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CLINICAL ARTICLE

Assessment of HPV infection among female university students in Honduras via Roche linear array

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ABSTRACT

Objective: To determine the prevalence of specific types of human papillomavirus (HPV), and the association with possible risk factors, among female university students at university in Honduras. *Methods:* In a cross-sectional study, cervical samples from 400 women aged 18–35 years were tested using a Roche HPV linear array to differentiate 37 genotypes of HPV. Associations with risk factors were assessed. *Results:* Of the 400 participants, 393 completed the study. HPV DNA was detected in 45% of these women, of whom 73% were infected with high-risk types of HPV and 46% had multiple infections. Overall, 36 HPV genotypes were identified, of which HPV types 16, 51, 84, 66, and 39 were the most common. There was a marked decrease in the prevalence of multiple and high-risk infections with age. The factors that were independently associated with risk of being infected were related to sexual behavior and smoking habits. *Conclusion:* The study showed that genital HPV infection is common among sexually active women at university in Honduras. In addition, the Roche linear array was shown to be a valuable tool for HPV genotyping, which will be useful for monitoring the future effectiveness of an HPV vaccine in the population.

1. Introduction

Human papillomavirus (HPV) is one of the most common sexually transmitted viruses among young women; most, if not all, sexually active women get infected by 1 or more types of HPV during their lifetime. Although most infections are transient, the potential health implications are obvious because HPV types 16 and 18 cause 70% of cervical cancers, and other types also have an important role in cervical carcinogenesis [1].

Although the process of developing cervical cancer can take decades, infection with high-risk HPV types and the presence of risk factors that promote persistence of the infection precede the development of precancerous lesions. These risk factors are either biological, such as co-infection with other sexually transmitted diseases, or behavioral, such as sexual habits [2].

Given the effect of HPV infection on public health, prophylactic vaccines against HPV 16 and 18 have been developed. To assure the efficacy of these vaccines, however, it is essential to gather baseline information on the acquisition of HPV by young women and the epidemiology of type-specific HPV infection arising from behavior. With the introduction of prophylactic vaccines, it is also important to monitor HPV infections in order to oversee changes in HPV epidemiology over time [3].

Although screening for cervical cancer precursors by cytology has been very successful, it is generally inefficient and unworkable in many parts of the world where the appropriate infrastructure is not achievable [4]. Accordingly, many ongoing international research projects are assessing the feasibility of introducing tests for high-risk HPV types as a marker for cervical cancer. For these screening purposes, several assays have been developed to detect high-risk HPV infections. Recently, Roche Molecular Systems Inc. (Branchburg, NJ, USA) launched their Line Array Assay that can identify 37 genotypes of HPV, providing an adequate test for epidemiologic studies among young women, who harbor more genotypes [5].

A previous HPV epidemiologic study performed in Honduras [6] used the SPF10-INNO LiPA assay (Labo Biomedical Products, Rijswijk, the Netherlands), a genotyping method that provides type-specific information for 25 different HPV genotypes. Both the SPF10-INNO LiPA and the Roche linear array are highly compatible with the detectable genotypes of HPV. Furthermore, the manageability of both assays is highly comparable [5].

The aim of the present study was to expand baseline information about the acquisition of HPV infections among young women and the

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epidemiology of specific HPV types in the context of the implementation of vaccination campaigns in Honduras. A descriptive and cross-sectional study was carried out among young university students attending the Universidad Nacional Autónoma de Honduras (UNAH) in Tegucigalpa to determine the prevalence of HPV, distribution of genotypes, and association with possible risk factors that might promote infection.

2. Materials and methods

The present prospective epidemiologic study was performed among 400 female students, aged 18 to 35 years, who were recruited at the Health Program of the UNAH on a voluntary basis. The study was reviewed and approved by the appropriate ethics committee and informed consent was given by all participants.

Each student filled out a standard validated questionnaire, which was used to obtain knowledge regarding their medical history, habits, and factors considered to increase the risk of cervical cancer. In addition, each participant was submitted to a general pelvic examination by the clinical gynecologist, and two samples were collected from the ectocervix and endocervix via an Ayre wooden spatula. One sample was immediately fixed for cytologic examination; the second sample was placed in 5 mL of sterile phosphate-buffered saline (PBS) containing 0.005% thimerosal, and transported to the laboratory for HPV analysis. On arrival, the cells were washed twice with 1 mL of PBS, and then stored in 0.5 mL of PBS at −20 °C until DNA analysis.

Purified DNA was obtained from a 250-μL aliquot of the cell suspension by using the DNA purification procedures, reagents, and columns provided in a QIAamp MiniElute Media kit for liquid media and a vacuum system from Qiagen (Valencia, CA, USA). Purified DNA was eluted from the columns into 100 μL of Promega (Madison, WI, USA) nuclease-free water.

Genotyping of HPV was performed by a Linear Array HPV Genotyping Test kit (Roche Molecular Systems Inc.), which uses biotinylated primers to define a sequence of nucleotides within the 450-bp fragment on the polymorphic L1 region of the HPV genome. The master mix of this test contains a pool of 37 HPV genomes, including 13 high-risk genotypes, and the gene encoding human β-globin to control for cell adequacy, extraction, and amplification.

