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Original Article

Analysis of prostaglandin-endoperoxide synthase-2 gene polymorphisms and risk of cervical cancer in an East Indian population: A case–control study

ABSTRACT

Background: The prostaglandin-endoperoxide synthase-2 (PTGS-2) gene appears to play a role in inflammation or tumor and mitogenesis. Genetic polymorphisms in PTGS-2 might contribute to differential PTGS-2 expression and subsequent interindividual variability in susceptibility to cancer.

Aim: The goal of this study is to identify genetic variants of PTGS-2 gene in women of East India, which may associate with risk of cervical cancer.

Materials and Methods: We enrolled 200 histopathologically confirmed patients with cervical cancer (age 18–60 years) (cases) and their corresponding sex-matched 200 normal individuals (controls). To identify genetic variants responsible for cervical cancer, we performed sequence analysis of PTGS-2 genes. Questionnaire survey was conducted to comprehend the demographic data, smoking status, and cancer stage of patients.

Results: The genotype frequency of rs689466 polymorphism was significantly different between case and control groups ($P < 0.001$). Compared with the wild-type genotype AA, the variant genotype GG was associated with 20-fold increased risk ($P < 0.001$; odds ratio = 20.76; 95% confidence interval [CI]: 2.86–160.73) for cancer patients. The rs5275: exon1-+837T>C polymorphism was not associated with cancer risk although this allele was correlated with decreased risk ($P = 0.701$; odds ratio = 0.71; 95% CI: 0.26–1.90). CC genotype was more frequently found in controls as compared with cases and showed an inverse association with the development of cervical cancer, thus suggesting a possible protective effect.

Conclusions: PTGS-2 genotype rs689466: —1195A/G gene polymorphism demonstrated strongly associated with cervical cancer disease. However, exon1-+837T > C polymorphism was not associated with cancer risk in East Indian women. Further studies evaluating the role of PTGS-2 gene polymorphisms in ethnically diverse populations and a larger cohort may help in understanding the etiopathogenesis of cervical cancer in women worldwide.

Keywords: Cervical cancer, East India, polymorphism, prostaglandin-endoperoxide synthase-2, susceptibility

INTRODUCTION

In worldwide, the second most common cancer in women is cervical cancer.[1,2] The high-risk human papillomaviruses (HPVs) (e.g., HPV-16 and HPV-18) are a major source of cervical cancer in women.[3] However, the underlying cellular and molecular mechanisms in the pathogenesis of cervical cancer remain largely unexplored. Genetic factors play important roles in initiation and progression of diverse diseases. Prostaglandin-endoperoxide synthase-2 (PTGS-2) has been implicated in cervical carcinogenesis.[4]

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Cyclooxygenases (COXs), also known as PTGS, are rate-limiting enzymes that convert free arachidonic acid into

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several prostaglandins, namely, prostaglandin E2 (PGE2),[5] inducing the immune response through the "inflammation pathway" [Figure 1].[6]

PTGS-2 is constitutively expressed in some tissues in physiological conditions, such as the endothelium, kidney, and brain and in pathological conditions, such as cancer. PTGS-2 is responsible for production of inflammatory prostaglandins. Upregulation of PTGS-2 is also associated with increased cell adhesion, phenotypic changes, resistance to apoptosis, and tumor angiogenesis. In cancer cells, PTGS-2 is a key step in the production of PGE2, which plays important roles in modulating motility, proliferation, and resistance to apoptosis. Recent studies have demonstrated that genitourinary carcinomas, including cancer of the uterine cervix, may be regulated by PTGS activity.[7-9]

COX-2 is an enzyme encoded by the *PTGS2* gene, which spans about 8.3 kb on chromosome 1q25.2-q25.3 and has 10 exons and 9 introns [Figure 2].

