Human Papilloma Virus detection through the cobas® 4800 system in women from Lima, Peru

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Abstract

Aim: Determine the prevalence of high-risk Human Papillomavirus (HR-HPV) infection in women from Lima between 2012 and 2013 using the Cobas 4800 test. **Method:** The study included 5324 women aged 30 or over, whose cervical samples underwent the Cobas 4800 molecular test. Positive samples were analyzed through liquid-based cytology. **Results:** The prevalence of HR-HPV infection was 15.2%. Genotype 16 was found in 16.2%, genotype 18 in 3.1% and genotypes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 in 71.2%. 62.8% of samples were identified without cytological changes, 19.8% with LSIL, 14% with ASCUS, 2% with HSIL, and 0.7% with ASC-H. **Conclusion:** The prevalence of HR-HPV in this study continues to be significant. In simple infections, the frequency of genotypes 16 and 18 stood out, and in coinfections, the group of the other twelve HPV-RA genotypes predominated.

Key words: Papillomavirus Infections. Human Papillomavirus DNA Tests. Prevalence.

Detección del virus de papiloma humano por medio del método de cobas® 4800 en mujeres de Lima, Perú

Resumen

Objetivo: Determinar la prevalencia de la infección del virus de papiloma humano de alto riesgo (VPH-AR) por medio del método cobas® 4800 en mujeres de Lima, durante el periodo 2012-2013. **Método:** Se incluyó a 5,324 mujeres de 30 años a más, a quienes se les realizó la prueba molecular cobas® 4800. Las pruebas positivas fueron sometidas a citología en base líquida. **Resultados:** La prevalencia de la infección del VPH-AR fue del 15.2%. Se encontró el genotipo 16 en el 16.2%, el genotipo 18 en el 3.1% y los genotipos 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 y 68 en el 71.2%. Se identificó al 62.8% de muestras sin cambios citológicos, un 19.8% con lesión intraepitelial de bajo grado, un 14% con células escamosas atípicas de significado indeterminado, un 2% con lesión intraepitelial de bajo grado y un 0.7% con células escamosas atípicas que no pueden excluirse una lesión de alto grado. **Conclusión:** La prevalencia del VPH-AR en este estudio sigue siendo una cifra importante. En la infecciones simples destacó la frecuencia de los genotipos 16 y 18, y en las coinfecciones predominó el grupo de los otros 12 genotipos del VPH-AR.

Palabras clave: Infecciones por papilomavirus. Pruebas de ADN del papilomavirus humano. Prevalencia.

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Date of reception: 24-05-2020

Date of acceptance: 31-01-2021

DOI: 10.24875/j.gamo.M21000210

Available online: 18-06-2021

Gac Mex Oncol. 2021;20(3):11-16

www.gamo-smeo.com

Introduction

Cervical cancer is the fourth most common neoplasm in the world, with 569,847 new cases and 311,365 cases of mortality in 2018 according to the report of the International Agency for Research on Cancer of the World Health Organization¹. In Latin America and the Caribbean, the mortality rate is three times higher than in North America, according to the Pan American Health Organization². In Peru, it ranks second as the cancer that occurs in more women, with 4,103 new cases and 1,836 cases of death in 2018, in addition to a prevalence of 11,155 women with this neoplasm in the last five years¹.

Human papillomavirus (HPV), declared more than 30 years ago to be a necessary causal agent for the development of cervical cancer, is responsible for 99.7% of disease progression³. There are more than 200 HPV genotypes, out of which 40 infect the vulva, vagina, cervix, anus, penis and oral mucosa, and are divided into high and low oncogenic risk. Genotypes 16 and 18 are responsible for 70% of the development of cervical cancer; the other high oncogenic risk types, which are found at lower percentages, are genotypes 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68^{4,5}. According to some studies, HPV infection is associated with patient characteristics such as age, smoking and sexual behavior, which includes the age of sexual activity initiation, number of sexual partners, use of hormone contraceptives and history of sexually-transmitted infections⁶⁻⁸.

