Prevalence of human papillomavirus in university young women

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Abstract. Cervical cancer is the second most prevalent female cancer worldwide. The majority of cases appear between the age of 30 and 50. Human papillomavirus (HPV) plays a central role in cervical cancer with 99.7% of HPV DNA identified in invasive cervical carcinomas. The prevalence of the HPV infection varies substantially among countries and according to age and lifestyle. HPV is a common sexually transmitted infection among males and females with a 70% higher incidence in sexually active females. This study aimed to determine the prevalence of human papillomavirus in young university women by analyzing the correlation between Papanicolaou (PAP)-stained cervical tests and HPV detection by genotyping, as well as other risk factors. A total of 200 women aged between 18 and 25 years were enrolled in this study, which took place between September 2008 and May 2009 at the Universidad de Tarapacá, Arica, Chile. Results of the PAP smears showed that 97.5% of cells had normal characteristics, although an inflammatory pattern was noted. The prevalence of generic HPV infection was 3.5% when testing for HPV DNA using the polymerase chain reaction (PCR) method. An analysis of the genotype of infected female individuals indicated that high-risk HPV types, such as HPV 16 and 31 were present in 42.84 and 14.29% of females, respectively, and low-risk types such as HPV 6, in 14.29%. Only one sample with differentiated non-HPV (14.29%) was found. A 95% correlation between PAP-stained cervical tests and the method of testing for HPV was observed. Using the PCR method, it was found that of the 195 negative PAP smears, 5 were positive for HPV and two of the samples that were positive for ASC-US were also positive. A significantly increased ($P<0.05$) HPV infection risk was observed in the 18-21 age group with a higher prevalence (71.40%) when compared to the 22-25 age group (28.6%). A significant ($P<0.042$) difference was found between smoking and HPV infection. In conclusion, a significant ($P<0.05$) correlation was found between PAP and PCR methods for HPV testing in young university women. A significant correlation between smoking and HPV was detected, whereas no difference was noted with other parameters.

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Introduction

Cervical cancer is the second most prevalent female cancer worldwide with 493,000 new cases occurring every year and 80% occurring in developing countries (1,2). The majority of cases appear between the age of 30 and 50. Human papillomavirus (HPV) plays a central role in cervical cancer with 99.7% of HPV DNA identified in invasive cervical carcinomas (3,4). The prevalence of HPV infection varies substantially among countries and according to age and lifestyle. HPV is a common sexually transmitted infection among men and women with a 70% higher incidence in sexually active females. The majority of HPV infections are asymptomatic and transient, especially in the young population. Up to 98% of cervical cancers in females are positive for HPV and more than 90% of new infections appear to induce high-grade cervical neoplasia (5,6). HPV prevalence is age-dependent with a peak in women below the age of 25 and a second peak in women over the age of 55 (7-10). Risk factors include smoking, drinking, education, number of partners, diet, nutrition, long-term oral contraceptive use, immunosuppression and age of first coitus. Reduced participation in screening is a probable cofactor that should be considered in an analysis of HPV in cervical female cancer (11-16).

There is an important public health concern for the control of HPV infection and the development of cervical female cancer in various countries. In the United States, cervical cytology screening has proven to be successful, since deaths from invasive cervical cancer have decreased by over 70%. In other countries with screening programs, cervical cytology screening has significantly reduced both incidence and mortality. Cervical cancer is currently considered to be completely preventable with universal cervical cytology screening and treatment. However, a number of limitations to current cervical cancer prevention programs exist such as racial and ethnic disparities in cervical cancer screening (17).