PTGS-2 +8473T/C (rs5275) is located at the 3'-untranslated region and could alter gene expression through both messenger RNA stability and translational efficiency *in vitro*.[10] The −765G/C polymorphism (rs20417) is located within a putative SP1-binding site that lowers promoter activity, thereby resulting in downregulation of COX-2 expression. As PTGS-2 is emerging as an important candidate gene in carcinogenesis, the present study was designed to evaluate the association of PTGS-2 +8473T/C and −765G/C gene polymorphisms with risk of cervical cancer. The biological location of these single-nucleotide polymorphisms (SNPs) advocates the importance of these variations, which may confer a modest effect on the gene product. The −1195A>G polymorphism is located in the promoter region of *PTGS-2*, characterized by an adenine (A) to guanine (G) transition at position −1195 from exon1. So far, functional studies have shown that the −1195A>G polymorphism regulates *PTGS-2* transcription activity and COX-2 expression, by creating different recognition-binding sites for nuclear proteins.

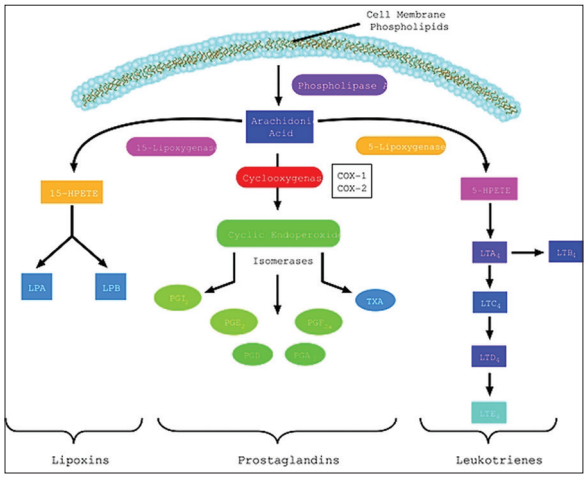

Figure 1: Arachidonic acid cascade pathway

To clarify the hypothesis that SNP variants of COX-2 are associated with the risk of cervical cancer, we analyzed the genetic polymorphisms of three PTGS-2 SNPs, namely, −1195A/G (rs689466), −765G/C (rs20417), and +8473T/C (rs5275), in a large eastern part of cervical cancer population (control/case = 200/200).

MATERIALS AND METHODS

Study design

We have designed a retrospective case–control study gathering patients with cervical cancer (200) and healthy controls (200).

Study population

This study included 400 participants: 200 histologically confirmed cervical cancer patients and 200 cancer-free controls, from the eastern region of India and recruited at the Obstetrics and Gynecology Outpatient Department of MAGS Medical and Research Center, West Bengal, Kolkata. Written informed consent was obtained from all recruited participants before their inclusion in the study, according to the Declaration of Helsinki. This research project was approved by the Ethics Committee (ref. 14/A; 667).

Sample collection and biological processing

Blood samples were collected from histopathologically confirmed patients of cervical cancer (Stages I, II, III, and IV) and age-matched, similar ethnicity, unrelated female controls free from cervical cancer, human immunodeficiency virus, and with no family history of malignancy, allergy, diabetes, or cardiovascular disease. Our control group did not have any chronic clinical problem and/or disease manifestation. Clinical diagnosis of cervical cancer patients was performed by trained medical personnel. Approximately 5 ml of peripheral blood samples was collected from the participants with the help of the collaborating doctors. Ethylenediaminetetraacetic acid (EDTA) was used as an anticoagulant. Genomic DNA extracted from fresh whole blood by QIAamp Blood Kit (Qiagen, Hilden, Germany). Genomic DNA used as a template in the polymerase chain reaction (PCR).

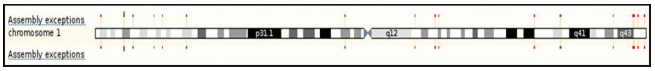

Figure 2: Human prostaglandin-endoperoxide synthase-2 gene (cytogenetic band: 1q25.2-q25.3)

