Detection of High Risk Human Papillomavirus Cervical Infections by the Hybrid Capture in Asunción, Paraguay

Laura Mendoza Torres, Malvina Páez, Ariel Insaurralde, Maria Isabel Rodriguez, Amalia Castro and Elena Kasamatsu

Department of Public Health and Epidemiology, Research Institute of Health Science, University of Asunción; Asunción, Paraguay

Cervical cancer is the most frequent malignant neoplasia in the female population of Latin American countries, among these, Paraguay occupies the third place in incidence with a rate of 53.2 x 100,000 women, only surpassed by Haiti and Bolivia [1,2].

Human Papillomavirus (HPV) is the most prevalent virus involved in sexually-transmitted diseases worldwide, being an important public health challenge. HPV is also considered the main cause of cervical cancer and squamous intraepithelial lesions (SIL) [3,4].

Approximately 40 distinct HPV types are known to infect the anogenital mucosa and they are grouped commonly into high risk (HR-HPV) and low risk (LR-HPV) categories on the basis of known epidemiological association with preneoplasic/neoplastic and benign lesions, respectively [5]. HR-HPV are detected in more than 95% of the cervical carcinoma cases [6,7].

Diagnosis of cervical disease, determined by the presence of abnormal cervical epithelial cells, is usually obtained by microscopic examination of Papanicolaou-stained smears (PAP). This is a simple and cheap method that allows detecting effects caused by HPV infection that consists in the observation of coilocytic cells, but does not permit to determine the presence of HR-HPV infections [8].

HPV could not be isolated in cells, therefore the diagnosis test depends on molecular techniques. The hybrid capture II assay (HCA II) is the only molecular technique approved by the Food and Drugs Administration (FDA) designed to detect 13 types of HR-HPV and 5 of LR-HPV, even in apparently normal tissues, due to the high sensitivity that permits the detection of 1 pg/mL of viral DNA [9].

In Paraguay, a country with a high incidence of cervical cancer, there are only two studies related to HPV typing in female genital invasive cancer by using Polymerase Chain Reaction (PCR) [10,11]. Due to the fact that the HCA II assay was recently introduced in our country, the goal of the present study was to determine the frequency of HR-HPV in cervical samples of women, in order to contribute to a better knowledge on HR-HPV cervical infections in Asuncion, capital city of Paraguay, with a population of 520,000 inhabitants. The findings were correlated with cytological diagnosis and age.

Material and Methods

Study Population

The population under study included 272 women which were attended at the HPV Laboratory that belongs to the Instituto de Investigaciones en Ciencias de la Salud from Universidad Nacional de Asunción, Paraguay, from May 2006 to December 2007.

The participants were included following a consecutive order by using the selection criteria previously established such as a previous cytological diagnosis realized by the specialized physician and without hysterectomy. The results of cervical cytology were classified using the Bethesda System, 2001 [12]. All the data were processed respecting the confidentiality of the participants.

Detection of HR-HPV by Hybrid Capture II Test

The smears were collected using a cervical cytobrush and then transported in Digene Specimen Transport Medium (Digene Diag, Md).

The kit detects HR-HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Following the manufacturer’s protocol, the specimens were treated with sodium hydroxide in order to hydrolyze specimen RNA and to denature the DNA.
A high frequency of HR-HPV was detected in women in the range of 11 to 30 years old and over 60 years old; with a notorious incidence in women under 21 years old (75%). Positive results of HR-HPV by HCA II assay by age ranges are shown in Table 3.

### Discussion

The World Health Organization reported that cervical cancer is the second cause of malignant neoplasia and death in women worldwide [4]. Epidemiological studies have demonstrated that HR-HPV is the main cause of this disease. However, it is necessary to use complementary diagnosis tools that allow a detection of HR-HPV genome, in order to increase the performance of conventional morphologic diagnostic methods usually used for screening cervical cancer [3].

In this study, cervical samples were analyzed using the HCA II assay in order to detect the HR-HPV genome. Viral infections were observed in 44% of the women. This finding agrees with previous studies where a comparable population and methodology of analysis was used [4,14]. Epidemiological studies showed that almost 50% of sexually active women between 15 and 49 years old are infected by HPV, becoming a worldwide public health problem [4,14].

A moderate concordance was observed between HCA II assay and cytology (kappa: 0.43 IC 95% 0.3 – 0.5). 25% of the women with N-SIL were positive to HR-HPV. This could be due to the high sensitivity of almost 100% by the HCA II assay, that allowed detecting the HR-HPV infection even in apparently normal tissues and revealing silent infections that may be responsible for the maintenance of HPV infections in the general population [8,15,16]. Previously, Koutsky et al. have described women with no cytological abnormalities presenting positive results for HPV genome, and they have also observed that 30% of the women analyzed developed CIN within the next two years, suggesting that there is a higher risk for these women for developing CIN [17].

Thirty-two percent and 22% of women with LSIL and HSIL, respectively, were negative for HR-HPV. These contrasting results could be due to a lower copy number of the HR-HPV genome infection; untested types of HPV HCA II assay or unidentified reasons, such as DNA testing errors [18,14].

