The Most Frequent HPV Genotypes in Women with Cervical Cancer in Southwest of Iran

Mojtaba Oraki Kohshour1,2, *, Manijeh Afra1, Abdolhossein Shakurnia1, Nepton Emad Mostofi3 and Behnaz Andashti4

1Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3Department of Pathology, Imam Khomeini Hospital, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
4Department of Genetics, School of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran

*Corresponding author: Mojtaba Oraki Kohshour, Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Postal Code: 6135713794, Tel: +98-9163902232, E-mail: mojtabao@gmail.com

Received 2018 February 07; Revised 2018 June 18; Accepted 2018 June 20.

Abstract

Background: Cervical cancer ranked as the most common cancer of women in developing countries. Human Papillomaviruses (HPVs) belongs to the Papillomaviridea family, which is able to infect humans. Some HPV types are the causes of getting cervix cancer and it is clear that persistent infection, with a high-risk HPV genotypes, is known to be a major carcinogenic factor.

Objectives: Determining the HPV genotypes distribution in women with cervical cancer in Khuzestan province, which is located in the southwest of Iran, to help design better preventive and also therapeutic strategies for promoting women health.

Methods: A total of 75 archival cervical carcinoma tissue samples were studied for the presence of HPV DNA and determination of the genotypes by PCR and Sequencing. Genomic DNA was extracted from the FFPE cervical tissues and then subjected for the PCR based amplification with subsequent direct sequencing of the HPV positive PCR products.

Results: The mean age of patients was 56. There were 42 samples with HPV DNA by using GP5+ and GP6+ primers. The result of HPV genotyping have been distinguished as follow: 33 samples as HPV-16, 4 samples as HPV-18, 1 sample for each HPV-54, 56 and 58 genotypes, and 2 samples remained unknown.

Conclusions: Detecting HPV genotypes absolutely shed light on guidelines cervical cancer screening and vaccination policy in our country and in other developing countries. Collectively, our results suggest that HPV-16 is the frequent high-risk HPV type in our geographical area. HPV genotype testing, as a primary screening tool, plays an important role in cervical cancer prevention and therefore, it is a strongly recommended vaccination against HPV, especially for high school girls be included in the national immunization program.

Keywords: Human Papillomaviruses, Genotypes, Cervical Cancer, Southwest of Iran

1. Background

The most common cancer in women in developing countries is cervical cancer. Despite the reduction rate of mortality by screening programs, 500,000 new cases of invasive cervix cancer are recognized each year, most of them live in developing countries (1). Human Papillomavirus (HPV) has an important role in human cancers (2). HPV, which belonged to the Papillomaviridae family, has more than 100 species and almost 40 types of Alpha Papillomavirus infect the anogenital tract (3, 4).

Several genotypes of HPV including HPV 16, 18, 31, 35, 39, 45, 51, 52, 56, 58, 59, and 66 involve cervical malignancy (1) and so divided as high risk genotypes (5). In Asia, HPV 16 and 18 genotypes are causes of 70% of cervical cancers, HPV 58 and 52 are the most prevalent after 16 and 18 (6, 7). Although HPV16 is the most common oncogenic genotype in the most regions of the world, distribution of HPV genotypes is various. It is clear that persistent infection with a high-risk HPV genotype is known to be a major carcinogenic factor, however, various high-risk HPV genotypes have different carcinogenic potentials (8). Therefore, finding out the genotype-specific features of HPV infection would facilitate the expanding of better strategies to prevent and manage cervical cancer (9).

HPV genotype testing, as a primary screening tool, play an important role in cervical cancer prevention. HPV infection and the risk of the development of cervical cancer are strongly related to cancer-associated HPV genotypes,
which are more than the known 15 types. Today, HPV DNA analysis such as PCR tests, due to high sensitivity to detect HPV DNA, which can reduce false negative results of Pap smear, has been recommended to recognize cervical cancer at the early stages in addition to Pap smear test (10, 11).

2. Objectives

In this study we determined the frequency of HPV genotypes in archival formalin paraffin embedded blocks of cervical cancer belong to women who referred to the Imam Khomeini Hospital of Ahvaz city (which located at southwest of Iran) from 2001 to 2010. The results of this study can help to design better preventive and also therapeutic strategies for promoting women health.

3. Methods

3.1. Sample Collection

We surveyed in report forms of the Pathology department of Imam Khomeini Hospital of Ahvaz from 2001 to 2010 to find the positive cervical cancer cases. We found 85 cases that had been reported positive as cervical cancer, which were then confirmed by a pathologist. After that, by going to sample bank section, we could collect 75 samples from archival formalin fixed paraffin embedded tissues (FFPE) of cervical cancer patients. The samples were transported to the Immunology department of Ahvaz Jundishapur University of Medical Sciences for doing molecular tests.

