Feasibility, validity and acceptability of self-collected samples for human papillomavirus (HPV) testing in rural Malawi

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Abstract

Aim
The World Health Organization (WHO) recently endorsed human papillomavirus (HPV) testing as a cervical cancer screening method in countries without established programs. Self-collection for HPV testing may be an effective way to expand screening. Our objective was to assess the feasibility, validity, and acceptability of self-collection for HPV testing in a population of care-seeking, unscreened women in rural Malawi.

Methods
We enrolled women reporting to a rural Malawian clinic from January to August 2015. Participants were offered the option to self-collect a vaginal sample and the study clinician collected a cervical sample for HPV testing. Using the clinician-collected sample as the reference standard, we calculated a kappa statistic, sensitivity, and specificity by hr-HPV type. Participants also received a brief survey assessing acceptability of the procedure.

Results
Among the 199 enrolled women, 22% had any high risk-HPV. Comparing self- and clinician-collected samples for HPV testing, we found generally high agreement (κ = 0.66-0.90) and high specificity (98%-100%), but varied sensitivity (50%-91%) for different types of hr-HPV. We also found that self-collection was acceptable, with 98% of women reporting it was easy to do and 99% reporting willingness to do so again.

Conclusions
WHO guidelines recommend that treatment is available immediately after a positive screening test for clinic-based cervical cancer screening programs. Our findings demonstrate that self-collection of samples for HPV testing is a feasible and acceptable method of cervical cancer screening in this rural Malawian population. High agreement between the self- and clinician-collected samples and high levels of acceptability among women in the study suggest that self-collection of vaginal samples for HPV testing may be effectively incorporated into screening programs among rural, largely unscreened populations.

Introduction
Effective and widespread cervical cancer screening has greatly reduced cervical cancer incidence and related morbidity and mortality. The most commonly used cervical cancer screening method worldwide is a Pap test, which involves the collection of cervical cells for examination under a microscope by a cytopathologist. Pap testing detects abnormal cells in the cervix and enables early detection and treatment of cervical cancer. However, Pap screening programs have low feasibility in limited-resource settings owing to a lack of infrastructure and trained personnel, limited health budgets and competing healthcare priorities.

To address these barriers, some national screening programs use alternatives to traditional cytology (Pap testing), such as visual inspection of the cervix with acetic acid (VIA). VIA involves unaided (naked eye) inspection of the cervix after application of acetic acid to identify abnormal tissue. While VIA eliminates some constraints of Pap testing, such as cost and need for multiple visits, there can be high variability by provider in the quality of VIA screening.

DNA testing for human papillomavirus (HPV) offers an accurate alternative to VIA. The WHO recommends hr-HPV DNA testing as the primary cervical cancer screening approach in places where Pap testing has not been established. Similar to other screening methods, cervical samples are typically gathered by a clinician during the course of a pelvic examination, but samples can also be self-collected by women themselves with a swab. WHO recommends screening with an HPV test and treatment over screening with VIA and treatment where feasible. HPV testing is also recommended as first line screening followed by VIA and treatment.

When successfully introduced, self-collection of samples for HPV testing can increase screening for hard to reach women or women who do not come in for screening tests. Self-collected samples have been shown to perform comparably to clinician-collected samples, but published findings suggest that the population and method of collection or testing are important to consider when assessing the utility of self-collected samples.
The recently lowered costs of HPV DNA testing may make this method of cervical cancer screening a viable screening method in low-resource settings. In a combined joint review of 21 countries throughout Africa and Asia, we aimed to evaluate how this method of sample collection performed in a low-resource setting. Previous research suggests that many women in rural Malawi would be willing to self-collect a sample at home, yet no research has examined self-collection in a clinical setting or whether women's hypothetical willingness to translate into actual behavior if offered an opportunity to provide a self-collected sample. We sought to assess the validity, feasibility and acceptability of using the GeneXpert HPV Assay to test self-collected vaginal samples in a rural clinic in Lilongwe District, Malawi.

**Methods**

**Study setting and population**

Women were recruited for this study as part of a larger clinic-based study examining sexual and reproductive tract infections. Briefly, from January to August 2015, any woman who presented to the study clinic in rural Lilongwe District, Malawi, with any genitourinary symptom (including abnormal menstrual cycle or patterns of bleeding; pain with urination, pain during sex, abnormal pain, lower back pain, or any type of pelvic pain; incontinence or unusual urinary odour, frequency or colour; unusual vaginal discharge in terms of quantity, odour, colour or consistency) was referred to a study clinic to be assessed for eligibility. Women were eligible if they were 18-49 years of age, spoke Chichewa, had at least one genitourinary symptom that was not consistent to be examined and give biological specimens for testing, and resided in Lilongwe District. Women who were pregnant or menstruating were ineligible. Women were provided written informed consent to participate either by signature or thumbprint.

