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Detection of *Toxoplasma gondii* in Iraqi Women with Breast Cancer by Real-Time PCR

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Abstract

A cross-sectional study was carried out in Kirkuk city from 15th of June 2018 to 15th of December 2018 to detect of *Toxoplasma gondii* DNA in sera of women with breast cancer by real-time PCR and comparing the results with IgG and IgM toward *T. gondii*. The number of breast cancer women understudy were 35. The ages of the patients were between 20-75 years old. These patients admitted to Kirkuk oncology center. The control group were included 50 healthy individuals. blood was collected from each patients and control for molecular tests of *T. gondii* using real-time PCR and serological testing for detection specific *Toxoplasma gondii* IgM and IgG by using ELISA technique. The study showed that the highest rate of anti *T. gondii* IgM+ IgG- antibodies 8.57% was recorded among women with breast cancer comparing with 8% in healthy control while 14.29% of women with breast cancer were IgM+IgG+ comparing 6% of the healthy control group. The highest rate of total *T. gondii* antibodies by ELISA 68.57% was noted among women with breast cancer and the lowest rate was among healthy control 20%. The study revealed that 54.17% of women with breast cancer with positive ELISA was positive by PCR comparing with 9.09% of patients with negative ELISA results. The result was highly significant with sensitivity and specificity of 54.17% and 90.09% respectively. The highest rate of anti *T. gondii* IgM+ IgG- antibodies 66.67% women with breast cancer was positive by PCR followed by 40% of patients with IgM+ IgG+ antibodies. It was concluded that *Toxoplasma gondii* more frequently associated with breast cancer and PCR was more accurate in detection of the parasite.

Key words: *Toxoplasma gondii;* breast cancer; ELISA and real-time PCR.

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الكشف عن المقوسات الكوندية في النساء العراقيات المصابات بسرطان الثدي بواسطة فحص تفاعلات البلمرة المتسلسلة

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الملخص

أجريت الدراسة في مدينة كركوك للفترة من 15 حزيران 2018 الى 15 كانون الأول 2018 للكشف الجزيئي عن المقوسات الكوندية في النساء المصابات بسرطان الثدي ومقارنته مع نتيجة البحث عن الاجسام المضادة تجاه الطفيلي. شملت الدراسة 35 امرأة مصابة بسرطان الثدي واللاتي كانت اعمارهن 20–75 سنة وكن يراجعن مركز كركوك للأورام السرطانية، كما وشملت الدراسة 50 فرداً سليماً كمجموعة سيطرة. أجريت فحوصات تفاعلات البلمرة المتسلسلة real- time PRC وفحص الاليزا ELISA للكشف عن الاجسام المضادة في جميع المرضى والاصحاء في الدراسة. أظهرت الدراسة أن نسبة الاجسام المضادة IgM+ IgG- كان 8،57% في النساء المصابات بسرطان الثدي مقارنة ب8% بمجموعة السيطرة كما وأن 14،29% من المرضى كن حاملات للأجسام المضادة نوع IgM+IgG+ تجاه الطفيلي مقارنة ب 6% في مجموعة السيطرة. كما سجلت الدراسة أن اعلى نسبة من مجموع الاجسام المضادة 67،57% سجلت في النساء المريضات مقارنة ب 20% في مجموعة السيطرة. أظهرت الدراسة أن 54،17% من النساء المصابات بسرطان الثدي واللاتي كن يحملن الاجسام المضادة تجاه الطفيلي كن فعليا ذوات نتيجة إيجابية بفحص PCR في الكشف عن الحمض النووي للطفيلي وبنسبة حساسية ودقة 54،17% و 90،09% على التوالي وأن اعلى نسبة من الاجسام المضادة كانت نوع IgM+ IgG- 66،67% تجاه الطفيلي مقارنة ب 40% من نوع IgM+ IgG+.

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الاستنتاج: يستنتج من الدراسة ان المقوسات الكوندية لها علاقة وارتباط مع سرطان الثدي ان فحص PCR هو الطريقة الأدق للكشف عن الطفيلي في المرضى

الكلمات الدالة: المقوسات الكوندية، سرطان الثدي، فحص الاليزا و تفاعلات البلمرة المتسلسلة.

