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The Association of Severe Toxoplasmosis and Some Cytokine Levels in Breast Cancer Patients

Maha Mustafa Assim*, Entsar Jabbar Saheb
University of Baghdad, College of Science, Biology Department, Baghdad, Iraq

Abstract

Toxoplasma gondii is an opportunistic pathogen in which the reactivation of a latent infection can cause death in congenitally infected fetuses, newborns, and immunocompromised patients. This study aimed to determine the seropositivity of toxoplasmosis infection and the possible association with Interleukin-12 (IL-12) and Interleukin-23 (IL-23) cytokines in breast cancer patients. In this study, 190 women were enrolled. All serum samples were tested for *T. gondii* immunoglobulins (IgG and IgM) antibodies and (IL-12, IL-23) levels using ELISA technique. The result of this study showed that breast cancer patients recorded the highest percentage of toxoplasmosis infection. There were no positivity rates for anti- *Toxoplasma* IgM in breast cancer patients while the positivity percentage for anti- *Toxoplasma* IgM among the control group was (7.00%). Furthermore, the seroprevalence of anti- *Toxoplasma* IgG was the highest in the age group (31- 40) years in patients with breast cancer while the highest mean titer of the IL-12 is restricted to ages (21-30) years in the control groups who are seropositive to anti- *Toxoplasma* IgG. Although, in patients with breast cancer who are seropositive to anti- *Toxoplasma* IgG, the highest mean titer of the IL-23 was in ages (21-30) years. Since most immunosuppressive patients are exposed to various possible risk factors including *Toxoplasma* primary infection or reactivation, so it is important to diagnose and treat toxoplasmosis in breast cancer patients to reduce the consequences of the infection.

Keywords: Breast cancer; Toxoplasmosis; Cytokines.

شدة الاصابة بداء المقوسات ومستويات بعض المحركات الخلوية في مرضى سرطان الثدي

مها مصطفى عاصم*، انتصار جبار صاحب
قسم علوم الحياه، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

تعد المقوسات الكوندية مُمْرِضًا انتهازيًا حيث يمكن أن يؤدي إعادة تنشيط العدوى الكامنة إلى الوفاة في الاجنة المصابة خلقيا والاطفال حديثي الولادة المرضى بالاضافة الى المرضى مثبطي المناعة. تهدف هذه الدراسة لتحديد إيجابية المصل لعدوى داء المقوسات ودراسة العلاقة المحتملة بين المحركات الخلوية الانترلوكين−12 و الانترلوكين−23 في مرضى سرطان الثدي. شملت هذه الدراسة (190) امراه. تم فحص جميع عينات المصل لقياس مستوى الاجسام المضادة لداء المقوسات الكوندية (IgG و IgM). كذلك ، تم اختبار العينات من اجل تحديد مستويات (IL−12) و (IL−23) بأستخدام تقنية الامتزاز المناعي. أظهرت نتائج هذه الدراسة أن مرضى سرطان الثدي سجلوا نسبة عالية من الإصابة بداء المقوسات. لم تكن هناك معدلات ايجابية للأجسام المضادة (IgM) في مرضى سرطان الثدي بينما مستويات الايجابية للاجسام

*Email: mahamustafa.bio@gmail.com

المضادة (IgM) بلغت (7.00%) في مجاميع السيطرة. علاوة على ذلك، كان معدل الانتشار المصلي (IgG) لداء المقوسات الكوندية هو الاعلى في الفئة العمرية (31−40) سنة في مرضى سرطان الثدي. في حين أن أعلى معدل لتركيز (IL−12) اقتصر على الأعمار بين (21−30) سنة في مجاميع السيطرة اللذين هم موجبي المصل (IgG) لداء المقوسات. على الرغم من ذلك، في مرضى سرطان الثدي اللذين هم موجبي المصل (IgG) لداء المقوسات كان اعلى معدل لتركيز (IL−23) والذي اقتصر على الاعمار بين (21−30) سنة. لان معظم المرضى مثبطي المناعة يتعرضون الى العديد من عوامل الخطر المحتملة ومن ضمنها التعرض للاصابة الأولية او اعادة تنشيط داء المقوسات الكوندية لذلك من المهم تشخيص ومعالجة داء المقوسات في مرضى سرطان الثدي لتقليل مضاعفات الاصابة بهذا الداء.

