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Investigating the Association between CYP1B1 C4326G Polymorphism and Cervical Cancer Risk

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Abstract

Cervical cancer is the fourth most common and deadly cancer in women around the world. One of the genes associated with many cancers is the CYP1B1 gene. Some studies have shown that polymorphisms in the CYP1B1 gene can induce hyperactivation of proteins and increase the incidence rate of several cancers. In the present study, the association between the CYP1B1 C4326G polymorphism and the cervical cancer risk was examined with 60 newly diagnosed patients with cervical cancer and 60 cancer-free controls. DNA was extracted using the salting-out method, and the restriction fragment length polymorphism (RFLP) technique was used to determine the genotype of the CYP1B1 C4326G polymorphisms. The frequencies of the three genotypes (CC, CG, and GG) of CYP1B1 C4326G were 68.0, 25.4, and 6.6% for cervical cancer cases, and 73.1, 21.4, and 5.5 % for cancer-free controls. A significant difference was found between the two groups ($\chi 2=7.41$, $P=0.02$).

INTRODUCTION

Cervical cancer is a common and deadly cancer in women around the world [1]. Current estimates indicate that every year, 917 women are diagnosed with cervical cancer, and 467 die from the disease [2]. Cervical cancer ranks as the 16th most frequent cancer in Iranian women and the 10th most frequent cancer in women aged 15-44 worldwide. About 2.8% of women in the general population are estimated to have harbor cervical HPV-16/18 infection at a given time, and 58.6% of invasive cervical cancers are attributed to HPVs 16 or 18 [3]. However, only a small proportion of such HPV-infected cases progresses to cervical cancer [5], indicating that HPV infection is a necessary but not sufficient condition to develop cervical cancer and that it may require other factors associated with increased susceptibility, such as genetic background [4]. Recently, host genetic factors, especially the single nucleotide polymorphisms (SNPs), have been considered to explain the individual differences of susceptibility to specific malignant neoplasms [5]. One of the genes associated with many cancers is CYP1B1 that belongs to the cytochrome P450 superfamily of enzymes [6]. The cytochrome P450 proteins are monooxygenases that catalyze many drug metabolism reactions and synthesis of cholesterol, steroids, and other lipids. The enzyme encoded by this gene is localized to the endoplasmic reticulum (ER) and metabolizes procarcinogens such as polycyclic aromatic hydrocarbons and 17beta-estradiol [7]. It is worth mentioning that conversion estrogen to 4-hydroxy estrogens induces DNA damage. Additionally, 4-hydroxyestrogens can activate the estrogen receptor, thereby increasing the quantity of estrogen within the cells [8]. Consequently, the metabolic conversion of estrogens to 4-hydroxy estrogens has been postulated to be a major factor in carcinogenesis. CYP1B1 variants are more efficient than wild types in the conversion and accumulation of carcinogenic catechol estrogens [9].

Furthermore, the ratio of product formation of 4-hydroxy estrogens to 2-hydroxy estrogens is higher for CYP1B1 variants than their wild type counterpart. Variations in the genes that control the production and metabolism of these hormones and their relationship with various cancers have not been clarified. Therefore, in the present study, the association between the CYP1B1 C4326G polymorphism and cervical cancer risk was evaluated in a case-control study.

METHODS AND MATERIALS

This study includes 60 newly diagnosed patients with

cervical cancer and 60 cancer-free controls. Written informed consent was obtained from each subject involved in the study according to the declaration. A 5-ml venous blood sample was taken from each subject for DNA preparation. DNA was extracted by the salting-out method, and its quality and quantity were evaluated on a 1.5% agarose gel by electrophoresis and spectrophotometry at 260/280 nm absorbance ratio. The restriction fragment length polymorphism (RFLP) technique was used to determine the genotype of the CYP1B1 C4326G polymorphisms. Each 20 ul of the PCR reaction contained 10 ng genomic DNA, 1.25 U Taq polymerase in PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 11 mM MgCl2, 0.1% gelatin), 1.5 mM MgCl2, 50 mM dNTPs, and 250 nM each primer sense, 5'-GCCTGTCACTATTCCTCATGCC-3; antisense, 5'-GTGAGCCAGGATGGAGATGAAG-3'. Thermal cycling conditions were initial denaturation step at 95°C for 10 min, at 94°C for 30 sec, at the 56°C annealing temperature for 30 sec, and at 72°C for 30 sec. The final extension was carried out at 72°C for 5 min. The 283 bp product was digested overnight with 5 U of the restriction enzyme BstXI. The C allele was digested into 171 and 112 bp fragments, and the G allele was intact. The distribution of genotypes and alleles between groups were analyzed using the χ^2 (chi-square) test.

Results

The frequencies of the three genotypes (CC, CG, and GG) of CYP1B1 C4326G were 68.3, 25, and 6.6% for cervical cancer cases and 78.3, 18.3, and 3.3 % for controls. There was a significant difference between the two groups ($\chi2$=7.41, P=0.02). The G allele frequencies of CYP1B1 C4326G SNP were significantly higher in cervical cancer cases (27.4 %) than in cancer-free controls (12.8 %). There was a statistical difference between the two groups ($\chi2$=8.54, P=0.002). Compared to the wild-type CC genotype, the variant GG genotype and genotypes containing the G allele (CG/GG) increased the risk of developing cervical cancer significantly. Only the variant GG genotype and the variant G allele increased the cervical cancer risk significantly.

Genotype Frequency	Case	Control	P-value
CC	68.3% (41)	78.3% (47)	0.078
GC	25% (15)	18.3% (11)	0.141
GG	6.6% (4)	3.3% (2)	0.028
Allele Frequency			
G	27.4%	12.8%	0.002
C	72.6%	87.2%	

DISCUSSION

The human CYP1B1 gene, located at the 2p21–22 chromosomal region, consists of three exons (one of which is non-coding) and two introns [10]. Some studies have shown that polymorphisms in the CYP1B1 gene can induce hyperactivation of proteins and increase the incidence rate of several cancers. To the best of our knowledge, in the present study, the association between the CYP1B1 C4326G polymorphism and cervical cancer risk was examined for the first time [11,12]. Although the exact biological mechanism remains to be explored, our findings suggest that the CYP1B1 C4326G polymorphism may play a role in developing cervical cancer. The change in amino acid from valine to leucine has been shown to increase the activity of the CYP1B1 enzyme on a variety of substrates, including procarcinogens and gonadal steroid hormones [13]. Moreover, for the polymorphism Leu432Val at codon 432 of exon 3, it was reported that the 432 G allele increases the mutation of p53 [14]. Therefore, it is reasonable to conceive that CYP1B1 Leu432Val polymorphism may affect the metabolism of environmental carcinogens and increase the mutation of p53 to alter susceptibility to cervical cancer.

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