The Effect of *Toxoplasma Gondii* on DNA Sequence Alteration among Breast Cancer Patients

تأثير المقوسة الكوندية على تغيير التتابع الجيني لدى مرضى سرطان الثدي

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المستخلص:

الهدف: تهدف الدراسة الى تحديد تأثير الأصابة بالمقوسة الكونيدية على التتابع الجيني لمرضى سرطان الثدي في مستشفى مدينة الطب /وحدة الاورام بغداد/ العراق.

المنهجية: تم جمع أربعين عينة من الأنسجة والمصول من المرضى الذين يشكون من سرطان الثدي والمصابين بالمقوسة الكوندية للفترة من ١ حزيران ٢٠١٦ الى ١٥ نيسان ٢٠١٧ وتم أخذ عينات المصل لمرضى المقوسة الكوندية والسليمين من الأصابات الورمية كمجموعة سيطرة . عملية تحليل البيانات تمت باستخدام أساليب الإحصاء الوصفي والاستدلالي.

النتائج: أظهرت نتائج الدراسة أن هناك تاثير معنوي من قبل المقوسة الكوندية على التتابع الجيني للمرضى المصابين بسرطان الثدي وكانت هناك فروقات معنوية لمرضى االسرطان من المصابين بداء المقوسات الكوندية مقارنة بالمجموعة الضابطة.

التوصيات: توصي الدراسة الى إجراء عمل تقنية التعبير الجيني بجهاز الزمن الآني (Real Time) لتحديد التطفير الجيني لتحديد التغيير الذي يحصل في تخليق البروتين لمرضى سرطان الثدي المصابين بداء المقوسة الكوندية.

Abstract:

Objective: The study aims to determine the effect of *Toxoplasma gondii* infection on the genetic sequence of breast cancer patients in the Medical City Hospital – Tumor Unit / Iraq-Baghdad.

Methodology: A study was carried out in the City of Medicine / Oncology Unit / Baghdad, during the period 1st June 2016 to 15th March 2017. Forty samples of tissue and serum were collected from patients who complaining from Breast cancer and infected with Toxoplasmosis. Forty sera samples were taken from patients complaining from parasitic infection only; without breast cancer as control group. Data is analyzed by using of descriptive and inferential data analysis methods.

Results: The results show that there is an effect for *Toxoplasma gondii* on the genetic sequence of patients complaining breast cancer, with significant differences among cancer patients with Toxoplasmosis in comparison with negative toxoplasmosis control group.

Recommendations: In view of the results, it is of importance to throw light on the gene expression using real-time technique to detect the genetic mutation and the change in the protein synthesis of patients with Toxoplasmosis.

Key Words: *Toxoplasma Gondii*, DNA Sequence, Breast Cancer patients.

Introduction

Toxoplasma gondii is a risky parasite it causes serious health problems within the human body. The parasite therefore often enters a dormant state forming small cysts in various body tissues [1]. This latent phase may provoke mild inflammation and may causes brain tumor [2]. Vital organs such as central nervous system (CNS) can be invaded by this parasite it is usually mild in human-beings, immunocompetent peoples it is asymptomatic [3]. The parasite may cause severe diseases such as breast cancer in immunocompromised individuals. It was reported that there may be a correlation between *T. gondii* and breast cancer [4]. A recent study in Iraq indicated that there were 89 cases of various cancers with toxoplasmosis in the Medical City Hospital – Unit of tumors/ Iraq-Baghdad [5]. In regard to detection of the mutation on breast DNA. It was found that BRCA1 gene can interact physically with certain growth inhibitory genes by *T. gondii* such as p21, p27 and Gadd45α, so as to regulate their promoter and expression [6]. BRCA1 play an important role in repairing DNA damage because it is a target of different upstream nuclear phosphoinositide kinase which are incorporated in DNA damage signal such as ataxia atelangiectasia mutated ATM and ATM- related ATR kinase [7]. It has also been found that there is an association between BRAC1+ BRAC2 RAD51 protein which is involved in the repair of HR of RDBs and in recombination during meiosis and mitosis [8].

squared was used to compare numeric data for healthy control and patients used to compare numeric data for healthy control and patients groups while Chi-square was used to assess the differences between the proportions. P-value <0.05 was considered significant [10].