HPV is a member of the papillomavirus family. It is 52-55 nm in size, and is a non-enveloped virus with circular

double-stranded circular DNA and a genome of approximately 7,900 base pairs with eight overlapping open reading frames. The late genes (L1 and L2) and early genes (E6, E7, E1, E2, E4 and E5) that are expressed in more than 100 different HPV genotypes have been identified based on differences in DNA sequence. These HPV types can be classified according to various criteria such as their tissue tropism, oncogenic potential and phylogenetic position using molecular biology techniques (18). At least 40 of these HPV genotypes infect the epithelial lining of the anogenital and aerodigestive tract, since different studies have shown that infection with high-risk HPV precedes the development of cervical premalignant disease in women with cervical cancer. Based upon epidemiological studies, HPV viruses are classified as high-risk (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56 and 58) and low-risk (6, 11, 13, 40, 42, 43, 44, 54, 59, 61, 70, 72, 81 and CP108). Several HPV types, such as 16, 18 and 59, have been implicated in cervical female cancer, and other types, such as HPV 6 and 11, are frequently detected in benign lesions such as condylomata. Female individuals infected with low-risk HPV types have a minimal possibility of developing cervical cancer. The World Health Organization has officially designated HPVs 16 and 18 as carcinogenic agents. In Asia, HPV 58 and 52 are the most common after HPV 16 and 18. However, HPV infection by multiple genotypes has been reported to occur in 10-20% of HPV-positive cases (19-22). HPV 16 is the most common oncogenic type in preinvasive and cervical cancer, detectable in 21% of women with low-grade squamous intraepithelial lesions (LSIL) and in more than 50% of women with cervical intraepithelial neoplasia grade 3 (CIN 3). HPV 18 causes 10-15% of CIN 3 and also causes more than 35% of cervical adenocarcinomas, which are difficult to detect by current screening methods (23).

Traditional screening for HPV infection is crucial, but early detection can be difficult for most cervical infections since HPV is asymptomatic. Many countries possess a screening program for HPV, involving the traditional protocol based on the Papanicolaou smear introduced in 1943, which uses a cytological staining technique. However, the high number of false negatives (between 15-50% for cervical premalignant lesions and cervical cancer) and false positives (30%) should be considered (24,25). Cytology examinations have limitations with regards to specificity and low predictive value for high-grade pathology (26,27). However, PAP smears have been shown to significantly decrease the incidence of cervical cancer in developed countries. The introduction of cervical screening programs is an important target in cervical cancer research since it involves improving the detection of precancerous lesions and reducing equivocal results by employing better collection, preparation, and testing methods (28).

Highly sensitive methods have been developed to detect HPV such as the polymerase chain reaction (PCR) method, which in *in vitro* conditions can amplify the DNA sequence present in a clinical specimen. This method can detect as little as one molecule of HPV DNA in 10^5 cells, and is considered the most sensitive HPV detection technique worldwide (29). PCR is based on the use of primers such as the MY09/MY11 primer set (MY-PCR), which is the most frequently used amplification system for the detection of the virus in clinical samples. This set is synthesized with several nucleotides in each primer and then mixed with 25 primers including HMB01M, which amplify a wide spectrum of HPV-types. MY09/MY11 has been used in studies predominantly in North and South America as well as in Asia (30-33). This study aimed to determine the prevalence of human papillomavirus in young Chilean university women by analyzing the correlation between PAP-stained cervical tests and PCR for HPV detection by genotyping and whether other risk factors were involved.

Materials and methods

A total of 200 females aged 18-25 with a mean age of 21.55 participated in the study between September 2008 and May 2009 at the Universidad de Tarapacá, Arica, Chile. Written informed consent was obtained from each female patient. Papanicolaou (PAP)-stained cervical tests (PAP) were included in this transversal study that was complemented with a standardized questionnaire. The information obtained included socio-demographic data such as age, marital status, use of contraceptives (condom, oral contraceptive), number of vaginal deliveries and sexual habits. Cervical cell samples were collected with a cytobrush from the ectocervix and endocervix of each woman and samples were preserved in SurePath® preservative fluid and methanol. The test tube was closed and sent to a molecular biology laboratory to be stored at -4˚C.

Papanicolaou (PAP)-stained cervical tests. PAP smears were obtained from the endocervix and ectocervix by scraping the squamous columnar cells with a wooden Ayre's spatula. Cervical scraping was then performed by using a cytology brush to spread the samples over designated slides for each patient. The slides were fixed with ethanol and colored by PAP technique. The samples were examined under a microscope by a pathologist and classified by the Bethesda system: ASC-US: consisting of atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion (CIN 1) and HSIL: high-grade squamous intraepithelial lesion (CIN 2 and CIN 3).

