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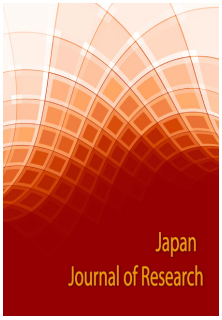

Frequency of the HLA-DRB1 and HLA-DRQ1 alleles in Mexican women with oncogenic HPV infection

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Abstract

Objective: The purpose of this study was to determine the expression of E6 / E7 mRNA of HPV-16 and HPV-18, along with the presence of HLA II-DRB1 and DQB1 variants in a Mexican population. **Methods:** The women who attended to the primary services in Morelos, Mexico, during 2019 were invited to participate in the conventional cytology and biomolecular testing for the detection of HPV. The mRNA assay method for detection of mRNA from 14 different HR-HPV using target capture of E6 and E7. Low-resolution PCR-sequence-specific priming (SSP) was used for amplification of HLA DR-DQ alleles in both cases.

Results: A total forty-eight women studies were included. The prevalence of HPV E6/E7 mRNA was 50%, and distributed as follows: 20% patients with the presence of E6/E7 mRNA from HPV-16, 24% patients with the presence of E6/E7 mRNA from HPV-18/45 genotype and 6% patients with the presence of E6/E7 mRNA from other HR-HPV. The most frequent allele in HPV-positive cases (32%;8/25) was DRB1*01 for the HLA DRB1 locus, followed by the HLA DRB1*04 allele (26%;13/48). It should be noted that for the group of HPV positive patients, DRB1*015 was detected, but not in the group of patients without HPV. Discussion: In this study, alleles HLA-DRB1*15 and HLA-DRB1*04 were identified in women infected by oncogenic HPV, but were not detected in absence of HPV infection. These findings are consistent with reports for populations of Asian, Caucasic, and Latin-American women, which suggests that this allele is a prognostic marker for CC development.

Introduction

Cervical cancer (CC) continues to be a global public health problem around the world. During 2018 a total of 528,000 new cases and 266,000 deaths were estimated, which represent 11% of malignant neoplasms for women in Latin America, and 8% (275,100) of cancer deaths in women [1,2].

Human Papillomavirus (HPV) infection is the main etiological factor for the development of CC; however, HPV-16/HPV-18 are considered high-risk (HR-HPV) genotypes since these can contribute to cell transformation and development of CC. The expression of E6 and E7 oncoproteins from HPV-16/-18 is associated with viral persistence and epithelial transformation [3,4].

Public policies for early detection of CC development have been strengthened by implementation of molecular biomarkers in screening and a vaccination scheme. Nonetheless CC remains as one of the main causes of death in women. Several studies have described the participation of the immune response and its relationship with the persistence of HPV and progression to CC. These studies suggest that the presence of DRB1 and DQB1 polymorphisms in the HLA-II alleles contributes to the development of carcinogenesis associated with CC [5-7]. Scientific evidence has shown that genetic and immunological factors of the host play an important role in the persistence of HPV, cell transformation, and development of CC. HLA class I and II are molecules involved in the antigen- presenting cell-mediated presentation of HPV peptides to T cells, and thus the immune response activation can vary according to the HLA polymorphism. The HPV-HLA peptide complex is necessary for efficient activation of the T cell response and subsequent elimination of HPV [8].

HLA-II plays a key role in tumor antigen presentation, immune response, and immune surveillance. Studies have reported that aberrant HLA-II expression is closely related to tumor pathogenesis, resulting in dysfunctional presentation of exogenous antigens to CD4+ T cells, allowing some tumorigenic cells to escape immune surveillance [9-10].

Therefore, it is necessary to analyze the presence of HPV-16/-18 mRNA, along with the expression

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of DRB1 and DQB1 polymorphisms in women, which could contribute to the persistence of these oncogenic viruses, favoring cell transformation and the development of CC.

The detection of DRB1 and DQB1 polymorphisms and its association with HPV-16/-18 mRNA can improve the screening in HR-HPV positive women. Consequently, this would contribute to provide timely treatment and to reduce costs for the health system, as well as to improve the management of premalignant cervical lesion cases and CC, which continue to be lately detected.