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1. Introduction:

A nuclear many-body problem Toxoplasmosis is a disease caused by the intracellular protozoan parasite *Toxoplasma gondii*. Most immunocompetent individuals who contract the parasite do not develop symptoms, or might experience nonspecific flulike symptoms including fever, headache, muscle pain, and lymphadenopathy [1]. Although one-third of the world's population is infected with the parasite, it often remains unrecognized, as most patients do not exhibit symptoms [2]. *Toxoplasma gondii* infections are prevalent in humans and animals worldwide. It has been estimated that one-third of the world population has been exposed to this parasite [3]. The infection is acquired by ingesting tissue cysts from undercooked or raw meat, consuming food or drink contaminated with oocysts shed by felids, or by accidentally ingesting oocysts from the environment [4]. Although the course of the primary infection is usually subclinical and the vast majority of infected human populations remain asymptomatic, the infection can cause significant morbidity and mortality in certain groups. This includes encephalitis, chorioretinitis, congenital infection and neonatal mortality [5]. The global annual incidence of congenital toxoplasmosis was estimated to be 190,100 cases [1]. Moreover, serological tests may fail to detect *T. gondii* infection in certain immunocompromised patients due to the fact that the titres of specific antibodies anti-Toxoplasma may fail to rise in this type of patient. Therefore, the high risk of congenital toxoplasmosis of a fetus may be undetected because the pregnant mother might test negative during the active phase of *T. gondii* infection[6]. Furthermore, the test may fail to detect *T. gondii* infection in certain immunocompromised patients due to the fact that the titers of specific anti-*Toxoplasma* IgG or IgM may fail to rise in this type of patient [7]. Toxoplasmosis of immunosuppressed individuals is most often the result of reactivation of latent infection, which presents neurological signs, including headache, disorientation,

drowsiness, hemiparesis, reflex changes, and convulsions [8]. Acute acquired *T. gondii* infection in immunocompromised patients may also occur and involve multiple organs. Pneumonia, retinochoroiditis, and other disseminated systemic diseases, can also be seen, but are not as common as encephalitis in immunocompromised patients [3]. The cancer can are activate latent *T. gondii* infection during antitumor treatment process [4]. The aim of this study was to detect of *T. gondii* DNA in sera of women with breast cancer by real-time PCR and comparing the results with IgG and IgM toward *T. gondii*.

2. Material and methods

A cross sectional study was carried out in Kirkuk city from 15th of Jane 2018 to 15th of December 2018. The number of breast cancer women understudy were 35. The ages of the patients were between 20-75 years old. These patients admitted to Kirkuk oncology center. The control group who were matched to the patients, included 50 healthy individuals (relatives of patients).

2.1 Methods:

Five ml of blood was collected by vein puncture using 5 ml syringe from each patient and the control group enrolled in this study. Blood samples were placed into two tubes, one of them containing anticoagulant EDTA for molecular tests of *Toxoplasma gondii* (using Sacace biotechnology-Iraly, Toxo-DNA Real-TM Qualit). The second part of the sample was 2 ml which placed in plane tubes, left for 30 minutes at 37 °C for clotting and centrifuged at 3000 rpm for 15 minutes, the obtained sera was aspirated using automatic micropipette and transferred to Eppendorf tubes and stored in deep freeze at -20°C for serological testing for detection specific *Toxoplasma gondii* IgM and IgG by using ELISA technique.

2.2 Statistical Analysis :

Computerized statistically analysis was performed using Anova version 11 statistic program. Comparison was carried out using; Chi-square (X^2).

3. Results

The nuclear study showed that the highest rate of anti *T. gondii* IgM+ IgG- antibodies (8.57%) was recorded among women with breast cancer comparing with 8% in healthy control while

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14.29% of women with breast cancer were IgM+IgG+ comparing 6% of the healthy control group.. Table 1.

Table 1: Rates of anti *T. gondii* IgM and IgG antibodies in women with breast cancer comparing with the control group.

Results of Toxoplasma IgM and IgG ELISA	women with breast cancer (n:35)		Healthy individuals (control group) (n:50)	
	No.	%	No.	%
IgM(+) IgG+ve	5	14.29	3	6
IgM(+) IgG-ve	3	8.57	4	8
IgM(-) IgG+ve	16	45.71	3	6
IgM(-) IgG-ve	11	31.43	40	80
Total	35	100	50	100

P. value: 0.043

The highest rate of total *T. gondii* antibodies by ELISA (68.57%) was noted among women with breast cancer and lowest rate was among healthy control (20%), Fig. 1.