3.2. DNA Extraction

Using a scalpel, the sections of 5-10 µm thick were cut from each sample block after discarding the first 2-3 sections. The sections were placed in a 1.5 ml microphage tube then, 160 µl Deparaffinization solutions (QIAGEN cat. No. 19093) was added, mixed vigorously, and centrifuged briefly to collect the sample in the bottom of the tube. DNA extraction was performed from 75 samples by using QiAamp DNA FFPE tissue kit (cat. No. 56404).

3.3. HPV Amplification

PCR test was applied for samples by using GP5+ and GP6+ primers to amplify 150 bp products from HPV L1 open reading frame (12) and 5 µl of each DNA sample mixed with 12.5 µl of AmpliconTaq DNA polymerase master mix (cat. No. 18031) to final volume of 25 µl. PCR profile was 5 min at 94 ºC for primary denaturation followed by 30 sec at 94 ºC, 1 min at 40 ºC, and 1 min at 72 ºC, which repeated 35 cycles. Final extension was 10 min at 72 ºC. A total of 5 µl of PCR products were analyzed by Agarose gel electrophoresis.

3.4. DNA Sequencing

Sequencing was done for HPV positive PCR products (Genefanavaran co. Tehran, Iran). Sequences results were aligned with Chromas software and confirmed by visual inspection.

4. Results

The mean age of patients was 56. There were 42 (56%) samples with HPV DNA by using general primers, GP5+ and GP6+. The result of HPV genotyping is shown in Table 1. As results show, 33 samples (78.5%) were distinguished as HPV-16, 4 (9.5%) samples were detected as HPV-18, 1 sample for each HPV-54, 56 and 58 genotypes (2.4% for each one), and 2 (4.8%) samples remained unknown.

<table>
<thead>
<tr>
<th>HPV Genotype</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-16</td>
<td>33/42 (78.5%)</td>
</tr>
<tr>
<td>HPV-18</td>
<td>4/42 (9.5%)</td>
</tr>
<tr>
<td>HPV-54</td>
<td>1/42 (2.4%)</td>
</tr>
<tr>
<td>HPV-56</td>
<td>1/42 (2.4%)</td>
</tr>
<tr>
<td>HPV-58</td>
<td>1/42 (2.4%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2/42 (4.8%)</td>
</tr>
</tbody>
</table>

5. Discussion

Cervical cancer is a major health problem in the world (13). The results of researches show about 99.7% of cervical cancers have HPV DNA in tissue lesions. Although HPV-16 genotype is the most common oncogenic genotype in different areas of Europe and America (14), Geographical distribution of HPV genotypes is various. In the present study, prevalence of HPV-16 genotype was 78.5%. In studies that have been conducted in different regions of Iran, HPV-16 genotype has shown the frequency of above 50%. For example, in the study published by Hamkar et al., in 2003, the prevalence of HPV-16 and 18 genotypes were reported 60.6% in North of Iran (15). Also in the research, which was performed by Mahmoodi et al., in 1997, the frequency of HPV-16 genotypes was 70% in the center of Iran (16). In a study published by Zandi et al., in 2010, the prevalence of HPV-16 genotype was almost 64% in the south of Iran (17). In a similar survey, which had been done by Hamidi - Fard et al., in 2012, 50% of the positive samples were genotype 16 (12). According to these studies, it may be reported that HPV-16 genotype is the most common HPV genotype in Iran; however, this conclusion needs more studies surely. According to the World Health Organization report, about 83% of
500,000 new cases of cervical cancer, which occur annually, are related to developing countries and these countries are known for the majority of cervical cancer cases that occur in the world (18, 19). Absolutely, performing powerful screening programs in these countries can seriously and continuously help decrease the incidence and also mortality rate greatly. In addition due to successful implementation of screening programs, the prevalence of cervical cancer in advanced countries is lower than the developing countries. The Society of Gynecologic Oncology and the American Society for Colposcopy and Cervical Pathology published an interim guidelines for screening with HPV test alone (without cytology) every 3 years for women ≥ 25 years old. Patients with positive test results for HPV 16/18 would undergo a colposcopy (20). It is strongly recommended that screening as well as vaccination against HPV, especially for high school girls, be included in the national immunization program.

Acknowledgments

We acknowledge all personnel of the Pathology department of Imam Khomeini Hospital of Ahvaz.

Footnote

Funding/Support: This research was supported by the Cellular and Molecular research center of Ahvaz Jundishapur University of Medical Sciences.

References