Comparison of Xpert® HPV and Hybrid Capture® 2 DNA Test™ for detection of high-risk HPV infection in cervical atypical squamous cells of undetermined significance

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Received 14 January 2016; received in revised form 5 April 2016; accepted 26 April 2016

Summary This study compares Xpert® HPV and Hybrid Capture® 2 High-Risk HPV DNA Test™ (hc2) for the detection of high-risk HPV infection in cervical smears. Papanicolaou smears with atypical squamous cells of undetermined significance (ASC-US) constituted the study specimens. Of the 168 ASC-US samples, 134 (79.8%) were from Saudi patients. The hc2 test was positive in 33 (19.6%) of the total patients, 20% among Saudi patients, and 17.6% among non-Saudi patients. Xpert® HPV produced positive results in 30 (17.8%) of the samples. The overall concordance rate between the two tests was 98.2%, and the positive concordance rate was 91%. There were three samples tested positive by hc2 that tested negative by Xpert® HPV. HPV 16, HPV 18/45 and HPV other types were the most

KEYWORDS
HPV;
Human Papilloma virus;
ASC-US;
Atypical squamous cells of undetermined significance

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http://dx.doi.org/10.1016/j.jiph.2016.04.017
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common types. Both tests have a reasonable positive concordance rate, although hc2 detected more cases than Xpert® HPV. Xpert® HPV provides a viable alternative to the hc2 test with similar detection results for samples with ASC-US.

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Introduction

Human papilloma virus (HPV) infection may lead to persistent infection and is the leading cause of cervical cancer. Genital HPV is a sexually transmitted infection and almost all cervical cancers are caused by high risk HPV (hrHPV) types. The risk of genital HPV infection includes the number of sexual partners, new partner, older sex partner, use of oral contraceptive, and condom use. HPV 16 and 18 are the most common hrHPV and responsible for 70% of cervical cancers. Screening for high risk HPV (hrHPV) is recommended for early detection of cervical cancer [1]. The U.S. Food and Drug Administration (FDA) approved four hrHPV tests: Hybrid Capture® 2 High-Risk HPV DNA Test™ (hc2) (Qiagen, Germantown, MD; 2003), Cervista (Hologic, Bedford, MA; 2009), Cobas HPV test (Cobas; Roche Molecular Systems, Pleasanton, CA; 2011), and Aptima (Gen-Probe/Hologic, San Diego, CA) [2]. The hc2 test detects hrHPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 [2]. The Cepheid Xpert® HPV assay takes one hour to complete and detects the same HPV high risk types in addition to type 66 [2]. A previous study exhibited similar detection results using Xpert® HPV and Cobas [2]. The hc2 test has been validated in large randomized trials with post-examination follow-up [3].

The use of molecular detection of hrHPV in women with atypical squamous cells of undetermined significance (ASC-US) was shown to detect cancer and carcinoma in situ better than cytology-based methods but at a cost of having lower specificity [4]. In this study, we examined the performance of Xpert® HPV and hc2 tests in patients with ASC-US. We also examined the prevalence of hrHPV among women in Saudi Arabia, where data on HPV is scarce [5–7].

Materials and methods

Study population and design

The study population included women of all ages who were screened for cervical cancer. Cervical cells obtained from Papanicolaou (Pap) smear specimens were collected with a Cytobrush, washed into PreservCyt collection medium (Hologic, Inc., Marlborough, MA), and maintained at room temperature. At Johns Hopkins Aramco Healthcare (JHAH), all samples were reviewed for integrity by a cytotechnologist and a pathologist confirmed the final diagnosis. At JHAH, only samples showing ASC-US were referred for hrHPV testing by hc2 assay (Qiagen, Gaithersburg, MD). These samples were subsequently tested by Xpert® HPV assay. Both assays were performed in the molecular diagnostic laboratory at JHAH between January and July of 2014. One hundred sixty-eight Pap smears with ASC-US were submitted to the molecular diagnostic laboratory for HPV screening.

