A Genetic Variant in CDKN2A Gene Predicts the Risk of Developing Cervical Cancer

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Abstract

Background: Cervical cancer is among the most commonly occurring cancers in women. Despite extensive efforts in the identification of prognostic and predictive biomarkers, only a very small number of markers have been identified. Several genetic variants in the 9p21 region have been found to be associated with an increased risk of developing cancers. The aim of present study was to explore the value of a genetic variant (rs10811661) in the CDKN2A in cervical cancer for the first time.

Methods: Data in computer-based patient dossiers (CPRs) of Mashhad University of Medical Sciences at Ghaem Hospital were applied to identify cervical cancer patients, between 2014 to September 2017. Sixty-four patients and 493 healthy subjects with no known history of infectious HPV, cancer, or family history of cancer were used. DNA was extracted, followed by genotyping using real-time-PCR. The genotype frequency and allele distribution of cases and controls were analyzed using χ², t-test or multivariate analyses.

Results: Our data showed the genotypic frequency for TT, TC and CC of the CDKN2A locus were 61.6%, 27.7%, 11.1% in the patient group and 70.7%, 25.9%, 3.6% in the healthy control group. Moreover, our data showed that the TT genotype of the rs10811661 polymorphism, using a recessive genetic inheritance model, was associated with an increased risk of cervical cancer (e.g., OR: 1.3, 95% CI: 1.1-2.9, p=0.04).

Conclusion: We have demonstrated that patients with a TT genotype for the rs10811661 polymorphism of the CDKN2A gene locus were associated with increased risk of cervical cancer. Further investigations are required in a larger population to explore the value of this gene marker in the management of cervical cancer.

Keywords: Cervical cancer; HPV; CDKN2B; rs10811661; Genetic variants; Polymorphism

Introduction

Cervical cancer is one of the most commonly occurring cancers in women in developing countries [1]. The major risk factor of cervical cancer is human papilloma virus (HPV) infection [2]. Other risk factors are: smoking habit, immune function, having several sexual partners, having first sexual intercourse at a young age, and oral contraceptive use [1]. There is growing evidence that the association of genetic variants and dysregulations of pathways with an increased risk of developing cervical cancer include the Wnt/b-catenin, PI3K/Akt, and c-MET/HGF pathways [3-6].

Recent genome-wide association studies have shown an association between a locus on chromosome 9p21 with various diseases [7]. Three genes are located in this region, that include cyclin-dependent kinase inhibitors CDKN2A CDKN2B and antisense noncoding RNA in the INK4 locus (ANRIL) [8], which have been reported to be an important susceptibility locus for various diseases. ANRIL transcribed from the string to CDKN2A/B. The absence or abnormal expression of CDKN2A and CDKN2B genes has been associated with many cancers and other conditions such as cardiovascular diseases [9]. Several genetic alterations have reported to be linked with CDKN2B and CDKN2A expression, such as deletion, amplification and genetic variants [8]. In our previous studies we have investigated the interaction of a genetic variant of the CDKN2A/B-rs10811661 with obesity as risk factor of different cancer with environmental-exposures (e.g., diet and physical activity) in individuals recruited from the Mashhad-Stroke and Heart-Atherosclerotic-Disorders cohort. Our data showed an association between this genetic marker with dyslipidaemia in a non-diabetic population. These data also showed that a low energy diet and high physical activity ameliorated the unfavourable effects of T allele of CDKN2A/B locus [10]. We have further investigated the value of this marker in breast and oesophagus cancers. In particular, we assessed...
the value of this genetic variant for the first time in 564 subjects with/ without breast cancer. Our results indicated that patients with a TT genotype for CDKN2A/B rs10811661 polymorphism had a higher risk of developing breast cancer [11]. Therefore, the purpose of this study was to examine the predictive value of a gene variant rs10811661 in the CDKN2A/B gene in women with cervical cancer.

