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Investigation of p16 gene promoter methylation in patients with cervical cancer and women with papilloma virus infection

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Abstract

Cervical cancer is the second most common cancer and an important cause of death in women worldwide. Objective biomarkers are needed to improve specificity for cervical cancer screening. The p16 gene is implicated in the cell cycle control, playing an important role as a tumor suppressor gene. In this study, the methylation of the P16 gene promoter was evaluated in people with cervical cancer and people with the papilloma virus. The study population included nine women with cervical cancer whose malignancy had been confirmed by a pathologist and ten patients with high-risk types of HPV virus. Methylation status was evaluated by MS-PCR. Cervical cancer patients showed a significantly higher methylation frequency for the p16 gene as compared to the control and the HPV group (p=0.001).

INTRODUCTION

Cervical cancer is the second most common cancer and an important cause of death in women worldwide (1). Cervical carcinogenesis is strongly associated with (high-risk) human papillomavirus (HPV) infections (2). Objective biomarkers are needed to improve specificity for cervical cancer screening (3). It is likely that host genetic and epigenetic events play an important role in cervical carcinogenesis. The term "epigenetic" refers to a heritable change in the pattern of gene expression that is mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene (4). One of the most important epigenetic changes that occurs in cells is the methylation of DNA, in which a methyl group binds to the cytosine nucleotide adjacent to guanine (CpG) (5). About 70% to 80% of CpGs are methylated. DNA methylation in the promoter has been shown to interfere with the binding of transcription factors to this area, thus inhibiting transcription. In normal cells, most DNA islands are non-methylated, and the process of gene transcription takes place naturally (6). In cancer cells, however, methylation of DNA on CpG islands inhibits gene expression. Significantly, many of the inactivated genes are tumor suppressor genes. Methylation of tumor suppressor genes is directly related to the onset and progression of cancer (7). Recently, aberrant methylation of promoter regions of several tumor suppressor genes has been reported in cervical cancer. The p16 gene is implicated in the cell cycle control, playing an important role as a tumor suppressor gene. This gene is located on 9p21.3 and generates several transcript variants which differ in their first exons (8), is capable of inducing cell cycle arrest in G1 and G2 phases, acts as a tumor suppressor, binds to MDM2, and blocks its nucleocytoplasmic shuttling by sequestering it in the nucleolus (9). This inhibits the oncogenic action of MDM2 by blocking the MDM2-induced degradation of p53 and enhancing p53-dependent transactivation and apoptosis. Several mechanisms for p16 gene inactivation have been described (deletion, promoter methylation, and point mutation), but their incidence depends on tumor type. In this study, the methylation of the P16 gene promoter was evaluated in people with cervical cancer and people with the papilloma virus.

METHODS AND MATERIALS

The study population included nine women with cervical cancer whose malignancy was confirmed by a pathologist, ten patients with high-risk types of the HPV virus HPV 16/18 virus confirmed by PCR tests, and ten healthy women. Samples included cells removed from the cervix by a sterile swab sampled by a gynecologist. The cells were kept in preservative and

at 4 °C until DNA was extracted. DNA samples were extracted by the QIAamp DNA Mini Kit genomic extraction kit according to the kit's protocol. The MSP method was used to investigate the methylation of the promoter area, so the process of bisulfite conversion of DNA samples was performed by the EpiTect Bisulfite Kits based on the kit's protocol. The PCR process was performed using GC TEMPase master mixes which were designed to replicate GC rich areas and specific methylate and non-methylate primers (Table 1). Samples of 5 ul of bisulfite DNA were mixed with 1 ul of specific primers and 12.5 ul of Mastermix and a volume of 25 was reached with PCR grade water. After the PCR process, the sample was run in 2% agarose gel, and the two methyl and non-methylene bonds were compared. Statistical analysis of this study was performed using SPSS software and the Mann Whitney u test.

Table1. MSP primer sequence

P16 primer	Sequence
Methylated	TTATTAGAGGGTGGGGTGGATTGT CAACCCCAAACCACAACCATAA
Non-methylated	TTATTAGAGGGTGGGGCGGATCGC GACCCCGAACCGCGACCGTAA

RESULTS

The status of p16 gene promoter methylation was assessed in nine patients with cervical cancer, ten patients with papilloma virus, and ten healthy women. The results showed that 66% of patients with cervical cancer had methylation of the p16 gene promoter region; however, only 20% of patients with the papilloma virus and none of the healthy women had methylation of this area of the promoter (Fig. 1). Cervical cancer patients showed a significantly higher methylation frequency for the p16 gene as compared to the control and HPV groups (p=0.001) (Table 2).

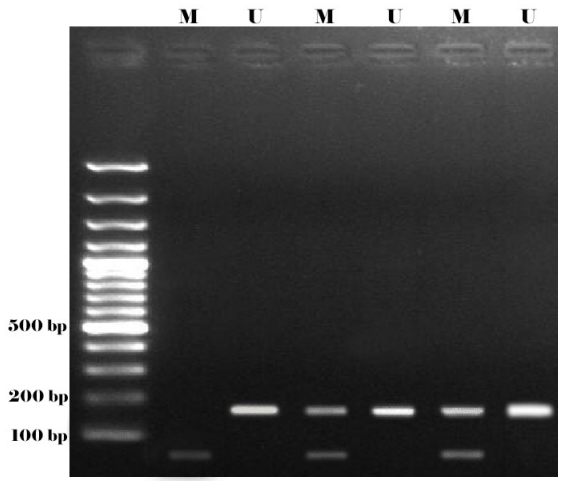

Fig 1. MSP results analysis on 2% agarose gel : Bands with a length of 176 are related to non-methyl samples and bands with a length of 48 are related to methylene samples.

Table2. P16 gene promoter methylation status in three groups: people with cervical cancer, people with human papillomavirus infection, and people with control

Group	Promoter status	-value
Cervical cancer	66% (6/9)	0.0001
HPV patient	20% (2/10)	
Control	0% (0/10)	

DISCUSSION

Epigenetic changes, especially methylation, play an important role in the formation of cancer. Promoter methylation of many tumor suppressor genes is closely linked to the inhibition of transcription and inactivation. In this study, the methylation status of the p16 gene promoter region was evaluated in people with cervical cancer, people with the papilloma virus, and healthy people (10). In previous studies, the frequency of promoter methylation of the p16 gene in cervical cancer was reported as 53%; in the present study, the promoter methylation frequency for the p16 gene in cervical cancer was 66% (6/9). The current results are similar to results from previous studies (11). The findings also suggest that p16 methylation occurs frequently and may be used as a marker for cervical cancer detection. In this study as in other studies, promoter methylation was observed in none of the controls. Also in this study, promoter methylation was observed in more people with the papilloma virus compared to healthy individuals. Larger prospective studies are necessary to establish the clinical applicability of these observations.

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