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Original Article

Serum Levels of Il-12 and Il-23 in Breast Cancer Patients Infected with *Toxoplasma gondii*: A Case-Control Study

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Abstract

Background: The possible risk factor of *Toxoplasma* infection and its possible correlation with Interleukin-12 (IL-12) and Interleukin-23 (IL-23) in breast cancer patients was investigated.

Methods: Overall, 190 female patients referred to the Oncology Teaching Hospital in the Medical City Hospital, Baghdad, Iraq were enrolled from 2017-2018. All serum samples were tested for *T. gondii* immunoglobulins (IgG and IgM)antibodies and IL-12, IL-23 levels.

Results: In patients with breast cancer, the results revealed a high positivity percentage for anti- *Toxoplasma* IgG. In breast cancer patients infected with *T. gondii,* the IL-12 level was lower than the controls while the mean level of IL-23 was higher than the controls. According to the cancer grade in breast cancer patients infected with *T. gondii*, the higher mean titer of IgG and IL-23 was in grade 3 in contrast the highest mean titer of IL-12 was in grade 1. Concerning the tumor stages in breast cancer patients infected with *T. gondii*, the higher mean titer of IgG, IL-12 and IL-23 was in stage (III). According to the tumor size in breast cancer patients infected with *T. gondii*, the highest mean titer of IgG, IL-12 and IL-23 was in size >3cm.

Conclusion: The levels of IL-23 could be a candidate as a non-invasive primitive marker for earlier prediction of breast cancer stage.

Introduction

Toxoplasma gondii is a coccidian protozoan parasite in man and animals (1). Toxoplasmosis can cause serious disease in patients with deficiencies in T cell function such as in AIDS patients, Hyper IgM Syndrome, cancer and transplant patients receiving immunosuppressive drugs (2). Latent infections of *T. gondii* reactivate in im-

munocompromised individuals (3, 4). This infection stimulates macrophage to secrete different cytokines such as TNF-α, IL-1, IL-12, and IL-23. IL-12 is required for resistance to acute and chronic toxoplasmosis due to its fundamental role in stimulating the production of IFN-γ (5-7). IL-12 plays the predominant role in resistance to toxoplasmosis, but in the absence of IL-12, IL-23 can supply a restricted mechanism of resistance to this infection (5).

The relationship between cancer and inflammation has been well established and many cancers originate at the site of chronic inflammation and inflammatory mediators are often produced in tumors (8, 9). Breast cancer is the widespread common cancer and the leading cause of cancer mortality among women in the world and there was an intense increase in breast cancer worldwide (10, 11). The high incidence of breast cancer have stimulated the need for new markers for earlier detection of metastases and better prognosis prediction (12). In recent decades, a lot of attention has been focused on the role of cytokines in breast cancer. Some cytokines stimulate breast cancer proliferation and some others modulate anti-tumor response (13). The IL-12 family of cytokines includes IL-12, IL-23, IL-35 and IL-27 (14). IL-12 is a proinflammatory, Th1-inducing and Th1-maintaining cytokine that links innate and acquired immunity (15). Th17 inflammatory cell populations are maintaining through IL-23 (16) thus, IL-23 could be an indirect marker of Th17 cells existence (17). The cytokines concentrations could be used as a marker for cancer prognosis (18).

The prognostic factors include the axillary lymph node status, the tumor size, and the nuclear grade and histological grade were significant indicators of breast cancer (19, 20). The assessment of histologic grade is a significant component in the evaluation of breast cancers and regard as a required parameter in the pathologic reporting of breast cancers (21). Breast tumors are divided into grade 1 (G1; well-differentiated), grade 2 (G2; moderately differentiated), and grade 3 (G3; poorly differentiated, highly proliferative) malignancies (22).

The immunocompromised persons are correlating with higher chances of *T. gondii* infection and most of these patients do not have detectable symptoms, thus we aimed to investigate the serum levels of IL-12 and IL-23 in breast cancer patients infected with *T. gondii*.

Materials and Methods

Subjects and Blood Collection

The study was permitted by the Ethical Committee of Iraqi Ministry of Health, in which 190 women were enrolled from Oct 2017 till Feb 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 the Medical City Hospital in Baghdad from different governorates in Iraq). Their ages range from 21-50 years. Some information such as cancer grade, stage, and size was gathered from each woman.

