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TERT Promoter Polymorphisms and Risk of Cervical Cancer

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Abstract

TERT It has been shown that *TERT* is overexpressed in 90% of human cancers, and genetic alterations in the proximal promoter of TERT are significantly associated with a variety of different cancer types. In recent years, a new mechanism of *TERT* regulation through the non-coding driver mutations (C228T and C250T) in the *TERT* promoter has been reported in several cancer types. In the present study, we investigated the relationship between the Single Nucleotide Polymorphism (SNP) rs2853669 and cervical cancer.

The study included 80 individuals, including 50 patients with cervical cancer and 30 healthy controls. The samples from the participants underwent sequencing and genotyping using the Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) method.

It was found that 16%, 24%, and 60% of the cervical cancer samples had the genotypes of AA, AG, and GG, respectively. In the control group, the frequencies were 13.33%, 50%, and 36.66% of the samples for the genotypes of AA, AG, and GG, respectively.

According to our findings, there was a significant association between the recessive model GG vs. AA+AG and cervical cancer susceptibility.

INTRODUCTION

Gynecological cancers are among the most common cancers in women, with cervical cancer being the most common gynecological malignancy (1). It is one of the major public health problems globally and one of the top causes with the highest Disability-Adjusted Life Years (DALYs). However, it is one of the most preventable malignancies (2). It is a common cause of death in women, especially in those from less developed countries. There are several risk factors for this cancer, including biological, socio-economic, and health factors (3). Many epidemiological studies have pointed out the role of sexually transmitted infections as well as reproductive, behavioral, and nutritional factors in cervical cancer development, of which being infected with certain types of the Human Papillomavirus (HPV) is the most important cause (4). This cancer is not highly prevalent in Iran and many Muslim countries, although the related mortality is remarkable (5). Adult cancers, in general, are age-related genetic diseases. They only manifest when normal cells accumulate genomic instability over a period of time and subsequently acquire the capability of replicative immortality (6). Telomere attrition during successive cell divisions induces chromosomal instability and contributes significantly to genomic rearrangements that can result in carcinogenesis. Telomeres are repetitive (TTAGGG) DNA-protein complexes at the ends of chromosomes and are crucial for the survival of cancer cells (7). In the vast majority of tumors, they are maintained by an enzyme called telomerase. This enzyme protects cells from senescence. The mechanisms underlying Telomere Length (TL) maintenance and telomerase expression involve transcriptional, post-transcriptional, and epigenetic regulation. An in-depth understanding of these mechanisms may provide novel biomarkers and targets for early detection of diseases, determination of disease prognosis, and the development of therapeutics (8). It has become clear that rather than maintaining long telomeres, telomerase protects cells from senescence by maintaining some critically short length, below which cells will cease to proliferate (9). It has been found that telomerase is overexpressed in 85%-90% of tumor cells, while the rest use the Alternative Lengthening of Telomeres (ALT) pathway to protect

their chromosomes. Thus, telomerase is an attractive target for antitumor therapy, and understanding its regulation would have relevance to many tumor models. The telomerase enzyme complex contains a protein core, *TERT*, which catalyzes Reverse Transcription (RT), and an RNA molecule, *TERC*, that serves as a template for synthesis (10). Recent advancement in next-generation sequencing has enabled the whole genome sequencing of tumors with their paired normal controls. The SNP rs2853669 of the *TERT* promoter and its association with cancer risk has been reported in various cancer types in different populations. In the present study, we investigated the relationship between the Single Nucleotide Polymorphism (SNP) rs2853669 and cervical cancer.

METHODS AND MATERIALS

A total of 80 individuals were enrolled in this study, including 50 cervical cancer patients and 30 healthy controls. Blood samples of the participants were collected at the time of diagnosis and before treatment initiation. Genomic DNA was isolated from the blood samples using the salting-out method. Extracted DNAs were solved in sterile, distilled water and stored at -20 °C for standby application. Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) method was used to genotyping rs2853669 polymorphism of the *TERT* promoter. The specific primer of F: 5′-CAGCGCTGCCTGAAACTC-3 and R: 5′-GTCCTGCCCCTTCACCTT-3′ were used for PCR. Then, each successful PCR product was digested by 10 U restriction endonuclease enzymes *SacI*. Digestion reactions were performed at 37 °C overnight, and digested fragments were electrophoresed on 3% agarose gel containing 0.5 µg/mL ethidium bromide and were visualized under UV illumination.

REFERENCE

In the present study, the promoter SNP rs2853669 (-245A>G)It was found that 16%, 24%, and 60% of the cervical cancer samples had the genotypes of AA, AG, and GG, respectively. In the control group, the frequencies were 13.33%, 50%, and 36.66% of the samples for the genotypes of AA, AG, and GG, respectively. The results are presented in Table 1. According to our findings, there was a significant association between the recessive model GG vs. AA+AGTable1. Frequency of genotype and cervical cancer susceptibility.

DISCUSSION

Unlimited proliferation is an important characteristic of cancer cells, and the activation of telomerase is a key process to achieve this characteristic in the vast majority of cancers, including cervical cancer. *TERT* encodes a key catalytic subunit of telomerase, maintaining telomere stability (11). Gene expression disorders always bring about abnormal telomerase activation, thereby resulting in unlimited cell proliferation and even malignancies (12). Considering the central role of TERT in oncogenesis, numerous studies have discussed the association between cancer susceptibility and SNPs in *TERT*. Accumulated evidence has suggested a significant association between these two (13). It has been shown that *TERT* is overexpressed in 90% of human cancers (14), and genetic alterations in the proximal promoter of TERT are significantly associated with a variety of different cancer types (15). In recent years, a new mechanism of *TERT* regulation through the non-coding driver mutations (C228T and C250T) in the *TERT* promoter has been reported in several cancer types with different frequencies. The relationship between the SNP rs2853669 of the *TERT* promoter with cancer risk has been reported in different populations (16). In the present study, we investigated the relationship between the Single Nucleotide Polymorphism (SNP) rs2853669 and cervical cancer in 50 patients with cervical cancer and 30 healthy controls. According to our findings, there was a significant association between the recessive model GG vs. AA+AG and cervical cancer susceptibility. In conclusion, within the limitations, the present study provides an insight into the significance of genetic variants present in the non-coding regions of genes and their association with the hotspot mutations. However, functional studies are warranted to establish the role of rs2853669 in cervical carcinogenesis.

Table1. Genotype and Allele Distribution of rs2853669 by group

FrequencyGenotype and Allele	Case Study group	Control group	P-Value
AA	8 (16%)	4 (13.33%)	0.089
AG	12 (24%)	15 (50%)	0.142
GG	30 (60%)	11 (36.66%)	0.058
A	28 (28%)	23 (38.33%)	0.178
G	72 (72%)	37 (61.66%)	0.094
AG+GG vs. AA	42	26	0.097
AA+AG vs. GG	20	19	0.001

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