which are more prevalent in women aged over 40 years [14]. T. *gondii* potentially increases the risk of brain cancer in humans or in adult patients with brain cancer aged 55 years or older [14, 23,25-27]. T. *gondii* infection has been reported that most women get infection before 25 years old [14, 28]. In addition, age is an important

associated factor in the epidemiology of Toxoplasma infection. Also, the incidence infection of breast cancer increases with age during the reproductive years [29]. Several studies have shown that the lowest positive rates were found in age <10 years and the infection rates were gradually increased with age, along with the peak level shown in >51 years in patients group and 41-50 years in control group [24]. Immunosuppressive patients are exposed to different risk factors, which might expose them to Toxoplasma infection [24]. This study demonstrated that Toxoplasma infection in cancer patients increase in age (26-35), this result is similar to that shown with a previous studies [20, 30]. It was shown that toxoplasmosis acquired in early life and the incidence increases with age and drop in later life [31].

In comparison between CA. Breast and CA.CRC patients whose are seropositivite to anti-T. *gondii* IgG, the results showed that the higher mean titer was in CA. CRC patients with dosage (0) followed by CA. Breast patients with dosage (0). There were significant differences (P< 0.01). Several studies demonstrated that the prevalence rate of anti- T. *gondii* IgG in patients under treatment and regular checkups was higher than newly diagnosed patients, but this difference was not statistically significant [14,15, 24]. Several factors could interfere with the anti-Toxoplasma antibodies production such as being under anti-cancer drug therapy which lead to change the production of circulating antibodies, and may decrease the titer to irrelevant levels [32]. These factors explain the absence or delay of a recent response of IgM antibody production in the cancer patients in the present study.

Moreover, patients being treated with anti-cancer drugs for solid malignant tumor such as the breast, ovary and lung have been associated with toxoplasmosis [33, 34]. On the other hand, patients under immunosuppressive therapy who had been previously infected with T. *gondii*, might display an altered serological response for this parasite compatible with reactivation, such as increased IgG antibody titers or, less frequently, increased titers of acute-phase antibodies thus, the patients with cancer undergo acute reactivation [18, 35, 36]. Patients have been treated with corticosteroids and cytotoxic agents which reduced immune system response and lead to reactivation of the dormant parasite [15]. Consequently, T. *gondii* has shown to be able to activate cellular immunity in the animals [37]. The high seroprevalence of T. gondii in cancer patients indicates a significant danger because the latent Toxoplasma infection may be prompted long term chemotherapy leading to the compromised immunity of the patients [30]. In patients with cancer, immune function is impaired and this is the main reason for the increase of Toxoplasma antibodies. This study disagree with other study concluded that toxoplasmosis infection can be due to the use of anti-cancer drugs in patients with Lymphoma, leukemia, malignant tumors and patients with breast, ovarian and lung tumors [38].

These finding suggests that incidental rate of toxoplasmosis is higher in cancer patients and the levels of IgG titer increase in untreated patients. Thus, the incidental rate of toxoplasmosis could be consider as an indication to the high risk of cancer due to the fact that the latent Toxoplasma infection may be trigger long term chemotherapy leading to the compromised immunity of the patients. In addition, anti- T. *gondii* IgG test has to be taken into consideration as markers for staging cancer disease.

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