The purpose of this study was to determine the expression of E6 / E7 mRNA of HPV-16 and HPV- 18, along with the presence of HLA II-DRB1 * 15 and DQB1 variants in a Mexican population that attends to the preventive care unit within the CC prevention program.

Materials and methods

An observational study was carried out in women from the Cervical Cancer Early Detection Program. This program is aimed to evaluate asymptomatic women between 25 and 64 years of age, or women under 25 and over 64 years of age that have reduced morbidity and the presence of some risk factors associated with CC. The women who attended to the primary, reproductive, or family planning services at the Institute of Security and Social Services for State Workers (ISSSTE, for its Spanish acronym) in Morelos, Mexico, during 2019 were invited to participate in the conventional cytology and biomolecular testing for the detection of HPV..

Cervical sampling

The endocervical exudates were inoculated in ThinPrep transport medium to perform conventional cytology and HR-HPV detection. Thereafter, samples and results were code-labelled and anonymized.

HR-HPV mRNA detection and genotyping in cervical exudate samples

All samples were HPV-tested with the mRNA Aptima assay. One milliliter of each sample was automatically transferred to an Aptima Specimen Transfer Tube and analyzed on the Panther system (Hologic). The mRNA Aptima assay is a qualitative method for detection of mRNA from 14 different HR-HPV (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) using target capture of E6 and E7. Capture oligomers target HPV mRNA, which is subsequently, amplified using a transcription based method. First, a DNA copy is produced and works as a template for extensive RNA amplification and then probe hybridization is used for luminescence detection (Relative Light Units, RLU). Controls are included to adjust cut-off levels and results are provided as signal- to- cut-off (S/CO). A S/CO above 0.5 is considered a positive test result as determined automatically by the assay software..

HLA genotyping

Peripheral blood samples were collected in tubes containing EDTA anticoagulant, and then processed and stored at -4°C within 24 hours of being drawn. DNA was extracted from peripheral blood mononuclear cells (Genomic DNA Purification Kit No: A1120). Final elution of DNA was performed in 100 µl of rehydration solution. Low-resolution PCR-sequence-specific priming (SSP) was used for amplification of HLA DR-DQ alleles in both cases (Protandim Cyclerplat System HLA-DRB1*,DQB1* Kit), according to the manufacturer's procedure.

Statistical analysis

Bivariate analysis was applied to compare HPV positive and HPV negative samples. Descriptive statistical analysis for allelic frequencies of HLA DR-DQ alleles was considered statistically significant $p<0.05$.

Results

Characteristics of the population with HR-HPV

The study population consisted of 48 women who arrived to the family planning service for a cytology sampling and analysis of HR-HPV infection during November 2019 to March 2020. The age group between 50 and 59 years was the most frequently screened group (Figure 1).

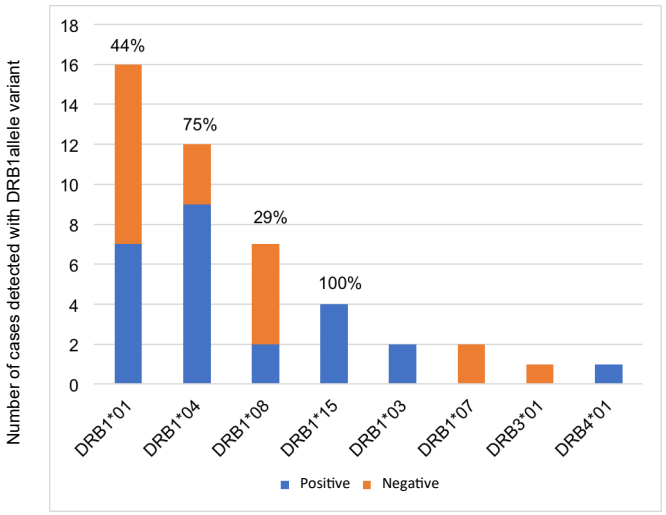
Prevalence of E6/E7 mRNA in HR-HPV infection

The prevalence of HPV E6/E7 mRNA was 50%, and distributed as follows: 20% (10) patients with the presence of E6/E7 mRNA from HPV-16, 24% (12) patients with the presence of E6/E7 mRNA from HPV-18/45 genotype and 6% (3/) patients with the presence of E6/E7 mRNA from other HR- HPV.