Samples of 5 ml blood were taken from women's vein. The samples were collected in sterilized Gel Clot activator vacuum tubes and left for 30 min at room temperature for clotting. Then, the samples were centrifuged at 3000 round per minute (rpm) for 10 min then dispensed into Eppendorf- tubes and stored at -20 ºC until the test day.

Serological Tests

Specific IgG antibodies were measured using commercial *Toxoplasma* IgG and IgM EIA Test Kit (ACON Laboratories, Inc. USA) (I231-1091) (I231-1101) based on the principle of ELISA capture (23), according to the manufacturer's instructions. The serum IL-12 and IL-23 levels were measured using the commercial ELISA kits SHANGHAI human IL-12 kits (Cat. No: YHB1704Hu) and the CUSABIO human IL- 23 kits (Cat. No: CBS-E08461h) according to the manufacturing

guidelines. The measurement range of the assay for IL-12 is 0.2 - 60 pg/ml and IL-23 is 6.25-400 pg/ml.

Statistical Analysis

Chi-square test was used to study the significant compare of the percentages and least significant difference –LSD test was used to significant compare between means in this study. Results are expressed as mean ± standard error of the mean. A *P*-value of less than 0.05 was considered significant.

Results

T. gondii seropositivity in control group and breast cancer patients

In this study all the samples were tested for anti-*T. gondii* IgG and IgM seroprevalence however, there were no positivity rates for anti-*T. gondii* IgM in breast cancer patients. Thus, the infection phase of *T. gondii* for all the results of this study was chronic (IgG seropositive).

The highest rate of toxoplasmosis in control group was in the age group 41-50 yr which was (45%), followed by age group 21-30 and 31-40 yr with (44%) and (40%) respectively (Table 1). The highest rate of toxoplasmosis in breast cancer patients was in the age group 41-50 yr which was (77.42%), followed by age group 31-40 and ۲۱-30 yr with (72.97%) and (63.64%) respectively (Table 2).

Table 1: The incidental rate of toxoplasmosis in control group with different ages

Age(yr)	Samples No.	%	Toxo(+)		Toxo(-)		Chi-square
			No.	%	No.	%	
۲۱-30	٥٠	50	22	44	28	56	5.027 *
31-40	٣٠	30	12	40	18	46.6	7.250 **
41-50	٢٠	20	9	45	11	55	4.392 *
Total	١٠٠	100%	43	43	57	57	5.419 *
Chi-square	---	9.066 *	---	2.077 NS	---	2.077 NS	---

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

Table 2: The incidental rate of toxoplasmosis in breast cancer patient with different ages

Age (yr)	Samples No.	%	Toxo(+)		Toxo(-)		Chi-square
			No.	%	No.	%	
۲۱-30	22	24.44	14	63.64	8	36.36	9.41 **
31-40	37	41.11	27	72.97	10	27.03	11.56 **
41-50	31	34.44	24	77.42	7	22.58	13.08 **
Total	90	100%	65	72.22	25	27.78	11.83 **
Chi-square	---	7.02 **	---	5.17 *	---	5.17 *	---

* (*P*<0.05), ** (*P*<0.01).

Anti- Toxoplasma IgG antibodies, IL-12, and IL-23 levels in breast cancer patients and controls

The present results revealed high positivity percentages of anti-*Toxoplasma* IgG in breast cancer patients with mean titer (266.36±18.52 IU/ml) compared with controls who are seropositive to anti-*Toxoplasma* IgG with mean titer (127.58±11.49 IU/ml) with a statistically significant differences (*P*<0.01). The mean level of IL-12 in breast cancer patients infected with toxoplasmosis was (11.52±0.73 pg/ml), being lower than the mean value in controls infected with toxoplasmosis which

was (21.08±1.26 pg/ml) with a statistically significant differences (*P*<0.01). Furthermore, the mean level of IL-23 in breast cancer patients infected with toxoplasmosis was (297.79 ± ١٦,٠٨ pg/ml), being higher than the mean value in controls infected with toxoplasmosis which was (149.79±9.54 pg/ml) with a statistically significant differences (*P*<0.01) (Table 3).