Numeric data were expressed as mean ± standard error of mean (SEM). Unpaired t-test and chi- square was

Methodology

Forty tissue and sera samples were collected from breast cancer patients were taken and stored in clean containers at -20ºC till use. Anti-TOXO IgG and IgM antibodies levels were measured by ELISA technique. Conventional PCR reaction was applied for the determination of the (BRCA1, ERBB2) genes, the agarose gel 2% was prepared in Tris- acetate-EDTA Na2 (1x TAE) with 8.3 pH Along with 100 bp DNA marker, have been run. At fixed current 100 mA, 40 minutes, Agarose gel. Electrophoresis was performed. The 2 primers of BRCA1 is F – AGTAAATCCAGTCCTGCCAATG R- GGTAGTATGAGTTCCATCAAGG. amplicon 363bp 60 ° C and the primer of ERBB2 is F – CTATCCACCACAAGGAGCTG R – TAGCCATCTTTCCTTGCAGTC. amplicon at 489bp 60°C. To evaluate the DNA Sequencing. All the data obtained from automated sequence was edited with a computer based program sequencer TM. These sequences were compared with the reference sequence (NCBI database) to determine the nature of mutations. The analysis for mutations, the synonymous mutations was analyzed for codon usage. The results from this study and the data from Bali and Bebokwere (2016) [9], analyzed to predict the amino acid changes by the computer program MEGA version 2.1. The program SPSS version 16 and Microsoft Office Excel 2007 were used for data analysis.

Results

Table (1): The mean age studied groups between patients and healthy control

Studied groups	N	Mean (Age/Year)	Std. Deviation	Std. Error	t-test (P-value)
A.H. Control	40	25.83	7.971	1.261	Non sign. (P>0.05)
Patients	40	29.03	10.001	1.581	
Total	80				

N= Sample size, Std Deviation = standard Deviation, Std Error = standard Error, P-value= probability value

No significant difference was seen between patients (25.845±7.971) in comparison with control group (29.03±10.001), (P>0.05).

Table(2): The mean of the level of antibody titration between patient and control group

Antibodies		N	Mean	Std. Deviation	Std. Error	t-test (P-value)
IgM level	A.H. Control	40	0.417	0.184	0.0291	Highly significant (P<0.01)
	Patients	40	1.346	0.647	0.1022	
	Total	80				
IgG level	A.H. Control	40	0.425	0.139	0.0221	Highly significant (P<0.01)
	Patients	40	1.357	0.638	0.1009	
	Total	80				

N= Sample size, Std Deviation = standard Deviation, Std Error = standard Error
A.H = apparently healthy, P-value= probability value
IgM= Immunoglobulin M
IgG= Immunoglobulin G

A highly significant deference in the level between Anti- *Toxoplasma* antibody IgM of patients (0.417±0.184) and the control group (1.346±0.647), (P<0.01), and there was a highly significant difference between Anti- Toxoplasma antibody IgG level of patients (0.425±0.139) and the control group (1.357±0.638), (P<0.01).

Table(3): Demographical picture of the studied groups (patients & Control groups).

Parameters			Studied groups: Control group (n= 40)	Studied groups: Patients (n= 40)	χ^2 test (P-value)
Gender	Male	N	6	4	Non Significant (P>0.05)
		%	15%	10%	
	Female	N	34	36	
		%	85%	90%	
Age groups / Year	15 - 30	N	29	23	Non Significant (P>0.05)
		%	72.5%	57.5%	
	31 - 45	N	11	17	
		%	27.5%	42.5%	
Residency	Rural	N	20	29	Significant (P<0.05)
		%	50%	72.5%	
	Urban	N	20	11	

Continues …

Table(3):To be continue…

		%	50%	27.5%	
Family history	Yes	N	0	11	Highly Significant (P<0.01)
		%	0%	27.5%	
	NO	N	40	29	
		%	100%	72.5%	
IgG Result	Positive	N	0	30	Highly Significant (P<0.01)
		%	0%	75%	
	Negative	N	40	10	
		%	100%	25%	
IgM Result	Positive	N	0	29	Highly Significant (P<0.01)
		%	0%	72.5%	
	Negative	N	40	11	
		%	100%	27.5%	