Polymerase chain reaction

PCR was carried out in a total reaction volume of 30 µl containing 50–100 ng genomic DNA, standard buffer, $MgCl_2$ (as appropriate), 0.2 mM of each dNTP, 0.5 µM of each primer [Table 1], and 0.8 units of TaqDNA polymerase (Invitrogen, Carlsbad, CA, USA) in a Veriti™ 96-well thermocycler (Applied Biosystems, Foster City, CA, USA). PCR conditions used were as follows: initial denaturation of 95°C for 5 min, 35 cycles at 95°C for 30 s, 65°C for 30 s, and 72°C for 30 s, followed by the final extension at 72°C for 5 min. The PCR products were analyzed by electrophoresis in 1.5% agarose gels and visualized under ultraviolet light. Only those PCR products that had a single amplification product with no evidence of nonspecific amplification were used for DNA sequencing. The PCR products free of contaminating bands due to nonspecific amplification were column purified using a Qiagen PCR purification kit (Qiagen, Hilden, Germany), and bidirectional sequencing was performed in an ABI PRISM 3130 DNA sequencer (Applied Biosystems, Foster City, CA, USA) using dye-termination chemistry. The sequences were analyzed using pairwise BLAST to examine if there was any change from the normal sequence available in the database. The *PTGS-2* polymorphisms included in this study (rs20417, rs689466, and rs5275) were selected based on: (1) previous evidence of association with cervical tumor risk, (2) biological plausibility, and (3) minor allele frequency of at least 15%.

Statistical analysis

Allelic and genotypic associations of each SNP were tested using Chi-squared or Fisher's exact test where appropriate. Hardy–Weinberg equilibrium of each SNP in the case and control individuals was also examined using a Chi-squared test. To calculate any statistically significant difference of continuous independent variables such as age, within the control and patient groups, we used Student's *t*-test. Mann–Whitney U-test was used to analyze nonparametric variables. All tests were done using GraphPad InStat software (GraphPad Software Inc., San Diego, CA, USA). The odds ratio and 95% confidential intervals were also calculated using the same software.

Power was estimated using genetic power calculator. The sample size was calculated using QUANTO software, version 1.2.4, May 2009. Continuous results are expressed as mean ± standard deviation.

Anthropometric and clinical characteristics

Cervical cancer patients and control participants were comparable in age (48.27 T 10.04 vs. 46.95 T 9.81 years). Stages I–IV were diagnosed in 200 cervical cancer patients (Stage I, $n = 21$ [10.5%]; Stage II, $n = 71$ [35.5%]; Stage III, $n = 104$ [52.0%]; and Stage IV, $n = 4$ [2.0%]). Clinical diagnosis/staging of cervical cancer cases was performed as per the guidelines of the International Federation of Gynecology and Obstetrics. Majority of cervical cancer patients had Stage III. Tobacco users, either smokers or chewers, constituted 27.5% of the patients.

Genes polymorphism and cervical cancer

Genotype and allele frequency distribution of PTGS-2 (+8473T/C; rs5275), PTGS-2 (−1195A/G; rs689466), and PTGS-2 (−765G/C; rs20417) gene polymorphisms between cervical cancer cases and controls is depicted in Tables 2 and 3. In control participants, the genotype frequencies for both polymorphisms were in Hardy–Weinberg equilibrium. Significant association was observed in PTGS-2 −765G/C and PTGS-2 −1195A/G gene polymorphism ($P \leq 0.001$; OR, 3.06; 95% confidence interval [CI], 1.94–4.82 and $P \leq 0.001$; OR, 14.87; 95% CI, 1.94–114.26). However, no significant association was observed in PTGS-2 +8473T/C gene.

Prostaglandin-endoperoxide synthase-2 (+8473T/C and J765G/C) gene polymorphisms and clinical stages of cervical cancer

Stages I–IV were diagnosed in 200 cervical cancer patients (Stage I, $n = 21$ [10.5%]; Stage II, $n = 71$ [35.5%]; Stage III, $n = 104$ [52.0%]; and Stage IV, $n = 4$ [2.0%]). To investigate any possible association between PTGS-2 (+8473T/C, −1195A/G, and −765G/C) gene polymorphisms and clinical stages of cervical cancer, we performed a case-only analysis; however, our findings did not reveal any significant association with PTGS-2 gene polymorphisms and clinical Stage I, II, III, or IV of cervical cancer. COX-2 (+8473T/C and −765G/C) gene polymorphisms and tobacco usage among cervical cancer patients in the present study comprising 200 cervical cancer patients, 55 (27.5%) were tobacco users and 145 (72.5%) were nonusers. The majority of women with tobacco usage had a chewing habit but even a small percent of smokers in the study reported considerable chewing of tobacco. A case-only study analysis did not demonstrate