The Hybrid Capture® 2 High-Risk HPV DNA Test™ (hc2; Qiagen, Gaithersburg, MD)

The hc2 test was performed on all ASC-US specimens. Briefly, 4ml total volumes of PreservCyt samples were processed using a Qiagen Sample Conversion Kit. The kit prepares cells for hybridization in the hc2 tests by converting the PreservCyt samples into hc2 compatible sample types. Samples were then tested with hc2 high risk RNA probes according to the manufacturer’s recommendations [8]. The hc2 probes detect 13 high risk genotypes as noted above. The test was done per package insert [8]. In summary, a specific hrHPV RNA probes hybridize target DNA resulting in RNA:DNA hybrids. These hybrids were then captured onto the surface of a coated microplate well plate. A specific alkaline phosphatase conjugated antibodies for the resultant RNA:DNA, is added and conjugated with a chemiluminescent substrate antibodies. The formed conjugated antibodies to each captured hybrid induce signal amplification. A light was formed as the chemiluminescent substrate was cleaved by the bound alkaline phosphatase. The emitted light was measured as relative light units (RLUs) by a luminometer. The light emitted intensity is relatively proportion to the amount of the target DNA in the specimen. A positive result is based on relative light units (RLU) as measured by
Comparison of Xpert® HPV and Hybrid Capture® 2 for hrHPV

Table 1  Results of hc2 and Xpert® HPV results based on nationality.

<table>
<thead>
<tr>
<th></th>
<th>Saudi (N = 134), n (%)</th>
<th>Non-Saudi (N = 34), n (%)</th>
<th>All (N = 168), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hc2 positive*</td>
<td>27 (20)</td>
<td>6 (17.6)</td>
<td>33 (19.6)</td>
</tr>
<tr>
<td>Xpert® HPV Any Positive*</td>
<td>25 (18.5)</td>
<td>5 (14.7)</td>
<td>30 (17.8)</td>
</tr>
<tr>
<td>HPV 16</td>
<td>6 (4.4)</td>
<td>0 (0)</td>
<td>6 (3.6)</td>
</tr>
<tr>
<td>HPV 18/45</td>
<td>4 (3)</td>
<td>0 (0)</td>
<td>4 (2.3)</td>
</tr>
<tr>
<td>HPV 16 and ‘others’</td>
<td>1 (0.7)</td>
<td>0 (0)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>(coinfection with HPV 16 and other types)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV other types</td>
<td>14 (10.4)</td>
<td>4 (11.8)</td>
<td>18 (10.7)</td>
</tr>
</tbody>
</table>

*p Value > 0.05, Fisher’s exact test.

Results

A total of 168 ASC-US samples were analyzed in this study. Of those samples, 134 (79.8%) were from Saudi patients. The mean age ± SD was 40.6 ± 11.6 years, with a range of 20–83 years.

Xpert® HPV was positive in 30 (17.8%; 95% CI of 12.02–23.58) of the samples, whereas 33 (19.6%; 95% CI of 13.6–25.6) were positive by hc2. There was no statistically significant difference in HPV-positivity between Saudi and non-Saudi women (Table 1). The most common HPV types were HPV other types (10.7%), HPV 16 (3.6%) and HPV 18/45 (2.3%). One patient had a coinfection with HPV 16 and other types (Table 1).

Table 2 shows the overall concordance rate (98.2%; 95% CI = 81–100%) between the two tests and the positive concordance rate (91%; 95% CI = 96–100%) (p value 0.08). Three specimens tested positive for hrHPV by hc2 but negative by Xpert® HPV, all of which exhibited carcinoma in situ neoplasms.

Discussion

We found high overall concordance and positive concordance rates between Xpert® HPV and Hybrid

Cepheid Xpert® HPV test (Cepheid, Sunnyvale, CA, United States)

All samples were tested by Xpert® HPV assay simultaneously with hc2 assay. Based on the manufacturer’s instructions, samples were shaken for several seconds, and 1 ml total volume of PreservCyt was poured into an Xpert® HPV cartridge and subsequently loaded into Cepheid Xpert Diagnosis instrument that uses a second generation real-time PCR [2]. Interpretation of assay results followed the manufacturer’s instructions as described previously [2]. This assay detects the previously mentioned 13 high risk types and possibly HPV type 66 [2,9]. Assay results were reported as negative or positive for HPV16, HPV 18/45, or other HPV types. A Probe Check Control and a Sample Adequacy Control were included in each cartridge.

Statistical analysis

The percentages of positive tests were assessed for significance using a Fisher’s exact test to measure the difference between Saudi and non-Saudi women. The McNemar χ² test was used to assess significance of discordance. A p-value of ≤0.05 was considered to be significant.

Table 2  Concordance between the results of hc2 and Xpert® HPV results.

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpert® HPV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>30</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>135</td>
<td>138</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>135</td>
<td>168</td>
</tr>
</tbody>
</table>

Positive concordance rate 30/33 = 91% (95% CI = 81–100%).
Overall concordance rate 165/168 = 98.2% (95% CI = 96–100%).
McNemar Chi-square statistic 3.0 (p-value is 0.083).
Table 3  Summary of the different studies from Saudi Arabia of the prevalence of HPV among different population.