Polymerase chain reaction for HPV. The primers used in the PCR assay for HPV were: MY09 (5'CGT CC (AC) A (AG) (AG) GGA (AT)AC TGA TC 3') and MY11 (5' GC(AC) CAG GG(AT) CAT AA(CT) AAT GG 3'). The primers used for β-globin were: PCO3 (5' ACA CAA ACT GTG TTC ATC AGC 3') and PCO5 (5' GAA ACC CAA GAG TCT TCT CT 3') to detect and genotype HPV DNA. Each primer is specific to a DNA segment and when the target sequence is present the primers anneal and allow *in vitro* replication of nucleotides. Cycles of heat denaturation, annealing and synthesis allow for the exponential accumulation of the specific target sequence.

Statistical analysis. Categorical data were analyzed for statistical significance by an analysis of all variables that included a Chi-square test. $P<0.05$ was considered to be significant, and when appropriate, the Fisher's exact test was included. The κ coefficient was used to assess the degree of agreement between the two tests. Odds ratio prevalence and 95% confidence intervals were calculated as approximations of the relative risk by logistic regression. The dependent variables

Table I. Papanicolaou (PAP) smear and HPV detection in 200 Chilean University women.

A, PAP smear		
Total no. of cases	N=200	%
Normal	195	97.50
Candida sp.	5	2.56
Trichomona vaginalis	1	0.51
ASC-US[a]	2	1.00
LSIL[b]	3	1.50
B, PCR		
Total no. of cases	N=200	%
Negatives	193	95.50
Positives	7	3.50
C, Type-specific HPV by PCR		
Total no. of cases	N=7	%
Low-risk		
HPV 6	1	14.29
HPV 59	1	14.29
High-risk		
HPV 16	3	42.84
HPV 31	1	14.29
HPV not differentiated	1	14.29

[a]ASC-US (atypical squamous cells of undetermined significance). [b]LSIL [low-grade squamous intraepithelial lesion (CIN 1)].

Table II. Correlation between Papanicolaou (PAP) smear and HPV detection in 200 Chilean University women.

	HPV analysis by PCR		
PAP smear	Positive	Negative	Total
Normal	5	190	195
ASC-US[a]	2		2
LSIL (CIN 1)[b]	0	3	3
HSIL (CIN 2-CIN 3)[c]	0	0	0
Total	7	193	200

κ coefficient (0.31); 95% correlation. [a]ASC-US (atypical squamous cells of undetermined significance); [b]LSIL (low-grade squamous intraepithelial lesion [CIN 1]) and [c]HSIL (high-grade squamous intraepithelial lesion [CIN 2 and CIN 3].

were the results of PAP smears and the PCR method. The independent variables were socio-demographic characteristics, such as marital status.

Results

The prevalence of human papillomavirus in young university women was analyzed by correlation between PAP-stained cervical tests and PCR for HPV detection by genotyping. Other risk factors were also considered and found to be involved. A total of 200 university women were enrolled in the study with a mean age of 21.55 years and an age range between 18 and 25 years. The mean age of first coitus was 17.22 years with an age range between 13 and 25 years and the mean number of sexual partners since the first coitus was 2.62 with a range of between 1 and 15 partners. PAP smears in 97.5% (195/200) of 200 samples were normal. However, the normal examinations showed an inflammatory pattern, which is a possible etiology agent that was found in 2.56% (5/195) of Candida sp. and 0.51% (1/195) of Trichomona vaginalis. Of the positive cases, 2.5% (5/200) showed an inflammatory pattern; 2 cases had atypical squamous of undetermined significance (ASC-US) and 3 had low-grade squamous intraepithelial lesion (LSIL), as shown in Table I. The prevalence of generic HPV infection, using the PCR method, was 3.5% (7/200) for HPV DNA (Table I). Genotyping showed high-risk HPV types; HPV 16 was detected in 42.84% (3/7) of infected women and HPV 31 in 14.29% (1/7). Low-risk HPV types were also identified, such as HPV 6 in 14.29% (1/7) and HPV type 59 in 14.29% (1/7). Only one sample with HPV was not differentiated (14.29%). The distribution of HPV-types in each positive sample using the PCR method is shown in Table I.

