

Association of single nucleotide polymorphism of transforming growth factor β 1 (T29C) in breast cancer patients: a case control study in Rawalpindi

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Abstract

Objective: To determine the association of single nucleotide polymorphism in three CC, TT and TC genotypes of transforming growth factor β 1 T29C in breast cancer patients.

Methods: The case-control study was conducted from April 2017 to April 2018 at the Islamic International Medical College, Rawalpindi, Pakistan, in collaboration with Nuclear Oncology Medicine and Radiotherapy Institute and Holy Family Hospital, Rawalpindi. Using convenience sampling, breast cancer cases and healthy controls were enrolled. All investigations were done using standardised laboratory protocols. The outcomes were determined in terms of association of single nucleotide polymorphism of transforming growth factor β 1 with breast cancer. Data was analysed using SPSS 21.

Results: Of the 150 subjects, 80(53.3%) were cases and 70(47.7%) were healthy controls. Among the cases, the most frequent genotype was CC 38(47.5%) followed by TC 26(32.5%) and TT 16(20%). Among the controls, the corresponding values were 50(71.42%), 13(18.5%) and 7(10%). Transforming growth factor β 1 TC genotype was strongly associated with the increased risk of developing breast cancer (odds ratio: 3.79).

Conclusion: The incidence of breast cancer was markedly lower among women with CC genotype compared to those with CT or TT genotypes.

Keywords: Breast cancer, Genotype, Single nucleotide polymorphism, Transforming growth factor. (JPMA 70: 390; 2020). <https://doi.org/10.5455/JPMA.296490>

Introduction

Breast cancer is the most common among fatal cancers, with high morbidity and mortality among women. Among all the cancer types, breast cancer is ranked second in terms of mortality¹ causing death of almost 350,000 women in both developing and developed countries annually.² Currently, one in every eight women has to face the diagnosis of breast cancer during her lifetime. Till a few decades ago, breast cancer was mostly linked to poor prognosis, but today the majority of patients may have a normal life expectancy and this is largely due to the improvements in early identification and availability of different treatments.³

According to the American Cancer Society (ACS), 232,340 new patients of invasive breast cancer and 39,620 deaths due to breast cancer were estimated among American

women in 2013. In the United States, one in every eight women will develop breast cancer in her life.³ From 2006 to 2010, the incidence of breast cancer increased slightly among African-American women, decreased amongst Hispanic women, and was unchanged among the whites and the Asian-Americans. Historically, the incidence of breast cancer among white women aged 40 years and older has had the highest rate.⁴ In Pakistan, prevalence of breast cancer is 2.5 times greater than bordering countries like India and Iran.⁵ Amongst Asian nations, Pakistan has the highest rate of breast cancer. Karachi cancer registry shows that the rate of breast cancer is 69.1 per 100,000 annually.⁶ In Lahore, 3,338 cases of breast cancer presented at Shaukat Khanum Memorial Cancer Hospital over 8 years.⁷ In cancer patients, over 90% of lethality is due to metastasis, and it severely restrains the prognosis of breast cancer.⁸ For breast cancer patients with localised disease, the 5-years survival rate drops from 98% to 23% for patients with metastasis.⁹

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Several risk factors are included in the development, progression and pathogenesis of breast cancer, involving biological, genetic, lifestyle and environmental factors.² Numbers of genetic mutations are identified to cause breast cancer. Transforming growth factor-beta (TGF- β) is a multifunctional, homodimeric, and pleiotrophic cytokine and a molecular weight of about 25kDa secreted by different cell types such as endothelial cells, Treg (T) cells, platelets and peripheral blood mononuclear cells. It performs several important functions in the modulation of cellular growth, differentiation and maturation, homeostasis, extracellular matrix formation, endothelial cell plasticity, angiogenesis, apoptosis and cancer development.¹⁰⁻¹² The human 25kb TGF- β is situated at 19q13 chromosome and comprises seven exons that encode three different molecular isoforms of TGF- β formed by alternative splicing and have been identified to have functions in normal mammary gland development as well as in breast neoplasm.¹³

The current study as planned to determine the association of single nucleotide polymorphism (SNP) in CC, TT and TC genotypes of TGF- β 1 T29C in breast cancer patients.

