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Association of *TGFB1* gene polymorphisms with cervical cancer in Bangladeshi women: A case-control study

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Abstract.
OBJECTIVES: Genetic susceptibility to cervical cancer in relation to transforming growth factor beta 1 (*TGFB1*) gene polymorphisms has not been investigated extensively among the women in Bangladesh. So, the aim of this study was to find out the correlation of the polymorphisms of *TGFB1* C509T (rs1800469) and T869C (rs1800470) with the risk of cervical cancer among the Bangladeshi women.
STUDY DESIGN: 134 cervical cancer patients and 102 age-sex matched healthy controls were included from two institutions in Bangladesh. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used for genotyping two *TGFB1* single nucleotide polymorphisms C509T (rs1800469) and T869C (rs1800470) in patients and controls.
RESULTS: No significant correlation was found between polymorphisms C509T (rs1800469) and T869C (rs1800470) of *TGFB1* gene with cervical cancer in Bangladeshi women. In case of the cervical cancer patients who had first degree relatives with cancer were prone to carry the polymorphic version of the *TGFB1* gene polymorphism at C509T (OR = 5.597, 95% CI = 1.224–25.597, $p < 0.05$) but may not result in the increase of developing cervical cancer.
CONCLUSION: In summary, two polymorphisms C509T and T869C of *TGFB1* gene may not be associated with cervical cancer risk in Bangladeshi women.

Keywords: *TGFB1* gene polymorphisms, C509T, T869C, Cervical cancer, Bangladesh

1. Introduction

Cervical cancer accounts for 3.2% of the total newly diagnosed cancer cases and 3.26% of cancer related deaths worldwide [1]. In Bangladesh, it is the second leading cause of cancer related death among women [2]. Although human papillomavirus (HPV) is considered as an etiologic agent for cervical cancer, many other risk factors exist, as only few women who are exposed to this virus develop cancer [3]. HPV-16 and HPV-18 are considered as prime subtypes, causing 70% of cervical

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cancer worldwide [4]. In terms of other causes, molecular alterations of tumor suppressor genes and genetic polymorphisms play a vital role in the development of cervical cancer [5].

Transforming growth factor-beta (TGFB) superfamily is a collection of multifunctional cytokines including activins, inhibins, and bone morphogenetic proteins which are involved in different crucial physiological functions like-proliferation and differentiation of cell, angiogenesis, immunosuppression, cell motility, apoptosis, wound healing, and embryonic development [6]. Moreover, it has also been hypothesized to be involved in the cancer pathogenesis where it works as a tumor suppressor or a tumor promoter depending on the stage of tumor [7]. During early stages of carcinogenesis it functions as a tumor suppressor gene due to its ability to arrest cell cycle and induce apoptosis [7, 8].

Among three homologous isoforms of TGFB present in human and other mammals, *TGFB1* is the most abundant [9]. It is encoded by the *TGFB1* gene located in chromosome 19q13.2 and has been linked with the predisposition to cervical cancer. A study by Torng et al. revealed that expression of *TGFB1* mRNA is decreased in tumor cells during progression from cervical intraepithelial neoplasia to microinvasive carcinoma [10]. This is further corroborated by another study which reported decreased *TGFB1* protein in serum in patients with cervical carcinoma [11]. In contrast, reports of elevated expression of *TGFB1* protein in patients with cervical adenocarcinoma are also available [12]. Polymorphisms of *TGFB1* gene has been reported in different types of cancer including lung cancer and colorectal cancer [13, 14] but its relation to the risk of developing cervical cancer in Bangladeshi population is not yet available.

One study reported higher frequency of A allele of the polymorphism G800A (rs1800468) of *TGFB1* gene in Mexican cervical cancer patients compared to healthy groups [15]. Another study found that, *TGFB1* C509T (rs1800469) polymorphism significantly associated with decreased risk of early stage of cervical cancer but confers increased risk for stage II of cervical cancer [16]. Moreover, various polymorphisms of *TGFB1* gene have been recognized that might regulate plasma levels of TGFB1 [17].

The C509T (rs1800469) and T869C (rs1800470) are the two most common polymorphisms of *TGFB1* gene that have been linked with lung cancer, colorectal cancer and gastric cancer [13, 14, 18]. C509T polymorphism is located in the promoter site of the *TGFB1* gene and a previous study reported individuals homozygous for T allele had nearly double plasma *TGFB1* protein level compared to homozygous wild type C allele carriers [19]. The T869C SNP is located at codon 10 of exon 1 and causes in a missense mutation that codes for proline instead of leucine. Several studies have demonstrated that homozygosity of the C allele of T869C is related with higher production of *TGFB1* in the periphery in breast cancer patients [17, 20]. To the best of our knowledge, no study has been conducted so far regarding polymorphism of the C509T and T869C SNPs in the *TGFB1* gene and its association with cervical cancer in Bangladeshi population. So, the aim of this study was to find out if polymorphism of the C509T (rs1800469) and T869C (rs1800470) SNPs increases cervical cancer risk among the Bangladeshi women.

