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The role of single nucleotide polymorphism (C/T) of TGF-β1 gene at position -509 in the promoter region in progression of breast cancer among some Iraqi women

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Abstract: Risk of breast cancer incidence among women can influence by many genes polymorphisms that cause different variations in their expression. Transforming growth factor-β1 (TGF-β1) has clear role in progression and invasion of breast carcinoma. Specific polymorphic variants in TGF-β1 gene correlate with increased its expression or loss of inhibition. This study was aimed to find out if the single nucleotide polymorphism C/T at position -509 in the promoter region of TGF-β1 gene with its genotypes can be considered as predisposing factors of in 58 Iraqi women with confirmed breast cancer who were admitted to Alsader Teaching Hospital in Al-Najaf province. Polymerase chain reaction-restriction fragment length polymorphism technique was used to estimate genotyping of TGF-β1 gene polymorphism in the patients and 30 healthy women without any inflammatory disorders or clinical manifestation of any disease (control group). Genotype distributions of SNP (-509 C/T) showed no significant difference ($p>0.05$) between patients (CC: n= 45, 77.58%; CT: n=11, 18.96%; TT: n=2, 3.44%) and controls (CC: n= 25, 83.33%; CT: n=5, 16.66%; TT: n=0). That means the SNP C/T in position 509 in the promoter region of TGF-β1 gene plays no significant role in the progression of breast cancer among Iraqi female patients.

Key words: Polymorphism , TGF-β1 gene , breast cancer.

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Introduction:

Breast cancer (BC) is the most frequent diagnosed malignancy in women worldwide. Over the next 25 years cancer is predicted to increase by more than 70 percent in the developing countries, BC represents 25% of all cancer cases and is a major leading cause of cancer death among women(1).

Breast cancer (BC) progression is influenced by many genetic factors. Genetic changes in BC ranges from single nucleotide or point mutations (substitutions or deletions or insertions of oligonucleotides) to chromosome abnormalities which collectively may cause disturbance in expression of gene that responsible for cellular proliferation and functions (2).

Transforming Growth Factor-β (TGF-β) is a cytokine with many receptors in all tissues that regulate epithelial cells behavior and their transformed carcinoma cell derivatives in addition to other effects on hormonal synthesis, reproduction, embryogenesis, and tissue repair (3-5)

TGF-β promote the apoptosis in cancer initiation process (suppressive

role), while impaired or loss of its function has been detected in in human diseases, inflammation, cancer formation or hyperproliferative, and autoimmune diseases (6-8).

In carcinoma cell TGFβ plays two roles: firstly as a tumor suppressor which includes hypoproliferative effects of normal cell, promote the apoptosis in early cancer cells (9, 10).

Second role of TGFβ is a tumor promoter which includes induction in tumor progression to secondary distant sites or metastasis, and invasion (11).

Cancer cells in advance stages secrete high levels of TGFβ which inhibit the natural adhesion of cells, destroy the matrix between cells, and promote angiogenesis (12, 13).

This high levels of TGFβ of TGF*β* gene are expressed in many malignancy cells, including breast, prostate, brain, and liver (14).

Multiple mutations in the TGFβ pathway or its receptors have been recorded as a critical cause for inhibit TGFβ role as cancer suppressor in many types of carcinomas (15).

There are six SNPs have been studied in TGF-β1 gene on chromosome 19 (q13.1- 13.3) (16-18). Two of these SNPs are (-509C/T and 869T/C) have an important effect that influence gene expression in breast cancer (19).

The first polymorphism (C-509T) is located in the region that represent major start site for transcription when the transcription factor initiate the transcription of TGF-β1 gene to form the precursor of TGF-β1(a 390 amino acid pro-peptide), so this SNP is relative to the TGF-β1 gene expression and its concentration in blood (20,21).

This study is focused on investigate the possible correlation between TGF-β1 SNP (C-509T) (with its combined genotypes) and breast cancer progression.

Materials and Methods:

This study was carried out during the period from January 2016 till February 2017, in Alsader Teaching Hospital in Al-Najaf Al-Ashraf province and in the Biology Department/ laboratory of molecular biology at Faculty of Science – University of Kufa.

1. Subjects:

a) Study group: This study was included 58 Iraqi women with confirmed breast cancer who were admitted to Alsader Teaching Hospital in Al-Najaf province .The diagnosis was confirmed by histological examination under the supervision of pathologist from the hospital, who determines the breast cancer histological grade, cancer stage (TNM staging system: tumor, node, metastasis), and identifies other specific *criteria* of the carcinoma on Scarff-*Bloom*-Richardson *grading* scheme (22).

All these women were diagnosed with invasive breast carcinoma (regardless of cancer cell origin, ductal or lobular carcinomas) in which cancer cell spread to the surrounding tissues of breast from inside the milk ducts or lobules (23).

b) Control group: consists from 30 healthy women without any inflammatory disorders or clinical manifestation of any disease.

2. Collection of Blood Samples:

Two ml of venous blood was collected in EDTA tubes from

breast cancer patients and store at -20 °C until used for PCR test.