Table 1: Primer sequences used for the analysis of human prostaglandin-endoperoxide synthase-2

SNPs	Forward	Reverse
rs689466	5′-CCC TGA GCA CTA CCC ATG AT-3′	5′-GCC TTC ATA GGA GAT ACT GG-3′
rs5275	5′-GTT TGA AAT TTTAAAGTACTT TTG AT-3′	5′-TTT CAA ATTATT GTT TCATTG C-3′
rs20417	5′-TAT TAT GAC GAG AAT TTA CCT TTC GC-′3	5′- GCTAAG TTG CTT TCA ACA GAA GAA AT-′3

SNPs – Single-nucleotide polymorphisms

Table 2: Genotype distribution of prostaglandin-endoperoxide synthase-2 (−1195A/G; rs689466), prostaglandin-endoperoxide synthase-2 (+8473T/C; rs5275), and prostaglandin-endoperoxide synthase-2 (−765G/C; rs20417) gene polymorphisms in cervical cancer cases and controls

SNP	Genotype	Case (n=200)	Control (n=200)	OR (95% CI)	Adjusted OR (95% CI)*	P
rs20417: −765 G >C	GG	116	162	Reference	Reference	
	GC	82	37	GG versus GC: 3.10 (1.96-4.88)	GG versus GC: 3.07 (1.94-4.86)	<0.001
	CC	2	1	GG versus CC: 2.79 (0.25-31.17)	GG versus CC: 2.63 (0.23-21.70)	0.745
				GG versus GC + CC: 3.09 (1.97-4.85)	GG versus GC + CC: 3.06 (1.94-4.82)	<0.001
rs689466: −1195A/G	AA	86	130	Reference	Reference	
	AG	99	69	AA versus AG: 2.17 (1.44-3.27)	AA versus AG: 2.18 (1.44-3.31)	<0.001
	GG	15	1	AA versus GG: 22.67 (2.94-174.82)	AA versus GG: 20.76 (2.86-160.73)	<0.001
				AA + AG versus GG: 16.14 (2.11-123.37)	AA + AG versus GG: 14.87 (1.94-114.26)	<0.001
rs5275: +8473T/C	TT	78	98	Reference	Reference	
	TC	115	90	TT versus TC: 1.61 (1.07-2.41)	TT versus TC: 1.53 (1.02-2.31)	0.060
	CC	7	12	TT versus CC: 0.73 (0.28-1.95)	TT versus CC: 0.71 (0.26-1.90)	0.701
				TT versus TC + CC: 1.50 (1.01-2.24)	TT versus TC + CC: 1.43 (0.96-2.14)	0.077

*OR was adjusted for age, sex, and BMI. Chi-square test was used to compare the genotype frequencies between cases and controls. OR – Odds ratio; CI – Confidence interval; SNP – Single-nucleotide polymorphism; BMI – Body mass index

Table 3: Allele distribution of prostaglandin-endoperoxide synthase-2 gene polymorphisms in the study

SNP	Allele	Allele frequency		OR (95% CI)	P
		Case	Control		
rs20417: −765 G >C	G	0.79	0.90	Reference	<0.001
	C	0.21	0.10	2.54 (1.69-3.81)	
rs689466: −1195A/G	A	0.68	0.82	Reference	<0.001
	G	0.32	0.18	2.21 (1.58-3.07)	
rs5275: +8473T/C	T	0.68	0.73	Reference	0.164
	C	0.32	0.28	1.25 (0.93-1.70)	

Chi-square test was used to compare the genotype frequencies between cases and controls. OR – Odds ratio; CI – Confidence interval; SNP – Single-nucleotide polymorphism

any significant association between PTGS-2 (+8473T/C, −1195A/G, and −765G/C) gene polymorphisms and modulation of cervical cancer risk due to tobacco usage.