Distribution of HLA-DRB1* and HLA-DQB1* alleles

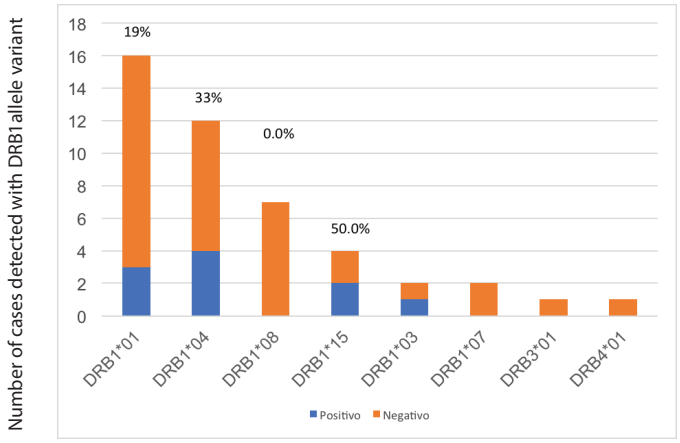
HLA DRB1 and DQB1 genotyping was completed in all 48 patients. The allelic distribution for the HLA DQB1 locus revealed that DQB1*01 was the most frequent allele (32%;16/48), followed by the HLA DRB1*04 allele (26%;13/48). The most frequent allele in HPV-positive cases (32%;8/25) was DRB1*01 for the HLA DRB1 locus, followed by the HLA DRB1*04 allele (26%;13/48). It should be noted that for the group of HPV positive patients, DRB1*015 was detected, but not in the group of patients without HPV (Figure 2).

A possible relationship was also established for the DRB1*04 allele (50%;5/10) and *15 allele 20% (2/10) regarding HPV-16



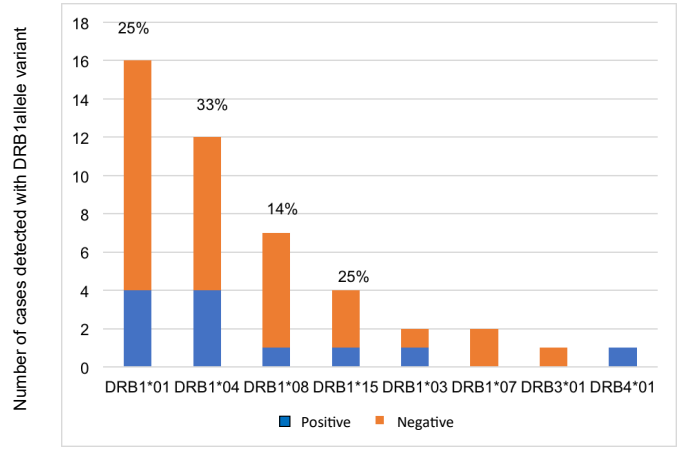
Distribution of HLA-DRB1* alleles in study population. 45 HLA-DRB1 alleles were identified among 48 women, including 25 HPV E6/E7 mRNA positive patients and 23 HPV E6/E7 mRNA negative subjects from Morelos. The HLA-DRB1*04 was the most frequent allele in HR-HPV E6/E7 mRNA positive cases (75%; p=0.212); in contrast on the allele DRB1*08, which showed a lower frequency in HPV positives cases, 29% (p=0.250). The allele DRB1*15 was detected only in HPV positives cases (p=0.178).

Figure 1. Distribution HLA-DRB1 alleles related with HR-HPV E6/ E7 mRNA*



The frequency of HPV-16 E6/E7 mRNA in the population studied was 20% (10/50 samples). DRB1*15 was detected more frequently in HPV positive cases; there was not a single HPV positive case with the DB1*08 allele (p = 0.296).