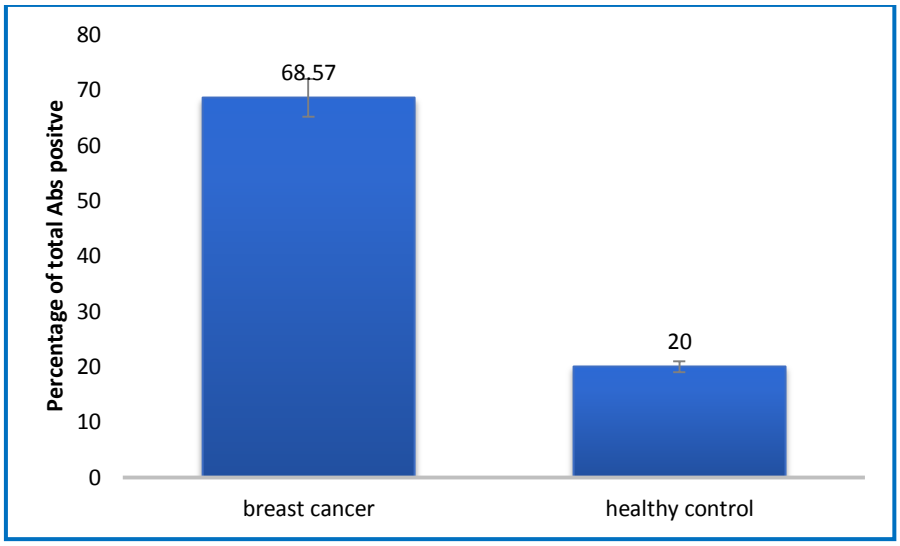

Fig. 1: Distribution of total anti- *T. gondii* antibodies PCR positive.

The study revealed that 54.17% of women with breast cancer with positive ELISA was positive by PCR comparing with 9.09% of patients with negative ELISA results. The result

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was highly significant with sensitivity and specificity of 54.17% and 90.09% respectively, Table 2. The highest rate of anti *T. gondii* IgM+ IgG- antibodies (66.67%) women with breast cancer was positive by PCR followed by 40% of patients with IgM+ IgG+ antibodies, Table 3 .

Table 2: Comparison between *Toxoplasma* total antibodies (IgM and IgG) by ELISA and real-time PCR in women with breast cancer.

Results of ELISA	No.	Results of RT-PCR				P. value	Sensitivity	Specificity
		Positive		Negative				
		No.	%	No.	%			
Total positive	24	13	54.17	11	45.83	0.008 HS	54.17	90.9%
Negative	11	1	9.09	10	90.91			
Total	35	14	40	21	60			

Table 3: Comparison between *Toxoplasma* IgM and IgG antibodies by ELISA and real-time PCR in women with breast cancer.

Results of ELISA	No.	Results of RT-PCR				P. value
		Positive		Positive		
		No.	%	No.	%	
IgM(+) IgG+ve	5	2	40	3	60	
IgM(+) IgG-ve	3	2	66.67	1	33.33	0.25 NS
IgM(-) IgG+ve	16	9	56.25	7	43.57	
IgM(-) IgG-ve	11	1	9.09	10	90.91	
Total	35	14	40	21	60	

The study found that the maximum rate of toxoplasmosis was recorded among breast cancer women within the age group 50-59 year while the lowest rate was in the age group 20-29 year and the rate was significantly increase with increase of patients age, Table 4.

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Table 4: Distribution of positive PCR of *T. gondii* in women with breast cancer according to age.

Age groups (years)	Total No.(35)	PCR +ve	
		No.(12)	%
20-29	14	2	14.29
30-39	3	1	25
40-49	4	2	50
50-59	8	5	62.5
≥60	6	2	33.33

P. value: 0.57

4. Discussion:

A variety of malignancies, including lymphoma, leukemia, and myeloma, can reactivate toxoplasmosis [1]. Robert-Gangneux *et al* [9] found that toxoplasmosis can be complicated and is considered a serious disease in immunocompromised patients, in which the reactivation of a latent infection can be fatal. People with immunocompromised systems, especially those with higher chronic infection risk due to cellular immune deficiency, as well as patients with cancer are more susceptible to be infected with *T. gondii* [4].