PCR amplification was performed in accordance with guidelines provided by the Linear Array HPV Genotyping Test kit. Each experiment was performed with a separate positive and negative PCR control. The biotinylated amplicons were denatured and transferred to a tray containing hybridization buffer and the linear array strip, on which they hybridized with oligonucleotides containing a complementary sequence. This step was completed by washing the strip with a stringent solution to remove unbound material.

After hybridization, a conjugate that ensures binding of the biotinylated amplicons to the oligonucleotide probes was added to the array. The test was completed by addition of a substrate solution that forms a blue complex where hybridization occurred. The linear arrays were manually interpreted via the HPV reference guide.

The results were analyzed via the software EpiInfo 6 (Centers for Disease Control and Prevention, Atlanta, GA, USA). To assess the association between the characteristics of subjects and HPV infection, a crude odds ratio (OR) with a 95% exact confidence interval (CI) was used. In addition, comparison of the frequencies of HPV infection between age groups was performed by the χ^2 test. $P<0.05$ was considered statistically significant.

3. Results

A total of 400 female students were recruited; however, 7 did not complete the questionnaire properly and were consequently withdrawn from the study. HPV DNA was detected in 176 (45%) of the 393 women. High-risk HPV genotypes were detected in 129 (73%) of the 176 HPV-positive samples, whereas 47 (27%) of the 176 samples showed only low-risk HPV genotypes. Samples infected with both high- and low-risk HPV genotypes were included in the high-risk group (Fig. 1).

There was a strong inverse relation between age and high-risk HPV incidence (Table 1 and Fig. 1), whereas the proportion of low-risk HPV infection remained similar regardless of the age of the women. A considerably lower prevalence of high-risk HPV infection was observed in women older than 25 years.

Infection with a single HPV type was found in 54% (95/176) of the positive samples, whereas 46% (81/176) of the positive HPV samples were infected with more than 1 type. Frequently, multiple infections included at least 1 type of high-risk HPV (88%, n = 71). Both multiple and single HPV infections showed an inverse relation with age, although this was more pronounced in women older than 25 years with multiple HPV infection.

Of the 37 genotypes on the linear array, 36 were detected. Among the high-risk HPV-positive group, the most prevalent HPV types were 16 (18%, n = 31), 51 (14%, n = 24), 66 (11%, n = 19), and 39 (11%, n = 19) (Fig. 2); in addition, types 18, 31, 33, and 45 were detected in 3% (n = 6), 6% (n = 10), 1% (n = 2), and 3% (n = 5), respectively, of the women. By contrast, the most prevalent types in the low-risk HPV-positive group were 84 (11%, n = 20), 61 (9%, n = 16), and 62 (9%, n = 16).

Associations between the presence of HPV DNA and several potential risk factors were assessed by multivariate analysis (Table 1). The factors that were independently associated with a risk of being HPV positive, excluding age and Pap smear result, were related to sexual behavior and smoking habits.

Among the strongest risk factors for HPV DNA prevalence and high-risk HPV infection were the number of lifetime sexual partners ($\chi^2 = 12.44$, $P<0.001$), smoking habits ($\chi^2 = 11.12$, $P<0.001$), number of sexual partners before 21 years ($\chi^2 = 5.36$, $P<0.05$), and age at first intercourse ($\chi^2 = 5.26$, $P<0.05$). In addition, among the factors related to screening and birth control habits, the number of lifetime pap smears was significantly associated with prevalence of HPV DNA ($\chi^2 = 4.89$, $P<0.05$) (Table 1).

4. Discussion

In the present study, the prevalence of HPV in the female Honduran university students was 45%. Of the infected women, 73% were positive for high-risk HPV genotypes and 46% had multiple infections. This prevalence is similar to that found in a previous study in Honduran college women [6].

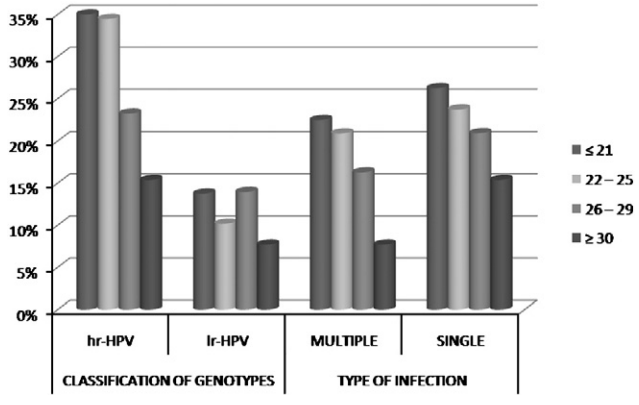

Fig. 1. Prevalence of low-risk and high-risk HPV types, and multiple and single infection by age. Shown is a chart comparing the age-specific prevalence of infection with high-risk (n = 129) or low-risk (n = 47) types of HPV, and single (n = 95) or multiple (n = 81) HPV infections among the HPV-positive women (n = 176).