Patients and Methods

The case-control study was conducted from April 2017 to April 2018 at the Islamic International Medical College (IIMC), Rawalpindi, Pakistan, in collaboration with Nuclear Oncology Medicine and Radiotherapy Institute (NORI) and the Holy Family Hospital, Rawalpindi. After approval from the IIMC ethics review committee and the heads of the participating institutions, the sample size was calculated using EpiTool¹⁴ while keeping confidence interval (CI) at 95%, power at 80%, with the percent of control exposed anticipated as 5% and odds ratio (OR) as 4.56.¹⁵ Patients with diagnosed breast cancer were subsequently recruited followed by healthy controls with no history of malignancy and any other hereditary disease. Cases were then matched to one or more controls based on age, gender and menopausal status which is believed to be a confounder. Patients with any other malignancy and pervasiveness of any other hereditary disease were excluded. After getting informed consent from the subjects, 5 ml venous blood samples were collected into Thomas scientific sterile vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA.) Genomic deoxyribonucleic acid (DNA) was extracted using chelex method¹⁶ from whole blood-lymphocytes samples and

Table-1: Primers and sequences.

Sr#	Primers	Sequences
1	T allele (F)	5-CTCCGGGCTGCGGCTGTGCT-3
2	C allele (F)	5-CTCCGGGCTGCGGCTGTGCC-3
3	Common primer (R)	5-GTTGTGGGT TTCCAC CATTAG-3

was stored at -70 Celsius.

TGF- β 1 T29C (rs1800470) was genotyped by single stranded polymorphism-polymerase chain reaction (SSP-PCR)(17) using specific primers (Table-1).^{17,18}

The PCR reaction was performed in two tubes in which each tube contained forward primer specific to one allele in addition to the generic primer. The final total volume for each PCR reaction was 18 μ L. PCR reaction ingredients were 13.5 μ L distilled water, 2 μ L 5x Firepol Master Mix (7.5mM MgCl) (Solis Bio Dyne, Estonia), 1 μ L of each primer (M/s Macrogen) and 1.5 μ L of DNA. The PCR cycling comprised one cycle of 94°C for 5 minutes followed by 35 cycles of 96°C for 30 seconds, 59°C for 30 seconds, 72°C for 55 seconds, and a final extension step of 5 minutes. PCR reaction was performed in thermal cycler of major science (model: CYCLER-25). PCR products were visualised on 2% agarose gel, prepared by 1.6 g agarose

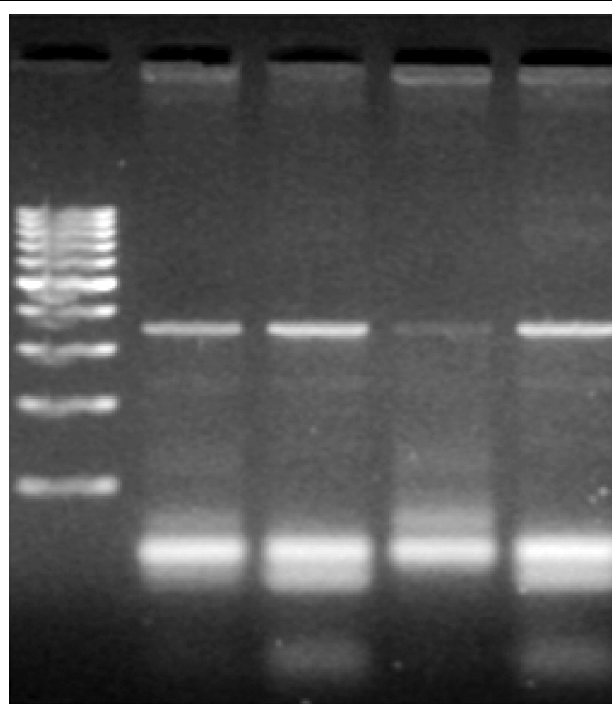


Figure: TGF- β 1 (T29C) PCR products of two samples. Lane 1 is 100 bp ladder. Sample 1 in lane (2 and 3) TC genotype and sample 2 in lane (4 and 5) CC genotype.

and 80 ml of 10x Tris/Borate/EDTA (TBE) buffer, 3 to 5 drops of 1% ethidium bromide and estimated in comparison with 100bp DNA ladder. The size of PCR product for TGF- β 1 T29C primers was 346bp for T or C allele.

The gel was placed on ultraviolet (UV) transilluminator. The amplified DNA fragments were seen as whitish bands. A permanent record of the gel was kept by taking a picture with camera and G:Boxgel document system (Figure).