According to scientific evidence on the role played by HPV in cervical cancer carcinogenesis, molecular tests have been shown to be effective for detecting HPV⁹; therefore, they are necessary in the prevention of cervical cancer. The cobas® 4800 test is a qualitative molecular test that typifies HPV by detecting viral DNA of genotypes 16 and 18, and of the other 12 high oncogenic risk genotypes; it was authorized by the Food and Drug Administration in 2011 as the only molecular test for cervical cancer screening, and its importance was demonstrated in the Addressing The Need for Advanced HPV Diagnostics (ATHENA) trial conducted in the USA¹⁰. In this investigation, 47,000 women were tested, with a prevalence of high-risk HPV (HR-HPV) of 6.7% being found; 11.4% of women aged 30 years and older with normal cytological results were carriers of genotypes 16 and 18¹¹.

In Denmark (2013), Canada (2013), Paraguay (2015) and Ecuador (2016), 5,072, 4,330, 170 and 1,581 cervical samples were studied using the cobas® 4800 system, with 26.8, 10.1, 16 and 25.3% of samples,

respectively, being found to be positive for HR-HPV¹²⁻¹⁵. In Peru (2014), 775 HR-HPV-positive samples were obtained in women aged 17 to 79 years, out of which 242, 45 and 631 had genotypes 16, 18 and the other high risk genotypes, respectively, according to the cobas® 4800 test¹⁶.

The purpose of the present study is to determine the prevalence of HR-HPV infection using the cobas® 4800 method in women from Lima, during the 2012-2013 period.

Method

Population

In the 2012-2013 period, 5,324 cervical samples were randomly obtained from women aged 30 years and older, collected by obstetricians belonging to the South and Central Health Directorate in order to detect and typify the human papillomavirus genotypes by means of the cobas® 4800 test. The women included in the study were volunteers and approved the informed consent; those with a history of and/or known precancerous cervical lesions, pregnant women and those who underwent any ablative or excisional treatment for cervical dysplasias were excluded.

HEALTH PROFESSIONALS' TRAINING

Prior to obtaining the samples, 200 midwives from the South and Central Health Directorate were trained for eight hours in two days. The training method was in charge of a geneticist physician, two medical oncologists and an midwife specialized in primary care for gynecological cancer prevention, based on theory and practice. The professionals responsible for the development of the study were explained concepts related to the test and to the importance of its execution. During practice, multimedia devices, gynecological models and cobas® 4800 real-time test kits were used. The procedure for obtaining the sample was in charge of the geneticist physician and midwife specialist.

Instrument

Data collection sheets, approved by experts from the National Institute of Neoplastic Diseases (INEN – *Instituto Nacional de Enfermedades Neoplásicas*) of Peru, were used, which were delivered to midwives for the collection of the following information: current age, origin according to health establishment, age of sexual

activity initiation, number of sexual partners and cobas® 4800 test results and the respective liquid-based cytology in positive cases classified according to 2012 Bethesda system.

Sample collection

During sample collection, the trained professionals were supervised. A cervical sample was collected per woman for the cobas® 4800 test by trained midwives belonging to each health facility of the South and Central Health Directorate. Said samples were sent to the INEN Molecular Biology Laboratory for processing on a machine that was loaned by Roche laboratories.

LIQUID-BASED CYTOLOGY FOR COBAS® 4800 HPV TEST

Using the ThinPrep 2000 system user's manual¹⁷, the sampling technique consisted of placing the central bristles of the brush inside the endocervical canal, in such a way that the short bristles were in contact with the ectocervix, thus exerting pressure against the cervix; the brush was then rotated clockwise five times to obtain the cervical sample. The brush was subsequently rinsed in the vial with PreservCity solution, generating pressure against the bottom of the vial five times, plus a last rotation to release more material and, finally, the brush was removed and the vial was closed with its corresponding cap.

Laboratory techniques

Acquired cervical samples that were positive for any type of HPV according to cobas® underwent liquid-based cytology in order to know if, at the cellular level, the viral infection would be generating damage; this procedure is called co-testing. Ninety-six samples were processed per run in approximately five hours. General practitioners or gynecologists of the health establishments belonging to the South and Central Health Directorate were instructed to implement the respective treatment in those patients with positive results during the study. Patients with negative results were instructed to repeat the screening in five years.