<table>
<thead>
<tr>
<th>Reference number</th>
<th>Population</th>
<th>Test used</th>
<th>Number</th>
<th>Number (%) positive</th>
<th>Most common types</th>
</tr>
</thead>
<tbody>
<tr>
<td>[3]</td>
<td>Histopathologically proven, locally advanced, cervical cancer</td>
<td>Linear Array HPV Genotyping Test (LA HPV GT; Roche Diagnostics).</td>
<td>100</td>
<td>82 (82)</td>
<td>HPV-16 (71%); HPV-31 (7%)</td>
</tr>
<tr>
<td>[4]</td>
<td>Paraffin-embedded cervical tumors</td>
<td>Linear Array kit (Roche Diagnostic)</td>
<td>100</td>
<td>89 (89)</td>
<td>HPV-16 (65.2%), 31 (7.9%), 45 (6.7%)</td>
</tr>
<tr>
<td>[5]</td>
<td>Cervical cancer and carcinoma in situ</td>
<td>DNA sequencing and reverse line blot hybridization assay</td>
<td>90</td>
<td>86 (95.5)</td>
<td>HPV-16 (63.4%); HPV-18 (11.1%); HPV-45 (4.5%)</td>
</tr>
<tr>
<td>Current study</td>
<td>Atypical squamous cells of undetermined significance</td>
<td>Xpert® HPV and HC2 Hybrid Capture® 2 (HC2)</td>
<td>168</td>
<td>Hc2 test: 33 (19.6); Xpert® HPV gave: 30 (17.8)</td>
<td>HPV 16 (3.6); HPV 18/45 (2.3); HPV 16 and other types (0.6)</td>
</tr>
</tbody>
</table>

Capture® 2 High-Risk HPV DNA Test™ (hc2) assays. High concordance rates suggest that either test would be effective diagnostics in clinical practice. In a previous study, the positive rate of hrHPV using Xpert® HPV was 64.1% (95% CI = 60.4–67.7%) compared to 58.2% (95% CI = 54.5–61.9%) when using hc2 in women referred for colposcopy [2]. The prevalence of hrHPV has been determined as 39.3% and 45.6% by Cobas Amplicor and hc2, respectively, with an overall agreement of 89.2%. [10–12] in different populations. In this study, positive rates of hrHPV were 17.8% for Xpert® HPV compared to 19.6% for hc2. In previous studies of Saudi women with cervical cancer, HPV infection was detected in 89%-95.5% of the cases [5–7], and the most common genotypes were HPV-16 (63.4%), HPV-18 (11.1%), HPV-45 (4.5%), and HPV-33 [5]. These studies differ from ours in the type of specimen used and pathology, which may give rise to differences in the prevalence of HPV infection (Table 3).

There is discordance between hc2 and Xpert® HPV assays. False-positives can arise from insufficient denaturation of the cervical samples from PAP smears in the hc2 test procedure. Partial or incomplete denaturation of non-specific RNA/DNA hybrids that are present in the cervical sample may cause a false positive result [11]. False-positives could also occur due to contamination of the hc2 HPV DNA specimen with non-specific RNA/DNA hybrids [11]. It has been shown that hc2 probes cross-react with low risk HPV-6 and HPV-42 types at concentrations ≥4 ng/ml [8]. Cross-reactivity in the hc2 assay may occur due to the presence of bacterial plasmid pBR322 in cervical samples [8] and the presence of genetically similar HPV types [13]. The reason for discordance among three samples in the current study is not clear. The presence of interfering substances, such as anti-fungal cream, contraceptive gel, or douche, may account for this discrepancy. The difference in volume used in both tests or low HPV DNA copies in analyzed specimens may have an effect on the detection of HPV.

Several HPV-negative samples analyzed by two PCR assays have been shown as positive by hc2 [8]. The DNA sample used in the PCR assays may not be representative of the HPV types present in the sample used in the hc2 High-Risk HPV DNA assay [10]. Further studies are needed to characterize the three Hc2-positive/Xpert® HPV-negative cases in this study using alternative technologies for further evaluation of high risk targets. In conclusion, Xpert® HPV provides a viable alternative to the hc2 test with good agreement for samples with ASC-US. Further studies are needed to provide outcome data such that sensitivity and specificity for underlying pre-cancer (histologically confirmed CIN2+) may be derived for either assay.

**Funding**

No funding sources.

**Competing interests**

None declared.
Ethical approval
The Study was approved by the Johns Hopkins Aramco Healthcare Institutional Review Board.

Acknowledgment
Xpert® HPV kits were provided by Cepheid Company for the validation purposes.

References