The correlation between the PAP smear and PCR methods of detecting HPV DNA was significantly high (95%) ($P<0.05$). Using the PCR method it was found that of 195 negative PAP smears, 5 were positive for HPV and 2 of the samples that were positive for ASC-US were also positive. Results of the PAP smears showed that 3 samples were positive for LSIL, but these samples were in fact found to be negative using the PCR method (Table II). To investigate the relevance of risk factors other than HPV, a statistical analysis was performed by adjusting the HPV prevalence by PCR using unconditional logistic regression. Table III indicates risk factors associated with the number of sexual partners. The age group was an important risk factor for HPV infection ($P=0.05$) since a significantly increased risk was observed in the 18-21 age group (OR=2.47; 95% CI=1.02-13.06) with a higher prevalence (71.40%) when compared to the 22-25 age group (28.6%). No correlation was found with the age of first coitus ($P>0.05$); even though the risk factor was important (OR=2.03; 95% CI=0.27-19.82) with the first coitus (<18 year old), no statistical difference was observed. A non-significant risk was observed with the other parameters. Table IV shows the risk factors associated with lifestyle. A significant ($P<0.042$) difference was found between smoking and HPV infection. An increased risk was found in females who reported to be tobacco smokers (OR=6.87; 95% CI=1.95-40.57). No significant correlation was found with other parameters.

Discussion

The prevalence of human papillomavirus in 200 young Chilean university women was analyzed with regards to the correlation between the PAP smear and PCR method of HPV detection by genotyping. Other risk factors involved were

Table III. Papanicolaou (PAP) smear HPV detection and risk factors in 200 Chilean University women.

Variables	Negative		Positive		OR	CI 95%
	N=193	%	N=7	%		
Age group						
22-25	96	49.70	2	28.60	1	-
18-21	97	50.30	5	71.40	2.47	(1.02-13.06)
Age of first coitus						
>18 year old	54	28.00	1	14.30	1	-
<18 year old	139	72.00	6	85.70	2.03	(0.27-19.82)
Number of sexual partners since first coitus						
>3 partners	84	43.50	2	28.60	1	-
1-2 partners	109	56.50	5	71.40	1.93	(0.37-10.18)
Number of sexual partners during last 12 months						
>2 partners	40	20.70	1	14.30	1	-
1 partner	153	79.30	6	85.70	1.62	(0.19-13.82)

OR, odds ratio. CI, confidence interval. P<0.05, significant difference.

Table IV. Correlation between Papanicolaou (PAP) smear HPV detection and risk factors in 200 Chilean University women.

Variables	Negative		Positive		OR	CI 95%
	N=193	%	N=7	%		
Condom use (partner)						
Occasionally	177	91.70	6	85.70	1	-
Never	16	8.30	1	14.30	1.84	(0.21-16.28)
Physical activity						
Yes	71	36.80	2	28.60	1	-
No	122	63.20	5	71.40	1.45	(0.28-7.70)
Smoking (P=0.047)						
No	103	53.40	1	14.30	1	-
Yes	90	46.60	6	85.70	6.87	(1.95-40.57)
Drinking						
No	61	31.60	1	14.30	1	-
Yes	132	68.40	6	85.70	2.77	(0.32-23.53)

OR, odds ratio. CI, confidence interval. P<0.05, significant difference.

