

Effects of Two Common Promoter Polymorphisms of Transforming Growth Factor- β 1 on Breast Cancer Risks in Ahvaz, West South of Iran

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Abstract

Background: Transforming growth factor- β 1 (TGF- β 1) has a critical role in breast cancer initiation and progression.

Objectives: We have investigated the possible differences in two promoter polymorphisms (-509C/T and -800G/A) of TGF- β 1 gene between breast cancer cases and controls.

Patients and Methods: A total of 100 patients with confirmed breast cancer and 100 subjects without breast cancer was selected. Two promoter polymorphisms (-509C/T and -800G/A) of TGF- β 1 gene were genotyped using PCR-based restriction fragment length polymorphism (RFLP) method.

Results: The allele frequencies were 63% for C allele and 37% for T allele of SNP -509C/T and 66% for G allele and 34% for A allele of SNP -800G/A. Although no significant difference has observed between two groups, according to the genotype distribution, However, the TT genotype of -509 and AA genotype of -800 was significantly associated with breast cancer risk [odds ratio (OR) = 2.409; 95% confidence interval (CI) = 1.087 - 5.337, P = 0.030; and OR = 2.383; CI = 1.039 - 5.40, P = 0.040, respectively]. In addition, a multinomial logistic regression model shown, homozygous of -800 G/A (OR = 0.570; 95% CI = 0.362 - 0.896, P = 0.015); and HDL-C (OR = 0.935; 95% CI = 0.906 - 0.965, P < 0.001) were the selected variables associated with the presence of breast cancer. Haplotype analysis has shown no significant association between TGF- β 1 haplotypes and breast cancer risk.

Conclusions: Our results indicated that among two promoter polymorphisms of the TGF- β 1 gene, -800G/A compared to -509C/T is more associated with breast cancer.

Keywords: Transforming Growth Factor- β 1, Breast Cancer, Polymorphism

1. Background

Transforming growth factor beta (TGF- β), a super family of growth factors has shown implicated in the regulation of cellular and molecular processes which have involved in cancer initiation and promotion (1). Moreover, the function of TGF- β through specific signal transduction pathway had a critical and significant effect in many cellular processes which have changed in several human cancers (2-5). The dual effect of TGF- β 1 on the carcinogenesis such as tumor suppressor in early stage, and tumor promotion and metastasis propagation in later stages of breast cancer has demonstrated in several studies (6-9). The significance effect of TGF- β for initiation and progression of cancer have examined in stem cells (10, 11) and the molecular mechanism of TGF- β signal transduction downstream modulators have evaluated in-vitro on the breast cancer cell line model (12). Lastly, TGF- β has suggested as a possible purpose for managing of several cancers (13-15) and

for cancer treatment the inhibition of signal transduction pathway though downstream modulators related to TGF- β , has evaluated, but results have been conflicting (16-21).

The human TGF- β family has comprised three isoforms including TGF- β 1, TGF- β 2, and TGF- β 3 which were very homologous and each isoform has encoded by a distinct gene (22). As the main isoform, TGF- β 1, found abundant in the circulation and epithelial area of the mammals breast cancer tissues (22). It has suggested that there was an association between increased serum levels of TGF- β 1, and increased metastasis of tumor cells to lymph node, poor histological grades, and advanced grade of breast cancer (23).

The human TGF- β 1 gene, which has encoded by 7 exons to be found on chromosome 19, had more than 100 single nucleotide polymorphism (SNP) and its mRNA to be expressed by many cell types (24, 25). Numerous polymorphisms in the TGF- β 1 gene have been studied, that two of the most reviewed were 800G/A and -509C/T which have located in the promoter area, and affecting the amount

serum levels of TGF- β 1 protein (26). A study has conducted by Grainger et al. obtained by medical record review using a standard protocol, as previously found the polymorphism at position -509C/T has significantly associated with increased (27) and substitution at position -800G/A with decreased production and serum levels of TGF- β 1 (28). The impacts of TGF- β gene polymorphism and breast cancer risk have been examined in several populations and due to the controversial results has been attention topic for further investigations (29-32).

2. Objectives

The aim of the present study was to investigate the effect of two common polymorphisms (TGF- β 1-509C/T and -800G/A) and their haplotypes on breast cancer risk in a sample of Iranian population.

3. Patients and Methods

3.1. Study Population

The subjects in this study have consisted of 100 unrelated women with histologically confirmed breast cancer, attending to the department of radiation and oncology of Ahvaz Golestan university hospital, Ahvaz, Iran. The cancer diagnoses for all patients have confirmed by pathologist through evaluation of pathologic slides. Clinical characteristics of breast cancer patients including tumor grade, cancer treatment, estrogen and progesterone status, have obtained by medical record review using a standard protocol, as previously described elsewhere (33). The control group consisted of 100 ethnically matched women have recruited from the same geographic area during the same period and have matched with the breast cancer cases by age and BMI. The control subjects have randomly selected among the health volunteers without history of breast cancer whom admitted to the same hospital. Details of data collection, processing and relevant corresponding characteristics of studied population, anthropometric and biochemical results have described elsewhere (34).