Figure 2. *Distribution HLA-DRB1* alleles related with HPV-16 E6/E7 mRNA*



The frequency of HPV-18 E6/E7 mRNA, in the population studied was 24% (12/50 samples). DRB1 * 04 allele was detected more frequently.

Figure 3. *Distribution HLA-DRB1* alleles related with HPV-18 E6/E7 mRNA*

infection. The DRB1*01 allele in HPV-18 infection was the most frequent allele in HPV-16 infection (33%; 4/12). Compared to the negative HPV cases, DRB1*01 allele was the most frequent (40%;10/25). (Figure 3).

In this study, only four samples with SIL-BG were identified by histological studies; in three HPV- 16 samples and one HPV-18 sample. In two cases, there was a relationship with the presence of the DRB1*15 allele in patients with a history of HPV infection and electrosurgery treatment.

Discussion

Studies related to HPV infection frequency in women in the state of Morelos have described a high infection rate, which is comparable to the high prevalence of CC cases in this region. It is important to highlight that 80% of the population is exposed to HPV, which disappears in 70 to 90% of infected women [10]. Only less than 4% of infected women develop a persistent infection, with an estimated 1-2% of women presenting cellular changes that progress towards cellular transformation and development of cervical cancer. Oncogenic genotypes HPV-16 and -18, have been associated to a higher risk for developing cervical cancer [11-12].

In this study, the higher prevalence of human papillomavirus infection was identified in the city of Cuautla, which had the highest number of positive cases in the entire Morelos State. Attention should be paid to other high-risk genotypes that could be of local epidemiological importance.

In this study, we identified that HPV-18/45 was the most frequent at 24% (12/50), followed by HPV-16, at 20% (10/50). Unlike other studies, in this case HPV-18 was the predominant serotype [13].

The age range for patients infected by HPV-18/45 and HPV-16 was 35 to 60 years old, as indicated by previous studies. 78% (37/47) of patients presented negative conventional cytology, and only 27% (10/37) of SIL-BG. Of these, 9 samples were related to the presence of HPV-18/45 (5/9) and HPV-16 (2/9).

Immune response against HPV is crucial during the clinical course of infection and natural developmental history of CC. Several studies have documented that the presence of certain alleles is associated with persistent HPV infections, increasing the risk of CC.

In this study, alleles HLA-DRB1 * 15 and HLA-DRB1 * 04 were identified in women infected by oncogenic HPV, but were not detected in absence of HPV infection. These findings are consistent with reports for populations of Asian, Caucasic, and Latin-American women, which suggest that this allele is a prognostic marker for CC development [14,15]. According to previous studies, there is evidence of alleles as risk markers for CC, where HPV-18 viral peptides have a higher chance of persistence when presented by DRB1 * 04: 02, DRB1 * 13: 03 and DRB1 * 15: 02, and for HPV-16 when presented by DQB1 * 03: 02 y DQB1 * 05: 02.

Some studies indicate a significant association between the distribution of homozygote HLA alleles and presence of CC. Haplotype DRB1*15-DQB1*06 has been reported as a protection marker for this oncological pathology [16].

A limitation of this study is its design, sample size and it is necessary to carry out further studies to strengthen this association between HLA-DRB1 alleles and CC.

Identification of specific HLA alleles associated to cervical cancer, and the polymorphisms that encode them, may help to predict the susceptibility of the individual to HPV infection and prioritize the population as high-risk.

Molecular testing for HPV genetic material is crucial to detect women at risk for cellular changes in the cervix or for progression of high-risk intraepithelial lesions that contribute to in situ carcinoma.

Ethics approval and consent to participate

The aims of the study were explained to the invited women, and everyone who agreed to participate signed an informed consent letter. A structured questionnaire was applied regarding gynecological- obstetric characteristics and sexual behavior, and the information in each questionnaire was entered into a database. Blood and endocervical samples were obtained from each participant. This study was approved by the ethics committee of ISSSTE (No: 122.2019). All the investigations were conducted according to the guidelines of The Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the British Medical Journal (18 July 1964).

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Conflict of interest

The authors declare no conflict of interest

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