**Data collection**

**Screening**

Women were examined in a private clinic room by the study clinician. At the start of the exam, each woman was offered the option to self-collect a vaginal sample for HPV testing. If she agreed, she was given a sterile, cotton-tipped swab and instructions on how to collect the vaginal sample. The clinician remained in the study room, on the other side of a privacy screen, in case the participant had any questions about collecting the sample. After collection, the clinician placed the swab in 20 ml of Preservcyt solution (Hologic, California). HPV DNA testing was performed at the end of the study (Cepheid, Sunnyvale, California). The swab was placed in 20 ml of Preservcyt solution and was tested using the GeneXpert assay as the gold standard.

Women were examined in a private clinic room by the study clinician. After the clinical exam, the patient was sent to a separate study room where a research assistant, who did not provide clinical care, administered a brief questionnaire capturing demographic characteristics and the acceptability of the self-collection procedure. The questionnaire included items about the ease of collecting samples and understanding instructions, using a five-point scale ranging from very easy to very difficult. Using the same scale, we also assessed women's confidence in their ability to self-collect a sample, their preferences for collection of samples for HPV testing, whether they would recommend self-collection to a friend, and concerns about self-collection. Questions were developed based on previous literature and work of the study authors. All survey questions were recorded directly into the Magpi data collection system (Mappi, Washington, DC) and uploaded to an internet-based storage system daily.

**Questionnaire**

Testing of clinician- and self-collected samples was not performed at the same time. A sample was collected by the clinician, placed the swab in 20 ml of Preservcyt solution (Hologic, California). HPV DNA testing was performed at the end of the study (Cepheid, Sunnyvale, California). The swab was placed in 20 ml of Preservcyt solution and was tested using the GeneXpert assay as the gold standard.

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We screened 234 women to enroll 200 in the parent study, of whom consented to HPV testing. Women without hr-HPV were slightly older than women with hr-HPV (median age 34 vs. 32 years) and more likely to be married (97% vs. 77%; Table 1). Sixteen women (8%) were initially noted as having a positive VIA screen although two of these were later determined to be false positives. Of the remaining 184 women, 10 presented with abnormal VIA results and four with inconclusive results that required additional screening. All 14 women were referred to the district hospital for HPV 18/45 at 91% (95% CI: 59%, 100%) and lowest for HPV 16 and additional hr-HPV types.

When restricting the analyses to women older than 30 years, based on current HPV testing guidelines, we found that there was increased sensitivity for detection of all hr-HPV types combined, HPV 16 and additional hr-HPV, but a decrease in sensitivity in detection of HPV 18. Restricting to younger women also led to an increase in most kappa values characterizing agreement between clinician- and self-collected samples, including for all categories of hr-HPV (κ ≤ 0.83), HPV 16 (κ ≤ 0.85) and the additional hr-HPV category (κ ≤ 0.77). Restricting to older women (who are likely to have fewer transient infections) we found that in general there was increased kappa agreement and sensitivity although it was not consistent across all hr-HPV types.

**Results**

**Study population and prevalence of hr-HPV infections with clinician-collected sampling**

All women who presented to the study clinic for cervical cancer screening were enrolled in the parent study. We screened 234 women to enroll 200 in the parent study, of whom consented to HPV testing. Women without hr-HPV were slightly older than women with hr-HPV (median age 34 vs. 32 years) and more likely to be married (97% vs. 77%; Table 1). Sixteen women (8%) were initially noted as having a positive VIA screen although two of these were later determined to be false positives. Of the remaining 184 women, 10 presented with abnormal VIA results and four with inconclusive results that required additional screening. All 14 women were referred to the district hospital for HPV 18/45 at 91% (95% CI: 59%, 100%) and lowest for HPV 16 and additional hr-HPV types.

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**Agreement between clinician-collected and self-collected samples**

We found high agreement between HPV results from self- and clinician-collected samples as measured by the kappa statistic, which measures agreement beyond chance alone. The highest agreement between self- and clinician-collected samples was for HPV 18 (κ = 0.90). The agreement between self- and clinician-collected samples for any type of hr-HPV and the additional hr-HPV types were similar (κ ≤ 0.77, x ≤ 0.74; Table 5). Overall compared to clinician-collected samples, the self-collected samples were highly specific and varied in sensitivity by type of HPV (Table 3).

**Table 2: HPV results, by collection modality**

<table>
<thead>
<tr>
<th>Clinician</th>
<th>n (%)</th>
<th>Self</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV 18</td>
<td>66</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>HPV 16</td>
<td>66</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>Additional hr-HPV</td>
<td>66</td>
<td>66</td>
<td>66</td>
</tr>
</tbody>
</table>

*Note: HPV results grouped by GenXpert category.*
Table 4: Concordance between HPV test results by self- and clinician-collected samples in women older than 30 years of age

<table>
<thead>
<tr>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Kappa1 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All types of hr-HPV</td>
<td>79 (57, 93)</td>
<td>99 (95, 100)</td>
</tr>
<tr>
<td>HPV 16</td>
<td>75 (19, 99)</td>
<td>100 (97, 100)</td>
</tr>
<tr>
<td>HPV 18/45</td>
<td>83 (36, 100)</td>
<td>99 (95, 100)</td>
</tr>
<tr>
<td>Other hr HPV</td>
<td>75 (18, 93)</td>
<td>98 (94, 100)</td>
</tr>
</tbody>
</table>