χ^2 test = Chi- Square test, P-value= probability value, IgM= Immunoglobulin M IgG= Immunoglobulin G

A significant relationship between the rural 29(72.5%) compared with control group 20(50%) and urban 11(27.5%) in comparison with the healthy group 20(50%) according to residency (P<0.05). While there was a highly significant relationship between cancer patients which positive in 11(27.5%) compared with control group 0(0%), and negative 4(100%) in compared with control group 29(72.5%), (P<0.01). However, there was a highly significant relationship in the level of IgG positive 30(75%) and negative results 10(25%) and the level of IgM positive 29(72.5%) and negative results 11(27.5%) of Ant- TOXO. antibodies (P<0.01).

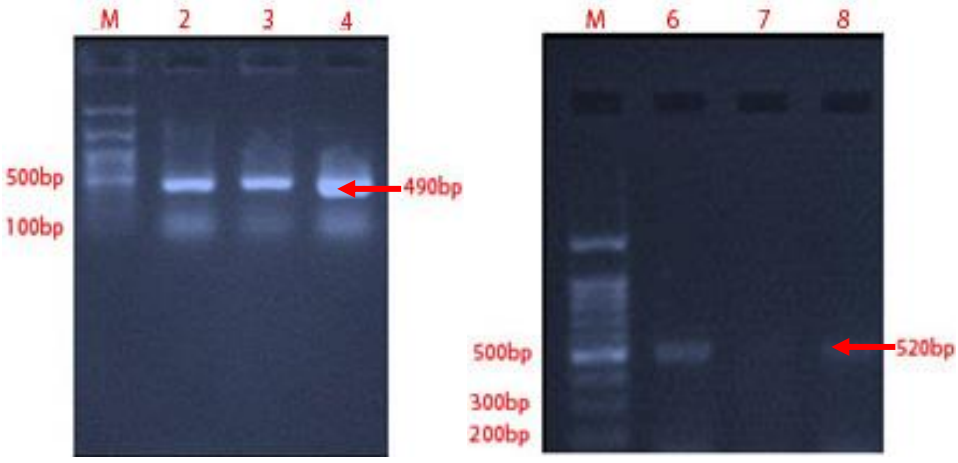

Figure 1: Detection the DNA of (*BRCA*1, *ERBB2*) genes

Lane 1 markers correspond to 100 bp ladder (fermintus), lanes 2 & 3and 4 the BRCA1 gene bands with 490 bp. Lane 4 markers correspond to 100 bp ladder (fermintus), lane 5 correspond to 100 bp ladder (fermintus) lanes 6, 7 and & 8the ERBB2gene bands with 520 bp.
The electrical current = 60 volts at 30 min, time when using the agarose gel (2%) with At 2.5% with ethidium bromide (0.5µg/ml).

DNA isolated by FFPE tissues was done by using QIAamp DNA FFPE tissue-DNA Extraction Kit (Qiagen,USA). The agarose gel 2% was prepared in Tris-acetate- EDTA Na2 (1x TAE) with 8.3 pH Along with 100 bp DNA marker, have

been run. At fixed current 100 mA, 40 minutes, Agarose gel. Electrophoresis was performed. The primers of BRCA1 gene is F –AGTAAATCCAGTCCTGCCAATG R –GGTAGTATGAGTTCCATCAAGG amplicon 363bp 60°C, and the primer of ERBB2 F –CTATCCACCACAAGGAGCTG R – TAGCCATCTTTCCTTGCAGTC amplicon at 489bp 60°C.