3.2. PCR and RFLP Analysis

For DNA extraction, venous blood has collected into K3-EDTA-treated tube and has stored at -20°C. Genomic DNA has extracted from whole blood using the salting out method. The PCR primers for both SNPs were as follow: 5'-CAGTTGGCGAGAACAGTTGG-3' (forward) and 5'-ACCAGAGAAAGAGGACCAGG-3' (reverse). Conditions for PCR amplification were 12.5 μ L commercially available PCR premix (AccuPower PCR PremiX; BIONEER, Daejeon, Korea) contained (dNTP, TaqDNA polymerase, MgCl₂, 10x buffer), 2.0

μ L (10 pmol/ μ L) forward and reverse primers, 100 ng of genomic DNA, and 6.5 μ L sterile nuclease free water. The PCR cycle conditions have consisted of an initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 seconds, at 60°C for 30 seconds, and a final elongation at 72°C for 5 minutes. Genotype determination has performed after restriction enzyme digestion (*Eco8II* for TGF- β 1-509C/T and *Tail* for TGF- β 1-800 G/A). Digested fragments was separated by electrophoresis on 3% agarose gel and stained with ethidium bromide for visualization under UV light. Fragment with 670 bp length of -509 C/T with C allele was digested into two fragments with 486 and 184 bp, whereas products with T allele could not cut by *Eco8II* restriction enzyme (New England Biolabs, Beverly, MA). Also, Fragment with 670 bp length of -800G/A with G allele have digested into two fragments with 473 and 197 bp, whereas products with A allele could not cut by *Tail* restriction enzyme (New England Biolabs, Beverly, MA).

3.3. Statistical Analyses

Data have expressed as mean \pm standard deviation, and all statistical analyses have performed using SPSS software for Windows version 20.0 (IBM Corporation New York, USA). The statistical significance difference of genotype distributions between cases and controls has determined by Chi square analysis. In order to estimate odds ratios (ORs) for breast cancer risk and the corresponding 95% confidence intervals (CI) logistic regression model was used. The haplotype distribution in two groups was estimated according to the two-stage iterative method named expectation maximization algorithm (EM algorithm) by using the software SNPStats (<http://www.bioinfo.iconcologia.net/SNPstats>). The risk for every haplotype has compared respect to the reference category; it is the most frequent haplotype. Multinomial logistic regression analysis has also examined, and results have expressed as p value, OR and 95% CI. A P value of < 0.05 has considered as the criterion for statistical significance.

4. Results

4.1. General Characteristics of the Study Subjects

General characteristics of breast cancer cases and controls was presented elsewhere (34). Briefly, there were no statistically significant differences between the breast cancer cases and controls for age and BMI. In addition, there were no statistically significant differences between two groups for the means of biochemical characteristics including total cholesterol, triglyceride and LDL-C. However, there was a statistically significant difference between two groups for the means of HDL-C ($P < 0.001$).

4.2. TGF- β 1-509C/T and -800G/A Genotype Analysis

Amplification of DNA consisting of the two SNPs by using specific primers has resulted in a fragment with 670 bp length. The observed genotype distribution of two SNPs in the both breast cancer cases and controls, were in accordance with the Hardy-Weinberg laws of equilibrium. After digestion of the amplified fragment with *Eco8II* restriction endonuclease for -509C/T, fragments with 486 bp and 184 bp (CC genotype), 670 bp, 486 bp, 184 bp (CT genotype), and 670 bp (TT) was observed. The frequencies of the CC, CT, and TT genotypes were 31%, 50%, and 19% in breast cancer cases and 21%, 48%, and 31% in controls, respectively (Table 1). Between two studied groups, the frequency of genotypes were not different (Table 1, $\chi^2 = 4.844$, $P = 0.089$). Likewise, regarding to the allele frequency, which for C and T alleles were 77.6% and 22.4% in breast cancer cases, and 75.9 and 24.1 in controls, respectively, no significant difference between two groups was observed (Table 1, $\chi^2 = 1.073$, $P = 0.300$). The OR for breast cancer was 2.409 (95% CI = 1.08 - 5.337, $P = 0.030$) for TT genotype, which was significant.