DISCUSSION

PTGS-2 is emerging as an important candidate gene in carcinogenesis, including cancer of the uterine cervix. To date, the effect of SNP of PTGS-2 on cervical cancer susceptibility has not been evaluated in the East Indian population, especially in West Bengal subpopulation. However, the precise immunobiological mechanisms associated with cervical cancer susceptibility in women are not yet fully elucidated. The present study aimed to investigate the role of PTGS-2 gene polymorphisms with the risk of developing cervical cancer in East Indian women. Our results demonstrated that the significant association was observed in PTGS-2 −765G/C and PTGS-2 −1195A/G gene polymorphism ($P \leq 0.001$; OR, 3.06; 95% CI, 1.94–4.82 and $P \leq 0.001$; OR, 14.87; 95% CI, 1.94–114.26). However, no significant association was observed in PTGS-2 +8473T/C gene.

We did not find any significant association between clinical stages of cervical cancer and tobacco usage. To the best of our knowledge, this is the first study to explore the role of PTGS-2 (+8473T/C, −1195A/G, and −765G/C) gene polymorphisms in cervical cancer susceptibility in East Indian women.

The polymorphism has been reported to alter gene expression through both messenger RNA stability and translational efficiency.[10] Lee *et al*.[11] have previously investigated the role of PTGS-2 gene polymorphisms with risk of cervical cancer in Korean women; however, their study did not reveal any significant association between PTGS-2 +8473T/C gene polymorphism and cervical cancer susceptibility. Significant association was observed in PTGS-2 −765G/C and PTGS-2 −1195A/G gene polymorphism ($P \leq 0.001$; OR, 3.06; 95% CI, 1.94–4.82 and $P \leq 0.001$; OR, 14.87; 95% CI, 1.94–114.26). However, no significant association was observed in PTGS-2 +8473T/C gene. Subbaramaiah and Dannenberg[12] have indicated that PTGS-2 transcription is regulated by HPV-16 E6 and E7 oncoproteins by induction of a corepressor/coactivator exchange and is mediated by the activation of epidermal growth factor receptor-Ras-MAPK-AP1 pathway. A recent study by Kim *et al*.[13] has implicated the involvement of nuclear factor-JB and activator protein-1 in PTGS-2 upregulation by HPV-16 E5 oncoprotein.

The role of promoter region polymorphism PTGS-2 −765G/C (rs20417) for cervical cancer susceptibility revealed a significant association in our study. Previous studies evaluating the association of PTGS-2 gene polymorphisms in cancer susceptibility in ethnically disparate populations worldwide have demonstrated inconsistent findings, thereby suggesting the relevance of host genetic factors in interindividual differences to disease susceptibility.

Both +8473T/C, −1195A/G, and −765G/C polymorphisms are located in the same gene, and we carried out disease risk at the haplotype interface. The complexity associated with the clinic-pathological events in cervical cancer progression is indeed interesting. We investigated any possible association between PTGS-2 (+8473T/C, −1195A/G, and −765G/C) gene polymorphisms and clinical stages of cervical cancer. Tobacco carcinogens have been associated with the risk of cervical malignancy. However, our data did not find any significant association between PTGS-2 (−1195A/G, +8473T/C, and −765G/C) gene polymorphisms and modulation of cervical cancer risk due to tobacco usage.

There are some limitations to the present study. First, we conducted a study with limited sample size and cervical cancer samples from a single cohort that might have influenced our findings. It is also plausible that limited study samples might lead to a relatively lower statistical power in certain subgroups, especially in case of tumor stage stratification. On the other hand, the strength of our study is that we have been able to explore the relationship between habitual substance use and the risk of cervical cancer from East India to provide us adequate information to well design the questionnaire and to adjust the influence from those potential confounding factors for the future studies. Future studies exploring PTGS-2 associations among diverse populations with sundry ethnicity may enhance our current understanding of the complex cellular and molecular mechanisms in cervical cancer susceptibility.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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