2. Materials and methods

2.1. Subject selection and sample collection

A total of 236 women were recruited for conducting this study where 134 were cervical cancer patients and 102 were healthy controls. Cervical patients were recruited from the Bangabandhu Sheikh Mujib Medical University (BSMMU) and National Institute of Cancer Research and Hospital (NICRH), Dhaka, Bangladesh in between October 2018 and June 2019. All patients were histologically diagnosed with cervical cancer and categorized according to the International Federation of Gynecology and Obstetrics (FIGO) staging system [21]. All the cervical cancer patients were non-smokers and abstinent

from consuming alcohol throughout their life. After performing physical assessment, age matched healthy controls were recruited. Healthy controls with head injury, trauma, history of psychiatric illness, pregnancy, alcohol intake, smoking, substance abuse were not recruited for the study.

All participants were informed about the study aim and experimental technique and written consent forms were taken prior to the study. This study was conducted according to the Declaration of Helsinki and its further amendments [22]. Laboratory experiments were carried out in the Pharmacogenetics and Pharmacokinetics lab at the Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka. Finally, approval for the study protocol was taken from the ethical review committee of the Bangabandhu Sheikh Mujib Medical University (BSMMU) and National Institute of Cancer Research and Hospital (NICRH), Dhaka.

2.2. DNA extraction and genotyping

3 ml of blood samples were collected from all the participants in potassium-EDTA sterile tubes (Becton, Dickinson and Company, NJ, USA) and stored at –80°C prior to DNA extraction. Genomic DNA was extracted following previously published method [23]. The selected polymorphisms of *TGFB1* gene, C509T (rs1800469) and T869C (rs1800470) were genotyped by PCR-RFLP (Polymerase Chain Reaction–Restriction Fragment Length Polymorphism) method. By following this method, the PCR products of rs1800469 (418 bp) and rs1800470 (277 bp) were digested with restriction enzymes *Bsu36I* and *MspA1II* at 37°C respectively for 24 hours. Following that DNA fragments were visualized through gel electrophoresis technique on 3% agarose gel mixed with ethidium bromide. Details of this newly developed PCR experiment protocol (primers, PCR condition, temperature, PCR length fragments) has been provided in the supplementary Table 1.

2.3. Statistical analysis

Chi-square (χ^2) test and unpaired *t*-test were conducted to compare demographic data, clinical data, and genotype frequencies between the case and control groups. Furthermore, Chi-square (χ^2) test was used to measure the deviation of genotype frequencies in the control group from cases to measure the Hardy-Weinberg Equilibrium (HWE). Multivariate logistic regression was used to calculate adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for age. SPSS software, version 21.0 was used for conducting all the statistical analyses.

3. Results

3.1. Characteristics of the study population

A total of 236 samples were taken in this study where 134 were cervical cancer patients and 102 were healthy controls. Table 1 contains the detailed demographic and clinicopathological features of the patients and the controls.

Correlation between *TGFB1* gene polymorphisms with the clinicopathological characteristics of the patients can be found in Table 2 where we found that age, menstrual status, parity, contraception were not significant cofactors for cervical cancer.

However, in case of C509T (rs1800469) polymorphism, patients who had first degree relatives with cancer were more prone to carry the polymorphic T allele compared to the patients with no first degree relative with cancer ($p = 0.026$).

Table 1
Distribution of clinicopathological features of cervical cancer patients and controls

Characteristics	Cases ($n = 134$) (%)	Controls ($n = 102$) (%)	p value
Age, years			
≤45	64 (47.76)	61 (59.80)	0.0868
>45	70 (52.24)	41 (40.20)	
Dwelling			
Urban	30 (22.39)	98 (96.08)	<0.00001
Rural	104 (77.61)	4 (3.92)	
Menstrual Status			
Pre-menopause	61 (45.52)	52 (50.98)	0.4319
Post menopause	73 (54.48)	50 (49.02)	
Parity			
0–7	126 (94.03)	100 (98.04)	0.1938
>7	8 (5.97)	2 (1.96)	
Contraception			
Oral Pills	66 (49.25)	41 (40.20)	0.1675
Others*	12 (8.96)	18 (17.65)	
Combination#	8 (5.97)	4 (3.92)	
None	48 (35.82)	39 (38.23)	
Family History of Cancer (First Degree Relatives)			
Yes	17 (12.69)	17 (16.67)	0.4553
No	117 (87.31)	85 (83.33)	
Stage of Cancer (FIGO)			
IA-IB	20 (14.93)		
IIA-IIB	79 (58.95)		
IIIA-IIIB	35 (26.12)		
Histopathology			
Squamous Cell Carcinoma	114 (85.07)		
Adenocarcinoma	18 (13.43)		
Others	2 (1.50)		
Tumor Grade			
I	10 (7.46)		
II	115 (85.82)		
III	9 (6.72)		

#Combination: Oral pills+combined injectable contraceptives (CIC); Oral pills+condom (male)
*Others: Barrier (cervical cup, diaphragm, female condom)+intrauterine device (IUD).