1. Introduction

Cancer is the major cause of death in economically developed countries, and in developing countries, it is the second main cause of death [1]. Despite good prognosis of this malignancy, it was also the most common cause of death related to cancer [2]. Within the last two decades, there has been a plain increase in the incidence rates of breast cancer. A lot of cases in Iraq tend to be diagnosed at advanced stages [3]. The epidemiologic relationship between cancer and inflammation has been well established [4]. Chronic inflammation may basically contribute to cancer development. The release of free radicals during inflammation may induce the gathering of genetic mutations leading to the onset of dysplastic cells [5].

IL-12 and IL-23 are proinflammatory cytokines [6]. Despite the similarities between IL-23 and IL-12, they head divergent immunological pathway [7]. IL-12 leads to the development of 'classical' interferon IFN-γ producing T helper 1 (Th1) cells, while IL-23 promotes the proinflammatory function of a memory T cell subset termed Th17 that is characterized by the production of the cytokine IL-17 [8].

In patients with cancer, immune function is impaired and this is the major reason for the increase of *Toxoplasma* antibodies and duration of chemotherapy [9]. In most breast cancer cases, the patients do not have detectable symptoms [10] thus, it is important to diagnose toxoplasmosis infection in cancer patients to decrease the burden on the immune system when both diseases are present.

2. Material and Methods

2.1. Subjects and Blood Collection

In this study, 190 women were enrolled from October, 2017 till 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 the Medical City Hospital in Baghdad from different governorates in Iraq). Their ages range between 21-50 years.

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, and the samples were centrifuged at 3000 rounds per minute (rpm) for 10 minutes for serum aspiration and dispensed into Eppendorf- tubes and stored at -20 ºC. ELISA kits (Acon *Toxoplasma* IgG ELISA (I231-1091), IgM ELISA (I231-1101) was used to determine the anti- *T. gondii* antibodies (IgG and IgM). All serum samples then were divided into 4 groups: healthy women, women infected with toxoplasmosis, breast cancer women infected with toxoplasmosis, breast cancer women not infected with toxoplasmosis. As well as, samples were tested for serum mean titer of IL-12 and IL-23 by using 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

3.1: The incidental rate of toxoplasmosis infection in breast cancer patients in different governorates

The results of this study showed that Baghdad- Rasafa recorded the highest incidental rate of toxoplasmosis infection in breast cancer patient with 36 **(**85.71%), followed by Baghdad- Karkh with 21 (60.00%). The other governorates Wasit and Diyala showed the lowest incidental rate of toxoplasmosis infection in breast cancer patient Table-1.

Table 1-The incidental rate of toxoplasmosis infection in breast cancer patients in different governorates

Governorate	Samples No.	%	Toxo(+)		Toxo(-)		Chi-square
			No.	%	No.	%	
Baghdad- Rasafa	42	**46.67**	36	**85.71**	6	**14.29**	13.56 **
Baghdad- karkh	35	**38.89**	21	**60.00**	14	**40.00**	7.25 **
Wasit	6	**6.67**	3	**50.00**	3	**50.00**	0.00 NS
Diyala	7	**7.78**	5	**71.43**	2	**28.57**	13.00 **
Total	90	**100**	65	**72.22**	25	**27.78**	11.83 **
Chi-square	---	11.62 **	---	13.78 **	---	13.78 **	---

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

3.2 Serological examination of anti *T. gondii* antibodies IgG and IgM in breast cancer and control group

The results showed higher positivity percentages for anti-*T. gondii* IgG antibodies in sera of 65 cancer patients (72.22%) compared with the sera of a control group which was 48 (48.00%) with a statistically significant difference (P<0.01). There were no positivity rates for anti-*T. gondii* IgM in cancer patients while the positivity rates for anti-*T. gondii* IgM among the control group was 7 (7.00%) Table-2.