3. DNA isolation and Genotyping:

Genomic DNA was isolated by using Genomic DNA Mini Kit protocol, which depend on for DNA isolation from frozen blood (Geneaid Biotech. Ltd., Taiwan Company, Cat. No. GB100, LOT. No. TJ21207).

A sequence of variants (C/T) at position -509 in the promoter region of TGF-β1 gene was amplified using these primer pairs (synthesized by AccuOligo® Bioneer Corporation. USA) sequences:

5'- CCCGGCTCCATTTCCAGGTG -3'
5'- GGTCACCAGAGAAAGAGGAC -3'

These primers were published previously (19, 24).

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method has been used for TGF-β1 genotyping.

Amplification was done in a thermal cycler (Bibby scientific Ltd/UK) programmed for 35 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min, these cycles preceded by an initial denaturation of 7 min at 95°C. The final extension step was for 10 min at 72°C.

The PCR amplified 808-bp product (which confirmed by gel electrophoresis method on 2% agarose gel) in 20 µl tube of PCR PreMix Reaction Mixture (Bioneer Corporation, USA) containing template DNA (5µl), DNA polymerase (1 unit), reaction buffer (2µl), stabilizer and loading-dye (2µl), dNTPs (2µl), and 2µl of each primer (2µl forward and 2µl reverse), and finally, distilled water was added to reach to the final volume of 20 µl.

The 808-bp DNA fragment was digested overnight at 37 °C with 10 unit of Bsu361 restriction enzyme (Source: from *Bacillus subtilis* 36) (synthesized by New England Biolabs, UK, Cat.No. R0524S). After digestion, the yield was one of these results:

a) Two fragments for allele C (617& 191bp DNA fragments).
b) One fragments for allele T (808-bp DNA fragment, not digested).

Finally, the gel electrophoresis method was done according to Sambrook and Russell (25), and 5 µl of each samples was loaded onto 2% agarose gel.

4. Statistical Analysis:

All statistical analyses of results were performed by the help of SPSS software version 17 for Microsoft Windows using the test of chi square (P value was considered significant at level less than 0.05) for the analysis of association between the study variables studied. Data were recorded as mean± SD (standard deviation), percentage or range. Direct gene counting was depended for calculation of genotypic frequency of C-509T polymorphism.

Results:

This study included 58 Iraqi women with confirmed breast cancer who were admitted to Alsader Teaching Hospital in Al-Najaf province and 30 healthy women without any inflammatory disorders or clinical manifestation of any disease (control group).

The Clinical characteristics:

The clinical characteristics of subjects are listed in Table 1. There are

no significant statistical different ($p>0.05$) of age and BMI between the women with breast cancer and the 30 healthy control group (the mean age was 51.03±9.34 and 49.35±10.21 years, respectively). The mean of Body mass index (BMI) in women with breast cancer is 30.28±5.13 kg/m^2, while 29.51±7.73 kg/m^2 for the healthy controls women. Of these cases, 21.69 were premenopausal and 78.31 were postmenopausal women. No significant difference ($P > 0.05$) was recorded between patients and controls in menopausal status.

Specific pathological features of the breast cancer cases were presented in detail in table 1, which include breast cancer stage (TNM staging system: tumor, node, metastasis), and histological grade.

Table (1): The results of clinical characteristics and some specific *criteria* of the cancer cases in breast cancer patients and controls.

Parameter		Breast Cancer Patients	Controls.
Age (*years*), n = 88		51.03 ± 9.34	49.35±10.21
Body mass index (BMI kg/m^2), n = 88		30.28 ± 5.13	29.51 ± 7.73
Menopausal status, n = 88	Premenopausal (%)	21.69	30.84
	Postmenopausal (%)	78.31	69.16
TNM stage (%): n = 58	0	0	
	I	36.2	
	II	51.72	
	III/IV	12.06	
Histologic grade (%): n = 58	Well	24.13	
	Moderate	34.48	
	Poorly	41.37	

* Data were recorded as mean± standard deviation or percentages. Abbreviations: ER: estrogen receptor; PR: progesterone receptor; TNM staging system: tumor, node, metastasis (the stage of the breast cancer).

The -509 C/T SNP and breast cancer:

a) Controls: Among the 30 healthy women (controls) 25 (83.33%) had found as homozygous wild (CC, normal alleles), and 5 (16.66%) found as heterozygous genotype (with the normal (C) and mutant (T) alleles (CT); (CC: n= 25, 83.33%; CT: n=5, 16.66%; TT: n=0). (Table 2 & Figure 1).

Table (2): The results of genotypic frequencies of -509C/T single-bp polymorphism at TGF-β1 gene in patients and controls.