HUMAN PAPILLOMAVIRUS DETECTION AND TYPING

The cobas® 4800 method used a fully automated system by means of the cobas® x 480 instrument and

the cobas® z 480 analyzer, thus allowing the two fundamental processes to develop the detection and typing of high-risk HPVs. High-temperature precipitation and lysis of the sample was carried for the release of HPV nucleic acids. Target DNA and β -globin DNA (control) underwent a purification, washing and separation process for their amplification and detection by polymerase chain reaction. In the cobas® z 480 analyzer, denaturation, exposure, replication cycles multiplication were carried out by generating an amplicon and target and β -globin DNA molecules, followed by amplification of only the HPV genome and/or β -globin gene region using the appropriate primer pair. Subsequently, due to the fluorescent oligonucleotide probes binding action to the complementary polymorphic regions of the target and control gene sequences, HPV 16 amplified signal emitted its own fluorescent dye; similarly, HPV 18 and the other 12 high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) were grouped with the same different color, and β -globin as well, so that each group could be individually identified¹⁸.

Statistical analysis

The analysis, which was descriptive in nature, was carried out with the SPSS program, version 25. Absolute and relative frequencies, mean and standard deviation were used for quantitative variables such as age, sexual intercourse initiation and number of sexual partners. Frequencies and percentages were used for qualitative variables such as cobas® 4800 test results and liquid-based cytology results, which were classified according to the Bethesda System.

Ethical considerations

Confidentiality of the information obtained from patients was respected. The study project was evaluated and approved by the INEN Ethics Committee, and registered with INEN 18-92 code.

Results

Of the total of 5,324 studied women, average age was 37.9 years. Age at sexual activity initiation ranged from 8 to 48 years, with an average of 18.7 years. According to the number of sexual partners found in the study, average was 1 (Table 1).

The prevalence of HR-HPV that was found using cobas® 4800 was 15.2%. With a total of 802 positive samples, 16.2 and 3.1% were found to have the HPV

Table 1. General characteristics

Characteristics	n	%
Age (x) ± SD	37.9 ± 6.8	
Range	(30-49)	
– 30-39	3,114	58.5
– ≥ 40	2,210	41.5
Sexual activity initiation (x) ± SD	18.7 ± 3.8	
	(8-48)	
Range	1.0 ± 0.9	
	(1-5)	

x: mean; SD: standard deviation.

16 and HPV 18 genotypes, respectively. The group where other HR-HPVs (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) were distributed was larger (71.2%). Coinfection with HPV 16 plus the group of other HR-HPVs (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) was found in 7.7%; coinfection with HPV 18 plus the group of other HR-HPVs (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) was observed in 1.1%; coinfection with HPV 16 plus HPV18 was identified in 0.4%; and coinfection with HPV 16, HPV 18, plus the group of other HR-HPVs (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) was present in 0.2% (Table 2).

Among HR-HPV-positive samples, 62.8% showed no cytological changes; however, 2% are observed to have a finding consistent with high-grade squamous intraepithelial lesion (HSIL), which corresponds to a group of users with a higher risk of progression to cancer. Atypical squamous cells of undetermined significance (ASCUS), which were observed in 14%, show ambiguity between a possible negative and positive result, as opposed to atypical squamous cells-cannot rule out high-grade squamous lesion (ASC-H), found in 0.7%, which could be between a precancerous lesion or cancer. The low-grade squamous cell intraepithelial lesion (LSIL) result was observed in 19.8%, which gives an idea of regression or progression according to the follow-up of the user where management of the trained health professional intervenes. Only 0.4% of HR-HPV-positive samples were inadequate (Table 3).

Discussion

The prevalence of HPV in Africa (22.1%) and America (12.9%) is estimated to be higher, in contrast to Europe (8%) and Asia (7.9%)¹⁹. According to the findings of the present study, the prevalence of HR-HPV is 15.2%, which is similar to that found in Paraguay (16%), lower

Table 2. Detection results and high-risk human papillomavirus (HR-HPV) genotypes distribution

Detection results and high-risk human papillomavirus (HR-HPV)		
Cobas® 4800 test results	n	%
Positive	802	15.2
– 30-39	525	9.9
– ≥ 40	277	5.3
Negative	4,461	84.8
– 30-39	2,552	48.5
– ≥ 40	1,909	36.3
HR-HPV genotypes distribution		
HPV 19	130	16.2
HPV 18	25	3.1
HPV 16, HPV 18	3	0.4
Other HR-HPVs	571	71.2
Other HR-HPVs: HPV 16	62	7.7
Other HR-HPVs: HPV 18	9	1.1
Other HR-HPVs: HPV 16, HPV 18	2	0.2

Other HR-HPVs: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.