1Kappa measures expected vs. observed agreement

Table 5: Acceptability of self-collection

<table>
<thead>
<tr>
<th>Concerns about self-collection</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Would self-collect multiple times</td>
<td>158 (74)</td>
</tr>
<tr>
<td>Might hurt</td>
<td>22 (12)</td>
</tr>
<tr>
<td>Might not be accurate</td>
<td>20 (11)</td>
</tr>
<tr>
<td>Might not do it correctly</td>
<td>14 (7)</td>
</tr>
</tbody>
</table>

Discussion

To our knowledge, this is the first study to examine self-collected samples for HPV testing using the GeneXpert HPV assay. Our findings suggest that self-collection of samples for HPV testing is a feasible and acceptable method of cervical screening in this rural, Malawian population. Although the specificity was high, we found that some HPV-positive samples were missing. While the specificity of the self-collected tests was very high in our study, sensitivity varied by type of HPV. In other words, HPV testing using self-collected samples accurately detected HPV-negative women, but the ability to detect HPV-positive women using self-collected samples was more variable. When younger women were excluded from the sample, sensitivity was more comparable across the different types of hr-HPV, although the sensitivity for detection of HPV 18/45 was slightly reduced. This overall pattern of lower sensitivity but higher specificity of self-collected vs. clinician-collected samples is similar to findings from a study of Gambian women, where self-collected samples had a sensitivity of 0.64 (95% CI: 0.52, 0.83) and specificity of 0.94 compared to clinician-collected cervical samples16.

Our findings suggest that self-collection of samples for HPV testing was widely acceptable, easy to perform, and preferred by women. Combined with results from other research, our study provides evidence that self-collection could be used in an outreach capacity to increase screening in hard-to-reach populations. For example, among women in Argentina, women who were offered the opportunity to self-collect a sample in the home community health workers were four times as likely to be screened for cervical cancer than women who were not offered the option to self-collect17. In a randomized controlled trial conducted in Uganda, 98% of women in the HPV self-collection arm were screened for cervical cancer while in the control arm (VIA), only 48% of women received cervical screening18.

To be successfully implemented, screening programs using HPV testing will need to consider clinician and laboratory perspectives alongside other programmatic considerations. For example, in our project, the study clinician found that after a small number of participants had enrolled, it was very simple to collect cervical samples using the cervical brush. On the other hand, he identified challenges explaining the self-collection procedure to women, suggesting that a future screening program must provide detailed instructions or have a present a healthcare provider to answer questions. The laboratory technician found it easy and fast to test samples using the GeneXpert HPV test. However, some samples required a repeat test due to machine error and added to costs of HPV testing. We also experienced challenges in procuring the transport medium – an expensive part of the testing procedure, and this supply issue must be addressed before a larger rollout of any HPV testing program is possible in this region. Studies of other polymerase chain reaction (PCR) based HPV DNA tests suggest that more commercially-available and inexpensive transport media (e.g. Scope mouthwash)19 or the collection and storage of dry samples, may perform comparably20, although to date these approaches have not been validated for the GeneXpert HPV DNA test.

Our findings must be interpreted in light of important study limitations. As our project was undertaken as a secondary arm of a larger study, the inclusion excluded women aged under 30 for whom HPV testing is not currently recommended. However, subgroup analyses with older women in the recommended range suggest that kappa of self-collected samples and sensitivity/specificity findings were valid for both groups. As this project was nested in a pilot study, we had a relatively small sample size, especially when excluding women under age 30. The self-collected samples were taken during the lower menstrual cycle21. While the study population was nested in a larger study, we determined the feasibility of self-collection compared to clinician-collected samples. Generalizability of our results may also be limited as we enrolled care-seeking women who presented with genitourinary symptoms to a medical facility. As HPV infections and early cervical lesions do not lead to noticeable symptoms, it will be important to determine whether HPV screening (via self-collected samples) remains acceptable to women with genitourinary-symptomatic illness. We also enrolled women presenting at a clinic, so we cannot extrapolate our results to cervical cancer screening programs in an outreach capacity. Additionally, our study clinician was in the Northern region of the country in which the self-collection option may not be generalizable to an outreach setting where there are not trained personnel available. Our study also only assessed the acceptability of self-collection compared to clinician-collected sampling, but we note that WHO guidelines recommend that immediate treatment be available for any clinic-based cervical cancer screening program. Lastly, the acceptability questions may be influenced by response bias with participants voicing more favorable views on self-collection than they actually felt. We attempted to minimize this bias by having a non-clinician research assistant deliver the questions in a separate, study room.

While the rates of cervical cancer incidence and mortality have been reduced precipitously in the last 40 years globally, the burden of disease falls disproportionately on women in low-resource settings without accessible screening programs. Although a number of HPV screening programs using self-collected samples for HPV testing may be a feasible, valid, acceptable, and effective cervical cancer screening method in this rural Malawian population.

References

11. Gik M, van Kemmeland FJ, Heideman D, A, Berkhof J, Rozendaal TL1TR001069 from the National Center For Advancing Translational Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center For Advancing Translational Sciences or National Institutes of Health.

Conflict of interest

Authors have no conflicts of interest to disclose.


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