Data was analysed using SPSS 21. Chi square test was used for proportion and then logistic regression was run for association.

Results

Of the 150 subjects, 80(53.3%) were cases and 70(47.7%) were healthy controls. Among the cases, 47(58.75%) women were aged 50 years or below, and 33(41.25%) had age above 50 years. Among the controls, 49(70%) women had age 50 years or below and 21(30%) had age above 50 years. In the cases, 25 (31.25%) women had positive family history of breast cancer with first-degree relative and 55(68.75%) were sporadic cases. Pre-menopausal women in cases were 44(55%), and 36(45%) women were post-menopausal. In controls, 33(47.14%) women were pre-menopausal and 37(52.85%) were post-menopausal (Table-2).

Among the cases, the most frequent genotype was CC 38(47.5%) followed by TC 26(32.5%) and TT 16(20%). Among the controls, the corresponding values were

50(71.42%), 13(18.5%) and 7(10%) (Table-3).

Genotyping of TGF- β 1 T29C showed a decrease in the distribution of TC genotype in controls 13(18.5%) compared to the cases 26(32.5%). Also, cases with TT genotype were 16(20%) compared to 7(10%) controls ($p=0.012$). Genotyping of TGF- β 1 T29C showed decreased risk of developing breast cancer with CC genotype compared to TC or TT genotypes (OR: 0.38) TGF- β 1 TC genotype was strongly associated with increased risk of developing breast cancer (OR: 3.79). TGF- β 1 TT genotype was also associated with the risk of developing breast cancer (OR: 2.63).

Discussion

the study had 80 diagnosed cases of breast cancer, and 38 of them were with CC genotype, 26 with TC, and 16 with TT genotype. Earlier studies¹⁸ concluded that TGF β 1 T29C T polymorphism was significantly associated with breast cancer risk in European countries which is similar to our findings. Histida et al.¹⁹ found that CC genotype was strongly associated with reduced risk of breast cancer compared to the TT genotype which matches the findings of the current study.

A study comprising Japanese men and women found that CC genotype was associated with higher serum level of TGF- β 1 than either the TT or CT genotypes.²⁰ Thus, increased serum level in subjects with CC genotype may contribute to long-term suppression of mammary epithelial growth and may lead to lower risk of breast cancer. This finding was similar to those of the current study.

The first prospective cohort study reported that individuals with CC genotype in the TGF- β 1 T29C polymorphism had a decreased risk of breast cancer, especially among women.²¹ CC genotype, which codes for proline in the Leu¹⁰→Pro polymorphism, has been associated with a decreased risk of breast cancer among white women.²¹ The same association between breast cancer cases and controls was found in Japanese pre-menopausal women. but not in post-menopausal women.¹⁹ These findings support our results.

In the present study, the most frequent genotype was CC 38(47.5%) followed by TC 26(32.5%) and TT 16(20%) among the cases compared to the controls who had corresponding values of 50(71.42%), 13(18.5%) and 7(10%). In a cohort study involving 3075 women aged 65 years or older, Ziv et al.²² reported that subjects with the CC

Table-2: Demographics characteristics of cases and controls.

	Case (n=80) n (%)	Control (n=70) n (%)
Age (Years)		
<50	47 (58.7)	49 (70)
>50	33 (41.2)	21 (30)
Menopausal Status		
Premenopausal	44 (55)	33 (47.1)
Postmenopausal	36 (45)	37 (52.8)
Family History		
Positive family history	25 (31.2)	
Sporadic cases	55 (68.7)	

Table-3: Association of transforming growth factor β 1 genotype (T29C) with study population.

Study groups	Transforming growth factor β 1 genotype (T29C)			p-value
	CC	TC	TT	
Case	38 (47.5%)	26(32.5%)	16 (20%)	0.012
Control	30 (71.5%)	13 (18%)	7 (10%)	

TGF- β 1 genotype had a 64% reduced risk of developing breast cancer compared to women with CT or TT genotypes. The current study also indicated the same risk parameters.

Conclusion

The risk of breast cancer was markedly lower among women with CC genotype when compared with women who had CT or TT genotype. The CC genotype at nucleotide 29 of TGF- β was associated with reduced risk of breast cancer, and the CT genotype was significantly associated with increased risk of breast cancer.

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Conflict of Interest: None.

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