Similarly, after digestion of the amplified fragment with *TaqI* restriction endonuclease for -800G/A, fragments with 473 bp and 197 bp (GG genotype); 670 bp, 473 bp, 197 bp (GA genotype), and 670 bp (AA) was observed. The frequencies of the GG, GA, and AA genotypes were 39%, 46%, and 15% in breast cancer cases and 24%, 54%, and 22% in controls, respectively (Table 1). Between two studied groups, the frequency of genotypes were not different (Table 1, $\chi^2 = 5.536$, $P = 0.063$). Likewise, regarding to the allele frequency, which for G and A alleles were 69.5% and 30.5% in breast cancer cases, and 62% and 38% in controls, respectively, no significant difference between two groups was observed (Table 1, $\chi^2 = 2.498$, $P = 0.114$). The ORs for breast cancer cases were 1.908 (95% CI = 1.003 - 3.628, $P = 0.049$) for GA genotype and 2.383 (95% CI = 1.039 - 5.407, $P = 0.040$, Table 2) for the TT genotype, respectively, which were significant.

4.3. Haplotype Distribution

Table 3 shows two-locus TGF- β 1 haplotype analysis which have stratified by study subjects. Of the four possible TGF- β 1 haplotypes, none was associated with breast cancer. A 'double-mutant' haplotype (-509T/-800A) was uncommon, and present at very low frequencies in both controls and breast cancer cases. Using the haplotype including the wild allele of each SNP (-509C/-800G) as reference, no significant association between the two remaining common haplotypes and breast cancer was observed.

4.4. Risk Factors for Breast Cancer

Predictors of breast cancer have examined by performing multinomial logistic regression model, with the de-

pendent variable being breast cancer, and the independent potentially confounding variables being age, BMI, serum levels of LDL-C, HDL-C, total cholesterol, triglyceride and TGF- β 1-509C/T and -800G/A genotypes (Table 4). Among the congenital risk factors, homozygous for -800G/A (OR = 0.570; 95% CI = 0.362 - 0.896, $P = 0.015$) was the selected variable associated with presence of breast cancer (Table 4). Among the no-congenital risk factors, HDL-C (OR = 0.935; 95% CI = 0.906 - 0.965, $P < 0.001$) was associated with presence of breast cancer (Table 4).

5. Discussion

As a case-control study model, we could not find differences between breast cancer cases and controls for polymorphism of two promoter TGF- β 1 gene which has either positively or negatively associated with the risk of this disease in several previous studies.

In fact, gene polymorphisms were mechanisms through which individuals might reveal variations within the range of what has considered as genetically normal (18). Further than 100 single nucleotide polymorphisms (SNPs) and other genetic variations have identified among the genes controlling TGF- β signaling pathway, and a few important of these have related to several diseases (19).

There was a strong motivation for investigating the effect of this genetic variation as a possible source for many cancer predispositions (26, 27, 35, 36). Experimental studies have shown that TGF- β was an important regulator of several cellular and molecular processes in the normal and malignant mammary epithelial cell lines (1). Acting through its specific downstream signal pathway molecules, particularly Smad proteins and its receptors, TGF- β has inhibited cell proliferation and motility in lobular and ductal of mammary tissues, consequently had tumor suppressor effect in the early stages of breast cancer development (2-4). As a result of changes in tumor cell sensitivity, in the later stages, TGF- β has acted as a promoter by enhancing tumor cell proliferation and metastasis (17). This dual effect of TGF- β has clearly assessed among several transgenic animal models (18) some of them have highly related to the initiation and progression of human breast cancer (6). Also, suggestive of a role for signaling pathway of TGF- β in the progression of human breast cancers were the results of mutations in type I and II TGF- β receptor genes (TBR1 and TBR2) in persistent or metastatic breast tumors (19, 20). It has suggested the expression of TGF-RII has inversely correlated with breast tumor propagation, which as determined by stage of tumor, cell division count, and change the clinical characteristics (21).

It has reported there were three common polymorphisms in the promoter region of the TGF- β 1 gene at locus

Table 1. Genotype Distribution and Allele Frequencies of Two Studied SNPs in Breast Cancer Cases and Controls^a

SNP	Genotype Frequency			χ^2	P Value	Allele Frequency		χ^2	P Value	
	CC	CT	TT			C	T			
-509C/T	Controls	21	48	31	4.844	0.089	121	79	1.073	0.300
	Cases	31	50	19						
-800G/A	Controls	24	54	22	5.536	0.063	124	76	2.498	0.114
	Cases	39	46	15						

Abbreviation: SNP, single nucleotide polymorphism.

