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Gene Polymorphism of Human Lymphotoxin Alpha in Iraqi Breast Cancer Women

Saadia Othman Muhammed[1], Nawal Mohammed Utba[*1], Yaala Saady Raof[2]
[1]*Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq, 10071*
[2]*Ministry of health, Al-Amal National hospital for Cancer patients, Baghdad, Iraq*

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Abstract

The lymphotoxin alpha is a highly polymorphic gene and any genetic variation in it may lead to an increased production of cytokine LTA thus helping tumor development and progression. The aim of this work was to investigate the association of LTA polymorphism with the risk of breast cancer among Iraqi women. The findings of this study demonstrated that the age group > 50 years old formed 52% of the breast cancer patients (P <0.001). Hardy–Weinberg equilibrium analysis revealed that genotype frequencies of most SNPs in BC patients and HC were consistent with HWE. No association was found between LTA polymorphisms and BC. Moreover, seven haplotypes were detected in BC group. However, only one of them developed significant increase; T -A -C -C -G -C –G (0.20 *vs*. 0.04, OR: 5.931, P = 0.015). Markedly, some SNPs were in a strong LD, while others showed weak or no LD. We can conclude that an insignificant association was found between LTA polymorphisms and breast cancer in Iraqi women.

Keywords: Breast cancer, LTA, Polymorphism, Haplotype, Iraq

تعدد الاشكال الوراثي لجين اللمفوتوكسين الفا البشري في النساء العراقيات المصابات بسرطان الثدي

سعدية عثمان محمد[1] ، نوال محمد عتبة*[1] ،علا سعدي رؤوف[2]
[1]قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق
[2] وزارة الصحة، مستشفى الامل الوطني لمرضى السرطان، بغداد، العراق

الخلاصة

يعد جين ليمفوتوكسين ألفا جينا متعدد الأشكال للغاية ويحتوي على تغايرات جينية تؤدي إلى زيادة إنتاج الحركي الخلوي لمفوتوكسين الفا الذي يساعد على تطور الورم وتقدمه. هدفت هذه الدراسة الى التحري عن علاقة تعدد الأشكال الوراثي للمفوتوكسين الفا مع خطر الإصابة بسرطان الثدي بين النساء العراقيات. أظهرت نتائج هذه الدراسة أن الفئة العمرية الأكبر من 50 سنة شكلت 52٪ من مرضى سرطان الثدي (P<0.001) ، كشف تحليل توازن هاردي–واينبرغ أن ترددات النمط الوراثي لـ SNPs في مرضى سرطان الثدي والسيطرة الاصحاء كانت متوافقة مع توازن هاردي–واينبرغ. لم يتم العثور على ارتباط بين تعدد الأشكال الوراثي للمفوتوكسين الفا ومرضى سرطان الثدي. علاوة على ذلك، تم اكتشاف سبعة أنماط فردانية في مجموعة المرضى واحد منهم فقط؛ T –A –C –C –G –C –G اظهر زيادة كبيرة لديهم (0.20 مقابل 0.04 ،

*Email: anooutba2014@yahoo.com

5.931 =OR، 0.015 = P). وأظهرت بعض SNPs ارتباطا غير متوازن قوي بشكل ملحوظ، بينما أظهر البعض الآخر ارتباطا غير متوازن ضعيف أو عدم وجوده على الإطلاق. تم الاستنتاج انه هناك ارتباط غير معنوي بين تعدد الأشكال الوراثي للمفوتوكسين الفا وسرطان الثدي لدى النساء العراقيات.

Introduction

Generally, cancer is a very heterogeneous multifactorial disease [1]. A strong association between breast cancer (BC) and several predisposing factors including hormonal levels such as oestrogen and progesterone, mutation, polymorphism, and some behavioural choices breastfeeding, physical activity, and diet has been reported [2, 3]. Nearly, 2.3 million females are diagnosed with BC worldwide out of which only 10% are hereditary [4].