Several studies have reported that the seroprevalence rate of *T. gondii* infection was higher in patients with different types of cancer including brain, hematologic and breast cancers and indicating that the seroprevalence of toxoplasmosis is significantly higher in patients with cancer than non-cancer patients, including breast cancer [10-12]. Detecting *Toxoplasma* DNA in breast cancer patients was done by several works. For example, Robert-Gangneux *et al* [9] and Kalantari *et al* [11] found the DNA *Toxoplasma* in tissue of breast cancer with rates less than in the current study. It seems that successful amplification of *T. gondii* DNA even in blood samples in addition to the technique of PCR depends on the time of sampling post infection because of the transient nature of parasitaemia [12]. Ahmed *et al* [10] found that 77.50% of breast cancer women have IgG antibodies against *Toxoplasma* comparing with 22% of healthy control. Immunocompromised hosts, especially those with deficient cellular immunity, are at risk of recrudescence of chronic infection and dissemination, with the occurrence of fulminating disease [13]. In

immunocompromised patients, the infection most often involves the nervous system, with diffuse encephalopathy, meningoencephalitis or cerebral mass lesions [4]. Assim *et al* [14] demonstrated that the seropositivity rate of anti-*T. gondii* antibodies (IgM) was seen in breast cancer patients IgM antibodies were found 10.3%. By contrast, the assessment of PCR methods is far more difficult in immunocompromised patients, as deep sites of infection may be difficult to reach by biopsy, thus definite diagnosis usually relies on the association of imaging findings, molecular diagnosis, serology, and response to specific therapy [6].

5. Conclusion

It was concluded that *Toxoplasma gondii* more frequently associated with breast cancer and PCR was more accurate in detection of the parasite.

References

[1] D. E. Hill and J. P. Dubey, ***"Toxoplasma gondii. In: Foodborne Parasites"***, 1st Ed., Springer, Cham (2018).

[2] D. W. Kimberlin, M. T. Brady, M. A. Jackson and S. S. Long, ***"Red Book, Report of the Committee on Infectious Diseases",*** American academy of pediatrics, (2015)

[3] R. H. Yolken and E. F. Torrey, ***"Exposure to Microorganisms and Adult Psychiatric Disorders: The Case for a Causal Role of Toxoplasma gondii. In: Immunology and Psychiatry",*** 1st Ed., Springer, Cham (2015).

[4] D. S. Lindsay and L. M. Weiss, ***"Opportunistic infections: Toxoplasma, sarcocystis, and microsporidia"***, 8th Ed., Springer Science and Business Media (2004).

[5] O. L. McGovern and V. B. Carruthers, ***"Toxoplasma retromer is here to stay",*** Trends in parasitology, 32(10), 758 (2016).

[6] J. R. Flegr, ***"Host Manipulation by Toxoplasma gondii, In: Encyclopedia of Parasitology",*** 2nd Ed., Springer Berlin Heidelberg (2016).

[7] B. J, Bogitsh, C. E. Carter and T. N. Oeltman, **"*Human parasitology*"**, 5th Ed., Academic Press (2018).

[8] D. Bass, G. D. Stentiford, D. T. Littlewood and F. H. Hartikainen, **"*Diverse applications of environmental DNA methods in parasitology*",** Trends in parasitology, 31(10), 499 (2015).

[9] F. Robert-Gangneux, Y. Sterkers, H. Yera and I. Accoceberry, **"*Molecular diagnosis of toxoplasmosis in immunocompromised patients: a three-year multicenter retrospective study*",** Journal of clinical microbiology, 11: JCM-03282 (2015).

[10] D. F. Ahmed and E. J. Saheb, **"*The Association of Toxoplasma gondii Infection in Breast and Colorectal Cancer Patients*"**, International Journal of Clinical Oncology and Cancer Research, 2(4), 86 (2017).

[11] N. Kalantari, S. Ghaffari and M. Bayani, **"*Preliminary study on association between toxoplasmosis and breast cancer in Iran*",** Asian Pacific Journal of Tropical Biomedicine, 5, 44 (2015).

[12] M. N. Khabaz, L. M. Elkhateeb and J. R. Al-Alami, **"*Reactivation of latent Toxoplasma gondii in immunocompromised cancer patients*",** Comparative Clinical Pathology, 20, 183 (2011).

[13] P. Bastien, **"*Molecular diagnosis of toxoplasmosis*",** Transactions of The Royal Society of Tropical Medicine and Hygiene, 96 (1), 205(2002).

[14] M. M. Assim and E.J. Saheb, **"*The Association of Severe Toxoplasmosis and Some Cytokine Levels in Breast Cancer Patients*",** Iraqi Journal of Science, 59(3), 1189 (2018).

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