Table 3. Liquid-based cytology results

Liquid-based cytology	n	%
NILM	504	62.8
ASCUS	112	14
LSIL	159	19.8
ASC-H	6	0.7
HSIL	16	2
AGC	1	0.1
Inadequate sample	4	0.4
Total	802	100

NILM: negative for intraepithelial lesion or malignancy; ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; ASC-H: atypical squamous cells-cannot rule out high-grade squamous lesion; HSIL: high-grade squamous intraepithelial lesion; AGC: atypical glandular cells.

than that reported in studies from Ecuador (25.3%) and Denmark (26.8%), but higher than that identified in investigations carried out at the USA (6.7%) and Canada (10.1%)¹¹⁻¹⁵.

In the national context, a prevalence of 34.4% was found, including all regions of the country (coast, mountains and jungle)¹⁶. In another investigation carried out in the department of Cajamarca, 20.5% of HPV infection was found²⁰. The differences could be related to methodological characteristics of the studies, which included a population with a broader age range (from

17 years on) and detection of high- and low-risk genotypes, unlike the present study, which only considered HR-HPV.

Regarding the characteristics of the study population, average age was 37.9 years, sexual activity initiation was at 18.7 years, and women generally reported one sexual partner. In the collected studies, it was observed that, the older the age, the lower the prevalence of HR-HPV^{11-13,16}, which could be related to cofactors that play an important role in viral persistence, especially in women older than 30 years, given that only 10% progress to a precancerous lesion or cancer¹⁹. Similar values to those reported in a study from Paraguay on HR-HPV were found for sexual behavior⁷; mean age of sexual activity initiation was 18.6 years, and mean number of sexual partners, two. Among the investigations that explore factors associated with HR-HPV infection, the PATRICIA trial stands out⁶, where sexual activity initiation is at less than 15 years of age (odds ratio [OR]: 1.88; 1.25-2.84), and having four or more sexual partners (OR: 7.62; 4.55-12.79) increased the likelihood of infection. Furthermore, a study in Mexico⁸ reported that having six or more sexual partners (OR: 1.79; 1.590-2.034) had a statistically significant association.

HR-HPV typing results and those of the referred international investigations showed that there is a higher prevalence of the group of other HR-HPVs (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68); however, in single infections, the values reported for HPV 16 stand out, followed by HPV 18^{11-12,14-15}. HPV vaccine implementation, an intervention that was incorporated into the National Vaccination Schedule since 2011 in Peru²¹, and that currently has a coverage of 98% for the first dose and 91.2% for the second, applied to girls aged from 9 to 13 years²², could generate a decrease in infection with certain HPV genotypes.

In Peru, cervical cancer screening is enhanced with Papanicolaou; however, the authors recommend that HR-HPV genotype testing should be included in national screening policies. Differences have been observed in the cost of screenings that include conventional cytology as initial test (\$ 4,795,900), HPV molecular test (\$ 3,472,320) and co-testing: cytology and molecular test (\$ 5,853,660), for a three-year period²³. Considering that cobas® 4800 test added value is HPV genotyping, it can clarify the epidemiological profile of the disease in terms of post-vaccination behavior. Therefore, in the future, the group of other HR-HPVs (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) will likely be predominant; consequently, surveillance will be required.

It is concluded that HR-HPV prevalence is lower in comparison with national studies; however, it remains an important figure. In single infections, the frequency of genotypes 16 and 18 stood out and, in coinfections, the group of other HR-HPVs (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) predominated. It is necessary to continue strengthening primary and secondary cervical cancer prevention interventions.

Acknowledgements

The authors thank the financial support by Roche laboratories, as well as the obstetricians of the Ministry of Health who participated in the collection of samples for the study.

Conflict of interests

The authors declare that they have no conflicts of interest.

Funding

The present investigation was financed by Roche laboratories, which donated the devices for sampling and provided a processing machine on loan.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this research.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained informed consent from the patients and/or subjects referred to in the article. This document is in the possession of the corresponding author.

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