Inflammatory reactions in certain organs may increase the risk of cancer development. However, these reactions represent the seventh hallmark of cancer [5]. As a cytokine belongs to the TNF family, lymphotoxin alpha (LTA) has been assigned as an antitumour factor. Thereafter, LTA was demonstrated to possess immunologic activities [6].

The gene encoding LTA is located in chromosome 6p21.3 [7]. There is a potential effect of single nucleic polymorphism (SNP) on the expression of cytokine being a significant mediator of cancer [8]. Meta-analysis data has revealed that SNPs related to *LTA* polymorphisms were associated with an increased risk of cancers [9]. Yet, others found a negative correlation between *LTA* polymorphisms and cancer [10-12]. Furthermore, Zhou, *et al.* [13] found an insignificant association between the LTA polymorphism and BC in Caucasian populations and a positive association was observed in Asian populations [13].
In view of the aforementioned controversial findings and the shortage in literature scrutinizing the association between LTA polymorphism and breast cancer in Iraqi women, the current work aimed to investigate the potential association of SNPs in TNF-LTA locus with breast cancer among Iraqi women.

Materials and Methods

Ethical Statement

This work was approved by the ethics committee of the College of Science, University of Baghdad (Ref. CSEC/1020/0054). All patients were enrolled in this study via informed consent.

Study Groups

A total of 50 women, aged between 21-70 years, with BC admitted to Al-Amal National Hospital for oncology and Al-Andulus Private Hospital in Baghdad province, were enrolled in the present study under the supervision of an expert oncologist. Moreover, another 50 apparently healthy women without a family history of BC, visiting Women's Health department in Al-Elwya Hospital, represented the control group (HC). Their age range matched that of the patients group. The diagnosis of the control group as free from breast cancer was confirmed by the consultant medical staff in these hospitals and the results of ESR and CRP tests were negative. All participants belonged to the same ethnic group of the Iraqi population. Before starting the treatment, 2 ml of blood sample was collected in EDTA tubes from patients with different stages of cancer and from control.

Detection of *LTA* SNPs

DNA was extracted from 200 µl blood samples using ABIOpure^{TM} Total DNA kit (USA). Whereas, its concentration was measured using Quantus Fluorometer (Promega, USA).

Two LTA SNPs (rs2229094, rs1041981) were determined in the first region (930 bp) using the forward primers 5`-TGTAAAACGACGGCCAGTAGAGGCAAACACCAGAATG-3` and the reverse primer 5`-CAGGAAACAGCTATGACAGAGAGAGATCGACAGAGAAG-3`. Moreover, five SNPs (rs2844482, rs2071590, rs1800683, rs2239704, and rs909253) for the second region (1032 bp) were investigated using the forward primer 5`-TGTAAAACGACGGCCAGTTCCTGTCTCTCTCTGTCT-3` and the reverse primer 5`-CAGGAAACAGCTATGACCCCTGGATACACCATCTT-3 `, all previous primers were prepared in the present work.

Monoplex PCR assays were performed in a 20 µl volume containing 10 µl of 2X GoTaq Green Master Mix (Promega, USA), 1 µl of each of 10 pmol forward and reverse primers, 6 µl nuclease-free water, and 2 µl of template DNA. PCR cycling was performed using BioRad (USA) thermocycler as follows: 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 30 sec; annealing at 60°C for 30 sec; and extension at 72°C for 30 sec. And the final extension for 7 min at 72°C. The amplicons were sequenced by the Sanger method using an ABI3730XL automated DNA sequencer (Macrogen, Korea). Each SNP genotype of the investigated *LTA* was ascertained in the DNA sequences upon alignment with a reference sequence of the SNP that was downloaded through Geneious software version 10.2.2.