3.2. TGF-beta 1 gene polymorphisms C509T and T869C

Genotype frequencies of *TGFB1* SNPs in the case and the control groups can be found in the Table 3.

For the polymorphism C509T (rs1800469) the frequency distribution of genotypes was 42.16% CC, 45.10% CT, 12.74% TT, 57.84% CT+TT in controls and 38.81% CC, 43.28% CT, 17.91% TT, 61.19% CT+TT in patients which predicts of no statistically significant risk of cervical cancer (heterozygous, CT: OR = 1.083, 95% CI = 0.612–1.915, $p > 0.05$; mutant homozygous, TT: OR = 1.216, 95% CI = 0.829–1.785, $p > 0.05$).

Table 2
Correlation of *TGFB1* gene polymorphisms with clinicopathological characteristics of the patients

Characteristics	C509T carrier $n = 82$ (%)	C509T non-carrier $n = 52$ (%)	OR (95% CI)	p-value	T869C carrier $n = 82$ (%)	T869C non-carrier $n = 52$ (%)	OR (95% CI)	p-value
Age, years								
>45	43 (52.44)	27 (51.92)	1.021 (0.509–2.048)	0.954	45 (54.88)	25 (48.08)	1.314 (0.655–2.636)	0.443
≤45	39 (47.56)	25 (48.08)	Ref.	1.000	37 (45.12)	27 (51.92)	Ref.	1.000
Menstrual Status								
Pre-menopause	40 (48.78)	21 (40.38)	1.406 (0.696–2.840)	0.342	32 (39.02)	29 (55.77)	0.508 (0.251–1.027)	0.059
Post-menopause	42 (51.22)	31 (59.62)	Ref.	1.000	50 (60.98)	23 (44.23)	Ref.	1.000
Parity								
>7	6 (7.32)	2 (3.85)	1.974 (0.383–10.171)	0.416	7 (8.54)	1 (1.92)	4.760 (0.568–39.867)	0.150
0–7	76 (92.68)	50 (96.15)	Ref.	1.000	75 (91.46)	51 (98.08)	Ref.	1.000
Contraception								
Oral Pills	43 (52.44)	23 (44.23)	1.335 (0.621–2.871)	0.459	44 (53.66)	22 (42.31)	1.429 (0.662–3.082)	0.363
Others	6 (7.32)	6 (11.54)	0.714 (0.201–2.540)	0.603	5 (6.10)	7 (13.46)	0.510 (0.141–1.841)	0.304
Combination	5 (6.10)	3 (5.77)	1.191 (0.255–5.565)	0.825	5 (6.10)	3 (5.77)	1.191 (0.255–5.565)	0.825
None	28 (34.14)	20 (38.46)	Ref.	1.000	28 (34.14)	20 (38.46)	Ref.	1.000
Family History of Cancer (First Degree Relatives)								
Yes	15 (18.29)	2 (3.85)	5.597 (1.224–25.597)	**0.026***	9 (10.98)	8 (15.38)	0.678 (10.244–1.887)	0.457
No	67 (81.71)	50 (96.15)	Ref.	1.000	73 (89.02)	44 (84.62)	Ref.	1.000

*Result is statistically significant ($p < 0.05$)

In case of T869C (rs1800470) polymorphism, the frequency distribution showed similar percentage with 49.02% TT, 40.20% TC, 10.78% CC in controls and 38.81% TT, 47.01% TC, 14.78% CC in patients which predicts of no statistically significant risk of cervical cancer (heterozygous: OR = 1.309, 95% CI = 0.742–2.310, $p > 0.05$; mutant homozygous: OR = 1.316, 95% CI = 0.888–1.949, $p > 0.05$). Different haplotypes from C509T and T869C polymorphisms combination also didn't reveal any significant risk association with cervical cancer (Table 4).