Table 3: Anti-*Toxoplasma* IgG antibodies, IL-12, and IL-23 levels in breast cancer patients and controls

Group	*Toxo (-)*	*Toxo (+)*	*P-value*
IgG (IU/ml)			
Control	3.20 ± 0.15	127.58 ± 11.49	0.0001 **
Breast cancer	2.13 ± 0.06	266.36 ± 18.52	0.0001 **
P-value	0.0772 NS	0.0082 **	
IL-12 (pg/ml)			
Control	8.17 ± 0.52	21.08 ± 1.26	0.0001 **
Breast cancer	6.35 ± 0.44	11.52 ± 0.73	0.0001 **
P-value	0.219 NS	0.0073 **	
IL-23 (pg/ml)			
Control	41.10 ± 2.56	149.79 ± 9.54	0.0002 **
Breast cancer	161.48 ± 11.83	297.79 ± 16.08	0.0364 *
P-value	0.0001 **	0.0026 **	

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

Anti- Toxoplasma IgG antibodies, IL-12, and IL-23 levels in breast cancer patients and controls according to grade of cancer

According to the grade status, the highest mean titer of IgG Abs in breast cancer patients who are seropositive to anti-*Toxoplasma* IgG was shown in grade (G3) (166.01 ± 11.05 IU/ml), compared with patients who are seronegative to anti-*Toxoplasma* IgG which was (2.49±0.07 IU/ml) with a statistically significant differences (*P*<0.01). While the highest mean titers of IL-12 in patient who are seropositive to anti-*Toxoplasma* IgG was shown in grade (G1) which was (16.47 ± 0.93 pg/ml) and it was higher than those who are seronegative to anti- *Toxoplasma* IgG with (G1) which was (9.16±0.82 pg/ml) with statistically significant differences (*P*<0.01). Furthermore, the highest mean titers of IL-23 in patient who are seropositive to anti- *Toxoplasma* IgG was shown in (G3) which was (266.05 ± 21.07 pg/ml) and it was higher than those who are seronegative to anti-*Toxoplasma* IgG with (G3) which was (163.21±12.08 pg/ml) with statistically significant differences (*P*<0.01) (Table 4).

Anti-Toxoplasma IgG antibodies, IL-12, and IL-23 levels in breast cancer patients and controls according to stage of cancer

In regard to the tumor stages, the highest mean titer of IgG Abs in patients who are seropositive to anti-*Toxoplasma* IgG was in stage III (250.31±19.62 IU/ml) compared with patients who are seronegative to anti-*Toxoplasma* IgG which was (4.05±0.17 IU/ml) with a statistically significant differences (*P*<0.01). While, the highest mean titer of IL-12 in patients who are seropositive to anti-*Toxoplasma* IgG was in stage III (14.08±0.95 pg/ml) and it was higher than those who are seronegative to anti-*Toxoplasma* IgG with stage III which was (7.35±0.57 pg/ml) with statistically significant differences (*P*<0.01). Furthermore, the highest mean titer of IL-23 in patients who are seropositive to anti-*Toxoplasma* IgG was in stage III (294.22±22.86 pg/ml) and it was higher than those who are seronegative to anti-*Toxoplasma* IgG with stage III which was (163.42±15.66 pg/ml) with a statistically significant differences (*P*<0.01) (Table 5).

Table 4: Anti- *Toxoplasma* IgG antibodies, IL-12, and IL-23 levels in breast cancer patients and controls according to grade of cancer

Grade	Toxo(-)	Toxo (+)	P-value
Number of grade (G1= 13, G2= 47, G3= 30)			
	IgG (IU/ml)		
G1	1.96 ± 0.04	89.44 ± 8.35	0.0001 **
G2	2.02 ± 0.07	164.97 ± 13.71	0.0001 **
G3	2.49 ± 0.07	166.01 ± 11.05	0.0001 **
P-value	0.1398 NS	0.0266 *	---
	IL-12 (pg/ml)		
G1	9.16 ± 0.82	16.47 ± 0.93	0.0027 **
G2	6.55 ± 0.37	10.84 ± 0.76	0.0484 *
G3	7.69 ± 0.52	11.44 ± 0.81	0.0472 *
P-value	0.0477 *	0.0037 **	---
IL-23 (pg/ml)			
G1	123.28 ± 8.92	184.56 ± 15.77	0.044 *
G2	156.99 ± 13.57	211.50 ± 18.61	0.037 *
G3	163.21 ± 12.08	266.05 ± 21.07	0.225 **
P-value	0.093 NS	0.0357 *	---