^aAllele frequencies and genotype distribution of the studied SNPs in breast cancer cases have compared to those in controls by chi-square (χ^2) test.**Table 2.** Genotypic Association Between SNPs-509C/T and -800G/A of TGF- β 1 Gene and Breast Cancer Risk

SNP	Group		OR (95% CI)	P Value	
	Controls	Cases			
-509C/T	CC	21	31	1.0	
	CT	48	50	1.417 (0.717 - 2.80)	0.316
	TT	31	19	2.409 (1.087 - 5.337)	0.030
-800G/A	GG	24	39	1.0	NA
	GA	54	46	1.908 (1.003 - 3.628)	0.049
	AA	22	15	2.383 (1.039 - 5.407)	0.040

Abbreviations: CI, confidence interval; OR, odds ratio.

Table 3. Estimation of Haplotype Frequencies and Haplotype Association With Breast Cancer

Haplotype	Controls	Cases	OR (95% CI)	P Value
-509T/-800G	0.277	0.294	1.00	
-509C/-800G	0.343	0.215	0.64 (0.34 - 1.22)	0.18
-509C/-800A	0.217	0.234	1.02 (0.58 - 1.80)	0.94
-509T/-800A	0.163	0.255	1.64 (0.76 - 3.54)	0.21

-988, -800 and -509 relative to the transcription start codon (29, 30). In our study, the genotype distribution and allele frequencies at locus -509C/T and -800G/A were not significantly different between breast cancer cases and controls. Mu et al. in a study suggested -509C/T polymorphism of TGF- β 1 gene was significantly associated with the plasma levels of TGF- β 1 (31). Serum levels of TGF- β 1 have increased among the dose-dependent model, with differences in TGF- β 1 levels in TT homozygotes being twice, in comparison with the subjects with TC genotype when CC genotype considered as reference. It has suggested that polymorphisms in the promoter region of TGF- β 1 resulted in altered transcriptional regulation, and so might affect the extension and seriousness of diseases which have associated with

TGF- β 1. The -800G/A polymorphism has seemed to down regulate a common binding site of the CRE-binding protein as a transcription factor, resulted to a lower serum levels of TGF- β 1 (32). Whereas, the association of T allele of the -509C/T polymorphism with a higher transcriptional activity of TGF- β 1 was observed (37) and therefore consider as higher producer of total and active TGF- β 1 (31, 32). In our study, although, the frequency of genotype and allele of two studied polymorphisms were not significantly different between breast cancer cases and controls. However, the T allele frequency of -509C/T polymorphism in controls was higher than patients, and this might be consistent with findings of Grainger et al. (27) in which subjects bearing T allele of this polymorphism have increased serum levels

Table 4. Results of Multinomial Logistic Regression Analysis

Variables	OR	95% CI	P Value
Age, y	1.011	0.975 - 1.049	0.554
BMI, kg/m ²	1.054	0.981 - 1.132	0.150
Total cholesterol, mg/dL	1.003	0.996 - 1.010	0.377
Triglyceride, mg/dL	1.003	0.998 - 1.008	0.228
HDL-C, mg/dL	0.935	0.906 - 0.965	< 0.001
LDL-C, mg/dL	0.993	0.973 - 1.013	0.480
TGF- β 1-800G/A	0.570	0.362 - 0.896	0.015
TGF- β 1-509C/T	0.686	0.443 - 1.060	0.090

of TGF- β 1. The -509C/T polymorphism in the promoter region of the TGF- β 1 gene might alter TGF- β 1 expression levels, whereas the downstream component involved in this pathway was unclear till now. Since the -509C/T polymorphism has found among the negative regulatory region of the TGF- β 1 promoter (37), thus, C allele of -509C/T polymorphism might selectively down regulate expression of TGF- β 1, and amplified levels of TGF- β 1 might related to T allele of -509 polymorphism due to the loss of negative regulation (38). The association between T allele of the -509C/T polymorphism and increased risk of diseases such as breast cancer, endometriosis, and asthma was reported (39-41). However, a recent meta-analysis reported TGF- β 1-509C/T variants might not involve in the risk of breast cancer. Though, they have found T allele might be a possible protecting allele for the developing of breast cancer in the subjects with estrogen receptor positive (42).

In conclusion, although, our findings have indicated there was no significant difference for genotype distribution of two promoter polymorphisms in TGF- β 1 gene between breast cancer cases and controls. However, the TT genotype of -509C/T and AA genotype of -800G/A have significantly associated with breast cancer risk in a sample of Iranian population. This was the first study to investigate the association between these two polymorphism and breast cancer risk in Ahvazian women as a sample of Iranian population.

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Footnotes

Authors' Contribution: Ghorban Mohammadzadeh coordinated the study, designs the project, analyzed the data and prepared the manuscript. Somayeh Parvizi has been carried out the molecular methods and all the other experiments. Maryam Karimi participated in the all experiments. Mozghan Noorbehbahani and Alireza Jafary participated in the preparation of the material and methods. All authors have read and approved the content of the manuscript.

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