Statistical Analysis

Data of ages, alleles, and genotypes of LTA gene SNPs was demonstrated as numbers and/or percentages. Hardy–Weinberg equilibrium (HWE) was applied to test the differences between study variables and expectations. Odds ratio (OR) and 95% confidence interval (CI) were calculated by Logistic regression analysis. P value less than 0.05 was considered significant. DNA sequences were analysed by Geneious software version 10.2.2 and the significance was analysed using the Chi-square test. SHEsis software version 4.2 was used to assess linkage disequilibrium (LD) between SNPs and to estimate haplotype frequencies. The LD coefficient (D') was used to define LD.

Results

Age Group Distribution of Breast Cancer Patients

The present findings demonstrated that 52% of the BC patients aged more than 50 years (P <0.001). Patients aged 30 – 50 years made up 38% and those younger than 30 years constituted 12%.

3.3 *LTA* Gene Polymorphisms

3.3.1 Hardy-Weinberg Equilibrium Analysis

HWE analysis revealed that genotype frequencies of most SNPs in BC patients and HC were consistent with HWE. However, insignificant differences were found between the observed and expected genotype frequencies. Nonetheless, with one exception of this trend; the rs909253 SNP in HC group (P = 0.005).

3.3.2 Genotype and Allele Analysis of *LTA* Gene SNPs Associated with Breast Cancer Disease

Regarding the results summarized in Table 1, an insignificant association was observed between the studied SNPs and the corresponding disease.

Table 1: Logistic regression analysis of *LTA* gene SNPs in breast cancer patients and control

LTA gene SNPs	Genotypes and Alleles	BC (N:50)		HC (N: 50)		OR	95% CI	*P* value
		N	%	N	%			
rs2229094	TT	36	72	32	64	1.45	0.45 to 4.67	0.762
	TC	10	20	12	28	0.79	0.21 to 2.95	1
	CC	4	8	6	8	0.64	0.10 to 4.03	1
	T	82	82	76	76	1.44	0.55 to 3.76	0.624
	C	18	18	24	24	0.61	0.20 to 1.85	0.567
rs1041981	CC	24	48	24	48	1	0.34 to 2.97	1
	CA	20	40	20	40	1	0.33 to 3.03	1
	AA	6	12	6	12	1	0.19 to 5.32	1
	C	68	68	68	68	1	0.44 to 2.30	1
	A	32	32	32	32	1	0.44 to 2.30	1
rs2844482	CC	36	72	44	88	0.35	0.08 to 1.51	0.239
	CT	14	28	4	8	4.47	0.86 to 23.38	0.138
	TT	0	0	2	4	-	-	-
	C	86	86	92	92	0.53	0.15 to 1.93	0.525
	T	14	14	8	8	2.04	0.53 to 7.90	0.496
rs2071590	GG	26	52	24	48	1.17	0.40 to 3.48	1
	GA	24	48	22	44	1.17	0.39 to 3.50	1
	AA	0	0	4	8	-	-	-
	G	76	76	70	70	1.36	0.56 to 3.27	0.653
	A	24	24	30	30	0.74	0.31 to 1.77	0.653
rs1800683	GG	22	44	30	60	0.52	0.17 to 1.58	0.396
	GA	16	32	14	28	1.21	0.37 to 3.97	1
	AA	12	24	6	12	2.32	0.52 to 10.23	0.463
	G	60	60	74	74	0.53	0.23 to 1.22	0.202
	A	40	40	26	26	1.90	0.82 to 4.39	0.202
rs2239704	CC	18	36	18	36	1	0.32 to 3.10	1
	CA	24	48	20	40	1.38	0.46 to 4.15	0.776
	AA	8	16	12	24	0.60	0.15 to 2.40	0.725
	C	60	60	56	56	1.18	0.54 to 2.59	0.840
	A	40	40	44	44	0.85	0.39 to 1.86	0.840
rs909253	AA	30	60	36	72	0.58	0.18 to 1.86	0.551
	AG	10	20	6	12	1.83	0.40 to 8.41	0.702
	GG	10	20	8	16	1.31	0.32 to 5.44	1
	A	70	70	78	78	0.66	0.27 to 1.61	0.495
	G	30	30	22	22	1.91	0.63 to 5.74	0.396

HC and BC denote to control group and breast cancer patients group respectively.