Table 2-Serological examination of anti *T. gondii* antibodies IgG and IgM in breast cancer and control group

Antibodies	Control N=100		Breast cancer N=90		Chi-square
	No.	%	No.	%	
IgG(+)	**48**	48.00	**65**	72.22	8.912 **
IgG(-)	**52**	52.00	**25**	27.78	8.912 **
IgM(+)	**7**	7.00	**0**	0.00	2.873 NS
IgM(-)	**93**	93.00	**90**	100.00	2.873 NS

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

3.3 The mean levels of IgG, IL-12, IL-23 in different ages of studying groups according to toxoplasmosis infection

According to the age groups, the seroprevalence of toxoplasmosis was the highest in the age group of (31- 40) years in patients with breast cancer (282.95 ± 19.31 IU/ml) compared with the control (113.92 ± 8.41 IU/ml) who are seropositive for anti- *Toxoplasma* IgG (P<0.01).

The results reveal that the highest mean titer of the IL-12 is restricted to ages between (21-30) years in control who are seropositive for anti- *Toxoplasma* IgG (17.25 ± 0.82 pg/ ml) compared with breast cancer patient (15.73 ± 0.81 pg/ ml) who are seropositive for anti- *Toxoplasma* IgG (P<0.01).

The highest mean titer of the IL-23 is restricted to ages between (21-30) years in patient with breast cancer who is seropositive for anti- *Toxoplasma* IgG (233.65 ± 14.62 pg/ ml) compared with the control (174.33 ± 11.75 pg/ ml) who are seropositive for anti- *Toxoplasma* IgG (P<0.01) Table-3.

Table 3-The mean levels of IgG, IL-12, IL-23 in different ages of studying groups according to toxoplasmosis infection

Age (year)	Control Toxo (-)	Control Toxo(+)	Breast cancer. Toxo (-)	Breast cancer. Toxo (+)	P-value
IgG (IU/ml)					
21 - 30	3.71 ± 0.11	156.87 ± 12.73	3.59 ± 0.12	230.58 ± 16.45	0.0001 **
31 - 40	3.74 ± 0.09	113.92 ± 8.41	2.23 ± 0.07	282.95± 19.31	0.0001 **
41 - 50	2.31 ± 0.12	103.23 ± 8.07	2.31 ± 0.07	175.79 ± 11.69	0.0001 **
P-value	0.277 NS	0.0397 *	0.307 NS	0.0361 *	---
IL-12 (pg/ml)					
21 - 30	7.82 ± 0.47	17.25 ± 0.82	7.51 ± 0.45	15.73 ± 0.81	0.0001 **
31 - 40	6.91 ± 0.35	13.11 ± 0.75	7.76 ± 0.51	12.24 ± 0.63	0.0001 **
41 - 50	7.01 ± 0.55	6.61 ± 0.36	6.31 ± 0.29	9.84 ± 0.66	0.0001 **
P-value	0.1448 NS	0.0001 **	0.2783 NS	0.0001 **	---
IL-23 (pg/ml)					
21 - 30	48.74 ± 2.68	174.33 ± 11.75	163.57 ± 9.21	233.65 ± 14.62	0.0001 **
31 - 40	29.02 ± 1.96	127.48 ± 8.82	154.71 ± 8.50	191.32 ± 11.97	0.0001 **
41 - 50	50.67 ± 2.62	96.39 ± 4.67	136.21 ± 8.14	194.65 ± 14.05	0.0001 **
P-value	0.0248 *	0.0261 **	0.1339 NS	0.082 NS	---