also analyzed. Using the PCR method, it was found that there was a 3.5% prevalence of HPV infection in students, which is lower than the data reported in other countries in South America, such as Mexico with 14.5%, Costa Rica with 16% and Colombia with 14.8% (10,34-35). Prevalence of the HPV infection was 26 and 20% in Nigerian and Taiwanese women, respectively (35). However, the prevalence in the present study is similar to that found in Spanish and Bolivian studies, at 3 and 5.2%, respectively (36,37). The lower prevalence found in this study can be explained by differences in the characteristics of the population, where variability exists between HPV prevalence as described in numerous studies (38). It can be observed that each study has a different experimental design in different parts of the world, such as sample collection and different methods are used for HPV detection and typing. In the United States, 64% of teenagers were found to be infected, a percentage similar to that found in Korean prostitutes and female individuals with cervical lesions in Brazil

(47 and 43%, respectively) (42). In contrast, a prevalence as low as 3-14% has been found in married women from Spain and Amazonian women from Bolivia (36,37,39-42). In Chile, the incidence rate was 29 per 100,000 and the prevalence was 14%, which compares to data from studies in Mexico (14.5%) and Colombia (14.8%), but the prevalence in Chile was higher than in many other parts of Europe (10,34,43,49).

Results of the present study showed four types of HPV in six out of seven patients who were positive, diagnosed using the PCR method, the other patient being of unknown type (14.29%). Four patients (57.13%) were infected with one of the high-risk HPV types; three with type 16 (42.84%) and one with type 31 (14.29%). Only two patients (28.58%) were infected with a low-risk type; one with type 6 (14.29%) and one with type 59 (14.29%). No mixed-type infections were detected by the PCR method. The high-risk HPV types found in this study have also been described in other studies in association with cervical cancer (2,44) with a predominant HPV type 16 infection. This type of virus has also been considered of high-risk for cervical carcinoma by other authors (45). The prevalence of HPV, diagnosed using the Papanicolaou method, was 2.5% (5/200). Among these samples, two (2/200) were considered as ASC-US and three (3/200) as LSIL by the Bethesda system. Five patients (5/195) had Candida sp. and one person (1/195) had Trichomona vaginalis. The 2.2% prevalence found in our study using the Papanicolaou method is similar to that found in Mexican women (46).

Clearly the PCR method (3.5%) is more effective than the PAP method (2.5%) in detecting the HPV infection since PCR assays can detect DNA sequences in the cells, whereas the Papanicolaou test can only analyze cellular changes. The differences found between the two methods were similar to those found in the study carried out in Mexico (46) due to the sensitivity of each method. PCR has a high level of sensitivity and specificity but the Papanicolaou test takes into consideration many variables such as the sample collection and reading process. However, a low correlation was found between two methods with a κ coefficient of 0.31, but the agreement was 95%. Of the samples, 195 were normal with the Papanicolaou method and 193 were negative for HPV infection using the PCR method. The results showed a significant κ coefficient between PCR and PAP test (0.45), which correlates (74%) with another study in Sweden (47).

The age of the group is an important risk factor for HPV infection. Female individuals of 18-22 years old had a higher risk of infection than those of 22-25 years old. It is possible that the mechanism of acquired immunity due to previous exposure affected the minimal chance of HPV infection in older women (48). The age of the first coitus was also observed and a significant correlation was noted in female individuals who had been sexually active when under the age of 18. In this study, a 3.5% prevalence of HPV infection was found using the PCR method. This prevalence is lower than the figure reported in another study of Chilean women in Santiago (49), where a prevalence of 14% was reported. This is similar to the data in other studies (10,34,35); however, 26 and 20% HPV infection was identified in Nigerian and in Taiwanese women, respectively (35). However, when sexual activity commenced after 18 years of age, a 2.03 higher risk factor was noted as compared with other female individuals younger than 18 years old, as confirmed by other authors (49). This discrepancy is significant in increasing the risk of HPV infection. Smoking is an important risk factor. The female patients who smoked were at a significantly higher risk than those who did not smoke. It appears that smoking affects the immune mechanism response of the cervical tissue when the virus is in contact with the tissue (50,51).

In conclusion, a significant ($P<0.05$) correlation was found between the PAP and PCR methods of testing for HPV in young university women. A significant correlation was also found between smoking and HPV infection, whereas no difference was noted with other parameters.

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