3.3.4 Haplotype Analysis between Alleles of *LTA* gene SNPs

Only haplotypes having a frequency greater than 3% were recorded. Based on this criterion, it was possible to assign seven haplotypes; only one of them T -A -C -C -G -C –G (0.20 *vs*. 0.04; OR: 5.931; P = 0.015) significantly increased in BC patients than in control (Table 2).

Table 2: Estimated haplotype frequencies of *LTA* gene SNPs (rs2229094 T/C, rs1041981 C/A, rs2844482 C/T, rs2071590 C/A, rs1800683 G/A, rs2239704 C/A and rs909253 A/G) in breast cancer patients and control groups.

Haplotypes	BCBT (N=25)		HC (N=25)		OR	95% CI	*P* value
	N	Frequency	N	Frequency			
C C C C G C A	3.0	0.060	5.75	0.115	0.469	0.109-2.027	0.302
C C T C G C A	4.0	0.080	2.37	0.047	1.696	0.321-8.962	0.530
T A C C A C G	7.0	0.140	6.0	0.120	1.152	0.354-3.748	0.814
T A C C G C G*	10	0.20	2.0	0.040	5.931	1.217 – 28.892	0.015
T C C A A A A	3.0	0.060	2.51	0.050	1.166	0.207-6.581	0.861
T C C A G A A	5.0	0.10	8.87	0.177	0.488	0.149-1.600	0.230
T C C C G C A	2.0	0.040	3.25	0.065	0.576	0.094-3.530	0.546

Pairwise analysis of linkage disequilibrium (LD) between the seven SNPs studied in this work revealed different values of D' (Coefficient of LD). Some SNPs were in a strong LD, while others showed weak or no LD. Such profile was different in BC patients (Figure 1) and controls (Figure 2).

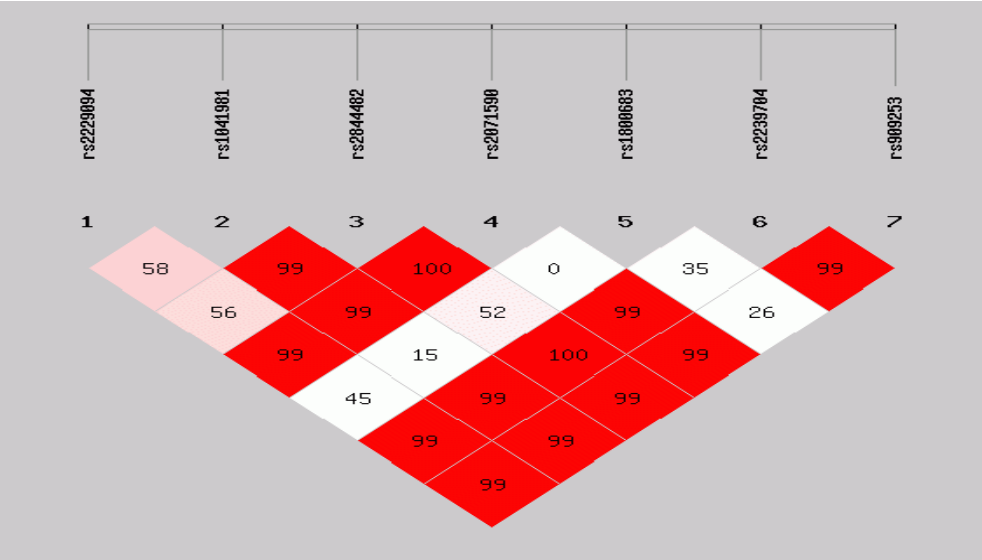

Figure 1: Schematic plot of pairwise linkage disequilibrium (LD) between LTA gene SNPs in breast cancer patients generated by SHEsis software. The LD was defined in terms of the LD coefficient (D`). The colour density of squares is proportional to the degree of LD (Red: Strong LD; White: No LD). The numbers in squares are the value of D` multiplied by 100.