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

Discussion

Toxoplasmosis was raised after Iraq occupation with a frequency of infection more than 40% compared to 2% in the eighties [11]. In Iraq during 2016, 335 patients infected with *Toxoplasma* in all Iraqi governorates were recorded [12]. The prevalence of toxoplasmosis differs because of many factors including the variation in climate and cultural practices in different regions of Iraq [13]. In Iraq, a study showed that the proportion of breast cancer in women was 33.81%. In comparison with the other Arabic countries, the proportion of breast cancer was lower in various Arab countries such as Kuwait, Jordan, and Bahrain. In contrast, the rate was higher in other Arab countries such as Saudi Arabia, Oman, UAE, Qatar and in Arab neighboring countries such as Iran and Turkey [14, 15]. Little is known about the epidemiology of *T. gondii* infection in patients who are immunocompromised that undergoes neoplastic disease or immunosuppressive therapy in Iraq [16]. Recently, a study revealed that the incidental rates of toxoplasmosis in breast cancer patients was (77.50 %) [17].

Chronic inflammation commonly stimulates carcinogenesis and may prompt an individual to cancer [18]. The present study displayed that (72.22%) of breast cancer patient and (48.00%) of the control group were confirmed to be positive for *Toxoplasma* IgG antibodies, and there were no positivity rates for anti-*T. gondii* IgM in breast cancer patient while the positivity rates for anti-*T. gondii* IgM among the control group was (7.00%). High levels of IgG and the absence of IgM antibodies are correlated with a chronic latent infection which acquired in the past [19]. On the other hand, other previous studies recorded that the seropositive cases for anti-*Toxoplasma* IgG were (29.9%), while (0.7%) was seropositive for anti-*Toxoplasma* IgM in cancer patient [20]. Another study recorded (24.4%) of toxoplasmosis infection on cancer patients. None of the subjects tested had anti-*Toxoplasma* IgM antibodies [21]. The chronic infections possibly persist throughout the life and may remain undiagnosed until it is reactivated as a result of acute immune suppression [22].

According to other study, the level of IgG among breast cancer patient was (220.72 IU/ml) but in control groups with *Toxoplasma* infection was (140.58 IU/ml) [23] while other study revealed the total levels of IgG antibody in patients with *T. gondii* group, cancer group and the control group were (87.32 IU /ml, 105.80 IU/ml, 63.26 IU/ml) respectively [24]. It is proposed that when individuals are previously infected with chronic toxoplasmosis and then get the infection with any sort of cancer, the probability of reactivation of the latent infection will be high and at the same time, the opportunity for cancer to be more aggressive will also be high [16].

The present study showed that *Toxoplasma* infection in breast cancer patients does not seem to increase progressively with age, which is similar to that shown with a previous study [9, 17] but it is in disagreement with other studies demonstrated that the seroprevalence rate of toxoplasmosis increases with age and the peak level was seen in cases older than 50 years [19], this is could be due to the increasing exposure years as the humans get older or due to the multiple minor infections which at first produce low antibody levels and later higher levels [25].

In recent years the focus has been on the role of cytokines as prognostic factors. Both the innate and acquired immune system are supposed to play crucial roles in the anti-tumor response, and the interaction between host immune system and tumor cells has been the subject of intense research during the past decades [26]. The balance of proinflammatory cytokines interleukin IL-12 and IL-23 plays a key role in shaping the development of antitumor or protumor immunity [27].

Aging is accompanied by many changes in immune response, with the most consistent and dramatic alterations occurring within the T cell compartment. Since cytokines are central to immune cell communications, age-associated changes in cytokine production may contribute to these alterations [28]. According to the present study, the level of IL-12 decreased with age, while other study demonstrated that the age was not significantly correlated with the serum IL-12 [29]. In addition, the present study also demonstrated that the level of IL-23 decreased with age. Other study compared serum levels of IL- 12p40 and IL-23 in respect of age, none of the cytokine levels were affected by age among colorectal cancer [30]. Thus, the results of these studies demonstrate that age-associated change in cytokine production is not consistent.

Conclusion

This finding shows a higher prevalence of *T. gondii* infection in breast cancer patients with low levels of IL-12 and IL-23 with age progress, thus patients with breast cancer should be screened for *Toxoplasma* routinely. Clinicians should be more careful with this patients group to prevent the possibility of severe toxoplasmosis.

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