4. Discussion

In this study, we have found that there is no statistically significant correlation between polymorphisms C509T (rs1800469) and T869C (rs1800470) of *TGFB1* gene with cervical cancer in Bangladeshi women. Moreover, there was no statistically significant correlation of *TGFB1* gene polymorphisms with the clinicopathological characteristics of the patients such as age, menstrual status, parity and contraception. Only in case of the patients who had first degree relatives with cancer were more prone to carry the polymorphic version of the *TGFB1* gene in C509T (rs1800469) SNP as it

Table 3
Genotype frequencies of *TGFB1* gene polymorphisms in cervical cancer patients and controls

	Genotypes	Cases $n = 134$ (%)	Controls $n = 102$ (%)	Adjusted Odds Ratio (AORs)	95% CI	*p* value
C509T						
	CC	52 (38.81)	43 (42.16)	Ref.	-	-
	CT	58 (43.28)	46 (45.10)	1.082	0.612–1.915	0.786
	TT	24 (17.91)	13 (12.74)	1.543	0.695–3.428	0.287
	CT + TT	82 (61.19)	59 (57.84)	1.186	0.694–2.025	0.533
	T Allele	106 (39.55)	72 (35.29)	1.216	0.829–1.785	0.318
T869C						
	TT	52 (38.81)	50 (49.02)	Ref.	-	-
	TC	63 (47.01)	41 (40.20)	1.309	0.742–2.310	0.352
	CC	19 (14.18)	11 (10.78)	1.694	0.724–3.966	0.224
	TC+CC	82 (61.19)	52 (50.98)	1.390	0.817–2.368	0.225
	C Allele	101 (37.69)	63 (30.88)	1.316	0.888–1.949	0.171

*Result is statistically significant ($p < 0.05$).

Table 4
Haplotype frequencies of *TGFB1* gene polymorphisms (C509T and T869C) in cervical cancer patients and controls

Haplotype	Cases $n = 268$ (%)	Controls $n = 204$ (%)	Odds Ratio (ORs)	95% CI	*p value*
CT	116 (43.28))	101 (49.51)	Ref.	-	-
CC	46 (17.16)	31 (15.20)	1.292	0.762–2.190	0.341
TT	51 (19.03)	40 (19.61)	1.110	0.678–1.817	0.678
TC	55 (20.52)	32 (15.69)	1.497	0.898–2.494	0.122

*Result is statistically significant ($p < 0.05$).

showed the significant *p*-value (OR = 5.597, 95% CI = 1.224–25.597, $p < 0.05$). This leads to a possibility that polymorphic T allele may be part of a combination of SNPs that are in strong linkage disequilibrium with each other and thus getting transmitted in families and increasing the risk of cervical cancer. Family studies are needed to elucidate the exact mechanism of transmission of this risk allele in cervical cancer patients and their family members with large sample size.

Several studies showed that polymorphisms of the *TGFB1* gene may confer risk of developing different types of cancer such as colorectal cancer [24], gastrointestinal cancer [25], and breast cancer [26]. In this case-control study we did not find any significant correlation of the polymorphisms of *TGFB1* gene with cervical cancer in Bangladeshi women. According to a study conducted by Qi et al., there was no association of T869C polymorphism with breast cancer but in the subgroup analysis by ethnicity, an increased risk was observed in Caucasian population but not in Asian population [27]. Similarly, for C509T polymorphism, no significant association was found for breast cancer risk [27]. Another study done by Chen et al. concluded that the *TGFB1* T869C (rs1800470) and C509T (rs1800469) polymorphisms were not associated with lung cancer development and revealed the C509T (rs1800469) polymorphism diminishes the danger of lung cancer growth in patients with non-small cell lung cancer (NSCLC) [14]. Furthermore, a meta analysis study reported that in people of Asian ethnicity, C509T polymorphism was associated with lower risk of breast and gastric cancer, whereas,

T869C polymorphism showed higher risk of developing cancer in Caucasian population but not in Asian people [28]. Overall, majority studies have reported that C509T and T869C polymorphisms of the *TGFB1* gene may not confer cancer risk in Asian population and our findings are in support of those studies and further adding that these two polymorphisms may not confer cervical cancer risk in Bangladeshi women.

Our study has some limitations. The sample size of our study was relaively small and may lack enough power to strongly detect genotype-disease associations. Moreover, future studies in population from different ethnicity and larger sample size are required to validate our preliminary findings.

5. Conclusion

In conclusion, we report that C509T and T869C polymorphisms of the *TGFB1* gene do not confer significant risk of developing cervical cancer in Bangladeshi women.

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Conflict of interest

The authors declare no conflicts of interest.

Ethical considerations

The current study was in accordance with the declaration of Helsinki and its further amendments and the ethical committee of the participating hospital (Bangabandhu Sheikh Mujib Medical University and National Institute of cancer Health and Research, Dhaka, Bangladesh) approved the study protocol. Each patient and control subject signed an informed consent document after they were informed of the study objectives.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/TUB-200061.

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