Materials and Methods

Patients

This study comprises 64 cervical cancer patients recruited from Ghoem Hospital during 2014-2017. The control group were 493 healthy women without cervical cancer and recruited as part of the Mashhad Stroke and Heart Atherosclerotic Disorders (MASHAD) cohort study and were matched with case group. The control group had no known history of infectious HPV, cancer, or family history of cancer. The study was approved by local Hospital Ethic Committees of Mashhad University of Medical Sciences.

Genotyping

Genomic DNA was extracted from peripheral blood or tissue samples using a QIAamp® DNA Mini-Kit (Qiagen, San Diego, CA) according to the manufacturer’s protocol. The concentration and purity of DNAs were assessed by the NanoDrop®-1000-Detector (NanoDrop-Technologies, Wilmington, USA). Genotype analysis of CDKN2A/B-rs10811661 polymorphism was carried out using Taqman®-probes-based assay; PCR reactions were carried out in 12.5 µl total volume, using 10 ng of DNA in TaqMan® Universal Master Mix with specific primers and probes (Applied Biosystems Foster City, CA). The ABIPRISM-instrument equipped with the SDS version-2.0 software was used to evaluate the allelic content of the samples [10,11].

Statistical analysis

Data was analysed using SPSS-20 software (SPSS Inc., IL, USA). Descriptive statistics of cervical cancer patients was reported as the mean and standard deviations (SD) for continuous variables, while frequencies and percentages were used for categorical variables. Genotype and allele frequencies of CDKN2A/B rs10811661 polymorphism were assessed for deviation from the Hardy–Weinberg equilibrium (HWE) by using the Pearson χ² distribution. The associations between risk of cervical cancer for the CC and CT genotypes, relative to the risk genotype TT homozygote under recessive genetic model were assessed by different genetic model, Cervical cancer risk estimates were expressed as the odds ratio (OR) and its corresponding 95% confidence interval (CI). The relationships between the CDKN2A/B rs10811661 polymorphism and clinic pathological features were assessed using Pearson’s chi-square χ² test for categorical variables and continuous variables were evaluated using Student’s t tests. Quantitative variables and Pearson distribution for qualitative variables were used to determine the relationship between the risk of cervical cancer and its association with genotype. All the analyses were two-sided and statistical significance was set at p<0.05.

Results

Clinical characteristic of populations

Our data showed that the genotype frequency of TT, TC and CC were 61.6%, 27.7%, 11.1% in patient group while these values were 70.7%, 25.5%, 3.6% in healthy group respectively (Table 1). Table 2 shows the distribution of genotype frequencies of CDKN2A/B rs10811661 polymorphism, which was in Hardy-Weinberg equilibrium (HWE) (p>0.05). The frequencies of CC, and TT genotypes in total population for rs10811661 were 17.3% and 82.7%, respectively.

Association of the genetic variant with cervical cancer

We then evaluated the risk of developing cervical cancer across different genotypes of the variant rs10811661 residing on the CDKN2A/B gene in different genetic models (Table 3). This data showed that TT genotype of the rs10811661 polymorphism in recessive genetic inheritance model (CC+CT) against TT was associated with increased risk for cervical cancer patient (e.g., OR: 1.3, 95% CI: 1.1-2.9, p=0.04).

| Group (n, %) | Total | Genotypes |
|-------------|-------|-----------|
|             |       | CC (6/11%) | CT (15/27%) | TT (33/61%) |
| Patient     | 54    | 6         | 15          | 33          |
| Control     | 493   | 18 (3/6%) | 126 (25/5%) | 349 (70/7%) |
| Total       | 574   | 24 (4/4%) | 141 (25/7%) | 382 (69/8%) |

Table 1: Distribution of studied population in different genotypes of variant of CDKN2A/B.