Some of those conflicting cases between cytology and HR-HPV genome detection could be due to a mistake in the cytological diagnosis assay, which presented almost 20% of false negative results (lower sensitivity) and 15% of false positive results (lower specificity) [18,14].

Seventy-five percent of women with ASCUS were positive for HR-HPV genome. Previous studies have demonstrated the efficacy of HCA II assay in the orientation of unspecific cytological diagnosis of ASCUS, suggesting that a positive result for HR-HPV genome means a possible presence of SIL [15,19-21].

Regarding the age ranges, a high frequency of positive cases of HR-HPV was observed in women from 11 to 30 years old, mainly in women under 21 years old. This high percentage of infected women could be due to a young cervical tissue, with...
In women from 31 to 60 years old it was observed a decrease of positive cases by HR-HPV infection. This finding agrees with previous studies that showed a prevalence of HPV infection of almost 30% in women under 30 years old, which decreases to 10% between 30 and 50 years old [22].

The decrease in the rate of infection observed with the increase in age can be explained because from 80% to 90% of women less than 30 years old were already exposed to any HPV infection, developed a natural immunity and could eliminate the infection in a period of following 24 months. It is also interesting to note that the remaining 10% to 20% of women over this age that are persistent infected by HPV have a great potential of developing SIL if they are not correctly diagnosed and treated [22].

Hence, several authors concluded that the use of cytological diagnosis complemented with DNA test for detection of HR-HPV genome, helps to increase the efficiency of screening programs allowing the early detection of cervical lesions at age intervals over 30 years old [17].

Interestingly, it was observed an increase of HR-HPV infections in women over 60 years old (67%). This finding also agrees with previous studies realized in other Latin American countries that described an increase in the rate of infection caused by both HR and low risk HPV (LR-HPV) [23,24]. This fact could be due to a new exposition to HPV through a sexual partner; a decrease of the immune system associated to the age that results in an increase of the reactivation of latent infections or to other factors [24].

Summing up, the results obtained in this study corroborate that the use of cytology together with the detection of HR-HPV by HCA II assay could improve remarkably the efficiency of screening programs of cervical cancer in Paraguayan women over 30 years old, allowing the identification of a group of women with high risk of developing SIL and supporting the

**Table 1.** Positive results of high risk HPV (HR-HPV) by hybrid capture II assay (HCA II) according to the cytological diagnosis of women.

<table>
<thead>
<tr>
<th>Cytological diagnosis</th>
<th>Number of women</th>
<th>HR-HPV infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSIL</td>
<td>159</td>
<td>40 (25)</td>
</tr>
<tr>
<td>ASCUS</td>
<td>25</td>
<td>18 (72)</td>
</tr>
<tr>
<td>LSIL</td>
<td>79</td>
<td>54 (68)</td>
</tr>
<tr>
<td>HSIL</td>
<td>9</td>
<td>7 (78)</td>
</tr>
<tr>
<td>Total</td>
<td>272</td>
<td>119 (44)</td>
</tr>
</tbody>
</table>


**Table 2.** Results of high risk HPV by hybrid capture II assay (HCA II) from women with and without squamous intraepithelial lesions.

<table>
<thead>
<tr>
<th>Cytological diagnosis</th>
<th>HR-HPV infection</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive N (%)</td>
<td>Negative N (%)</td>
</tr>
<tr>
<td>SIL</td>
<td>61 (69)</td>
<td>27 (31)</td>
</tr>
<tr>
<td>NSIL</td>
<td>40 (25)</td>
<td>119 (75)</td>
</tr>
<tr>
<td>Total</td>
<td>101 (41)</td>
<td>146 (59)</td>
</tr>
</tbody>
</table>

SIL: squamous intraepithelial lesions. NSIL: negative for squamous intraepithelial lesions. Kappa 0.43 (IC 95% 0.3 – 0.5 ).

**Table 3.** Positive results for high risk HPV (HR-HPV) by hybrid capture II (HCA II) assay according to the age of the women (in years).

<table>
<thead>
<tr>
<th>Age range</th>
<th>Number of women</th>
<th>HR-HPV infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n (%)</td>
</tr>
<tr>
<td>11-20</td>
<td>12</td>
<td>9 (75)</td>
</tr>
<tr>
<td>21-30</td>
<td>107</td>
<td>62 (58)</td>
</tr>
<tr>
<td>31-40</td>
<td>79</td>
<td>21 (27)</td>
</tr>
<tr>
<td>41-50</td>
<td>53</td>
<td>19 (36)</td>
</tr>
<tr>
<td>51-60</td>
<td>13</td>
<td>5 (38)</td>
</tr>
<tr>
<td>61-70</td>
<td>3</td>
<td>2 (67)</td>
</tr>
<tr>
<td>Total</td>
<td>267*</td>
<td>118 (44)</td>
</tr>
</tbody>
</table>

*Age was not determinate in 5/272 women included in the present study.
necessity of follow-up by screening with the HPV test in older women groups.

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References

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