Subjects	Genotype distribution results			Total
	Homozygous wild CC	Heterozygous CT	Homozygous mutant TT	
Healthy controls	25 (83.33%)	5 (16.66%)	0	30 34.09%
Breast cancer group	45 (77.58%)	11 (18.96%)	2 (3.44%)	58 65. 9%
Total	**70 79.54%**	**16 18.18%**	**2 2.27%**	**88 100%**

P-value >0.05

b) Breast cancer patients: Among 58 breast cancer women, 45 (77.58%) had found as homozygous wild (CC, normal alleles), 11 (18.96%) found as heterozygous genotype (with the normal (C) and mutant (T) alleles (CT), and 2(3.44%) had found as homozygous mutant genotype (TT, mutant alleles); (CC: n= 45, 77.58%; CT: n=11, 18.96%; TT: n=2, 3.44%) (Table 2 & Figure 1).

That means the frequencies of -509C/T SNP in the TGF-β1 gene in the 58 Iraqi women with breast cancer in Al-Najaf province showed no significant differences compared to 30 healthy women in control group ($p>0.05$).

The association of TGF β1 -509C/T polymorphism and pathological features of the breast cancer cases was analyzed. The results of this study showed (Table 3) no evidence for association of -509C/T polymorphism with histologic grade and TNM of breast cancer.

Table (3): The association between TGF β1 -509C/T polymorphism and pathological features of the breast cancer cases.

Subjects		Genotype Distribution Results		
		CC	CT	TT
TNM stage	**0**	0	0	0
	I	19	2	0
	II	25	5	0
	III/IV	1	4	2
Histologic grade	**Well**	12	2	0
	Moderate	15	4	1
	Poorly	18	5	1

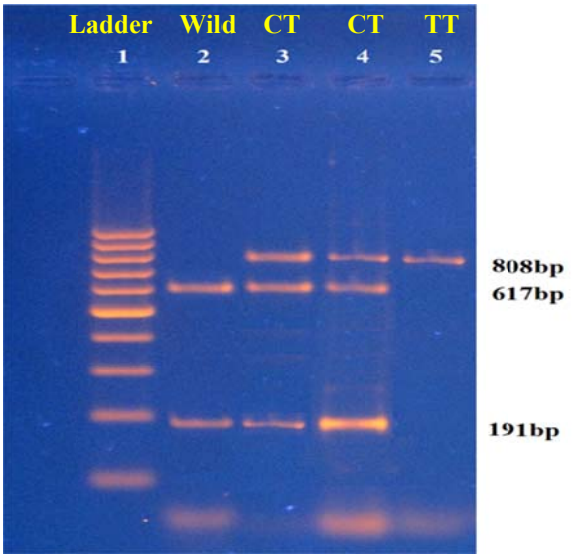

Figure (1): The gel electrophoresis image of PCR-RFLP results (Lane 1: 100 bp DNA Ladder; Lane 2: wild homozygous genotypes (CC; 617-bp and 191-bp); Lane 3&4: The heterozygous genotype (CT; 617-bp, 191-bp, and 808-bp. The last fragment which represented T allele is the original PCR fragment that not digested and remains intact because the Bsu361 restriction enzyme not found the specific cleavage position); Lane 5 homozygous mutant genotype (TT; 808-bp).

Discussion:

With the annually new cases in all over the world breast cancer is the most widespread invasive cancer in females. Breast cancer progression is influenced by many genetic factors.

This study is focused on investigate if there was any possible correlation between C-509T polymorphism in the promoter region of TGF-β1 gene and invasive breast cancer progression in Iraqi women from Al-Najaf Al-Ashraf province.

The healthy controls were selected to be matched by age with breast cancer cases. All of them were with age over 38 years old. That because the incidence of breast cancer increase with age. Najjar and Easson (26) found that the average age of breast cancer incidence in Arab countries was 45.4 years (these data from 11 articles from 8 Arab countries and included 5144 cases), and that incidence was obvious to be a 10 years earlier than in western world.

This study showed that 78.31% of patients were postmenopausal women, which confirm that the postmenopausal hormones are one of the most important breast *cancer* risk factor. Many studies reported a similar increasing risk of BC in postmenopausal women (27-29).

The -509C/T single-bp polymorphism in the TGF-β1 gene plays a major role in increase TGF-β1concentration and promotes BC invasion, and migration (5, 30-32).

In this study, frequencies of -509 C/T single-bp polymorphisms in the TGF-β1 gene in the 58 Iraqi women with breast cancer and the 30 healthy women in control group revealed no significant difference between them ($p>0.05$) in Al-Najaf province.

These results agreed with other studies found that no significant association between TGF-β1 gene polymorphism in BC patients and controls (33, 34).

The curious finding of this study is that, although not statistically significant differences between patient and controls, BC cases with CT or TT genotype for the-509C/T single-bp polymorphisms in the TGF-β1 gene had higher tumor grade, poorly differentiated, and advanced stage of cancer than GG genotype subjects.

That confirmed by other study which recorded that T allele in this polymorphism was associated with higher blood concentration of TGF-β1 in BC patient (17; 35, 36).

Qi *et al.* (33) performed ethnic subgroups analysis and found that the -509C/T polymorphism in the TGF-β1 gene significantly increased risk of BC in Caucasian population, but not in Asian population.

In this study, the SNP C/T at position -509 in the promoter region of TGF-β1 gene plays no significant role in the progression of breast cancer among Iraqi female patients.

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