| Gene       | SNP  | Allele Minimum/Maximum | Maximal Allele Homozygotes | Minimal Allele Homozygotes | HWE P-Value |
|------------|------|------------------------|-----------------------------|-----------------------------|-------------|
| CDKN2A/B   | rs10811661 | C/T                | 382 (82.7%) | 24 (17.3%) | P=0.21 |

HWE: Hardy–Wienberg Equilibrium; MAF: Minor Allele Frequency; SNP: Single-Nucleotide Polymorphism.

| Variant     | Risk allele | Gene | Additive model OR (95% CI) | Recessive model OR (95% CI) | Dominant model OR (95% CI) |
|-------------|-------------|------|---------------------------|-----------------------------|---------------------------|
| rs10811661  | T           | CDKN2A/B | 1.8 (0.7-3.4) P=0.1 | 1.3 (1.1-2.9) P=0.04 | 2.3 (0.3-3.4) P=0.3 |

Table 3: Association of rs10811661 residing on CDKN2A/B on the risk of cervical cancer.
Discussion

To the best of our knowledge this is the first study showing the association of a genetic variant in CDKN2A/B with increased risk of developing cervical cancer. In particular patients with a TT genotype had a higher risk of cervical cancer. This observation can be explained at least in part by its function in the regulation of cell cycle and apoptosis [12,13]. It has been shown that when CDKN2A/B is deactivated by methylation or ANRIL, it can suppress the activity of tumor suppressor p15/CDKN2B-p16/CDKN2A-p14/ARF encoded by these genes [14]. ANRIL regulates the expression of these genes via suppressor complexes of PRC5 via epigenetic mechanism. Cell proliferation is indirectly regulated by ANRIL through three methylations at histone 3 lysine 27 (3meH3K27) in chromosome 9p21 by PRC2 and consequent silencing of CDKN2A/B. PRC1 allows ubiquitination at histone (H2A) at lysine 119 and maintenance of silencing [15,16]. In other word, PRC1 and PRC2 suppress transcription of several genes, specifically genes involved in silencing CDKN2A/B position via ANRIL [17]. Several studies have identified that high ANRIL expression in tumor tissue. Also inactivation of the CDKN2A gene can be due gene mutations, homozygous deletion and promoter methylation [18]. Cunningham et al. [8] have demonstrated that ANRIL expression is strongly associated with genetic variants within the CDKN2A/B promoter. Nisha et al. [19] studied the association between polymorphisms rs151580 in RB1 gene with cervical cancer. Ellen and colleagues revealed that polymorphisms in CDKN2A/B were associated with ovarian cancer [20]. Similarly Healy et al. [21] showed that genetic variants in CDKN2A/C, CDKN2A/B, and CDKN1B were associated with the susceptibility to childhood leukaemia. Many studies have investigated the potential value of polymorphism rs10811661 in CDKN2A/B as a breast cancer susceptibility gene. Antoniou et al. [22] showed the association of the rs1011970, near CDKN2A/C, with increased risk of breast cancer. Similarly Düben et al. [23] investigated the potential value of CDKN2A/B as a breast cancer susceptibility gene. Kristy and colleagues found several polymorphisms within 13 genes involved in the cell cycle pathways with the risk of breast cancer. This study revealed a significant relationship between four genetics variants in the region of CDKN2A/B and breast cancer risk [24]. Another study by Lee et al. [25] revealed a correlation between rs3731239 in intron region of CDKN2A with ESCC cancer, gastric cancer and intestinal cancer. Another study in a cohort of 120 gastric cancer patients, found the higher expression of CDKN2A/B and CDKN2B-AS1 with ESCC [26]. Other studies have reported that genetic variants in CDKN2A/B were related to the presence of type 2 diabetes [27,28].

Conclusion

Our findings showed an association between CDKN2A/B rs10811661 with high risk cervical cancer. Further investigations are warranted in a larger population to assess the value of emerging marker in management of patients.

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Conflict of Interest

The authors have no conflict of interest to disclose.

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