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

Table 5: Anti- *Toxoplasma* IgG antibodies, IL-12, and IL-23 levels in breast cancer patients and controls according to stage of cancer

Stage	Toxo(-)	Toxo (+)	P-value
Number of stage (I= 18, II= 37, III=35)			
	IgG (IU/ml)		
(I)	1.20 ± 0.06	94.52 ± 8.92	0.0001 **
(II)	2.73 ± 0.11	104.34 ± 11.07	0.0001 **
(III)	4.05 ± 0.17	250.31 ± 19.62	0.0001 **
P-value	0.0492 *	0.0002 **	---
	IL-12 (pg/ml)		
(I)	6.47 ± 0.51	11.86 ± 0.76	0.0329 *
(II)	7.44 ± 0.64	10.46 ± 0.81	0.0385 *
(III)	7.35 ± 0.57	14.08 ± 0.95	0.0013 **
P-value	0.358 NS	0.0366 *	---
	IL-23 (pg/ml)		
(I)	119.56 ± 8.55	173.40 ± 14.63	0.0492 *
(II)	143.50 ± 11.02	245.28 ± 21.16	0.0038 **
(III)	163.42 ± 15.66	294.22 ± 22.86	0.0041 **
P-value	0.0294 *	0.0038 **	---

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

Anti-Toxoplasma IgG antibodies, IL-12, and IL-23 levels in breast cancer patients and controls according to size of cancer

According to the tumor size, the higher mean titer of IgG Abs in patients who are seropositive to anti-*Toxoplasma* IgG was in patients with tumor size (>3cm) (309.99±20.33

IU/ml). While the higher mean titer of IL-۱۲ was shown in patients with tumor size (>3cm) who are seropositive (14.70 ± 0.85 pg/ml). Furthermore, the higher mean titer of IL-23 was shown in patients with tumor size (>3cm) who are seropositive (257.45 ± 15.61 pg/ml) (Fig. 1).

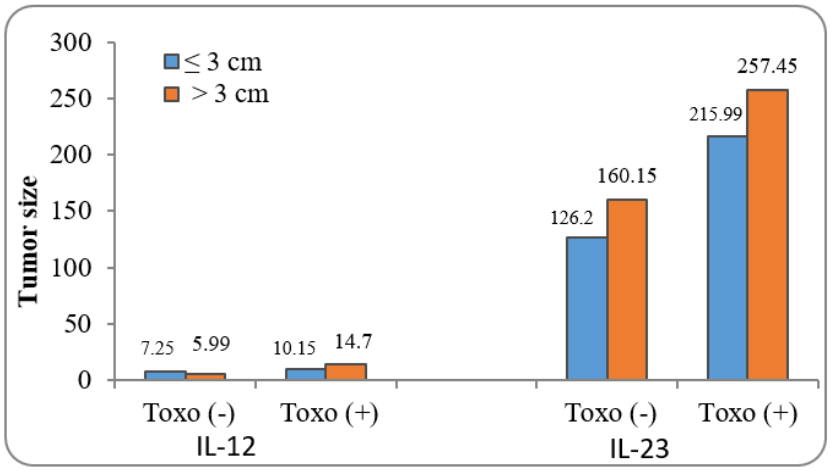

Fig. 1: The mean titer of IL-12 and IL-23 in breast cancer patients according to tumor size

Discussion

Age is a crucial factor for both breast cancer and toxoplasmosis which are more prevalent in women aged over 40 years (24). According to the current study, the highest rate of toxoplasmosis in patients with breast cancer was in yr 41-50. The seroprevalence rate of toxoplasmosis increase with age (25, 26). The seropositivity of toxoplasmosis is higher in age 40-84 yr, this is could be due to the increased exposure to the risk factors of *T. gondii* infection as the human gets older (27).

The role of *Toxoplasma* to cause severe symptoms in immunocompromised individuals has been studied widely, but toxoplasmosis in immunocompromised patients with cancer has not been considered well (24). The results of present study showed higher percentages of positivity for anti-*T. gondii* IgG titer in breast cancer patients compared with the controls groups. Similarly, the high titer of anti-*Toxoplasma* IgG was among patients with breast cancer compared with the controls (28). Regardless of the kinds of cancer, these findings are in agreement with other studies that reported that seroprevalence rate of toxoplasmosis in cancer patients was higher than that in healthy individuals (24, 25, 29, 30). When individuals are formerly infected with chronic toxoplasmosis and then acquire the infection with any type of cancer, the likelihood of reactivation of the latent infection will be high and at the same time the chance for cancer to be more aggressive will also be high (30).