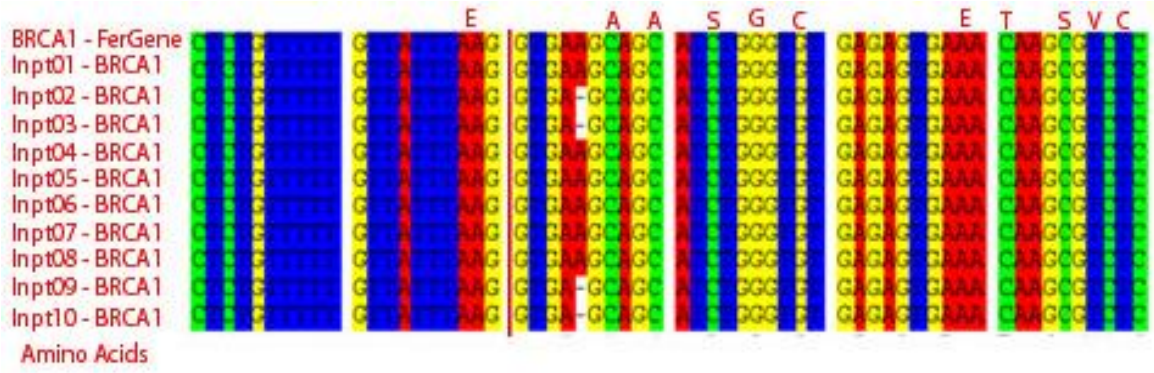

Figure (2): The sequence of BRCA1 Gene

Nucleotides changes showed in BRCA1 gene Exon 11 amplified from tumor breast cancer samples, the mutation occurred on nucleotides AC, CG, TC, and CT of BRCA1 gene

Sequencing of 10 samples of breasts cancer groups for the BRAC1 gene were carried out Ha Thuc Ai Hien, and his association [10], and the reference sequence which is taken from NCBI database was compared with these sequence. In order to edit the sequencer and to determine the mutant nature, a computer program sequencer (TM).

Discussion

Toxoplasma gondii invades different organs and tissue and causes modulation of host cells may develop into cancer. The distribution of breast cancer among Iraqi patients was among female 90% than male 10%, a survey presented supportive evidence to this result that found Al-Jabbar [5] who reported that 67(87.6%) females compared with males 10(12.94%), and were 52.24% infected from *T. gondii*. Also the distribution of breast cancer among Toxoplasmosis patients was a highly significant difference in rural patients than urban patients according residency [5]. While the family history plays an important role among *Toxoplasma* infection with breast cancer patients. A study presented supportive evidence to this result that found with Melvin, and his associates who reported that a family history (FH) of breast cancer (BC) is known to increase an individual's risk of disease onset[11]. The levels of Anti-*Toxoplasma* IgM and IgG were at its highest among breast cancer infected individuals. A study presented supportive evidence to this result that found Imam and his associates who reported that in Qassim region, *Saudia Arabia,* the antibody frequency seropositivity of *T. gondii* infection in a group of cancer patients, and they also found a relationship between *T. gondii* seropositivity and the

selected variables [12]. It has also been found that there association between *BRCA1* and *BRCA2* with Rad51protein which is incorporated the HR repair DSBs and recombination during meiosis and mitosis [8], Mutation occurred in 11 position of BRCA1 gene and this is an evidence of a deviation in the pathway of the gene sequence in this codon of gene expression of breast tissue that may cause cancer. A study presented supportive evidence to this result that found_Araya, and his associates who determined that cancer driver genes can be identified by the accumulation of protein- altering mutations[13]. The evolution in abnormal cell development is a phenomenon of ancient history and that the family has an important role in the emergence of those cases genetically. A study presented supportive evidence to this result that found Al-Jabbar, has concluded that the history of the families has an important effect in the emergence of the disease and it is associated with toxoplasmosis [5]. It also opens a new horizon that confirms the relationship of *Toxoplasma* with breast cancer was trivial**.** The change that occurred in these sites of BRCA1 gene protein, and changing the course of manufacturing in nucleotides is evidence of cellular deformation. A study presented supportive evidence to this result that found Amir M. and Mansoureh A. who reported that number of mutations in two predisposing genes (BRCA1 and BRCA2) that occurred in patients with a family history was investigated [14]. *T. gondii* possesses a number of proteins that in turn lead to breast tissue malformation that may cause cancer [15]. Thus, we conclude that there is a link between toxoplasmosis and breast cancer, and that this mutation in Breast cancer patients is due to Toxoplasma gondii infection which causes a risk of death.

Recommendations: In view of the above results it is of importance to throw light on the gene expression by real-time technique to detect the genetic mutation and the change in the protein synthesis of patients with Toxoplasmosis.

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