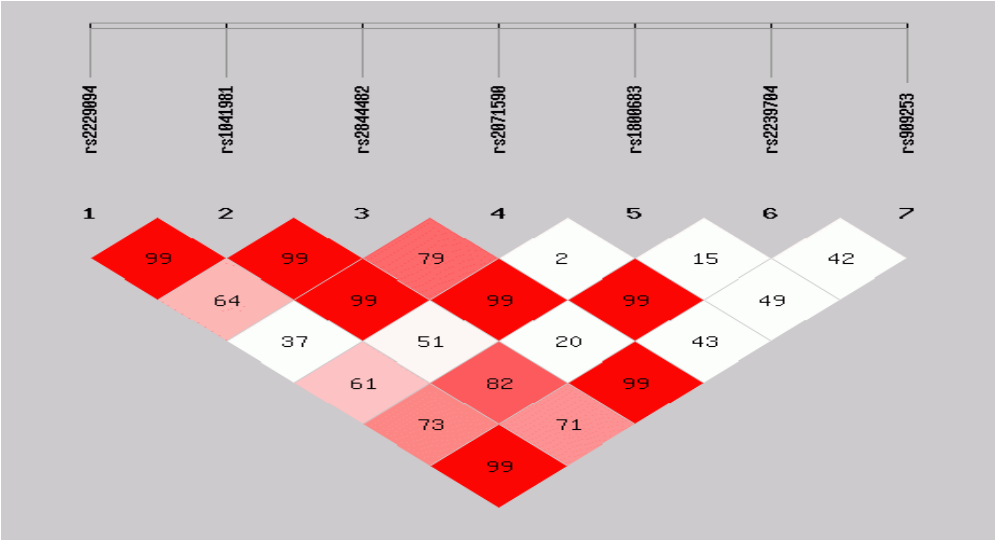

Figure 2: Schematic plot of pairwise linkage disequilibrium (LD) between LTA gene SNPs in healthy control group generated by SHEsis software. The LD was defined in terms of the LD coefficient (D`). The colour density of squares is proportional to the degree of LD (Red: Strong LD; White: No LD). The numbers in squares are the value of D` multiplied by 100.

Discussion

Women of > 40 years old were found to be at high risk of getting BC due to increasing opportunity for genetic damage that took place and lacking the repairing capability [14]. Aljubori [15] stated that BC patients aged ≤ 50 years in northern Iraq, while Mutar, *et al*. [16] found that 45% of the Iraqi female patients were ≤ 50 years. Locally, the mean age of the Iraqi women with BC was under 50 years old [15-19].

Cytokine genes implicated in the inflammation and metabolism have gained distinguished consideration in cancer genetics. Numerous investigations have been done to comprehend the association of polymorphism of cytokine genes such as LTA in BC. The polymorphism of *LTA* gene among ethnic populations has demonstrated conflicting findings. Studies in north India have revealed that *TLA* polymorphism has no significant association with BC [20]. However, Korean population of AA genotype of rs909253 has shown resistance for the BC [21]. An allele is associated with the production of high levels of TNF-α [22]. Such findings are compatible to designation of TNF as being an antitumour factor. Interestingly, it seems that *TLA* polymorphism presents its association with BC based on ethno-specific manner [20]. Many factors significantly participate in the pathogenicity and epidemiology of cancer, among which are the genetic determinants. Interestingly the gene expression of TNF can be regulated by its genetic polymorphism that is correlated with some inflammations and malignancies [23].

The carrier of the rare allele A of the SNP rs1041981 was reported to be significantly associated with protection from the BC. However, carrier of the rare allele C of the SNP rs3093543 has shown significant association with susceptibility to BC and insignificant association for rs2229094 with BC has been observed [24]. Huang, *et al.* [9] reported a significant association with an increased cancer risk for rs1041981, rs2229094 and rs2239704. Furthermore, several other studies have found no association between LTA SNPs and BC [20, 25-27].

Conflict of Interest:

No conflict of interests to declare

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