The balance of proinflammatory cytokines, IL-12 and IL-23, plays a main role in shaping the development of antitumor or protumor immunity (31). In this study, low serum IL-12 level was showed in breast cancer patients in comparison with controls according to toxoplasmosis. Other results revealed a significant deficiency in IL-12 levels in breast cancer patients than benign tumor patients and healthy subjects (32). In cancer, the macrophages would be hectic and unable to produce a sufficient amount of IL-12 (33). In contrast, other results recorded higher levels of IL-12 in

breast cancer patients than control subjects (34, 35).

IL-23 has a pro-carcinogenic activity, promoting inflammation and angiogenesis within the tumor microenvironment. Expression of IL-23 is increased in human tumors (36). The current study showed that the mean level of IL-23 in patients was higher than the mean value in controls according to toxoplasmosis. The measurement of IL-23 may be of important prognostic value in the assessment of survival. The IL-23 has shown significant higher levels in patients with breast cancer compared with healthy controls (17).

The result of this study showed that the highest means titer of IgG in patients who are seropositive to anti-*Toxoplasma* IgG was shown in grade (G3) while serum IL-12 levels were decreased with the progression of tumor grades. There were no significant differences in serum IL-12 levels among cancer groups classified by histopathological findings such as pathological grade (12, 33). The result of the present study also showed that the raised serum IL-23 levels are correlated with tumor grades.

Cancer is usually diagnosed at a late stage when prognosis is poor and therapy effectiveness is limited. Moreover, distinguishing two of three stages has problems including the TNM classification. Thus, there is tremendous opportunity to improve the outcome for people with cancer by enhancing detection and treatment approaches as well as continuous research and evaluation of biomarkers in relation to therapeutic efficiency and overall survival (37). The result of the study demonstrated the highest mean titer of IgG in patients who are seropositive to anti- *Toxoplasma* IgG was in stage III. The highest mean titer of IgG Abs in patients with breast cancer was in stage (IIIC) (28). In most breast cancer cases, the patients do not have detectable symptoms. Thus, it is important to find markers for early diagnose to reduce mortality (32). The present study showed raised serum IL-12 levels are associated with the progression of tumor stages. The IL-12 levels in breast cancer patients with stage I was significantly lower than those observed in the healthy group. There were no significant differences between breast cancer patients with tumor stages II, III, or IV and the healthy group concerning the mean serum level of IL-12. This finding represents that reduced IL-12 production may participate in the initial phase of tumor establishment and development (38). Advanced stage and grade were associated with higher IL-12 levels in breast cancer patients (32). Moreover, IL-12-based immunotherapy may be more efficient in cancer patients with defects in IL-12 production (39, 40). Besides, the present study showed raised serum IL-23 levels are associated with the progression of tumor stages. The levels of IL-23 were significantly increased in CRC patients with no significant differences between disease stages (37).

In regard to tumor size, this study revealed that the mean titer of anti-*Toxoplasma* IgG was associated with increasing tumor size. The prognostic factors including the axillary lymph node status, the tumor size, the nuclear grade and histological grade were a significant indicators of breast cancer (19, 20). The tumor growth can be effectively controlled by the host immune system (41) and interleukins are known to play a significant role in immune response regulation (12). This study showed that the decreased serum IL-12 and IL-23 levels are associated with increasing tumor size. Breast cancer is the most common cancer of female (10). Cytokines are central to immune cell communications (42). Tumor mediate changes in cytokine levels directly or indirectly so they are important parameters that affect the development of the disease (43).

Conclusion

High levels of IgG and IL-23 titers were shown in breast cancer patients infected with *T. gondii* in grade 3. The high levels of IgG, IL-12 and IL-23 titers were also shown in these patients with an advanced size and stage. *T.*

gondii infection affects IL-12, IL-23 levels in the advanced stage of breast cancer patients thus, *T. gondii* should be diagnosed and treated to decrease the burden on the immune system when both diseases are present. In addition, the levels of IL-23 could be a candidate as a non-invasive primitive marker for earlier prediction of breast cancer stage.

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Conflict of interest

The authors declare that there is no conflict of interest.

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